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Cytopathological and Numerical Changes in Haemocytes of *Periplaneta americana* after Treatment with Malathion

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Haemolymph plays a significant role in the detoxification of chemicals. (Patton 1961) observed a reduction in haemocyte numbers after the application of parathion to crickets and adjudged the haemolymph to be the main site of detoxification. (Arnold 1974) was of the opinion that haemocytes do not function in direct defense against the toxicants. (Rakitin 1974) found that haemocytes absorbed a considerable proportion of an administered radioactive insecticide, but he did not say how this affected the insects' response to the insecticide. (Feir 1979) reviewed the cellular and humeral responses of haemocytes to toxic substances. In this paper, the pathological and numerical changes in the haemocyte population of American cockroaches after treatment with malathion are described and the route of insecticide movement inside the insect body is discussed.

MATERIALS AND METHODS

Malathion (technical grade) was made up as a 2% solution in benzene.

Aliquots of 10 ul of this solution were applied separately to the forefemur, dorsal thoracic terga and ventral abdominal segments, in nonmelanized newly emerged adults (1 hour after eclosion) of both sexes. A metathoracic leg was severed after 15, 30 and 60 minutes of treatment at the COXO-sternal joint. The haemolymph was quickly drawn up to the 0.1 mark in a Thoma white blood cell dilution pipette and the sample was diluted 100 times with 0.2% glacial acetic acid in Ephrussi and Beadle saline (Buck 1953). The first two or three drops of this solution were discarded, small

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drops were then put on two Knaubers chambers which were immediately covered by a coverslip. Haemocytes were counted in the outer 4 chambers, each containing 16 squares of each chamber. The mean of the two chambers was taken as the haemocyte concentration. At each interval after treatment, four such sets from three different individuals were repeated to determine the standard error. In this way 12 counts were done at each interval. The haemocytes of both sexes were counted independently. The control counts were made in a similar fashion.

The pathological changes in the haemocytes were observed by preparing haemolymph smears after the same time intervals taken for the total counts. The haemocytes were fixed in 5% formalin and stained by the rapid Giemsa method.

The quantitative determination of the haemocyte abberations viz. mitotic counts was also taken into account. Nuclear and cell diameters of control and treated haemocytes (plasmatocytes) were calculated by ocular micrometer and have been expressed in m/u.

RESULTS AND DISCUSSION

The observations showed that females have significantly more haemocytes than males under the conditions of this experiment (Table 1). The total haemocyte count fell in each time interval after treatment in both sexes (Table 1).

Various cytopathological changes exhibited by haemocytes after malathion treatment included shrinkage of granulocytes, cytoplasmic vacuolization in plasmatocytes (Fig. 2) and mitotic divisions in prohaemocytes (Fig. 3), irregular shapes (Fig. 4) as compared to controls (Fig.1). A significant reduction in the nuclear diameter and a non-significant reduction in the cell diameter was observed after 60 minutes of treatment (Table 2). The percentage of mitotic figures was increased after 60 minutes of treatment (Table 3).

A fall in total haemocyte count in both the sexes after malathion treatment suggests that the haemocytes might have been used up in the process of detoxification. A substantial fall in numbers was also seen in the controls by 60 minutes. Parathion (Patton 1961) and chlordane (Gupta and Sutherland 1968) had been found to cause a fall in total haemocyte count. (Witten 1969) suggested that the detoxification process

Table 1. Haemocyte counts of P. americana after treatment with 2% malathion in benzene.

Place of Application	Time interval	Sex	Control* cells/mm^3		Treated* cells/mm^3
Forefemur	15 min	Male	34 ± 1.0	NS	20 ± 1
		Female	40 ± 4.4	P < 0.02	21.5 ± 1.5
	30 min	Male	32 ± .39	P < 0.02	24.7 ± 1.1
		Female	40 ± 4.4	P < 0.02	21.5 ± 1.5
	60 min	Male	21.6 ± 6	P < 0.01	14.2 ± 1.2
		Female	28.6 ± 1.1	P < 0.05	20.9 ± 1.8
Dorsal Thoracic Terga	15 min	Male	50.8 ± .96	P < .001	26.8 ± 1.20
		Female	67.4 ± 5.9	P < 0.02	42.3 ± 1.1
	30 min	Male	27.7 ± 1.8	P < 0.02	20.6 ± .08
		Female	60.3 ± 1.6	NS	52 ± 14.7
	60 min	Male	28.2 ± 1.1	P < 0.01	14.8 ± 1.7
		Female	26.8 ± .3	P < 0.05	21.1 ± 1.7
Central Abdominal Segment	15 min	Male	37.1 ± 11.5	NS	28 ± 1.0
		Female	43.6 ± 1.5	P < 0.05	32.3 ± 3.1
	30 min	Male	25.7 ± 1.4	NS	20.5 ± 2.4
		Female	59.9 ± 2.0	P < .01	37.5 ± 1.0
	60 min	Male	28.0 ± 3.5	NS	20.3 ± 3.0
		Female	26.4 ± 1.4	NS	23.9 ± 1.9

*Round off figures to nearest 1000.
NS = Non-significant.

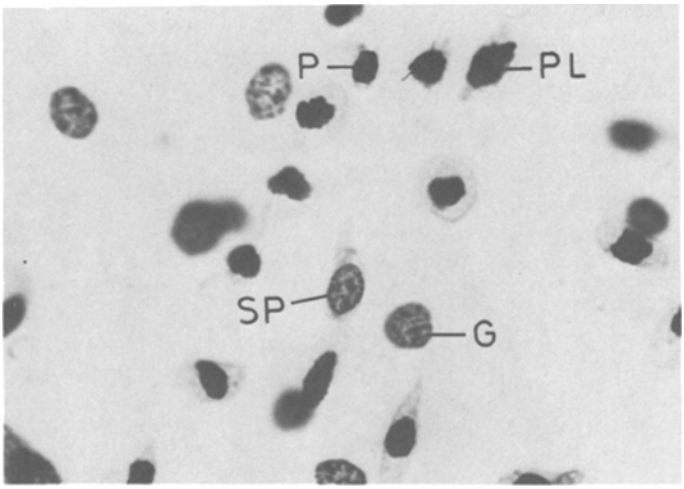

Fig.1 Normal haemocytes showing Prohaemocytes (P) Plasmatocyte (PL) Spherulocyte (SP) Granulocyte (G) x 400.

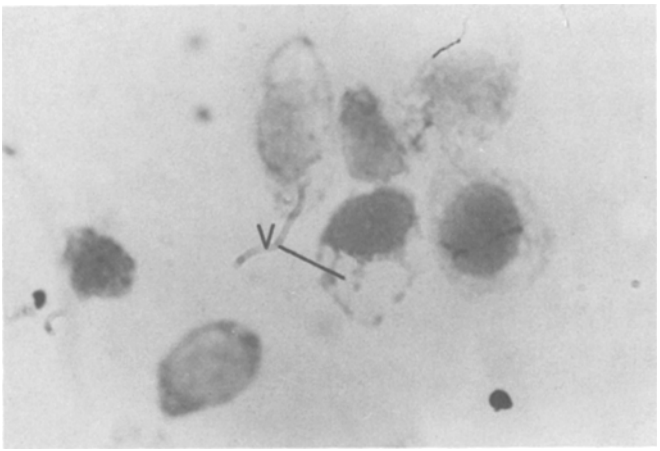

Fig.2 Treated insect showing Vacuolization in Plasmatocytes (V) x 950.

that sets in after insecticidal pentration through the tarsal cuticle caused a fall in total haemocyte population.

Cytopathological changes in haemocytes of Prodenia after arsenical and mercuric chloride poisoning (Yeager and Munson 1942) included rounding up of cells, cell agglutination and increased mitotic frequency. Such changes were also found in the present investigation, and can be interpreted as indicating the passage of malathion to the target organ (Saxena and Saxena 1984) through the haemolymph. Onset of the changes

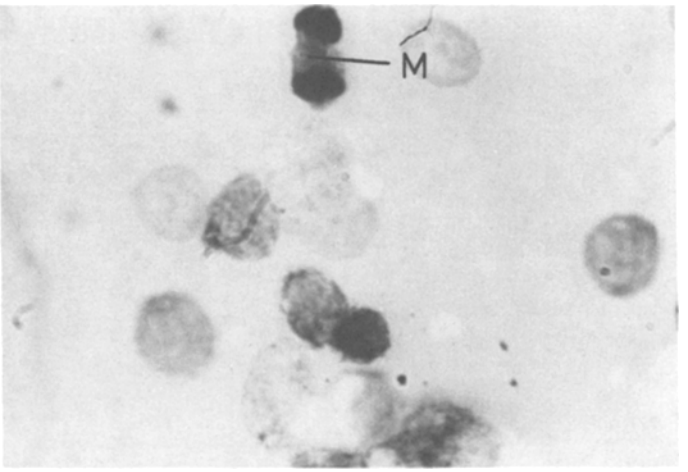

Fig.3 Treated insect showing Mitotic division in Prohaemocytes (M) x 950.

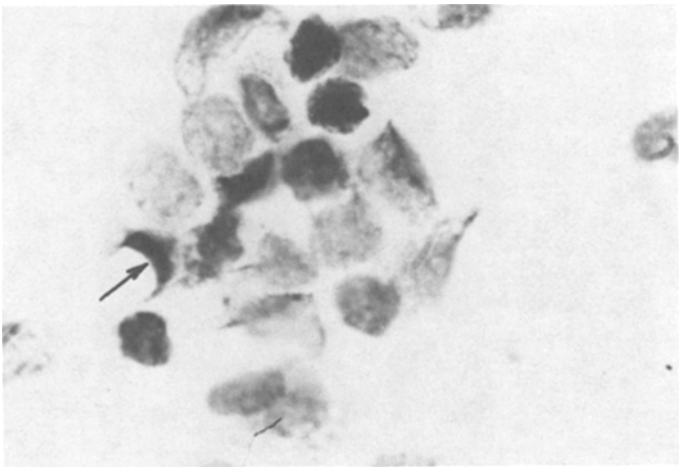

Fig.4 Treated insect showing change in shape of haemocytes (arrow) x 950.

was observed at the time paralytic symptoms appeared, namely 15 minutes after treatment. Based on these findings the present authors do not accept the view of (Gerolt 1969) that penetration of topically applied chemicals is solely via tracheae. Perhaps Gerolt might not have examined the effect of insecticides on haemocytes. Further evidence supporting the passage of malathion through the haemolymph to the target organs was the presence of malathion on thin layer chromatograms and gas liquid chromatograms of the haemolymph after 5 minutes of treatment (Saxena 1982).

Table 2. Quantitative determination of nuclear and cell diameter of plasmatocytes after treatment with 2% malathion (in benzene).

Total No.of plasmatocytes*	Time interval (min)	Nuclear Diameter (m/u)		Cell Diameter (m/u)	
		Control	Treated	Control	Treated
10	60	3.62±.067	3.35±.06*	6.71±.38	6.46±.26**
10	60	3.62±.058	3.34±.06*	6.72±.36	6.44±.22**
10	60	3.63±.070	3.32±.05*	6.73±.34	6.45±.25**

*3 sets were taken in each case.

*($P < .001$)

**($P > .05$)

Table 3. Mitotic counts of prohaemocytes in control (benzene) and treated (malathion 2%) cockroaches.

Number of Cells counted	Mitotic figures in (Percentage)	
	Control	Treated
50	4%	34%
50	2%	32%
50	2%	38%

Earlier (Burt and Lord 1968; Burt et al. 1971) found no evidence for involvement of tracheae in the penetration of pyrethrin I. (Moriarity and French 1971; Polles and Vinson 1972; Olson 1973) had similar findings and demonstrated that lateral spreading of the toxicant in the epicuticular lipids is unimportant and favoured haemolymph to be the chief distributor of toxicant.

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