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Effects of Treatment with Sodium Fluoride and Subsequent Starvation on Fluoride Content of Earthworms

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The two experiments described here originated during a long-term investigation into the occurrence and movement of pollutant fluoride in a terrestrial ecosystem. Moles (Talpa europaea) whose diet consists largely of various species of earthworm Lumbricidae (Funmilayo, 1979), are one of the species under investigation. Bone fluoride in moles (Walton, in press) was found to be higher, on average, than in foxes or small rodents (Walton 1983, 1984, 1985). Moles probably acquire fluoride from their earthworm diet. Earthworms do not have any readily identifiable tissue in which to store large amounts of fluoride but, for their size, they have a considerable amount of soil in their gut, up to 20% of their dry weight, according to Hartenstein and Amico (1983). Preliminary measurements of fluoride in whole earthworms suggested that observed levels could probably be accounted for by fluoride bound in the mineral part of contained soil and released during preparatory ashing.

Two experiments to investigate this situation are described below; their aims were: to expose earthworms kept in soil to different concentrations of sodium fluoride; to measure resulting fluoride in earthworms when soil was removed from their gut by starvation for varying periods of time; and to compare amounts of fluoride in whole starved earthworms with those in starved earthworms from which remaining soil had also been physically removed by dissection and washing.

MATERIALS AND METHODS

Soil from ground near the Research Station was roughly sieved. It was used to fill 12 plastic plant-pots of about 4 litres capacity. Earthworms, mostly *Lumbricus terrestris*, were extracted from a nearby lawn with 0.5% formalin solution. They were washed clean of residual formalin and 8 worms (5 experimental, 3 reserve) transferred into each pot with nylon netting over top and bottom to prevent escape.

In the first experiment, pots 1-4 were untreated; pots 5-8 were treated at intervals with sodium fluoride solution containing 50 mg/l fluoride (total added=17.5 mg/pot); those in pots 9-12 were treated with 500 mg/l fluoride (total added=175 mg/pot). All pots were kept in the dark for 20 days in a constant environment cabinet at 15°C and 70% relative humidity. Earthworms were then recovered, washed, transferred to empty plastic jars and returned to the cabinet, except for those to be analysed immediately. These were washed and killed. On each of days 2, 4 and 6, all worms were washed, egested earth was removed from the jars, and 3 groups (1 for each treatment) of 5 worms were removed and killed. On each occasion, soil had been egested into the jars. Subsequently all worms were oven-dried at 60°C and ground to pass a 0.6 mm sieve.

The second experiment details were decided after the findings of the first were available. Soil (from a slightly different place) and worms were collected as before. Only one concentration of fluoride was used; a solution of 1000 mg/l was added to pots 7-12 at intervals over 30 days (total added=350 mg/pot). The pots were kept in the dark in an outbuilding at 10-15°C. At the end of this time, worms were removed and put into plastic jars to eliminate soil from the gut, as before, but withdrawn only on days 0, 3 and 8. On each of these days,5 worms from 2 control and 2 treated pots were washed and killed. Worms from one control and one treated pot were oven-dried immediately. Worms from the other control and treated pot were cut along the body wall and gut. The inside gut wall was washed free of soil with a jet of water, keeping chloragogenous tissue intact as far as possible. This tissue has been suggested as a possible site for fluoride storage, by analogy with heavy metals (Garrec & Plebin, 1984). Worms were then oven-dried.

All material was prepared for chemical analysis by the ashing and fusion method of Allen *et al.* (1974). Fluoride was measured with a fluoride selective electrode and direct-reading Orion Ionalyzer.

At the end of each experiment, fluoride was measured in soil from all 12 pots. It was first oven-dried and passed through a 0.6 mm sieve. Total fluoride was determined after ashing and fusion as above. A sample of soil from each pot was also analysed for water-soluble fluoride, using a modification of the method described by Supharungsun and Wainwright (1982). Results are expressed as mg/l of fluoride in the filtrate, which reaches equilibrium when the soil:water ratio exceeds about 1:7, sometimes referred to as "available fluoride" (Larsen and Widdowsen,1971).

RESULTS & DISCUSSION

Results from the first experiment are summarized in Tables 1 and 2; trends are shown in Fig.1. As the number of days of starvation increased, the mean concentration of fluoride in all worm samples decreased significantly (for any treatment $r>=-0.81$, $df=18$, $P<0.001$). Possibly because of large variance and small sample size, differences between treatment means on days 0 and 2 (P around 0.1) were indicative but not significant. After 6 days of starvation, there was virtually no difference between mean fluoride content of treatment groups; all measured 22 mg/kg or less. It was deduced that the main material that had been measured up to this point was fluoride in soil particles contained in the gut. Even after 6 days of starvation, one of the treated groups (50 mg/kg) still had a range of 2.8-66 mg/kg of fluoride, suggesting that one animal, at least, had not completely eliminated soil from the gut.

Table 1. Fluoride concentrations (mg/kg dry weight, mean and 1 sd, N=5) found in whole experimental earthworms.

	No. of days worms starved before analysis			
	0	2	4	6
Untreated	156±36	131±31	102±47	5.6±2.8
50 mg/l (17.5 mg/pot)	163±17	94±25	77±43	22±23
500 mg/l (175 mg/pot)	219±64	192±41	48±21	10±6.2

Table 2. Total fluoride (mg/kg dry weight) and water soluble fluoride (mg/l in filtrate) found in experimental soil that earthworms were kept in for 20 days.(N=4)

	ControL		50 mg/l		500 mg/l	
	MEAN	SD	MEAN	SD	MEAN	SD
Total	267	12	298	37	327	21
Water Soluble	1.23*	0.05	1.75*	0.09	2.5*	0.38

* Differences between means (Student's t) were significant at P<0.02.

Soil from treated pots had more fluoride, both total and water-soluble, on average than soil from the control pots. Concentrations of total fluoride in treated pots were more variable, however, probably because of uneven application of fluoride solution (ANOVA gave F=4.06, df=2 and 9, P=0.05).

Results from the second experiment are summarized in Tables 3 and 4; they differ in a number of important ways from the results of the first. Trends are shown in Fig.2. Using an increased concentration of sodium fluoride produced a much clearer difference between treated and untreated samples. Untreated whole worms killed on day 0 had a mean fluoride concentration of 119 mg/kg, similar to the value found for day 0 in the first experiment (156 mg/kg), making allowance for slightly different soil. However, untreated worms from which gut soil had been removed contained less than 7 mg/kg, only 6% the amount of fluoride found in untreated whole worms. For the treated sample, whole worms on day 0 contained 276 mg/kg fluoride, more than twice as much as the whole, untreated sample on the same day. But the most striking difference was that treated cleaned worms, with 47 mg/kg, had more than 6 times the amount of fluoride found in the untreated cleaned sample; and 17% of the amount found in treated whole worms. Even after 8 days of starvation, mean fluoride concentration in treated, cleaned worms was still 4 times greater than in the comparable untreated group.

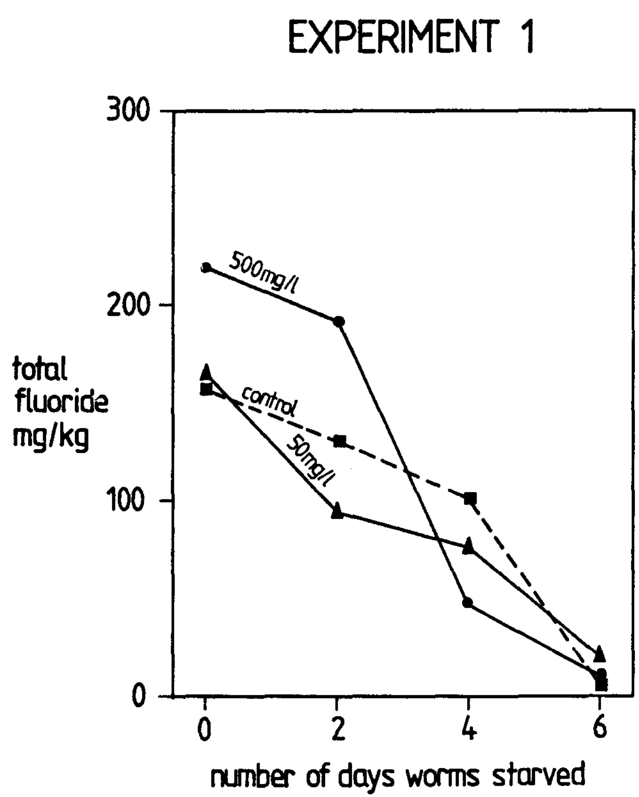

Figure 1. Mean concentrations of fluoride found in samples of 5 earthworms treated with 50 or 500 mg/l fluoride or untreated, after starving for various lengths of time.

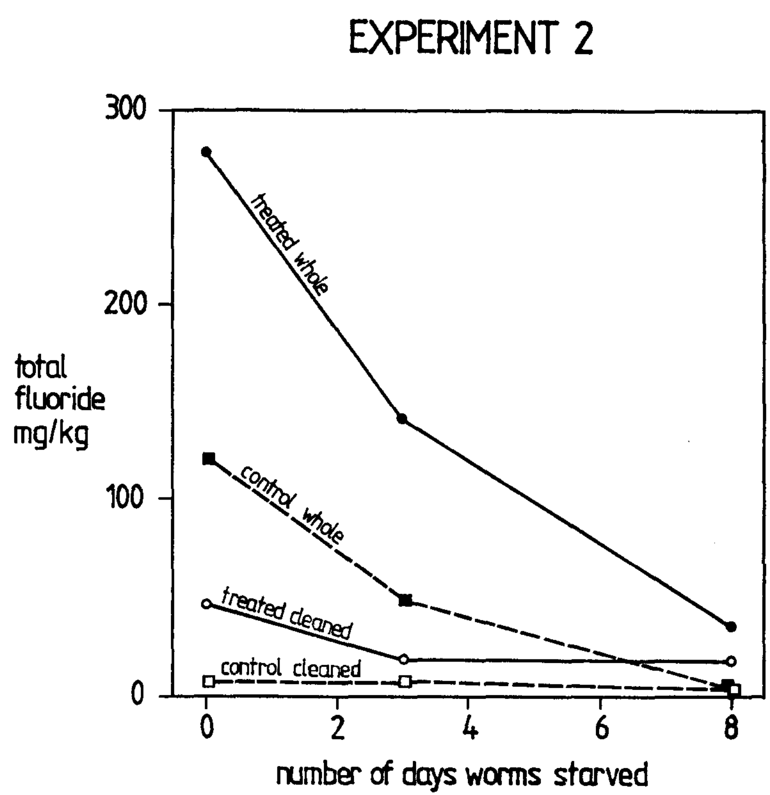

Figure 2. Mean concentrations of fluoride found in samples of 5 earthworms treated with 1000 mg/l fluoride or untreated. Worms were analysed whole or cleaned (i.e. with gut contents removed) after starving for various lengths of time.

Table 3. Fluoride concentrations (mg/kg dry weight, mean and 1 sd, N=5) found in experimental earthworms, analysed whole or with gut contents removed.

	No. of days worms starved before analysis		
	0	3	8
UNTREATED			
complete	119±15	48±7.2	4.9±3.2
gut contents removed	6.7±3.1	8.7±2.4	4.1±1.1
TREATED			
complete	276±51	139±34	35±13
gut contents removed	47±5.6	18±4.9	18±7.5

Differences between all row means (Student's t) for each of days 0, 3, and 8 were significant at P<0.01.

Table 4. Total fluoride (mg/kg dry weight) and water soluble fluoride (mg/l in filtrate) found in experimental soil that earthworms were kept in for 30 days. (N=6)

	Control		1000 mg/l	
	MEAN	SD	MEAN	SD
Total	246*	12	342*	23
Water Soluble	0.27*	0.03	1.87*	0.53

* Differences between control and treated means (Student's t) were significant at P<0.001.

This finding indicates that earthworms may have elevated amounts of fluoride in their tissues, as well as in their gut contents, under these experimental conditions. But it is worth remembering that the concentration of fluoride in the solution used to treat the soil in this experiment was many times greater than might be expected to occur naturally.

In neither experiment did any individual worm ever contain more total fluoride than soil from the pot in which it had been kept.

Only one report concerned with fluoride and earthworms gave details of preparation for analysis. Garrec & Plebin (1984) found a positive correlation between total fluoride in earthworms and soil at 17 sites near aluminium plants. They kept worms in flasks for 24 hours to void their gut contents which, in the light of my findings, seems too short to achieve proper clearance. Speculation by these authors about "accumulation" of fluoride in worms on the basis of these results seems premature. What can be deduced is that exposure of earthworms to greatly raised amounts of soluble fluoride (1000 mg/kg) can cause, at least, a temporary increase in the concentration of fluoride in body tissue. It was not clear whether amounts of fluoride measured in the treated, cleaned worms (hence, presumably, in body tissue rather than gut contents) were related to total or water soluble fluoride in soil, both of which differed significantly from soil control values. The decisive factor may well have been a transient increase in water soluble fluoride following application of the treatment solution; an increase subsequently reduced by adsorption onto the soil. Several authors have reported on the capacity of soils for adsorbing fluoride ions, mostly by exchange with hydroxyl in aluminium hydroxide (Bower & Hatcher, 1967; Omueti & Jones, 1977).

My current work is concerned with earthworms as a fluoride source for moles. It may make little difference to moles whether they absorb fluoride from earthworm tissue, or from the finely ground soil and vegetation in earthworm gut, but elucidation of the mechanism involved seems to be essential for a proper understanding of fluoride pathways.

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