RNase activity. The effect of ultrasonic waves was studied for two kinds of sections prepared from the 16 mm roots: 0–5 mm (from the apex, section I) and 5–10 mm (section II).

As shown in the Table, the RNase activity is obviously decreased for section I by the ultrasonic treatment, when the result is presented in terms of fresh weight. The water content of ultrasonicated roots is almost completely stable[3], whereas the PN level is significantly decreased[3]. As a consequence, no RNase activity change is shown when the result is expressed in terms of PN weight, because the variations of the enzymatic activity correspond to some extent to the PN level decrease. In regard to section II, no significant RNase activity change is observed, no matter what unit of reference is used. Therefore we can conclude that the ultrasonic waves do act on section I only (in the limits of the irradiation duration and the intensity level used).

It was often found that the ultrasonication, in the presence of oxygen, decreases the activity of several enzymes in solution, and no enhancement of enzymatic activity has ever been observed[8,9]. As regards RNase, the irradiation of solutions can lead to the detachment of low molecular peptides or of amino acids, generally without loss of activity[9]. However, inactivation is observed in the presence of OH radicals, which can be produced by cavitation[9,10].

In the present study, the mechanism of inactivation must be entirely different, since cavitation does not occur. Compared to water solutions, the cytoplasm is characterized by a higher viscosity. As a result, ultrasonic waves bring on significant thermal effects. The propagation of sound waves is essentially adiabatic, so that the resulting raising of temperature may cause enzyme denaturation. Since the ultrasonication is a non-cavitating one, changes of pH do not occur. They could be induced by electrochemical effects due to cavitation, and these changes would also explain denaturation.

Compared to section II, section I shows a higher cytoplasmic density[11] and a lower water content[3], which indicates a higher viscosity. The discrepancy between section I and II with respect to RNase inactivation can therefore be explained by the higher cytoplasmic viscosity of section I.

In spite of RNase inactivation, the PN level is decreased, as has been observed several hours after ultrasonication[2,3]. Therefore, the RNA biosynthesis must also be disturbed. Furthermore, the protein synthesis can be directly inhibited by the ultrasonically-induced ribosomes disintegration[12].

Résumé. L'irradiation ultrasonique de racines de *Lens culinaris* provoque une baisse de l'activité RNasique dans la zone apicale (0–5 mm). L'ultrason étant non-cavitant, cette réduction d'activité doit être causée par l'action dénaturante liée aux effets thermiques de la perturbation ultrasonique. La relativement haute viscosité cytoplasmique de la zone apicale expliquerait pourquoi celle-ci est la seule à être affectée.

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RNase activity (expressed as $10^2 \times$ OD increase) for the 2 sections prepared from non-irradiated (NIR) and irradiated (IR) roots

	NIR	IR	Change (%)[a]
Section I per 50 mg fresh weight	7.1	5.8	− 18.3
Section I per 50 µg PN	1.28	1.30	+ 1.6
Section II per 50 mg fresh weight	10.4	10.1	− 2.9
Section II per 50 µg PN	6.38	6.60	+ 3.4

[a] % = 100. (IR–NIR)/NIR. Each value is the mean of 4 analyses.

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Non-Specific Esterases in Females of *Aedes aegypti* (L.)

Previous work on non-specific esterases in mosquitoes was carried out comparing the isozyme patterns during development from egg to pupa in *Aedes aegypti*, *Culex pipiens pipiens* and *Culex pipiens fatigans* (BRIEGEL and FREYVOGEL[1]). It was shown that during the 4 larval stages, where growth processes take place, the pattern of enzymes is similar and shows most bands. Developing embryos differ from the larval stages as well as pupae and teneral adults. The last 2 were similar to each other within one species. Adults showed no sexual dimorphism with regard to these enzymes.

In order to extend knowledge on non-specific esterases during the lifespan of mosquitoes, the present paper deals with changes in this group of enzymes which occur during the adult life of female *Aedes aegypti* (L.). For this purpose

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the pattern-changes were examined at various ages after emergence; during the gonotrophic cycle, as it is initiated by a blood meal, experiments were done at daily intervals. Since preliminary experiments yielded no convincing sexual differences in esterases, this investigation was restricted to the female sex.

Materials and methods. Two strains of *Aedes aegypti* (L.) were used throughout this work: *A. aegypti*-Congo was the same as in previous work (BRIEGEL and FREYVOGEL[1]), whereas *A. aegypti*-Segemaganga is a new strain isolated in Tanzania from bamboo pot material (BRIEGEL and FREYVOGEL[2]). The new isolate represents a relatively young and unadapted strain. Maintenance of the colonies was according to the general methods for mass breeding.

All females used were allowed to feed on 10%sugar water ad libitum. The females sampled up to 10 days after emergence were not allowed a blood meal, whereas the older ones, of 40–50 days, had passed through several gonotrophic cycles; these contained neither eggs nor traces of blood at the time of homogenization. By the fourth day after blood meal in most cases oviposition had occured and females still carrying eggs were not used.

The methods of separating and staining the non-specific esterases were the same as before (gel-electrophoresis on 10% acrylamide, α-naphtyl-acetate, MAURER[3], BRIEGEL and FREYVOGEL[1]). 3 improvements were made: The tube size of the gels was reduced to 2 mm internal diameter, which enabled us to use less material. For desalting Sephadex G-25 medium (Pharmacia) was added to the sample, which was put on the spacer gel after polymerization. This procedure is less time-consuming and a better resolution of the proteins was achieved. Thirdly, the mosquitoes were homogenized in 20 µl of cold distilled water using a self-tuned ultrasonic disintegrator (MSE 100 Watt) for 2–4 sec.

Nomenclature of the bands is not the same as before (BRIEGEL and FREYVOGEL[1]). Experiments with mixed homogenates enabled the bands carrying esterase activity to be homologized among the different stages investigated.

Results. a) *Aedes aegypti*-Congo. Figure 1 represents all the information obtained on non-specific esterases in the female of this strain. The gel of a newly emerged female (0 days) is taken from Figure 2 in our previous paper (BRIEGEL and FREYVOGEL[1]), but the letters of the bands have been adapted to the recent identification.

The number of visible bands increases slightly (from 7 to 13)with progressing age of the females. Partly this seems to be due to the splitting of some of the enzyme bands, e.g. B and D and, partly, it is due to the appearance of bands, not visible in the earliest stage, e.g. A, E, F. The most prominent bands are D (9–17% of total activity), H (9–24%), and K+L (22–55%). Some bands have reduced activities with age but band H shows an increasing intensity in the older stages.

The total esterase activity per female is 0.45 shortly after emergence, it is reduced in the following days to 0.06 and rises again to 0.21 after 7 weeks.

b) *Aedes aegypti*-Segemaganga. The results obtained for this strain are shown in Figure 2. These zymograms bear similarities to the previous strain. Although no splitting can be observed, there is a slight increase in the number of bands. Further, the same bands bear the main activities: D (10–24%), K (9–25%), L (12–34%). Band H is less intense (5–15)% but shows a weak tendency to increase. Band A is visible from the third day onwards. The observation of the diminishing activities of bands D, K, L again is comparable to *A. aegypti*-Congo. The sum of all esterase extinctions calculated per female is at its highest after emergence (0.14); afterwards it becomes lower (0.07).

Band C is present throughout all stages observed, while it is missing in the Congo strain. Band F is missing here, but present there. In addition, it is surprising that in the Segemaganga strain no esterase activity was recognized at the origin of the gels, unlike in the Congo strain where some staining was always detected in this position. These seem to be the most conspicuous strain differences.

c) Changes depending on blood meal in *A. aegypti*-Segemaganga. Figure 3 shows the group of gel-columns which are based on the female homogenates made at daily intervals after blood meal. For comparison, a diagram of esterases in whole mouse blood is given, obtained applying the same methods. To begin with, the migration of all the bands is retarded. This is thought to be caused by the presence of blood material.

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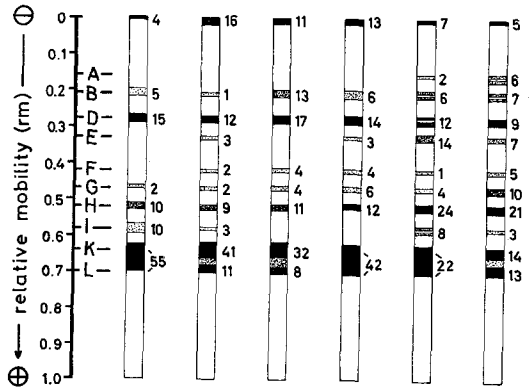
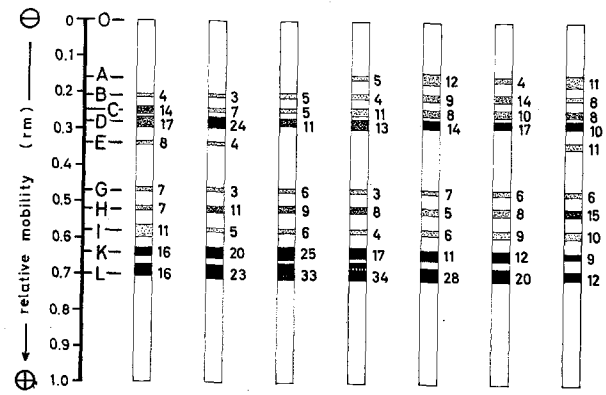


Days after emergence	0	1	3	5	10	50±
No. of experiments	1	3	2	2	3	7
Wet weight (mg/gel)	12.0	24.1	40.2	47.2	32.9	16.9
Total extinction per ♀	0.45	0.06	0.06	0.07	0.17	0.21

Fig. 1. Diagram of non-specific esterases in females of *Aedes aegypti* strain Congo during adult lifespan. For further explanation see text.



Days after emergence	0	1	2	3	5	10	50±
No. experiments (*n*)	4	4	6	2	3	5	15
♀♀ used per gel	3.0	3.7	3.0	3.0	3.3	3.0	4.1
Total extinction per ♀	0.14	0.08	0.11	0.07	0.08	0.09	0.07

Fig. 2. Diagram of non-specific esterases at several intervals after emergence in females of *Aedes aegypti* strain Segemaganga.

Shortly after the ingestion of the blood, the pattern of esterase enzymes is changed: bands G (15%) and H (12%) are now the most intense ones, whereas K (3%) and L (2%) are rather weak. At the origin some esterase activity was observed. The highly increased activity at the region of rm 0.50 is clearly caused by the bands which can be found in gels of blood at the same place. They represent so-called albumin associated esterases (HUNTER and STRACHAN[4]). In general, not much difference is seen the 1st and 2nd day after blood meal. By this time already 65% of the ovaries observed have reached stage IVa. The 3rd day after blood intake, the enzyme patterns change and begin to regain their earlier appearance; 85% of the ovaries are in stage V, i.e. ready for oviposition.

On the 4th day after blood meal, the blood is usually digested, its waste products discarded, and often the eggs are laid. The situation is almost the same as before the blood meal: the pattern of esterase enzymes, their relative mobilities, and the total amount of esterase activity calculated per female are comparable (Figure 2 and 3). The latter values reached a 40-fold increase by the intake of blood; they gradually decreased to about 0.09 during the period of digestion.

Although Sephadex was used, at times it was difficult to separate certain bands when blood was present in the homogenates. Band A which occurred in detectable amounts after blood meal was demonstrated to belong to the group of cholinesterases, by the method of KARNOWSKY (cit. in MAURER[3]).

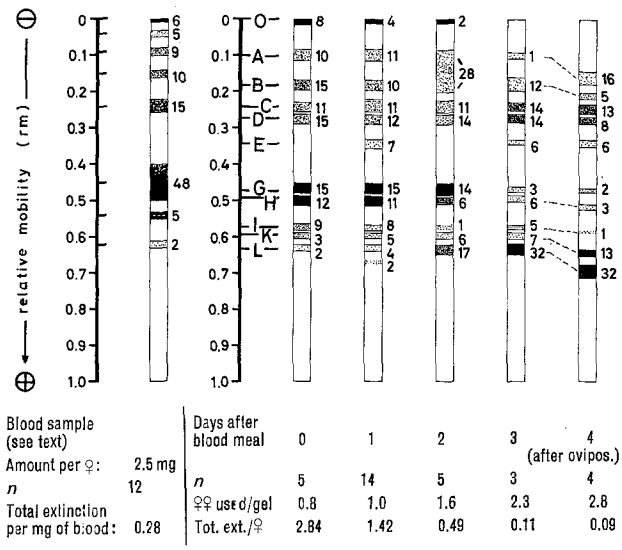


Fig. 3. Diagram of non-specific esterases at daily intervals after a blood meal in females of *Aedes aegypti* strain Segemaganga. For comparison, a diagram of whole mouse blood esterases is given.

Discussion. Based on the results reported, the following conclusions can be made. First of all, the patterns of esterase enzymes are very similar among the 2 strains of *A. aegypti* which were investigated. Exceptions are: activity at the origin, the splitting of certain enzymes with advancing age and the presence or absence of band C and F, respectively. Main activities are localized on bands D, K, L. Qualitative strain differences in esterases have also been stated by FREYVOGEL and MCCLELLAND[5] for *Aedes vittatus*.

Female mosquitoes were homogenized at different time intervals after emergence up till about 50 days, when they had completed about 7–8 gonotrophic cycles. This age may be considered old (KERSHAW et al.[6]). The number of esterase bands increased with age, their relative activity seems to stay or less constant while the total esterase activity per female is reduced. Obviously, these events must be taken as signs of ageing since they are found to occur in both strains checked for 50 days. SIMON[7] did not observe this behaviour of esterase enzymes in *Culex fatigans*, but his experiments comprised younger specimens.

The most dramatic changes of the non-specific esterases of whole female homogenates occur during the gonotrophic cycle, starting with the blood meal. Band G+H now show 27% of total activity whereas bands K+L, which were most prominent before, have a weak appearance (5%). On the 4th day when oviposition has occurred, the distribution of esterase activity as well as the total activity per female return almost to the same level as before the blood meal. These observations are very similar to those found during investigations on the protein content of *Culex pipiens* (BRIEGEL[8]). There it was called a residual value and this term could also be applied to the total esterase activity per female found after oviposition. This residual activity per female during resting time (0.07–0.08) is increased about 40 times after a blood meal. Further work on the localization of esterases in different organs at different times of the gonotrophic cycle is in preparation[9].

Zusammenfassung. Unspezifische Esterasen wurden während des Adultlebens von *Aedes aegypti* (L.) quantitativ bestimmt und zwischen Weibchen von 2 Stämmen verglichen. Alterung ist begleitet von einer Zunahme der Bänderzahl und einer Abnahme der Esterasenaktivität pro Weibchen; die relative Aktivität der einzelnen Esterasen-Isozyme bleibt aber mehr oder weniger konstant. Nach einem Blutmahl, dem Beginn eines neuen gonotrophen Zyklus, wurden auffällige Veränderungen im Bändermuster festgestellt. 4 Tage später, nach erfolgter Eiablage, ist dieses Muster wieder ähnlich wie vor der Blutaufnahme.

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