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Preventive Effect of Some Substances on Experimental Oxalic Calculogenesis in the Frog

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Summary. Rana esculenta tadpoles that are fed spinach develop an oxalic calculogenesis. The addition of cholestyramine, orthophosphate, citrate, allopurinol and tungstate to the tank water prevented calculi formation while succinimide, magnesium oxide, hydrochlorothiazide and tetracycline were ineffective. Methylene blue proved lethal to tadpoles, and its anti-lithogenic activity could not be assessed. These findings, except for the non-effectivness of magnesium oxide, are in agreement with both the theoretical expectations and the results obtained in other experimental models. Experimental frog calculogenesis seems to be a simple and valid method for evaluating anti-lithogenic activity.

Key words: Frog calculogenesis, Oxalate calculi, Anticalculous substances, Calculi prophylaxis.

The tadpoles of some frog species (R. pipiens, R. esculenta), that are fed a diet consisting exclusively of spinach, all develop oxalic calculogenesis (1, 10, 13, 14). We have used this model for a comparative assessment of the protective action performed by a number of substances.

MATERIALS AND METHODS

R. esculenta tadpoles, about one month old, divided into groups of 60 each, were kept in 2 litre tanks filled with a hypotonic solution containing NaCl 30 mg/dl, KCl 7.5 mg/dl, $CaCl_2$ 4 mg/dl and $MgCl_2$ 2.5 mg/dl. A first group of tadpoles, serving as control, was fed lettuce (standard diet), one group was fed spinach only (lythogenous diet), while all the others were administered, in addition to the lythogenous diet, the various substances to be evaluated by direct addition to the tank water, namely: succinimide 300 mg/l; cholestyramine 200 mg/l; tetracycline hydrochloride 625 mg/l; hydrochlorothiazide 6.25 mg/l; sodium citrate 1,000 mg/l; allopurinol 12.5 mg/l; sodium tungstate 2.5 mg/l; disodic orthophosphate 200 mg/l; magnesium oxide 200 mg/l; methylene blue 100 mg/l.

The duration of the experiment was 60 days; tank water was changed every 48 h. The kidneys of the frogs that died after metamorphosis (defined as when the four limbs were present and the tail had disappeared) were examined with the naked eye and microscopically. After 60 days all the frogs that had completed their metamorphosis and had survived were sacrificed and they also were studied. Some of the animals that were fed the standard diet had not completed their metamorphosis, while all those that were fed the lithogenic diet had turned into frogs.

RESULTS

The kidneys of the control frogs (standard diet) contained no calculi and no calcification in the tubules was observed. The kidneys of the frogs reared on a spinach diet alone were loaded with macroscopic stones and the collecting ducts were stuffed with calcareous precipitation (Fig. 1A, B). Identical appearances (no protective effect) were observed in the kidneys of the frogs treated with the lithogenic diet plus any one of the following drugs: succinimide, hydrochlorothiazide, magnesium oxide and tetracycline. The kidneys of the frogs treated with the lithogenic diet plus sodium citrate or allopurinol contained neither calculi nor intratubular precipitates (optimum protective effect). The kidneys of the frogs treated with the lithogenic diet plus cholestyramine, sodium phosphate or sodium tungstate

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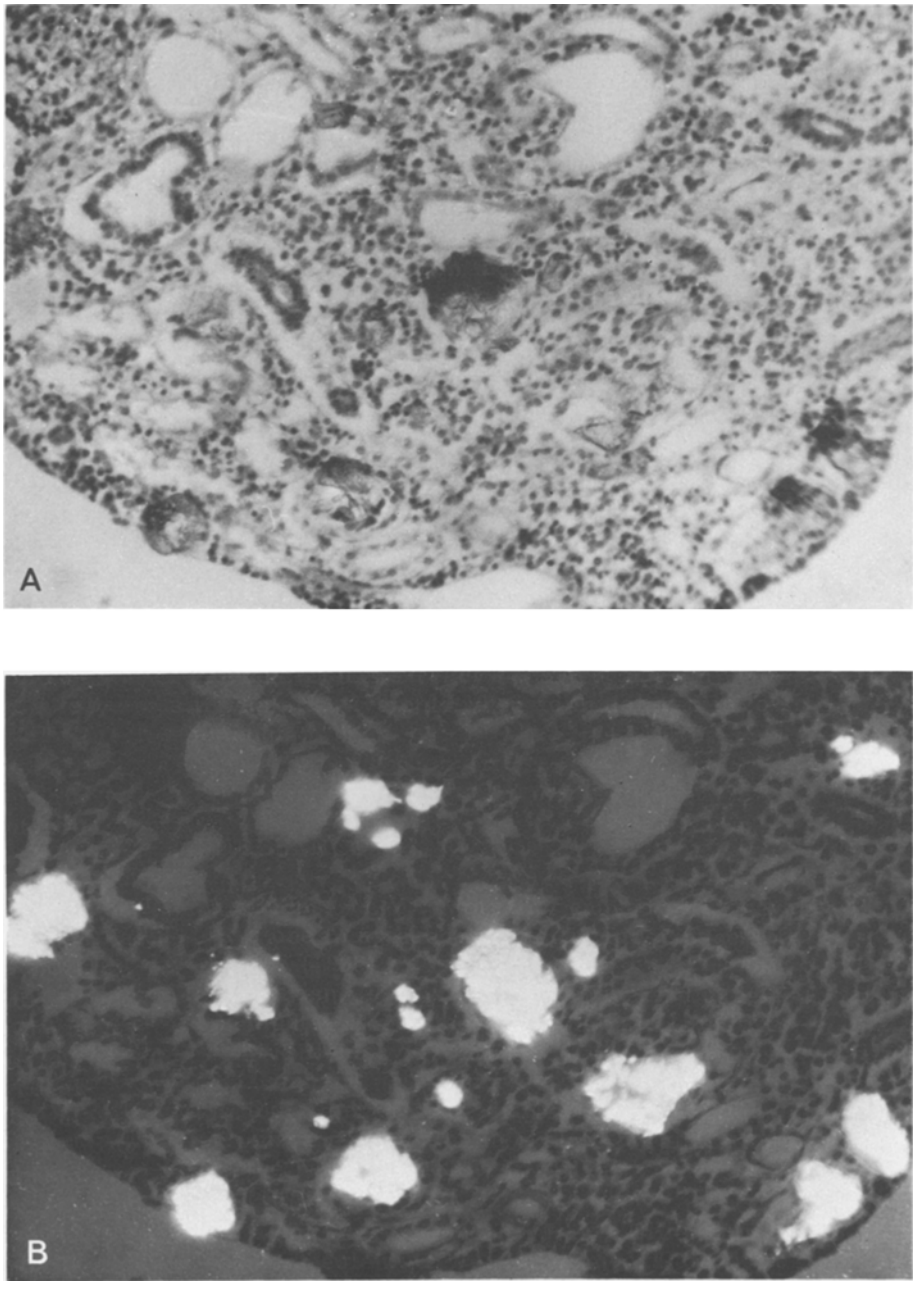

Fig. 1. A Aspect of a kidney of a frog raised on a diet of spinach (x 150). B The same picture under polarized light

contained no stones but there were occasional intratubular calcareous precipitates, no more than one per low power field (see Table 1). All the tadpoles treated with methylene blue died long before they had completed their metamorphosis, and its anti-lithogenic effect could not be estimated.

DISCUSSION

It is once again confirmed that a diet of spinach alone causes the formation of calculi and renal precipitations in the frog kidney. Previous research (10, 13) has shown that these calculi consist of the monohydrate and dihydrate of calcium oxalate (10, 13) and that the same result can be attained by feeding tadpoles a standard diet, but with the addition of sodium oxalate in the tank water (13).

In this model oxalate is already preformed in the food and so it was to be expected that those drugs which act, as in the case of succinimide, by depressing its endogenous formation (16), would have had no effect at all. On the other hand, it would be expected that those drugs such as cholestyramine (9), that act by causing the oxalate intestinal absorption to decrease, would prove effective.

Table 1. Preventive effect of various substances in oxalate calculogenesis of the frog

	Kidneys examined	Findings
Succinimide	18	a
Cholestyramine	12	b
Tetracycline	16	a
Hydrochlorothiazide	18	a
Sodium citrate	10	c
Allopurinol	14	c
Sodium tungstate	12	b
Sodium orthophosphate	12	b
Magnesium oxide	16	a

[a] Presence of calculi in the pelvis or of a number of intratubular precipitations per low power field

[b] Absence of calculi in the pelvis, presence of not more than one intratubular precipitation per low power field

[c] Absence of calculi in the pelvis and of intratubular precipitations

The effect of the drugs that act on urinary calcium such as tetracycline and hydrochlorothiazide, were more difficult to predict. Tetracycline could act by sequestering calcium, and was shown to be active in only one previous study on rats (8). Hydrochlorothiazide was at times effective in preventing experimental oxalic calculogenesis, and at times it even favoured it (3, 7). It cannot be established whether the absence of effects in frog calculogenesis is to be ascribed to a different behaviour in the renal tubule of the tadpole compared to that shown by adult mammals, or, instead, to the impossibility of preventing oxalate load precipitation by acting only on calciuria.

Drugs such as citrate, allopurinol and tungstate cause the urine inhibitory activity to increase. It is generally assumed that citrate fed by mouth, at least in mammals, does not appear in the urine, being completely metabolized. It is possible that in the tadpole, especially in view of the high dosage used, a proportion of citrate is eliminated unmodified, and that it is thus capable of carrying out its antilithogenic action.

It may be that allopurinol, in oxalate lithiasis, acts by causing a decrease in oxaluria (6), but its main action is enabling, through a reduction in uricosuria, a greater amount of inhibitors to prevent oxalate precipitation (5). Sodium tungstate acts by a similar mechanism to that of allopurinol. The administration of tungstate brings about a relative molybdenum shortage and, hence, a depression in the xanthine-oxidase activity. Some years before the introduction of allopurinol in clinical practice, Sorrentino demonstrated that sodium tungstate could cause a reduction of uricaemia and uricosuria in man (11, 12). Tungstate also has a certain preventive effect on oxalate calculogenesis in the rabbit (15).

Orthophosphate carries out its anti-lithogenic activity in two ways: by hindering calcium absorption at the intestinal level and by increasing pyrophosphate excretion. Which of the two mechanisms is more important in tadpole calculogenesis is impossible to assess.

Magnesium oxide has proved effective in some experimental calculogenesis (4) and has been used clinically for many years. We are unable to explain why it is ineffective in frog calculogenesis.

Methylene blue, at the dose used, proved lethal for the tadpoles, and its anti-lithogenic action could not be assessed. Our findings do not confirm those obtained by Smith (10), who, in R. pipiens, using the same doses, found a good preventive effect. In vitro, the colourant has proved very active in preventing calcium oxalate crystal precipitation, but, on the other hand, Borden (2) has already reported that, in vivo, it was effective only when administered in toxic amounts.

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