BOV 92

SEAC 7/3

SPONGIFORM ENCEPHALOPATHY ADVISORY COMMITTEE

SURVIVAL OF THE SCRAPIE AGENT IN THE SOIL

1. A copy of a recent Article in the Lancet by Brown and Gajdusek is attached.

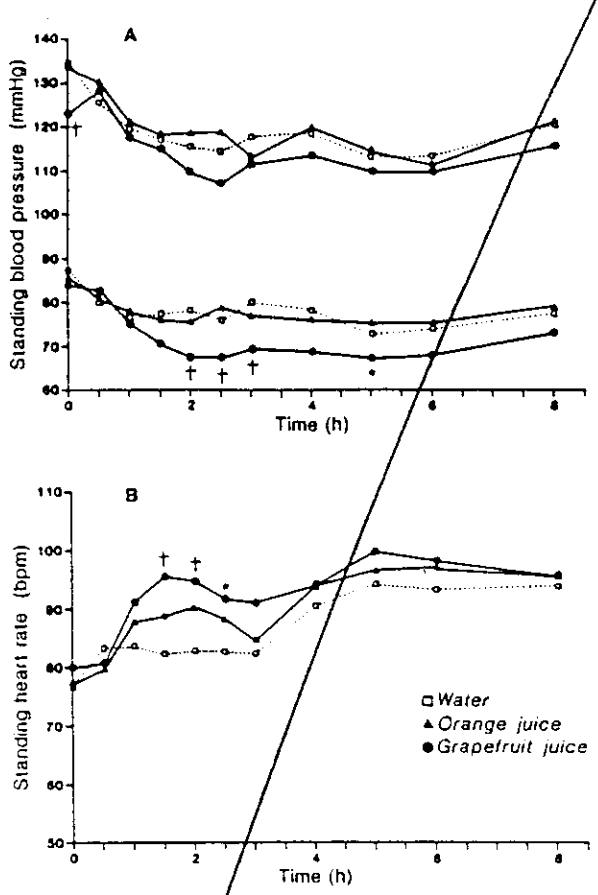
2. The following points need to be noted when considering this experiment:

(a) The Experiment

1 The experiment refers to scrapie not BSE.
2 It refers to passaged hamster scrapie infection assayed in hamsters (no species barrier).
3 Scrapie-like agents are very resistant to physical and chemical deactivation - we knew that already.
4 The reduction in titre was over 98%.
5 Leaching does not significantly occur.

(b) The Implications for BSE

1 No brains of cows with actual or suspect BSE are ever buried. All are examined histologically and the remains incinerated.
2 No tissues of cattle other than brain have so far been shown to harbour infectivity.
3 It is MAFF's policy to incinerate all cases but this is not fully achieved.
4 Before burial is permitted the water authority/local authority must sanction it.
5 It is illegal to exhume carcases.
6 Advice on fertiliser use from rendered material is under consideration. It could be treated with hypochlorite or reduced to phosphate or some other process could be adopted if it was regarded as a danger.



Standing systolic and diastolic blood pressure and heart rate after felodipine 5 mg with water, orange juice, or grapefruit juice in six men with borderline hypertension.

For comparisons between water and juice: *p < 0·025; †p < 0·01.

naringenin[10]) found in grapefruit juice but not orange juice.

Grapefruit juice augmented the bioavailability of nifedipine, though to a lesser extent than that of felodipine. The difference may be due to the lesser presystemic metabolism of nifedipine. The age difference in the study populations could have contributed to the differing pharmacokinetics[4] but not accounted for the size of the differences. It is important to find out whether this interaction can contribute to variability within and between patients in response to long-term felodipine treatment and whether it occurs with other foods and drugs.

PHARMACOKINETIC CHARACTERISTICS OF FELODIPINE, NIFEDIPINE AND PRIMARY PYRIDINE METABOLITES

	Mean (SEM)			
	AUC 0–8 (nmol.h.l^{-1})	Cmax (nmol/l)	Tmax (h)	Half-life (h)
Felodipine				
Water	41 (8)	13 (2)	1·1 (0·2)	3·0 (0·5)
Orange juice	44 (7)	16 (2)	1·9 (1·5)	2·3 (0·3)
Grapefruit juice	103 (15)†	29 (3)†	2·1 (0·2)†	2·3 (0·1)
Dehydrofelodipine				
Water	46 (5)	18 (3)	1·0 (0·1)	2·1 (0·2)
Orange juice	58 (10)	25 (4)	1·8 (0·5)	1·9 (0·2)
Grapefruit juice	80 (5)‡	26 (3)	2·1 (0·2)‡	1·9 (0·1)
Nifedipine				
Water	464 (92)	222 (54)	0·8 (0·1)	2·0 (0·2)
Grapefruit juice	627 (152)*	250 (42)	1·2 (0·1)*	1·8 (0·1)*
Dehydronifedipine				
Water	199 (22)	111 (23)	0·8 (0·1)	2·2 (0·1)
Grapefruit juice	242 (22)*	114 (20)	1·2 (0·1)*	1·8 (0·2)*

For differences between variables after juice and water *p < 0·05; †p < 0·01; ‡p < 0·001

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Survival of scrapie virus after 3 years' interment

PAUL BROWN D. CARLETON GAJDUSEK

Supernatant fluid from a scrapie-infected hamster brain homogenate was mixed with soil, packed into perforated petri dishes that were then embedded within soil-containing pots, and buried in a garden for 3 years. Between 2 and 3 log units of the input infectivity of nearly 5 log units survived this exposure, with little leaching of virus into deeper soil layers. These results have implications for environmental contamination by scrapie and by similar agents, including those of bovine spongiform encephalopathy and Creutzfeldt-Jakob disease.

Lancet 1991; 337: 269–70.

Laboratory procedures to inactivate scrapie virus have established its extraordinary resistance, and field experience suggests that the virus might also withstand environmental

exposure for several years.[1] We report a study in which the residual infectivity of a mixture of scrapie hamster brain and soil was measured after a 3-year interment.

263K strain hamster-adapted scrapie virus was used at its fourth passage level. Brains from fourteen terminally ill hamsters were removed (total weight 13 g), sonicated to homogeneity as a 10% suspension in phosphate-buffered saline (PBS) pH 7·4, and clarified by centrifugation at 150 *g* for 5 min. The 100 ml of supernatant fluid was added to 150 g dry screened topsoil. Samples of the supernatant fluid and supernatant-saturated soil were frozen and stored at −70°C. Two plastic petri dishes with holes drilled in their covers (and in the bottom of one dish) were used. Disks of nylon mesh ('Nitex' HC 3–160, 160 µm mesh opening, 52% open area; Tetco, Elmsford, NY) were placed in the bottom of the dishes and in the covers. The dishes were then packed with 90 ml of scrapie-soil mush, covered, and sealed at the sides. Two 15 cm diameter plastic flowerpots were lined with aluminium foil and plastic sheeting and filled with soil. The dishes were placed just below the upper level of each pot, covered with a small amount of additional soil, and the pots were sunk at ground level in a garden and loosely covered with mulch (fig 1). The ensemble was enclosed in a wire cage and left undisturbed from September, 1986 to August, 1989.

The Washington, DC area has a temperate climate with average annual high and low temperatures between 25°C and 3°C, short-term highs being up to 40°C and down to minus 20°C; the average annual rainfall is 100 cm, and several ground level freeze-thaw cycles occur each winter.[2]

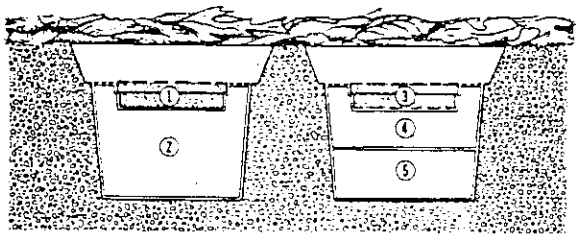

Diagram of experiment.

Supernatant fluid from virus-infected hamster brain homogenate was mixed with soil and packed into petri dishes (1 and 3) with holes drilled in the top (left) or both top and bottom (right). Dishes were embedded in soil-filled pots, buried at ground level, and left undisturbed for 3 years. Soil compartments 1–5 were assayed for residual infectivity.

In the laboratory the contents of the petri dishes surrounding soil within each pot were restored to their original consistency with distilled water and mixed with equal volumes of distilled water after the addition of gentamicin sulphate (0·2 mg/ml final concentration). The mixtures were agitated vigorously for 24 h, and samples were ground in a previously unused mortar and centrifuged at 1000 *g* for 15 min. The supernatant fluids were passed through 450 nm filters before inoculation into animals. End-point dilution titrations were done in 4-week weanling female LGV golden Syrian hamsters (Harlan Sprague Dawley, from the Frederick facility, Maryland); 33 µl serial 10-fold dilutions were inoculated intracerebrally into four to eight hamsters. Animals were then observed for 10 months (as were three cages of uninoculated sentinel hamsters), and the brains from all sick animals were examined histologically for spongiform change. Virus infectivity titres (method of Reed and Meunch) were expressed as logarithms of the median lethal dose ($\log_{10}LD_{50}$) per 33 µl inoculum volume.

The initial infectivity of 4·8 log units fell to between 2·2 and 3·0 log units of residual infectivity at the end of the 3 years, and an additional 1·3 log units had leached into the soil lying immediately under the petri dish that was perforated top and bottom. No infectivity was detectable in the lower layer of soil 4–8 cm beneath the bottom of the dish or in the soil surrounding the unperforated dish. None of the sentinel animals caged with the inoculated animals became ill. Before nitrocellulose filtration (to eliminate bacteria) the control virus-soil mixture had an infectivity of 7·5 log units, compared with 4·8 after filtration. If filtration losses were the same for all specimens, the buried samples may have contained nearly 1000-fold more infectivity than is shown below:

Material	*Infectivity titres**	*Specimen volume (ml)*	*Total infectious* units*
Control virus/soil mixture†	4·8	90	170 358 000
Top-drilled dish (1)‡	2·2	90	428 000
Soil under dish (2)	0§	1200	0
Top/bottom-drilled dish (3)	3·0	90	2700 000
Upper soil layer under dish (4)	1·3	600	359 000
Lower soil layer under dish (5)	0§	600	0

*Infectivity titre (per 33 µl inoculum) and total infectious units in $\log_{10}LD_{50}$.
†Specimen stored at −70°C during 3 year experiment
‡Numbers in parentheses correspond to those in the figure
§No virus detected in undiluted inoculum

This experiment establishes the durability of scrapie virus exposed to natural environmental conditions for 3 years, and also shows that most residual infectivity remains in the originally contaminated soil, with little leaching. Our data help to explain how, in Iceland, healthy flocks of sheep contracted scrapie after being brought to vacant farmland that had 3 years earlier been grazed by scrapie-affected flocks.[1] Other unconventional viruses, such as Creutzfeldt-Jakob agent,[3] all of which are highly resistant to ordinary methods of disinfection, may also survive for a long time in contaminated environments. We do not know whether such persistence has any role in the epidemiology of human disease[4,5]—for example, as a means of species-crossing transmission of scrapie or bovine spongiform encephalopathy (BSE) or as a mechanism for the spread of Creutzfeldt-Jakob disease—but our findings leave these possibilities open to consideration. We do suggest that the practice of ploughing-under carcasses of animals dying of scrapie or BSE, even with the addition of quicklime, be abandoned, and that such animals be excluded as a source of bone meal in fertilisers, unless it is first autoclaved.

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