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Effect of L-Thyroxine on Renal Excretion of Water and Electrolytes in Both Normal and Mercury-Intoxicated Rats

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Summary. The urine output and the change in excretion of electrolytes (sodium, potassium, calcium, chloride, inorganic phosphate) in rats following mercury-induced acute tubular lesions showed marked recovery during L-thyroxine therapy. The kidney mechanisms responsible for this effect are discussed, considering especially the observation that rats treated with L-thyroxine have a reduced urinary output, unlike other species, including man, which react with polyuria. The oliguric effect of thyroid hormone in rats is attributed to the greater length of the vasa recta and loops of Henle.

Key words: Acute renal failure - Mercury-induced tubular lesions - L-thyroxine therapy - Thyrogenic nephrotropic effects - Renal electrolyte excretion - Diuresis.

The strong influence which thyroid hormone has upon various aspects of renal function has been known for some time. An excess of L-thyroxine or L-triiodothyronine is accomplished by a marked increase in the rate of renal plasma flow (RPF) and glomerular filtration (GFR) (30, 35-37). Moreover, considerable stimulation of the energy-consuming epithelial transport of glucose, PAH, trypan blue, and sodium in the proximal tubule can be observed (7, 15, 16, 30, 36, 37). The upper part of the nephron shows cellular hypertrophy and hyperplasia (4-6, 8, 19, 28, 33), activation of numerous enzymes (2, 5, 14, 19, 31, 32, 42, 45), and increase of O_2 consumption (5, 19). A diuretic response is seen in man and other species, such as rabbits (36, review: 5, 19, 39).

In previous studies it has been demonstrated that thyroid hormone treatment also leads to an improvement of renal function in rabbits with severe mercury-induced tubular lesions as well as in patients with manifest acute renal failure (30, 35-37, 39, 40).

The thyroid-hormone-induced increase of RPF and GFR has been attributed in the past to the augmentation of cardiac output and arterial hypertension (review 5, 19), an interpretation recently rejected in view of the autoregulation of renal circulation (39). A more rational hypothesis, which takes into account the physiological mechanism of autoregulation of renal blood perfusion, has been proposed concerning the RPF- and GFR-raising effect of thyroid hormone (39). According to this hypothesis the filtered load of sodium chloride, and therefore the GFR and RPF, adapt to the high "active" (obligatory) sodium chloride reabsorption in the proximal tubule and in the loop of Henle, whereby the juxtaglomerular apparatus is in operation.

Furthermore, the relative length of the renal tubule between the glomerulus and macula densa determines the concentrating and reabsorption capacity of the kidney by influencing the action of the counter-current mechanism : the longer the nephron, the greater the concentrating capacity and ability of the nephron to react to thyroid hormone administration. In this study, the rat was chosen because of its especially long vasa rectae and loops of Henle. If our hypothesis is valid (39, 40), thyroxine administration in the rat should - in contrast to other species - reduce the volume of urine output, thus contradicting the original cardiac output/arterial hypertension theory stated above. This would also indicate that thyroxine administration directly affects renal function, i.e., it positively influences acute renal failure, apparently by improving sodium chloride reabsorption in the proximal tubules and loops of Henle.

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MATERIAL AND METHODS

Rats. 94 young male albino rats (Sprague-Dawley) were kept in "automatic" cages designed for metabolic studies, beginning 5 days prior to the start of the experiment. They were fed standardized "altromine-R" dried food and permitted free access to water.

Sublimate ($HgCl_2$). A watery solution of 0.25% sublimate was injected subcutaneously into the animal's backs.

L-thyroxine ($C_{15}H_{10}J_4$-$NNaO_4$; molecular weight 798.86; L-triiodothyronine: 0.3%). A watery solution of 0.005% L-thyroxine was injected as above.

62 rats were subjected to sublimate intoxication as follows: 1st day 0.2 mg; 2nd day 0.3 mg; 3rd day 0.5 mg; 4th day 0.8 mg $HgCl_2$ per 100 g body weight (L.D. 15 - 20%). Animals which died spontaneously were replaced by new rats. After this intoxication period, one group of 18 animals (group "S") received no further treatment; two other groups "S/T_7" (n = 18) and "S/T_{21}" (n = 9) were treated for 7 and 21 days with 5 µg L-thyroxine per 100 g body weight daily, starting 24 hours after the last injection of sublimate. Two additional groups without sublimate pretreatment ("T_7" and "T_{21}") were given daily injections of 5 µg L-thyroxine for 7 and 21 days. Five sublimate-intoxicated rats served as controls for histological examination in order to confirm acute tubular necrosis. They were sacrificed one day after termination of mercury injection. Five additional rats without any treatment were used as normal controls for functional investigation.

Body weight of the animals was determined every second day. 24 hour urine samples were harvested in the "automatic" cages every two or three days. For the serum investigations, groups of three rats were exsanguinated by heart puncture under mild ether anaesthesia after 2 days of intoxication and on days 3, 5, 8, 22 and 28 in the post-intoxication period. Blood specimens were carefully centrifuged and the sera stored at -20°C until analysed.

Sodium, potassium and calcium were determined by means of flame photometry, chloride by electrochemistry, inorganic phosphate by photometric analysis, and osmolarity by electronic half-micro-osmometry.

RESULTS

All rats treated with sublimate showed poor mobility on the second day of intoxication and loss of appetite. After the four days of mercury administration, the animals were apathetic and their fur had lost its sheen. A total of 12 animals died, most of them during the first two days after intoxication. Three rats of group "S" died between the 9th and 24th day, while there were no deaths in the L-thyroxine-treated groups. All animals receiving only L-thyroxine remained well.

Histological examination of the five control rats revealed marked tubular necrosis after sublimate treatment proving a strong toxic effect on the tubular epithelium. Typically, the pars rectae of the proximal tubules were primarily involved. Figure 1 demonstrates that body weight decreased impressively after sublimate intoxication. But also L-thyroxine treatment led as expected to a marked loss of body weight even in the sublimate intoxicated groups. After termination of hormone therapy, body weight increased, confirming the preceding metabolic effect of L-thyroxine administration.

Results concerning urine volume and osmolarity, renal excretion of sodium, potassium, calcium, chloride and inorganic phosphate, as well as serum concentrations of the electrolytes are presented in Figures 2 to 8. The significant diminution of urinary output and the retention of sodium (chloride) during thyroid hormone therapy are the most interesting findings.

DISCUSSION

The rise in urinary volume shortly after the intoxication period (Fig. 2) does not rule out a strong inhibition of glomerular filtration. In fact, the serum levels of urea and creatinine measured during the polyuric phase were clearly above normal: serum urea 188 mg% and serum creatinine 1.53 mg%. This corresponds to a creatinine clearance of 17.6% of the normal. Renal sodium output and urinary osmolarity (Figs. 4b and 3) were significantly reduced, suggesting that the primary decrease of sodium reabsorption in the damaged proximal tubules was answered, via the juxtaglomerular apparatus, by an adaptive decrease of RPF and GFR (44) in order to prevent life-threatening sodium and volume losses. This pattern of diminution was also found in experiments with rabbits (37). Low urine production following the primary polyuric phase (Fig. 2) may partly result from dehydration.

L-thyroxine treatment initiated immediately after complete sublimate intoxication totally inhibited the early polyuria (Fig. 2). The average osmolarity increase during the first seven days after intoxication was more than 200 mOsmol/l (Fig. 3b). On the other hand, the urinary volume immediately increased after termination of thyroid hormone administration. The same pattern was noticed in normal rats treated only with L-thyroxine.

This result is surprising, since exogenous L-thyroxine increases urinary output not only in oliguric hypothyroid patients (1, 3, 13), but also in euthyroid humans and rabbits (3, 13, 25, 26, 36, 38) and even in patients with diabetes insipidus (9, 11,

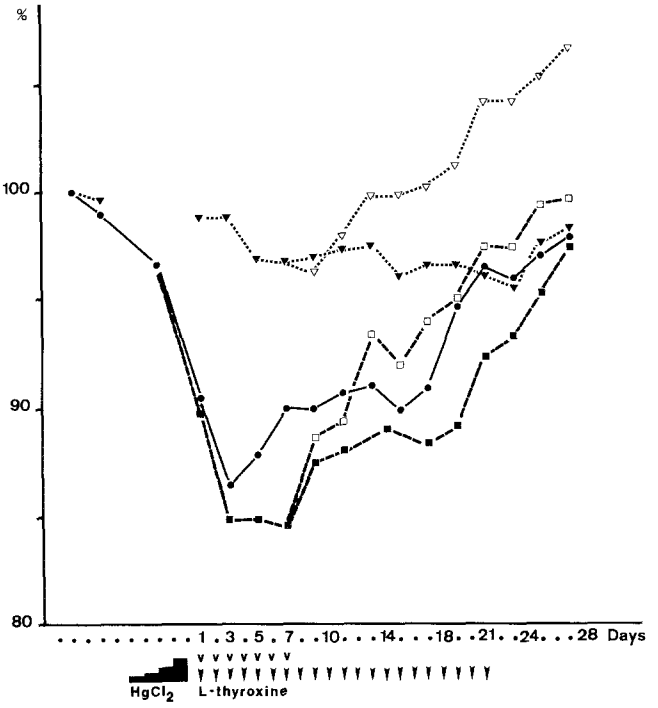

Fig. 1. Mean body weight expressed in percentage (%) of the values obtained before starting the experiment: ●——● : sublimate-intoxicated group "S"; □▬▬□ : group "S/T7", treated 7 days with L-thyroxine after mercury intoxication; ■▬▬■ : group "S/T21", treated 21 days with L-thyroxine after mercury intoxication; ▽▪▪▪▪▽ :group "T7", administration of L-thyroxine alone for 7 days; ▼▪▪▪▪▼ : group "T21", administration of L-thyroxine alone for 21 days. Sublimate intoxication is followed by a dramatic loss of body weight. L-thyroxine administration leads to an impairment of body weight gain

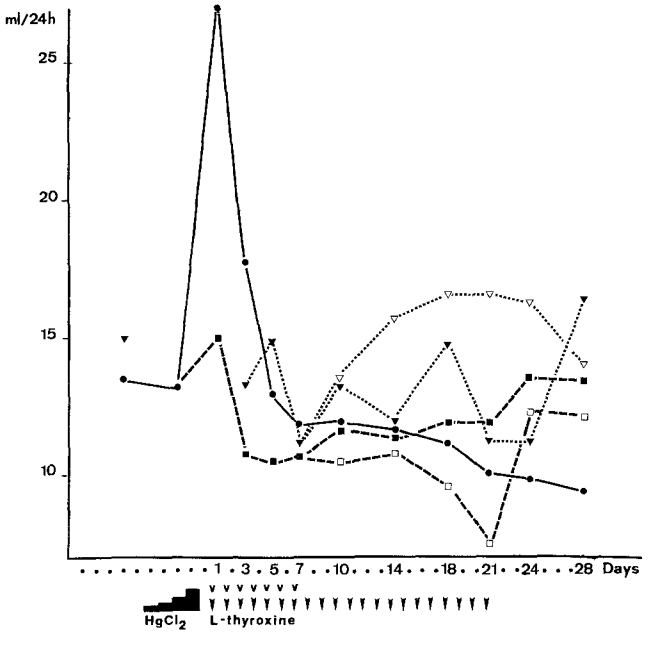

Fig. 2. Urinary volume in ml/24 h expressed as mean values. Symbols as in Figure 1. Sublimate intoxication leads to a strong increase of urinary volume. L-thyroxine administration totally reverses this effect

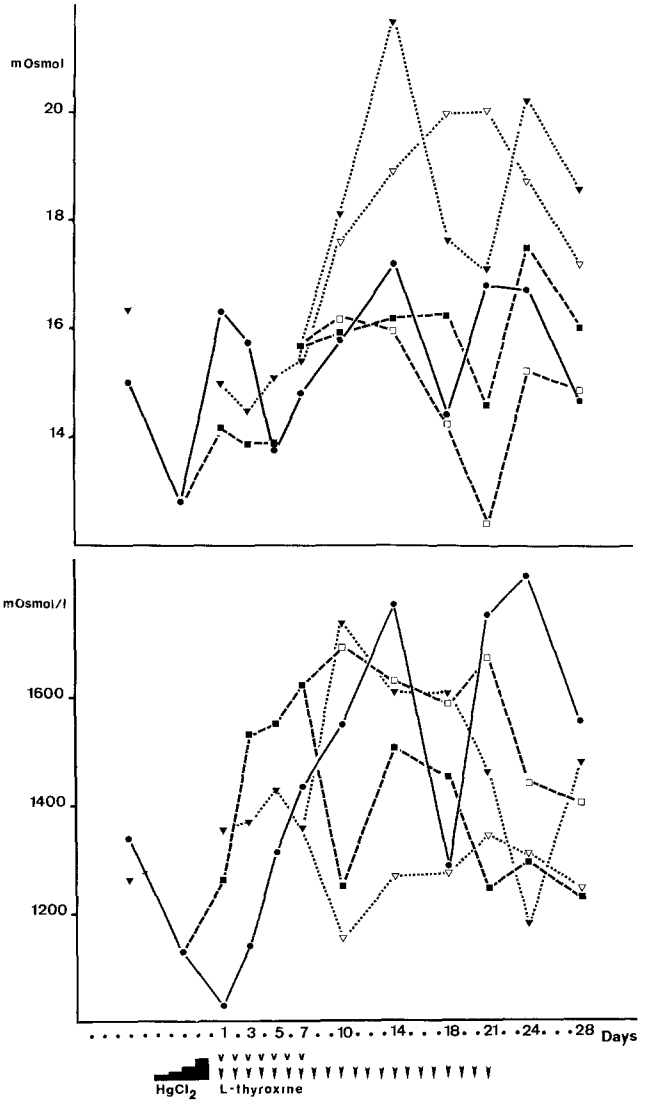

Fig. 3. Total urinary mOsmols per 24 h (upper) and mOsmolarity/l (lower). Symbols as in Figure 1. L-thyroxine administration effectively increases the mOsmolarity/l during the first week after the intoxication period (group "S/T" in comparison to group "S")

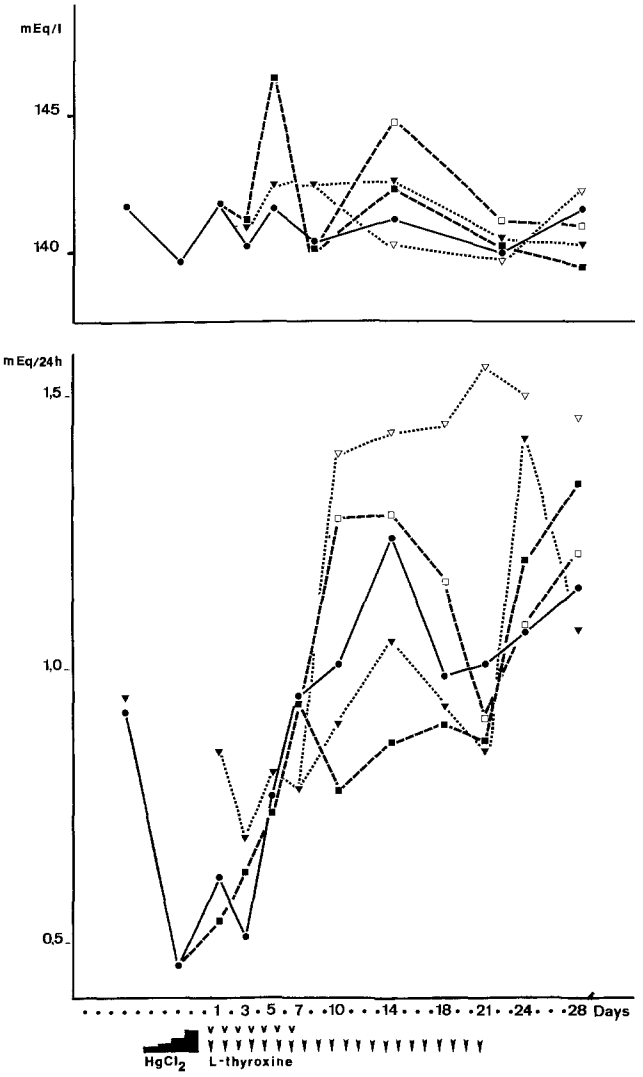

Fig. 4. Serum concentrations (upper) and urinary excretions of sodium per 24 h (lower). Symbols as in Figure 1. After low sodium excretion during and shortly after the sublimate intoxication period (acute renal failure) the sodium loss increases indicating further tubular dysfunction. L-thyroxine administration causes diminished loss of sodium probably because of improved sodium reabsorption in the tubules

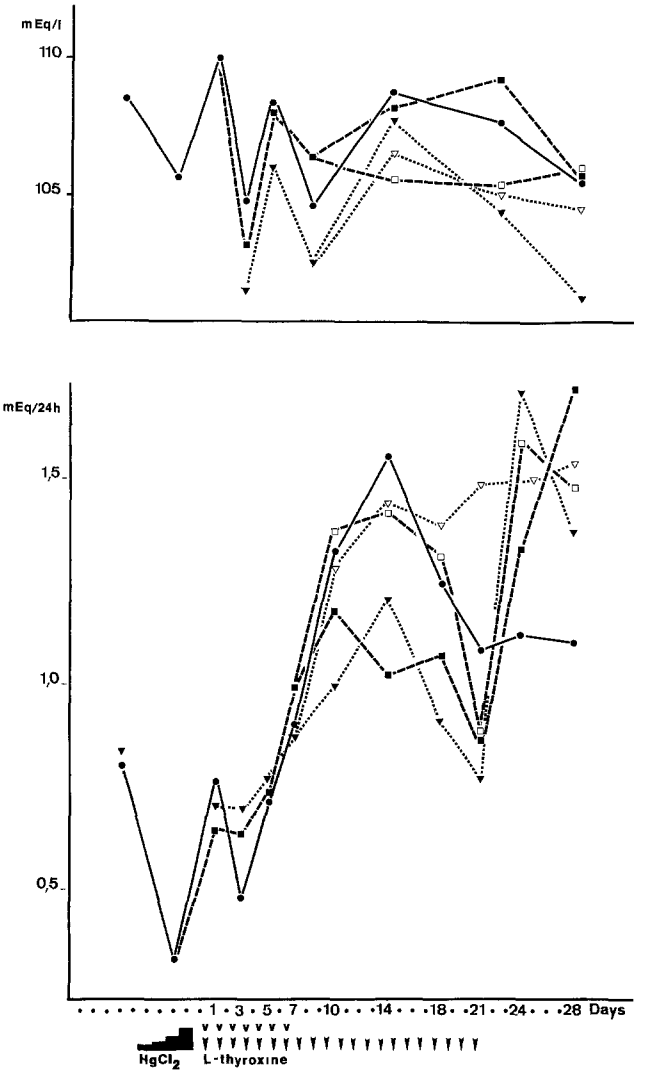

Fig. 5. Serum concentrations (upper) and urinary excretions of chloride per 24 h (lower). Symbols as in Figure 1. Chloride follows the pattern of sodium (Fig. 4). L-thyroxine possesses a sodium-chloride saving effect

13, 21). As far as the urinary volume is concerned, rats show an opposite reaction compared with other species although their glomerular filtration increases as usual (24).

This is in agreement with the results of Jahn et al. (17, 34), who found that hypothyroid rats have polyuria with reduced Tm_{H_2O} (18).

The diminution of urine output after thyroid hormone treatment can be explained by a high osmotic gradient in the medullary interstitium due to the H_2O-free sodium (chloride) reabsorption in the ascending branch of Henle's loop. This provides the motor for free water reabsorption in the distal tubule and collecting duct. Réville and Stéphan (27) were able to show that rats have, indeed, a high concentration of urea in the renal papilla during hyperthyroidism. The high osmolarity of the bladder urine during L-thyroxine treatment in group "T" as well as in group "S/T" (first seven days) is in agreement with this view (Fig. 3b). Furthermore, urine volume increased and osmolarity fell promptly after termination of hormone therapy (Fig. 2, 3b).

The thyroid hormone induced polyuria in other species, such as man or rabbit, probably reflects a decrease of the medullary osmotic gra-

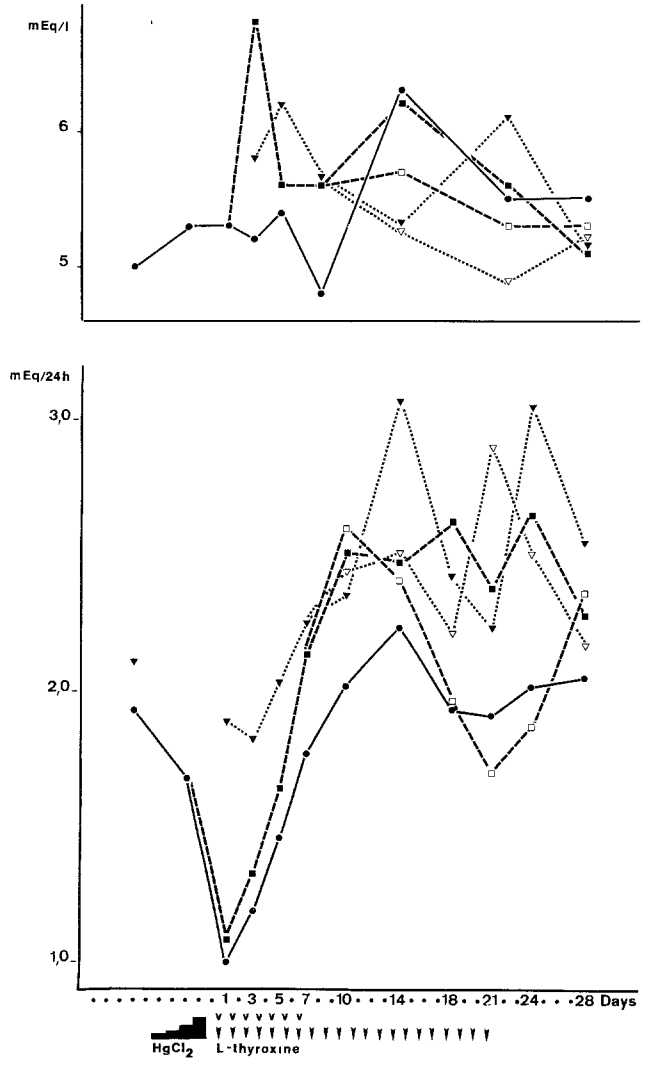

Fig. 6. Serum concentration (upper) and urinary excretions of potassium per 24 h (lower). Symbols as in Figure 1. L-thyroxine administration leads to a transient increase of serum potassium and a decrease of potassium excretion. The high transient hyperkalaemia in group "S/T" is obviously caused by the decrease of glomerular filtration (sublimate effect) as well as the loss of cellular potassium (thyroxine effect)

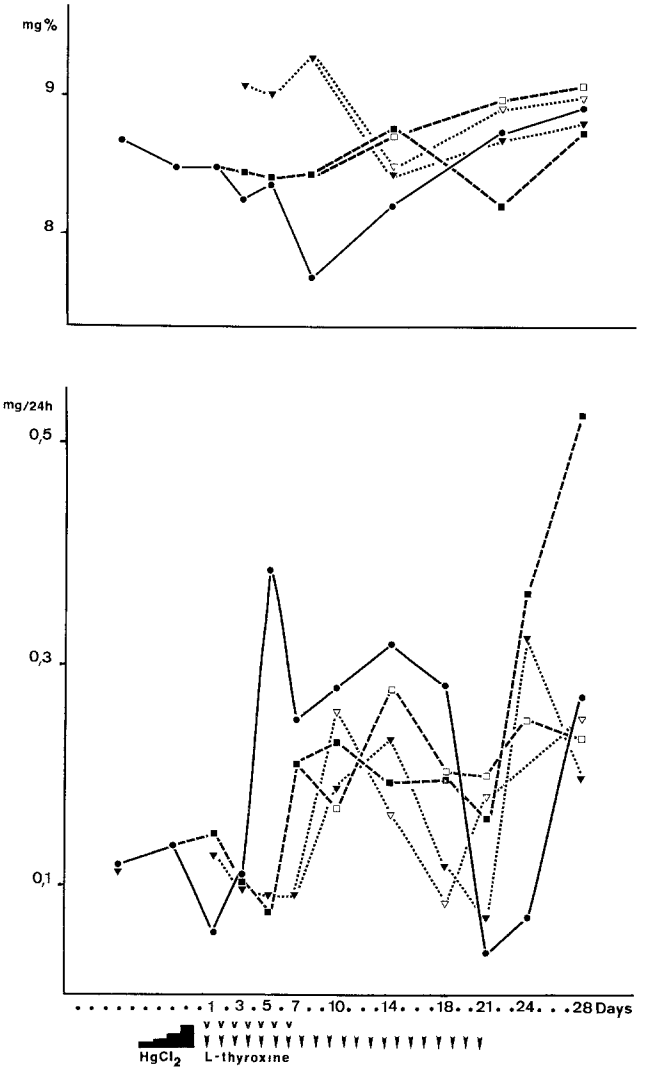

Fig. 7. Serum concentrations (upper) and urinary excretions of calcium per 24 h (lower). Symbols as in Figure 1. Sublimate intoxication leads to an impressive loss of calcium. L-thyroxine administration reverses this effect

dient, hence, attenuation of the activity of the counter-current multiplication. In contrast to the cortical renal blood circulation which is stabilized by the autoregulation mechanism, the medullary blood perfusion depends directly on cardial output and arterial blood pressure (40). Therefore, hypercirculation may diminish the concentration gradient in the medulla in spite of RPF- and GFR-raising enhancement of H_2O-free sodium (chloride) reabsorption in the ascending limb of Henle's loop. This "wash-out" effect is obviously absent in rats or is of minor importance, presumably because of the very long and more flow-resistant vasa rectae of these animals.

After a one week period, renal excretion of electrolytes was significantly above normal in sublimate-intoxicated rats, indicating a persisting inability of the nephron to handle the tubular fluid adequately. L-thyroxine therapy, however, was able to control urinary loss of sodium chloride (Fig. 4). This can also be observed in other species (36) and reflects a high sodium chloride reabsorption in the upper nephron. The increased loss of sodium chloride and water after termination of hormone therapy is obviously a sign of normalization of the previously expanded extra-

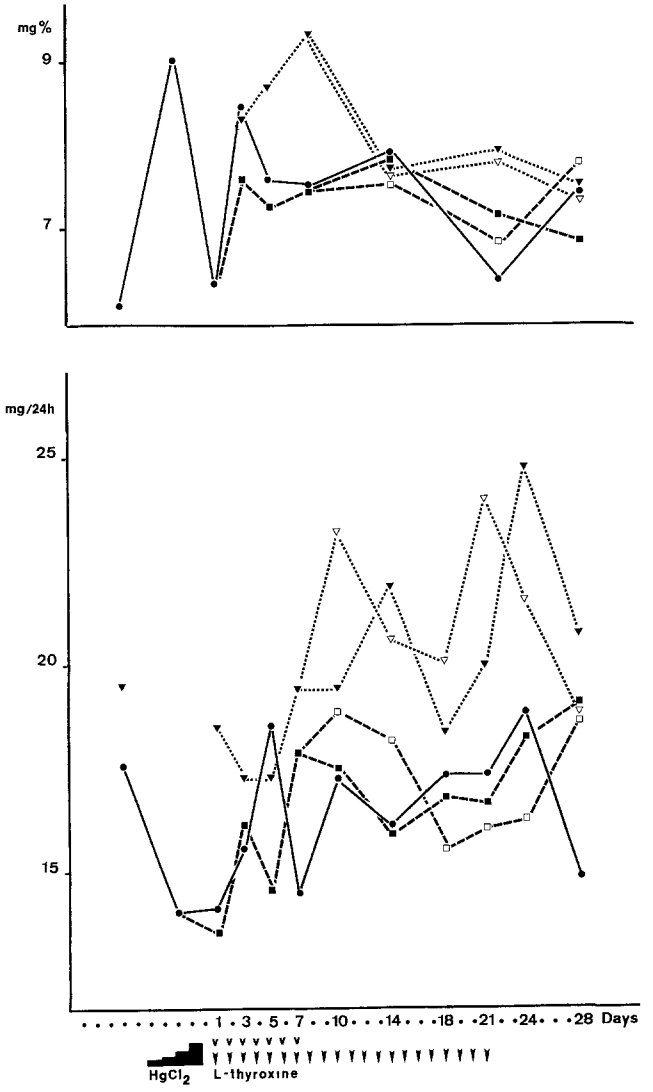

Fig. 8. Serum concentrations (upper) and urinary excretions of inorganic phosphate per 24 h (lower). Symbols as in Figure 1. The transient hyperphosphataemia in group "S" is probably caused by the decrease of glomerular filtration

cellular compartment (5, 10, 13, 19, 29, 41).

Only limited conclusions can be drawn with regard to the elimination pattern of potassium, calcium and inorganic phosphate. The role of cellular sodium and potassium exchange, as well as the very complex direct and indirect metabolic effects of thyroid hormones, have not yet been evaluated in detail (review: 5, 19). Non-renal mechanisms are apparently of relevance in the regulation of these electrolytes. For example, thyroid hormone causes uncontrolled loss of water and electrolytes after hypophysectomy (21, 25) and, interfering with calcium and phosphate metabolism, affects the parathyroid gland (1, 2, 5, 16, 20, 22, 23, 46) and indirectly the kidney. In addition, a functioning adrenal cortex is absolutely necessary for some thyrogenous effects upon the kidney (11, 13, 25).

The hyperkalaemia (Fig. 6a) and the hyperphosphataemia (Fig. 8a) of sublimate-intoxicated animals may be explained by the decrease of glomerular filtration, tubular insufficiency, acidosis and catabolism.

The transient hyperkaluria observed after mercury intoxication (Fig. 6b) results partly from the extracellular excess, whereas hyperkaluria observed in normal animals during L-thyroxine treatment (Fig. 6) is likely to be the consequence of cellular potassium leakage (review: 13).

Although the effects on diuresis and sodium are relatively well understood, additional investigations are needed to evaluate the direct and indirect effects of L-thyroxine upon the other electrolytes. For example, measurements of the antidiuretic hormone (ADH) and aldosterone activity during hyperthyroidism are underway to elucidate their influence.

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