

The Transmission of Tumours Induced in Cockroaches by Nerve Severance

Transection of the recurrent nerve has been found to induce tumour-like growths in the salivary glands, reservoirs, gastric coeca and midgut of the cockroach, *Periplaneta americana*^{1,2}. Such growths begin to appear within about 3 weeks in the salivary glands, and histologically comprise the glandular tissue and accumulated masses of haemocytes which often infiltrate into the surrounding tissues. Fragments of the tumour tissue transplanted into normal insects induce similar growths in the salivary glands (HEMA, unpublished observations). This report deals with the effects of injections of cell-free extracts or filtrates of tumour-bearing tissues into normal cockroaches.

Materials and methods. Tumour-like growths involving salivary glands, receptacles, gastric coeca and neighbouring adherent masses of haemocytes were removed from 6 experimental adult cockroaches of both sexes 8–10 weeks after severance of the recurrent nerve, and cell-free extracts were prepared, as follows: the tissues were homogenized in insect saline to give a 10% homogenate (w/v), which was centrifuged at $10,000 \times g$ for 1 h at 4°C in a Sorvall RC2-B refrigerated centrifuge. Some of the supernatant fluid was then centrifuged again at $20,000 \times g$ for 1 h. Some of the $20,000 \times g$ supernatant fluid was then filtered through a 450 ± 20 nm cellulose acetate filter using a Swinnex filter holder (Millipore); some of this filtrate was then filtered through a 100 ± 10 nm filter. Some of the $10,000 \times g$ supernatant fluid was frozen and thawed 5 times (at -70°C and 37°C) or heated at 56°C for 40 min. Insects were then given injections of cell-free extract ($10,000$ or $20,000 \times g$ supernatant fluid), cell-free filtrate (450 or 100 nm filtrate, frozen-thawed extract or heated extract. Similar tissues were removed from normal insects, homogenized and centrifuged at $10,000 \times g$ for 1 h, the supernatant fluid being injected as a normal tissue extract control.

0.05 ml of extract was injected into the haemocoel through the prothorax or abdominal tergite of adult

cockroaches of both sexes or last instar nymphs. Salivary glands and receptacles were dissected out 10–12 weeks after injection, examined under a stereomicroscope to note gross changes or the white spotting characteristic of haemocyte invasion. These tissues were then fixed in Bouin's fluid, and paraffin sections cut at $8 \mu\text{m}$ were stained with haematoxylin and eosin for histological examination. Ultra-thin sections of glutaraldehyde-osmic acid fixed material were cut with an Ultratome III LKB and stained with 3% uranyl acetate, and examined in an RCA EMU 3H electron microscope.

Results. Whatever the site of injection, it was found that in most treated insects, there was an accumulation of haemocytes scattered at various points on the lobes of the salivary glands. However, this accumulation was very pronounced in insects which had received injections of cell-free tumour extracts or filtrates, and in these

Haemocyte accumulation and infiltration with swelling in salivary glands of *Periplaneta americana*, following a single injection of 0.05 ml of cell-free tissue extract

Details of injection	Response (No. of animals)	
	Adults	Nymphs
Tumour extract ($10,000 \times g$ supernate)	+ (5)	+ (5)
Tumour extract ($20,000 \times g$ supernate)	— (6)	++ (5)
Tumour filtrate (450 nm filter)	++ (4)	+++ (4)
Tumour filtrate (100 nm filter)	— (4)	+ (4)
Frozen-thawed tumour extract	— (3)	+ (3)
Heated tumour extract ($56^\circ\text{C}/40'$)	— (2)	+ (1)–(3)
Normal tissue extract ($10,000 \times g$ supernate)	— (14) ? (2)	— (5)

¹ P. HEMA, *Curr. Sci.* 35, 624 (1966).

² D. J. SUTHERLAND, *Natn. Cancer Inst. Monogr.* 37, 399 (1969).

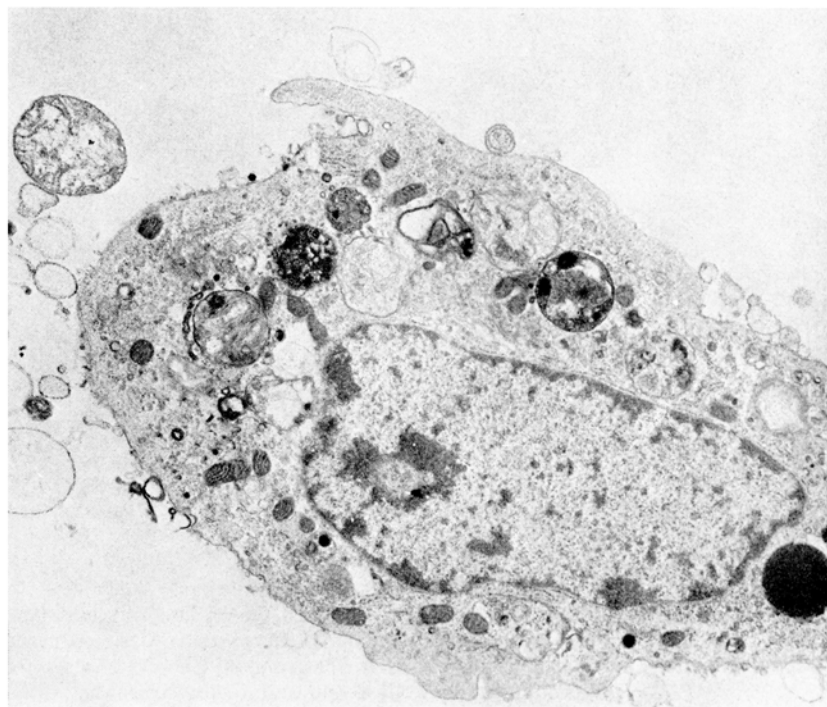


Fig. 1. Electron micrograph of a haemocyte attached to a lobe of salivary gland, showing cytoplasmic bodies and vesicular bodies. From an insect 50 days after injection of tumour extract. $\times 9,200$.

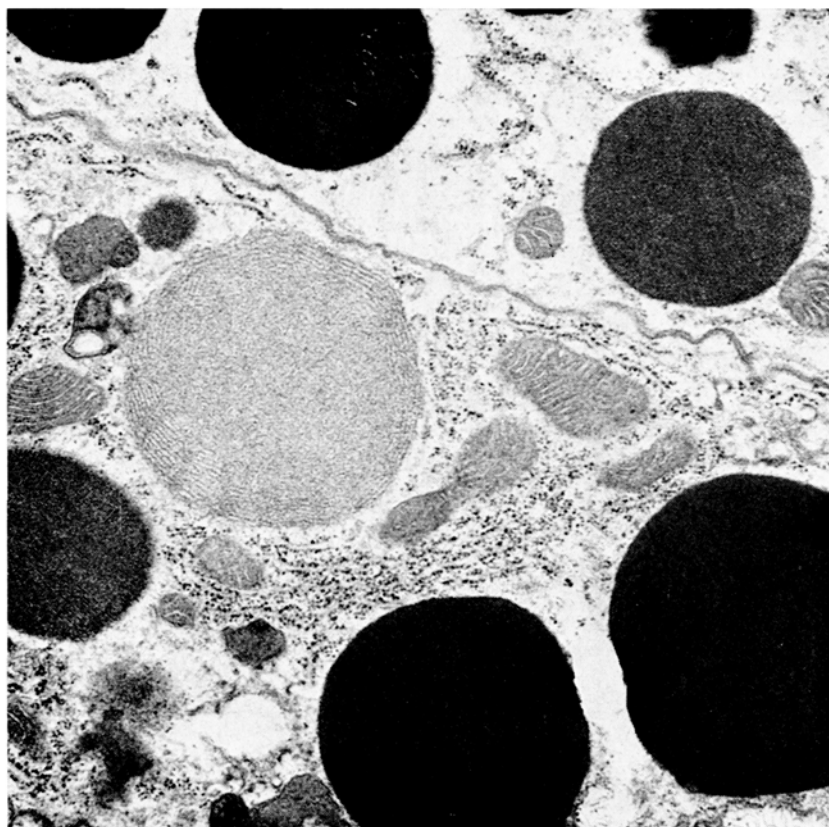


Fig. 2. Edge of salivary gland from the same animal, showing adipohaemocyte and electron-dense granules. $\times 20,700$.

cases there was marked infiltration of the blood cells into the glandular tissue similar to the reactions shown by nerve-sectioned insects. Nymphs showed the greater haemocyte accumulation and infiltration response. The results obtained are summarized in the Table, and the extent of the reaction is roughly quantitated using the symbols — + + + or + + + +.

Different types of cytoplasmic granules occurred in the haemocytes accumulated on the glands. Ultrastructural studies of the blood cells revealed irregularly-shaped mitochondria and many membrane-bound bodies containing characteristic structures (Figure 1). Granular bodies encroaching into the gland cells may represent haemocytic remnants³ and probably parts of adipohaemocytes and spherulites⁴ (Figure 2).

Discussion. The results indicate that the nymphs respond to injections of cell-free extracts or filtrates of tumorous tissues more readily and exhibit more positive reactions involving infiltration and invasion of the salivary glands than do adults, where the response, though present, is less well defined. The sensitivity of the salivary glands to such injections cannot yet be explained, but they are also the first organs to develop tumorous lesions after severance of the recurrent nerve^{1,5}.

Résumé. On a obtenu des tumeurs dans des blattes (*Periplaneta americana*) après la section du nerf récurrent. Des extraits ou des filtrats de ces tumeurs, exempts de cellules, ont provoqué des lésions semblables quand on les a injectés à des adultes ou des nymphes.

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Norwich NOR 88C (England), 13 July 1970.

³ R. L. TAYLOR, J. Inv. Path. 13, 167 (1969).

⁴ I. HARPAZ, N. KISLEV and A. ZELCER, J. Inv. Path. 14, 175 (1969).

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A Comparison of Ultrastructural Localization of Carnitine Acetyltransferase Activity in Mouse Liver Mitochondria with that in Cardiac Muscle

This communication describes the ultrastructural localization of carnitine acetyltransferase activity in the mitochondria of mouse liver, which to our knowledge has not been previously reported, compared with the activity in the cardiac mitochondria.

This enzyme catalyses the reaction; Acetyl-CoA + Carnitine \rightleftharpoons Acetylcarnitine + CoA. The cytochemical method of HIGGINS and BARNETT¹ utilizes the reducing property of the released SH group of free CoA to reduce potassium ferricyanide to potassium ferrocyanide, which