

**Observations of the effects of
formaldehyde on cockroaches and their flora: I. Survival of
vaccinia virus-infected cockroaches during
fumigation with formaldehyde**

BY C. A. BARTZOKAS, K. MCCARTHY, W. B. SHACKLETON AND
B. F. BAKER

*Department of Medical Microbiology, University of Liverpool,
P.O. Box 147, Liverpool L69 3BX*

(Received 5 July 1977)

SUMMARY

In these studies it is shown that the common 'British' and 'American' adult cockroaches can survive exposure to formaldehyde fumigation carried out at double the strength and for four times as long as is recommended for disinfection of rooms.

It is further reported that vaccinia virus ingested prior to the fumigation survives in the cockroach gut and may be excreted up to 5 days later.

Since cockroaches are ubiquitous and are to be found in most hospitals, laboratories and animal houses, these findings should be considered whenever fumigation is called for.

INTRODUCTION

A chance observation in one of our experimental animal rooms suggested to us that the standard formaldehyde fumigation procedure (Martindale, 1972; D.H.S.S., 1975) did not kill flies present in the room throughout the 24 h period, and a repeat experiment confirmed this.

Since formaldehyde fumigation is commonly used in experimental animal rooms, hospital wards and in various commercial activities as a presumptively reliable and wide spectrum biocide, we undertook the studies reported here using both *Periplaneta americana* and *Blatta orientalis* as representatives of the cockroaches commonly found, often in very large numbers, in animal houses and in hospitals. The Martindale fumigation method has been in frequent use in our animal house for 20 years and is known to be effective after 24 h in killing high titre vaccinia virus preparations dried on slides. We were concerned, however, that cockroaches might, like flies, be resistant to formaldehyde vapour and that, if so, ingested virus might persist in them throughout the fumigation process.

MATERIALS AND METHODS

Cockroaches

Adult *Periplaneta americana* (L.) and *Blatta orientalis* were kindly supplied by Professor C. J. Duncan, Department of Zoology, Liverpool University. Some of the *B. orientalis* were acquired from their natural habitat.

Eggs

Embryonated hen's eggs 12 days old were inoculated by the chorioallantoic membrane (CAM) route with an appropriate dilution of the Lister strain of vaccinia virus to give confluent pocks; CAMs were harvested at 48 h and shaken with glass beads in 28 ml (Universal) glass containers on a mechanical shaker for 30 min at 4 °C. The homogenate was centrifuged at 1000 rev./min for 5 min at room temperature. The supernatant fluid, referred to as 'CAM extract', was assayed for vaccinia virus in African green monkey kidney (Vero) cell monolayers.

Cockroach boxes

Transparent polystyrene lunch boxes, overall dimensions 115 × 175 × 52 mm, were modified as follows (Plate 1*a, b*): the lower half of one of the narrow ends was cut, and removed to allow insertion of a plastic faecal collection tray. A sheet of perforated zinc with 2 mm diameter holes (14 holes/cm²) was used as a platform separating the cockroaches from the collection tray. A lid was made out of the same zinc sheet. The centre well of an organ culture Petri dish (Falcon Plastics) proved to be a stable but small drinking trough for the CAM extract.

Formaldehyde fumigation

After humidifying the room with boiling water, vaporization of Formalin* for fumigation was brought about chemically by adding for each 28 m³ (1000 cubic feet) of air space 340 g (12 ounces) of potassium permanganate to 1 l (2 pints) of commercial Formalin (Formaldehyde Solution 40% (w/v) supplied by May & Baker, Dagenham: stored at +30 °C to avoid polymerization). This is double the quantities recommended in the 1975 Smallpox Memorandum and the Martindale *Extra Pharmacopoea* but will not necessarily result in a doubling of the concentration in air because polymerization is more active at the higher concentrations.

Maintenance and dilution medium

Parker 199 (Wellcome), containing 2% fetal calf serum with bicarbonate and penicillin 100 i.u., streptomycin 100 µg, gentamicin 40 µg and nystatin 50 i.u./per ml.

* In this country the name Formalin is a synonym of Formaldehyde Solution B.P. Formalin is by definition a colourless aqueous solution of about 40% (39–41%, w/v) formaldehyde, containing not less than 12% (w/w) methyl alcohol as a stabilizing agent to delay polymerization to paraformaldehyde. Formalin in some countries is a trade-mark.

Experimental methods

Sixty *Periplaneta americana* (L.) were anaesthetized with CO₂ gas and transferred to five cockroach boxes labelled A–E; 12 cockroaches per box. All insects were maintained on a water-only diet for 3 days. On the 4th day each group received in place of the water 1 ml of CAM extract with a titre of at least 10⁷ p.f.u./ml which was soon consumed; this was replenished *ad libitum*. No solid food was provided. Faeces were collected daily by picking individual faecal pellets out of the collection trays with forceps.

Faeces collected from each container were pooled and homogenized in 0.2 ml of diluent per faecal pellet and spun at 3000 rev./min for 30 min; 0.2 ml of undiluted, 10⁻¹ and 10⁻² dilutions of the homogenate were inoculated into each of 5 Vero tubes, adsorbed for 1 h at room temperature, re-fed with 1 ml of maintenance medium – without removing the inoculum – and incubated at 36.5 °C for 48 h after discarding growth media.

The collection trays were chemically disinfected with hypochlorite solution, well rinsed in sterile distilled water, dried and replaced immediately after each collection.

On the 6th day cockroaches received their last meal of CAM extract. Fumigation commenced on the 6th day, food receptacles being removed from all boxes. Four boxes with cockroaches were placed in a room in the isolation suite in the animal house, the 5th box (A) being kept in an adjacent unfumigated control room. Fumigation was carried out using double the recommended quantities of formalin and potassium permanganate. The initial concentration of formaldehyde in the air was measured and the room, which had already been carefully sealed internally, was closed and the door and keyhole sealed externally. Sealing was effected with 'plumbers' tape', a 7.5 cm wide fabric tape impregnated with heavy petroleum grease (Denso Tape, supplied by Winn & Coales (Denso) Ltd.). The sealed room was left for 24 h, after which air samples were withdrawn by a 30 ml syringe (containing 5 ml of distilled water) through thin rubber tubing passed through the keyhole. The syringe was shaken for about 2 min and the formaldehyde content of the water was estimated colorimetrically by the method of Nash (1953).

On the 7th day cockroaches in all boxes were changed to a diet of normal (uninfected) CAM extract. Faeces were assayed for virus daily, up to the 17th day.

RESULTS

All cockroaches survived the fumigation process with no evident damage. Assay of the formalin showed an initial concentration greater than 1 mg/l of air. Faeces collected daily up to the 4th day, i.e. before infection, did not yield any endogenous viruses. All faeces collected on the 5th and 6th day (before fumigation) were positive for vaccinia virus. Faeces collected subsequently up to the 11th day (5 days after fumigation) were intermittently positive. Unfumigated control cockroaches excreted virus intermittently up to the 17th day. Results are shown in Table 1.

Table 1. Presence (+) or absence (-) of vaccinia virus in faeces from unfumigated and fumigated cockroaches

Day	Procedure	Box A (control)	Box B	Box C	Box D	Box E
1	Starvation	-	-	-	-	-
2	Starvation	-	-	-	-	-
3	Starvation	-	-	-	-	-
4	Infected CAM extract	-	-	-	-	-
5	Infected CAM extract	+	+	+	+	+
6	Infected CAM extract	+	+	+	+	+
Fumigation						
7	Uninfected CAM extract	+	-	-	+	+
8	Uninfected CAM extract	+	+	+	+	+
9	Uninfected CAM extract	+	-	-	-	+
10	Uninfected CAM extract	+	+	-	-	+
11	Uninfected CAM extract	-	-	+	-	-
12	Uninfected CAM extract	-	-	-	*	-
13	Uninfected CAM extract	-	-	-	*	-
14	Uninfected CAM extract	-	-	-	-	-
15	Uninfected CAM extract	+	-	-	-	-
16	Uninfected CAM extract	-	-	-	-	-
17	Uninfected CAM extract	+	-	-	-	-

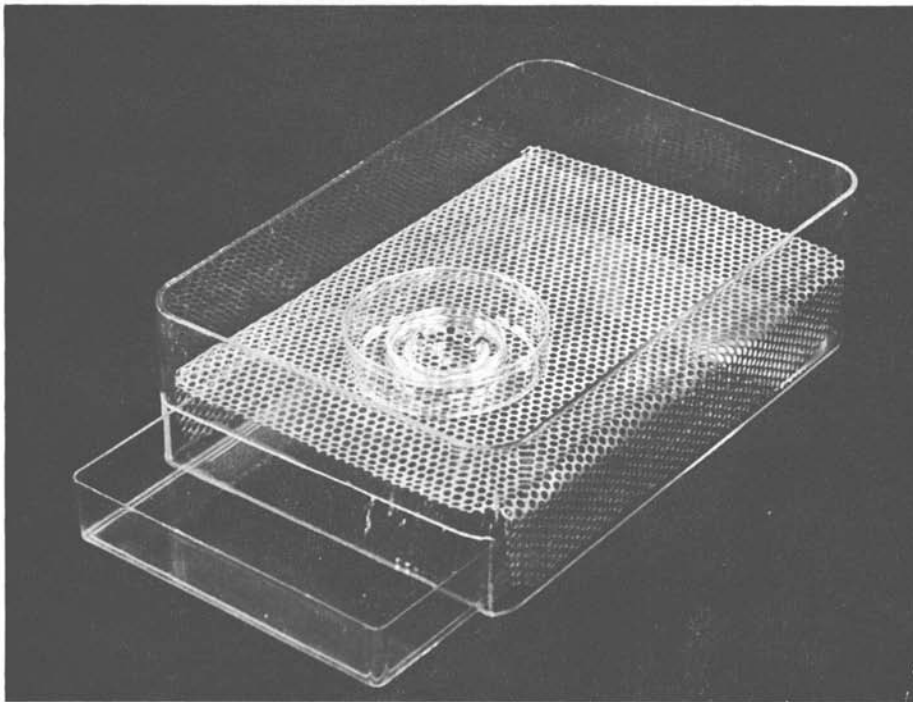
* No samples available.

DISCUSSION

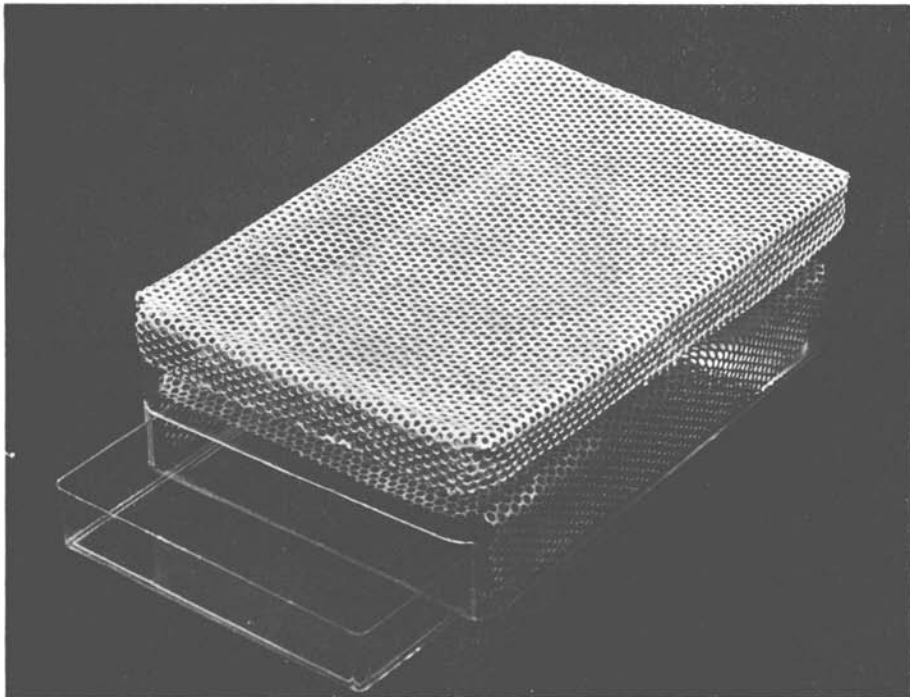
Neither the principal monograph on formaldehyde (Walker, 1964), nor the two classic volumes on the cockroach (Cornwell, 1968, 1976), nor any of the numerous works on disinfection give adequate indication of the tolerance of cockroaches to gaseous formaldehyde at concentrations of 1-2 mg/l of air demonstrated in these experiments. This observation, repeatedly confirmed, led to speculation about the mechanism involved in the resistance. Possibilities include tissue tolerance, chemical alteration or polymerization of the formaldehyde, and exclusion from the body by some altered respiratory mechanism.

The feeding experiment reported above seemed to indicate that virus could pass through the intestinal tract, but the possibility existed that the faeces might have become contaminated during defaecation by virus lodged on the body surface. Preliminary tests showed that virus did sometimes survive on the external surface of the cockroaches subjected to fumigation - presumably protected in crevices. We found, however, that after cockroach bodies had been washed in aqueous buffered (pH 7.5-8.5) 2% glutaraldehyde in Tween 80 (Cidex) in an ultrasonic bath for 15 sec, all residual surface virus was killed. These insects could then be dissected, residual glutaraldehyde neutralized with sodium metabisulphate, and live virus recovered from a homogenate of gut. These findings seemed to indicate that the virus found in faeces in the main experiment was indeed derived from the gut contents and not from surface contamination of the faeces during defaecation.

A third finding arising out of these experiments was that 2% glutaraldehyde



a



b

C. A. BARTZOKAS AND OTHERS

(Facing p. 129)

solution was regularly effective in rendering the surface of the insects virus-free, whereas 24 h exposure to formaldehyde vapour was not.

Perhaps the most important practical point is the frequent survival of ingested virus and its excretion in the faeces. It was possible to make only an approximate estimate of the intake of virus by individual insects but the quantity in the faeces did not suggest that virus had replicated to any significant extent.

Although natural transmission of animal or human viruses by cockroaches has not yet been proved, four unspecified strains of poliomyelitis virus were isolated from four lots of cockroaches captured on the premises of paralytic poliomyelitis patients (Syverton *et al.* 1952), and Roth & Willis (1957) in their review on the 'Medical and Veterinary Importance of Cockroaches' give further evidence that cockroaches are potential vectors of disease and can harbour Poliomyelitis, Coxsackie, Mouse Encephalomyelitis and Yellow-fever viruses experimentally administered.

These findings should be considered by those wishing to make formaldehyde fumigation an effective scientific procedure rather than – as sometimes seems to be the case – a symbolic catharsis, for our findings suggest that the Public Health view that cockroaches do not actually spread disease may be worth re-appraisal.

REFERENCES

- CORNWELL, P. B. (1968). *The Cockroach*, vol. I. London: Hutchinson & Co. (Publishers) Ltd.
- CORNWELL, P. B. (1976). *The Cockroach*, vol. II. London: Associated Business Programmes.
- GREAT BRITAIN DEPARTMENT OF HEALTH AND SOCIAL SECURITY AND THE WELSH OFFICE (1975). *Memorandum on the Control of Outbreaks of Smallpox*. London: Her Majesty's Stationery Office.
- MARTINDALE, W. (1972). *The Extra Pharmacopoea*, 26th ed. London: The Pharmaceutical Press.
- NASH, T. (1953). The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. *Journal of Biochemistry* **55**, 416.
- ROTH, L. M. & WILLIS, E. R. (1957). *The Medical and Veterinary Importance of Cockroaches*. Washington: Smithsonian Miscellaneous Collections 134, No. 10.
- SYVERTON, J. T., FISCHER, R. G., SMITH, S. A., DOW, R. P. & SCHOOF, H. F. (1952). The cockroach as a natural extrahuman source of poliomyelitis virus. *Federation Proceedings* **11**, 483.
- WALKER, J. F. (1964). *Formaldehyde*, 3rd ed. New York: Reinhold Publishing Corporation.

EXPLANATION OF PLATE 1a, b

Cockroach box (a) with cover removed, (b) with cover in place.