Amoebiasis and its effect on cell division in the midgut of the African migratory locust

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Malameba locustae (King & Taylor), a protozoan parasite of many laboratory-reared locusts, is known to damage epithelial cells of the host midgut regions. Mitotic and labelling indices in both caecal and ventricular regions of parasite-free and infected *Locusta migratoria migratoriodes* (Reiche & Faimaire) were investigated in the laboratory. Infected locusts were found to have significantly higher mitotic index values in both regions of the midgut than those found in parasite-free locusts. No significant differences were found in the labelling index between infected and parasite-free locusts indicating that one of the gap phases in the cell cycle becomes altered. This would account for the observed increase in cell division. A significantly larger number of cells was noted in the nidi of the ventricular regions of locusts infected with parasites.

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Dit is bekend dat *Malameba locustae* (King & Taylor), 'n protosoale parasiet van laboratorium geteelde sprinkane die epiteelselle van die gasheer se middelderm beskadig. Mitotiese en gemerkte indekse in die caeca en ventriculi van besmette en parasietvrye *Locusta migratoria migratoriodes* (Reiche & Fairmaire) is in die laboratorium ondersoek. Besmette sprinkane het betekenisvol hoër mitotiese indekse in albei streke van die middelderm getoon as die wat in die nie-besmette insekte gevind is. Geen betekenisvolle verskil in die gemerkte indeks is tussen die besmette en parasietvrye sprinkane gevind nie. Dit dui daarop dat die een G-fase in die selsiklus verander word wat die waarneming van 'n groter aantal selle in mitose verklaar. 'n Betekenisvol groter aantal selle is in die nidi van die middelderm van die besmette sprinkane waargeneem.

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Henry (1968) working with *Melanoplus differentialis* maintained that the epithelium of midgut regions was completely destroyed by trophozoites of *Malameba locustae* (King & Taylor), an amoeboid parasite commonly found in laboratory-bred locusts, and postulated that dividing cells in the nidi of the midgut epithelium were unable to produce new cells fast enough to replace damaged tissue. Such extremes of infection could not be confirmed in *Locusta migratoria migratoriodes* (Reiche & Fairmaire) by Hanrahan (1975) where even high infections caused relatively little cell damage. However, it seems unlikely that a parasite that penetrated host cells would play the role of harmless commensal. If cells were damaged and lost more rapidly from the infected midgut epithelium one would expect an increase in cell division to compensate for early cell death. Even if many of the *M. locustae* trophozoites only migrated between cells or between cells and basement membrane, life span of the cells could be shortened. The ability of nidal cells to replace dying epithelial cells must play an important part in the insect's ability to cope with infection.

A great deal of research has been done on the replacement of epithelia in the mammalian alimentary canal, (Leblond & Walker 1956; Cleaver 1967; Eastwood & Quimby 1982) but comparatively few investigations have been carried out on the insect midgut epithelium. The midgut of locusts comprises six gastric caeca and the ventriculus which are lined by a simple columnar epithelium of differentiated functional cells. Undifferentiated cells occur in groups called nidi which are situated below the free surface of the epithelium and bulge into the body cavity. Nidal cells undergo mitosis. Snodgrass (1935) assumed that cells formed here replaced dying cells sloughed from the epithelium. This process of epithelial renewal is known for other insects such as *Rhodnius* (Wigglesworth 1942), *Periplaneta americana* (Day & Powning 1949) and *Bombyx mori* (Gheorghiu 1971). Harry (1965) described the changes that occur in the caecal epithelium of *Schistocerca gregaria* just after the final moult. These changes involved an increase in size of the nidi and formation of chromatic granules in older cells which were basophilic prior to sloughing. No mention of mitotic activity was made.

Mitotic activity is known to be influenced by starvation and feeding in *Periplaneta, Blatella* and *Tenebrio* (Day & Powning 1949) and *Aeschna cynea* (Andries 1970a,b). Ecdysone plays a role in influencing both mitotic activity and epithelial cell differentiation (Andries 1970a).

M. locustae trophozoites have been observed in both the gastric caeca and ventricular regions of the midgut of *L. migratoria* and as these tissues undergo cell division the

present investigation was carried out to determine the possible correlation between parasitic infection and cell division.

Materials and Methods

Rearing of locusts

Parasite-free *L. migratoria* were obtained by using methods suggested by Harry (1965, 1969). Recently laid egg pods were obtained from the locust laboratory at the University of the Witwatersrand. Individual eggs were separated and rinsed, first in a jet of 70% ethanol for 30 s then in distilled water, and placed in sterilized covered glass dishes on damp filter paper and incubated at 22 °C. Damaged and discoloured eggs were discarded. Hatchlings were placed in 12,5-l perspex cages which were cleaned beforehand with running hot water and 70% ethanol. The removable perforated metal floor and lid of each cage were sterilized in boiling water. Cages were placed on aluminium foil which was replaced daily so as to remove faeces. These cages were covered by a second perspex cage. A 20-W light globe to provide illumination and maintain a temperature of approximately 30 °C was placed under the outer cage.

Hatchlings were fed fresh oat leaves and dry bran daily. One group was infected with *M. locustae* cysts which were placed on this food. The other group remained parasite-free. Fifth instar hoppers were kept under daily observation and the time and date of the final moult recorded, so that adults of known age could be obtained for experimentation. Since fledging locusts did not feed for 24 h after the moult, two days were allowed to elapse before they were used.

Techniques used to measure mitotic index and labelling index

Twenty locusts were given either 50 μl of 0,5% colchine made up in locust saline (Hoyle 1953) or 40 μl (40 Ci) ^{3}H thymidine per gram body mass, by means of an injection directly into the abdomen via the soft integument just posterior to the hind femur. Ten locusts which served as controls were injected with equal amounts of locust saline at the same time. Injections were given at 10h00 each morning, immediately before feeding. Four hours later the injections were repeated because examination of faeces by means of scintillation counting had shown that large quantities of injected ^{3}H thymidine were excreted rapidly. After eight hours the locusts were killed and eviscerated, the guts being fixed in Carnoy's fluid. Each gut was slit longitudinally and the contents were removed prior to dehydration and embedding in wax. Colchicine-treated specimens and controls were longitudinally sectioned at 10 μm and stained with Mayer's haematoxylon and eosin. Specimens destined for autoradiography were sectioned at 8 μm. Sections were hydrated, dipped in 0,3% sodium lauryl sulphate then washed in distilled water. Once dry, the slides were coated with formvar made up as an 0,1% solution in chloroform. The slides were then dipped in Ilford G5 nuclear emulsion and exposed for 17 days at 4 °C. The slides were developed in D19B developer and stained in Mayer's haematoxylon for 5 min.

Since gut length varied according to the distention caused by ingested food, a constant number of nidi was chosen as the best unit of comparison. Cells of both caecal and ventricular epithelium were counted over a distance equivalent to 35 nidi, anything from 1,75 – 2,00 mm, and replicated four times per locust gut. Average numbers of nidal cells, including cells in metaphase, and of mature epithelial cells were determined for locusts injected with colchicine and expressed as a percentage of the total cell number examined. Mitotic index was determined from the percentage of cells in metaphase. The locusts that received ^{3}H thymidine were treated similarly and the labelling index was determined from the percentage of labelled cells. Results from parasite-free and infected locusts were then compared statistically. The proportions of cells making up the midgut structures were compared by expressing the number of nidal cells as a percentage of the number of mature epithelial cells.

Results

Controls

The controls, which were parasite-free and which were given neither colchicine nor ^{3}H thymidine, were used for comparative purposes to evaluate the quality of processing of the tissues of the locusts that had been given the experimental treatment. In trial counts the mitotic index in the controls was found to be very low, varying from 0 – 1,9%. No serious attempt was therefore made to complete counts to calculate the mitotic index of these specimens. The midgut region of the locust was found to cause background when exposed to the nuclear emulsion but the use of a formvar film reduced the background substantially.

Cell composition of midgut regions

As expected from a microscopic examination of the midgut regions the number of cells in the nidal compartment was found to vary. The number of cells found in the nidi of ventricular regions (Figure 1) was smaller than the number of cells found in the nidi of the caecal regions (Figure 2). It was therefore interesting to find that the number of mature epithelial cells counted over a length of 35 nidi was remarkably constant and similar in both caeca and ventriculi. When this difference was noted the nidal cells were expressed as a percentage of the epithelial cells. It was found that in caeca of both infected and parasite-free locusts the nidal cells were equal to 63% of the mature cell number. In the ventriculi of parasite-free locusts, including those of the controls, nidal cells were almost equal in number to the mature epithelial cells (i.e. 96% Table 1 and 94% Table 2), whereas in the infected locusts there were significantly more cells in the nidal compartment (t = 2,95; 0,01 < p < 0,002; d.f. = 18). Either large numbers of cells are produced in the nidal compartment in the ventriculi of infected locusts or numbers of cells are retained in this compartment.

Colchicine experiment (Table 1)

Mitotic indices in both caeca and ventriculi were found to be similar for each group of locusts studied. A significant difference in mitotic index was found between locusts infected with *M. locustae* and those that were parasite-free. As expected, the mitotic index was found to have a greater variability in the infected animals, as parasites were distributed randomly along the epithelium. The results summarized in Table 1 show that the presence of *M. locustae* in the midgut can definitely be related to a significant increase in mitotic activity of the nidi of caeca and ventriculi (for caeca t = 3,48; 0,002 < p < 0,01 and for ventriculi t = 5,28; p > 0,001).

^{3}H-Thymidine experiment (Table 2, Figures 1 & 2)

Since the mitotic index was higher in infected locusts than in the parasite-free locusts it was expected that more cells would be synthesizing DNA and therefore incorporating the ^{3}H thymidine. As can be seen from Table 2 the percentage of labelled cells was roughly the same in the midgut regions of both infected and parasite-free locusts (p > 0,5).

In eight of the ten infected locusts examined labelled nuclei were found not only in the nidus but also in cells of the adjacent epithelium (Figure 1). These cells were few in number and were mostly found immediately adjacent to the nidus.

Discussion

Morphological damage to the locust midgut epithelium caused by *M. locustae* trophozoites has been observed to be of a minor nature in *L. migratoria* (Hanrahan 1975) and not as severe as originally described by Prinsloo (1960), using *Locustana pardalina* collected in the field, and Henry (1968) using *Melanoplus* sp. Nevertheless the results of the present study have shown that *M. locustae* infection in *L. migratoria* can be correlated with a higher mitotic index in the midgut regions which indicates that the parasite plays a significant role in decreasing the epithelial cell life span.

A number of authors working with various protozoan and other infections of chicken, mammalian or human intestine have discovered that an increase in cell number in the progenitor compartment (crypt) and a decrease in the cell number of the functional epithelial compartment (villus) occurs as a result of acute infection (Symons 1965; Fernando & McCraw 1973, 1977).

In the present study only an increase in the progenitor compartment (nidus) was noted. Functional epithelial cell number remained constant. Conditions found here appear to represent a state of chronic rather than acute infection. Reduction of size of the functional compartment is possibly a feature of acute infection.

One discrepancy found in these experiments was the small number of nidal cells in the caecal regions of infected locusts. This was surprising as the mitotic index was observed to be

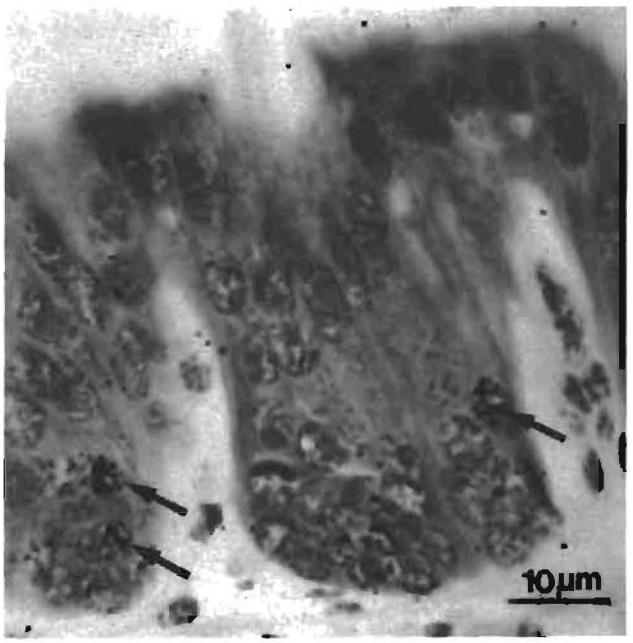

Figure 1 Longitudinal section through the ventricular region of the midgut. ^{3}H-Thymidine-labelled nuclei in and next to the nidus indicated by arrows.

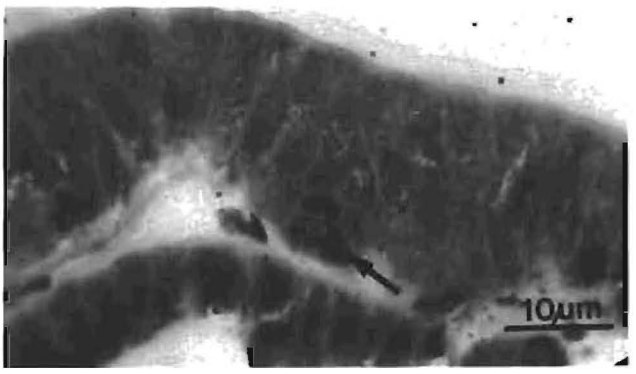

Figure 2 Longitudinal section through the ceacal region of the midgut showing nidi containing few cells.

Table 1 Effects of *M. locustae* on the mitotic index of locust midgut tissues

Locust	Tissue	Av. no. of epithelial cells (E.C.)	Av. no. of nidal cells (N.C.)	$\frac{N.C.}{E.C.} \times 100$	Av. no. of mitotic cells	Average mitotic index	Standard error	Coefficient of variation
Parasite-free (10)[a]	Caeca	374	239	64%	50	7,5	±0,36	5%
	Ventriculus	318	305	96%	41	6,1	± 0,28	5%
Infected (8)[a]	Caeca	364	229	63%	80	12,3	± 1,33	11%
	Ventriculus	363	378	104%	112	13,8	± 2,50	11%

[a]Number of locusts examined.

Table 2 Effects of *M. locustae* on labelling index in locust midgut tissues

Locust	Tissue	Av. no. of epithelial cells (E.C.)	Av. no. of nidal cells (N.C.)	$\frac{N.C.}{E.C.} \times 100$	Av. no. of labelled cells	% labelled cells	Standard error	Coefficient of variation
Parasite-free (10)[a]	Caeca	385	220	57%	75	12,6	± 1,15	9,13
	Ventriculus	354	333	94%	98	14,2	± 1,05	7,4
Infected (10)[a]	Caeca	391	251	64%	86	14,52	± 4,92	33,3
	Ventriculus	387	468	125%	122	14,21	± 1,27	31,6

[a]Number of locusts examined.

higher in these locusts. Previous observations (Hanrahan 1975) indicated that caecal epithelia were generally less severely infected with *M. locustae* trophozoites than ventricular epithelia. The increase in nidal cell number in the ventricular regions in the present investigation probably represents a localized response of the host tissue to the parasite.

The number of cells undergoing DNA synthesis within a given time period was found to be similar in both infected and control locusts. This raises the question of whether other phases of the cell cycle such as the gap phases, G_1 and G_2, are shortened so that cell production rate increases to cope with the cells lost as a result of infection. G_1 is known to be a highly variable phase in the cell cycle (Quastler & Sherman 1959; Symons 1965) so that this would not be unusual.

The functional epithelial cells that were labelled could either have taken up the ^3H thymidine during synthesis of DNA for repair or possibly could have completed DNA synthesis, division and differentiation within the 8-h period that elapsed between injection and killing the locusts. This suggests that the cell cycle in some locust nidal cells may be relatively short. A third possibility is that some of the locust midgut cells become polytene, duplicating DNA material within the nucleus. This is not usual for insect cells (Wigglesworth 1972).

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