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The stability of ergocalciferol in rodenticidal baits

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SUMMARY

Concentrations of the rodenticide ergocalciferol (vitamin D_2) in samples of rodent baits laid in foodstores and in the laboratory were monitored over several months. Bait samples were solvent extracted and ergocalciferol concentration determined by high pressure liquid chromatography (HPLC). Ergocalciferol levels were constant for more than 21 days in dry samples and did not fall by more than 30% in 100 days. When water (10% w/w) was added to the baits in the laboratory the ergocalciferol concentration fell by approximately 30% in 30 days. In these wet laboratory samples there was a rapid visible growth of fungus and in normal rodent control use baits should have been replaced when such deterioration became evident.

INTRODUCTION

A combination of ergocalciferol (0·1%) and warfarin (0·025%) was shown to be an effective combination for controlling anticoagulant poison resistant or susceptible rats and mice (Greaves, Redfern & King, 1974).

Initial studies on the stability of ergocalciferol in baits indicated a maximum storage period of 3 weeks (MAFF, 1973) but further studies indicated that baits remained effective when stored in closed containers away from light for up to 10 months (MAFF, 1974). More recently there has been concern about the stability or ergocalciferol, particularly in damp conditions, when baits are laid down for several months as part of a permanent baiting regime. This study was undertaken to examine ergocalciferol concentrations in baits that were laid for long periods in both wet and dry environments.

METHODS

Ready mixed bait (25 g; 0·1% ergocalciferol, 0·025% warfarin on canary seed) was laid in wooden trays in two unheated foodstores where such baits are normally used as a part of the rodent control regime. Foodstore 'A' was selected as a dry

building that is normally in darkness with only occasional low-level artificial lighting. Foodstore 'B' is a damp building with natural lighting although baits were never in direct sunlight. The trial commenced in October 1981 and the last set of samples was taken in March 1982. Average weekly temperatures ranged from −2 to 11 °C during this period.

For the laboratory studies premixed 0·1 % ergocalciferol, 0·025 % warfarin on canary seed and a rodenticide concentrate (2 % ergocalciferol in vegetable oil) were obtained from Rodent Control (Reading) Ltd. Bait samples were laid in the laboratory away from direct sunlight. Some samples were maintained in a wet condition by addition of 10 % (w/w) water every other day. Ergocalciferol was obtained from Sigma Chemical Co. (Poole, Dorset) for use as an analytical standard. All solvents were HPLC grade and were obtained from Rathburn Chemicals Ltd. (Walkerburn, Peebleshire) or BDH Chemicals Ltd. (Poole, Dorset).

Ergocalciferol analysis was carried out in subdued lighting by a method adapted from Vanhaelen-Fastré & Vanhaelen (1978). Bait samples (10 g) were finely ground (10 s) in a household coffee grinder. Aliquots (1 g) were extracted by sonication (30 min; 200 watts/5 l ultrasonic bath, Gallenkamp and Co. Ltd., London) with N,N-dimethylformamide (12 ml) in dry stoppered glass tubes. Studies on vitamin D concentration in vitamin tablets (Vanhaelen-Fastré & Vanhaelen, 1978) also used dimethyl sulphoxide for extraction. Preliminary experiments using this solvent for extraction of canary seed were not successful since a gel formed that could not be subsequently extracted. Ice-cold glass-distilled water (8 ml) was added to each sample after extraction with N,N-dimethyl formamide, and the ergocalciferol partitioned into 2,2,4-trimethylpentane (10 ml) by vigorous shaking (3 min). Extracts were centrifuged (10 min; 4000 rev./min) in a bench centrifuge and an aliquot (3 ml) of the upper phase taken for HPLC analysis.

The HPLC equipment used consisted of two M6000A pumps (Waters Associates, Instruments, Ltd., Hartford, Cheshire) a Waters M720 system controller, a Waters M710B autoinjector, a LC3 ultraviolet detector (Pye Unicam Ltd., Cambridge) and a 3390A recording integrator (Hewlett Packard U.K., Wokingham, Berks). Chromatography was carried out on a column of Zorbax ODS (4·8 × 250 mm, Du Pont Co., Wilmington, Delaware, USA) packed at a pressure of 6000 p.s.i. in dichlormethane using a CPIII Slurry packer (Jones Chromatography Ltd., Llanbradach, Glamorgan). Samples (20 μl) of standards or canary seed extracts were eluted (2 ml/min) with a convex gradient (Waters no. 2, 20 min) from 20 % water, 80 % methanol to 100 % methanol. The effluent was monitored at 265 mm and 0·16 absorbance units full scale deflection. Fresh ergocalciferol standards in 2,2,4-trimethylpentane and integrator calibrations curves were prepared on each analysis day. Calculations of ergocalciferol concentration were based upon the results of consecutive duplicate injections of standards and samples.

RESULTS

HPLC extracts of bait samples showed that material was present (Fig. 1*a*) that co-chromatographed (retention time 17·5 min) with standard ergocalciferol (Fig. 1*b*). When examined by scanning ultra-violet spectroscopy both standard ergocalciferol and the compound extracted from baits showed a maximum absorption

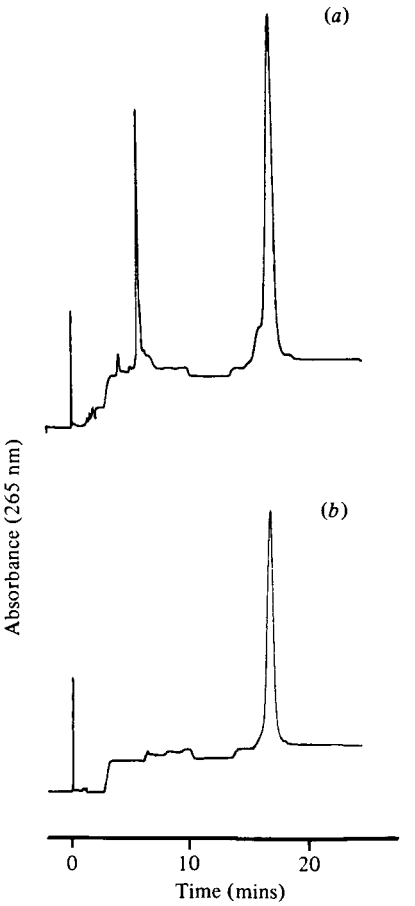

Fig. 1. HPLC elution profiles for u.v. absorption (265 nm) of (*a*) an extract of canary seed bait containing 0·1 % ergocalciferol and 0·025 % warfarin and (*b*) ergocalciferol. Details of the extraction procedure and HPLC system are given in the text.

at 265 nm. This compound was not detected in extracts of untreated canary seed and was assumed to be ergocalciferol. The amounts of ergocalciferol detected in freshly prepared canary seed baits was 710–740 μg/g bait (0·071–0·074 %). The bait contained 0·1 % ergocalciferol and analysis of the rodenticide concentrated by HPLC indicate that the correct amount of ergocalciferol had been mixed with the appropriate weight of canary seed. It was not possible to improve the recovery of ergocalciferol from canary seed above 74 % by increased sonication time or repeated extraction with 2,2,4-trimethylpentane. The freshly prepared bait was stored in the laboratory in a sealed container and regular ergocalciferol analyses carried out. Results of ergocalciferol analyses carried out over a 180 day period fell within the range of 710–740 μg/g bait, indicating little if any bait degradation under these conditions. Analyses of samples of bait laid in the two foodstores were carried out at intervals for 140 days. Results of analyses of bait samples from the 'dry' foodstore A (Fig. 2*a*) showed that the ergocalciferol concentration fell by only 5·5 % (40 μg/g bait) in a 100 day period. When baits were laid in the 'damp'

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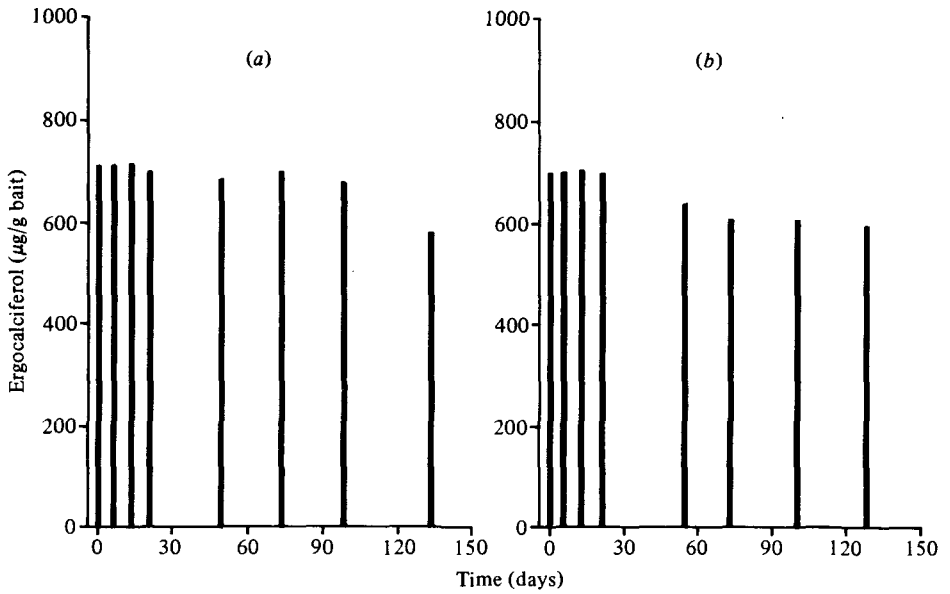

Fig. 2. Concentrations of ergocalciferol in samples of canary seed bait containing ergocalciferol and warfarin laid in (*a*) foodstore A (dry) (*b*) foodstore B (damp). Samples of bait were taken at intervals and analysed as described in the text.

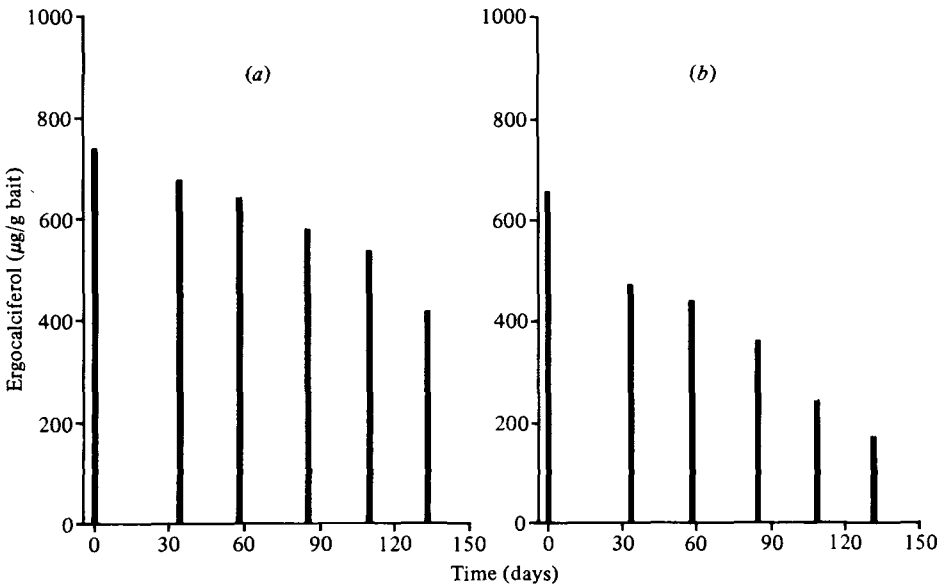

Fig. 3. Concentration of ergocalciferol in samples of canary seed bait containing ergocalciferol and warfarin laid in the laboratory (*a*) dry and (*b*) with the addition every two days of 10% water. Samples of bait were taken at intervals and analysed as described in the text.

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foodstore B the ergocalciferol concentration was constant for 21 days but approximately 14% was lost over 100 days (Fig. 2*b*).

Results of egocalciferol analyses of dry bait samples laid in the laboratory showed that the rate of degradation of ergocalciferol was greater than that in baits laid in foodstores (Fig. 3*a*). Addition of 10% water increased this rate of degradation and only 245 μg/g bait were detected after 110 days (Fig. 3*b*). Within one week of the addition of water to the bait an extensive fungal growth was observed. A similar experiment was carried out using pinhead oatmeal as a base for 0·1% ergocalciferol without warfarin. Baits were placed in a laboratory in direct sunlight, and 10% (w/w) water was added to one bait. Results of analyses of ergocalciferol in pinhead oatmeal over 80 days were essentially similar to those obtained in laboratory experiments using canary seed baits.

DISCUSSION

This study was initiated because of concern that baits containing ergocalciferol would not be effective against rodents when left down for long periods. The results indicated that in a cool, dry and dark environment, such as in a foodstore, there is very little loss of efficiency of rodent control, over several months. The rate of degradation of ergocalciferol in both dry and wet laboratory samples was faster than in baits laid in foodstores. This may reflect the average daytime temperature of 18–24 °C in the heated laboratory. However, even under the worst storage conditions studied of high temperature and the addition of 10% water the 'half-life' for ergocalciferol in bait preparations was 90 days.

Lund (1974) showed that baits containing 0·1% ergocalciferol without warfarin remained highly toxic for at least 7 weeks in feeding tests using several rodent species. One experiment with a 16 week old bait resulted in 87·5% mortality of *Rattus norvegicus* when given a choice between 0·1% ergocalciferol and an identical bait prepared without active ingredient (Lund, 1974). Unfortunately details of storage conditions for the baits were not reported. Other studies (Greaves *et al.* 1974; Bai, Krishnakumari & Majunder, 1978) indicated that bait containing 0·025% (250 μg/g bait) ergocalciferol, and 0·025% warfarin, was totally effective against *R. norvegicus*, *R. rattus* and *Mus musculus* in no-choice feeding tests. This applied to warfarin susceptible and resistant rodents except for animals of the species *R. rattus*. Only 90% mortality was obtained for warfarin resistant animals of this species with the higher concentration of 0·1% ergocalciferol, with 0·025% warfarin in a no-choice test (Greaves *et al.* 1974). These studies indicated that losses of up to 30% ergocalciferol from baits are unlikely to reduce their effectiveness for rodent control.

The results of the present study indicate that ergocalciferol in canary seed or pinhead oatmeal baits is more stable than anticipated. Baits that are laid in cool, dry conditions away from direct light should be as toxic after 3 months as when first laid down. When baits are laid in environments with higher temperature or humidity, ergocalciferol concentrations will probably fall quicker. However these baits should still control rodents for 3 weeks, the treatment period recommended for calciferol–warfarin baits (MAFF, 1980).

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