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# The Esterase Patterns in the Ovaries and the Embryonated Eggs of *Aedes aegypti* L.

K. GEERING and URS-P. OBERLIN

## *Abstract*

The esterases of *Aedes aegypti* were studied in the ovary before and during a gonotrophic cycle and also in fully embryonated eggs by means of disc electrophoresis. During oogenesis no significant changes can be observed besides a marked increase in the total esterase activity. A different esterase pattern is found in eggs after embryogenesis. The electrophoretic mobility of some esterase bands is highly increased in the ovary compared to other organs. One esterase fraction in the ovary and two in the fertilized egg were identified as acetylcholinesterases. All other enzymes are carboxylesterases. The results are compared to those of previous authors and are discussed in view of possible functions of esterases during reproduction.

## **Introduction**

The demonstration of the variation in the esterase patterns of different organs of *Aedes aegypti* has been the subject of some recently published papers (BRIEGEL & FREYVOGEL, 1973; GEERING & FREYVOGEL, 1974). The same isozymes were detected in all the organs studied, but considerable differences were found in respect to their quantitative distribution. Marked changes occur in the esterase patterns of several organs in connection with a blood meal. This fact points to a function of certain esterases in processes triggered by the blood uptake such as digestion and egg development.

Studies on the occurrence of esterases in the ovary during a gonotrophic cycle and in the eggs have been undertaken in several insects providing some information on the distribution and synthesis of these enzymes (SALKED, 1965; GUSS & KRYSAN, 1972; CHO et al., 1972; FREYVOGEL & BRIEGEL, 1973) and suggesting special functions in insect reproduction (WAN & HOOPER, 1967; HOOPER & WAN, 1969; SUDDERUDDIN & TAN, 1973).

An investigation of the esterase patterns, by polyacrylamide gel electrophoresis (PAG), of the ovary of *Aedes aegypti* was of further interest in view of new findings on the influence of the fungicide merthiolate on mosquito esterases (GEERING, 1973). This substance is occasionally used in PAG studies where it is added to the electrode buffer to prevent fungus contamination (ORTEC AN 32, 1970; BRIEGEL & FREYVOGEL, 1973). Thus, a re-examination of earlier results seemed to be expedient.

The present study includes clarification of the changes in the esterase pattern of the ovary during a gonotrophic cycle as well as a description of the esterase status of fertilized eggs. Attention was paid to a comparison with the earlier findings of BRIEGEL & FREYVOGEL (1973). The results are discussed in the context of the known facts of the function of the carboxyl and choline esterases during egg development and embryogenesis.

## Material and methods

In all experiments the strain 'Segemaganga' of *Aedes aegypti* was used, which was isolated in Tanzania (BRIEGEL & FREYVOGEL, 1971b) and maintained in this laboratory since 1970 according to usual breeding methods.

10–20 days old sugar fed females and females within 0–4 days after a blood meal were studied. Eggs were collected within 40–60 days after oviposition.

The preparation of tissue extracts was essentially the same as described by BRIEGEL (1972) and BRIEGEL & FREYVOGEL (1973). For enzyme separation conventional disc electrophoresis was used (MAURER, 1968), including modifications described by the authors mentioned above.

To detect the influence of merthiolate, the fungicide was added to the electrode buffer at a concentration of 1 g/10 litres (ORTEC AN 32, 1970). In all other experiments, merthiolate was omitted in the buffer solution.

The unspecific esterase activity was demonstrated by the method described in the ORTEC Instruction Manual 4200 (1970) using  $\alpha$ -naphthyl acetate as a substrate and Fast Blue RR salt as a coupling dye.

To demonstrate acetylcholinesterase activity acetylthiocholine iodide was used as a substrate according to the method of KARNOVSKY & ROOTS (1964) with slight modifications (GEERING & FREYVOGEL, 1974). For further distinction of these enzymes, inhibition tests were carried out with eserine, at a concentration of  $5 \times 10^{-5}$  M (GEERING & FREYVOGEL, 1974).

The homologization of the isozyme bands was achieved by mixing homogenates of different organs and stages and by treating longitudinally split gels with different staining methods. The nomenclature of the isozymes is the same as used in other publications (BRIEGEL & FREYVOGEL, 1973; GEERING, 1973) to permit direct comparison.

The quantitative estimation of the esterase bands was carried out as described by BRIEGEL & FREYVOGEL (1971a). In order to make a comparison with the ovary possible, the total extinction of the oviposited eggs was also calculated per female by assuming an average of 150 eggs per female.

## Results

### *Homologization of the esterase bands*

In order to homologize the esterase bands, the esterase pattern of the head described by BRIEGEL & FREYVOGEL (1973) was compared with the patterns of the ovary and with a mixture head/ovary. The material was obtained from females dissected 4 days after a blood meal. The results of these experiments are summarized in Fig. 1. Judged by the intensity of the fractions and their reaction with different substrates it is possible to state that the uppermost band of the mixture originates from the A-bands of the head and the ovary, respectively. The second band of the mixture is likely to include bands B and D of the head and band D of the ovary. Band B does not exist in the ovary. The esterases E–L can easily be homologized.

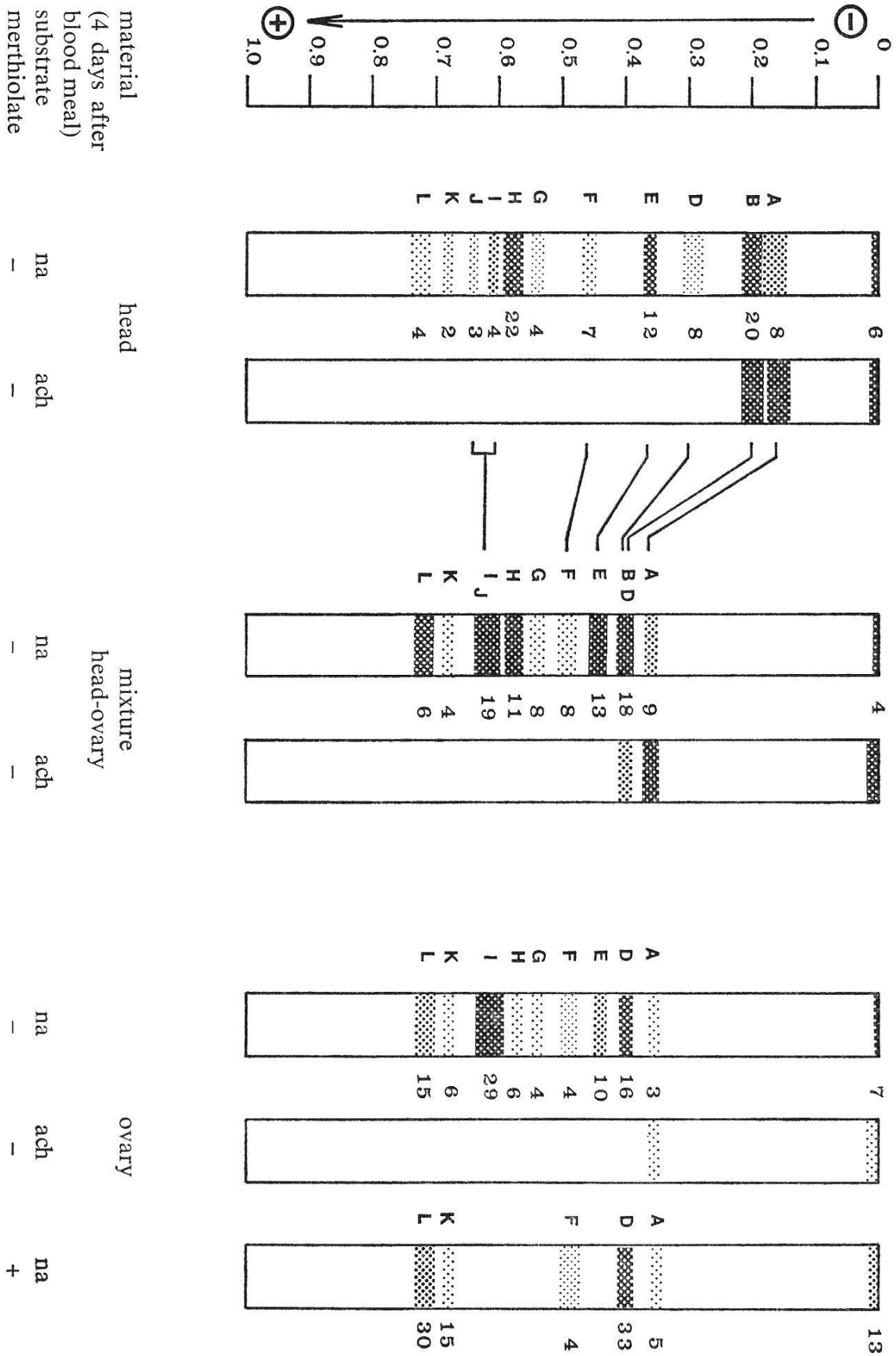


Fig. 1. Identification and homologization of esterases in the ovary of *Aedes aegypti* and specific inhibition of fractions by merthiolate. Na = *a*-naphthylacetate; Ach = acetylthiocholine iodine. Numbers to the right of the gel strips = relative extinction of the bands expressed as percentage of the total extinction.

The esterases A and B were identified as acetylcholinesterases; they are totally inhibited by eserine at a concentration of  $5 \times 10^{-5}$  M. The esterases D–L belong to the unspecific esterases.

The esterase bands A–F of the ovary showed increased *rm*-values compared to the homologous bands of the head. The same phenomenon occurred in the mixtures of the heads with the ovaries. Occasionally incomplete esterase bands were observed in the ovary.

### *Merthiolate*

The influence of merthiolate on the esterase pattern of the ovary is represented in Fig. 1. In all stages the bands E, G, H and I were totally inhibited by merthiolate. Band F showed a partial inhibition while bands A, D, K and L were not affected.

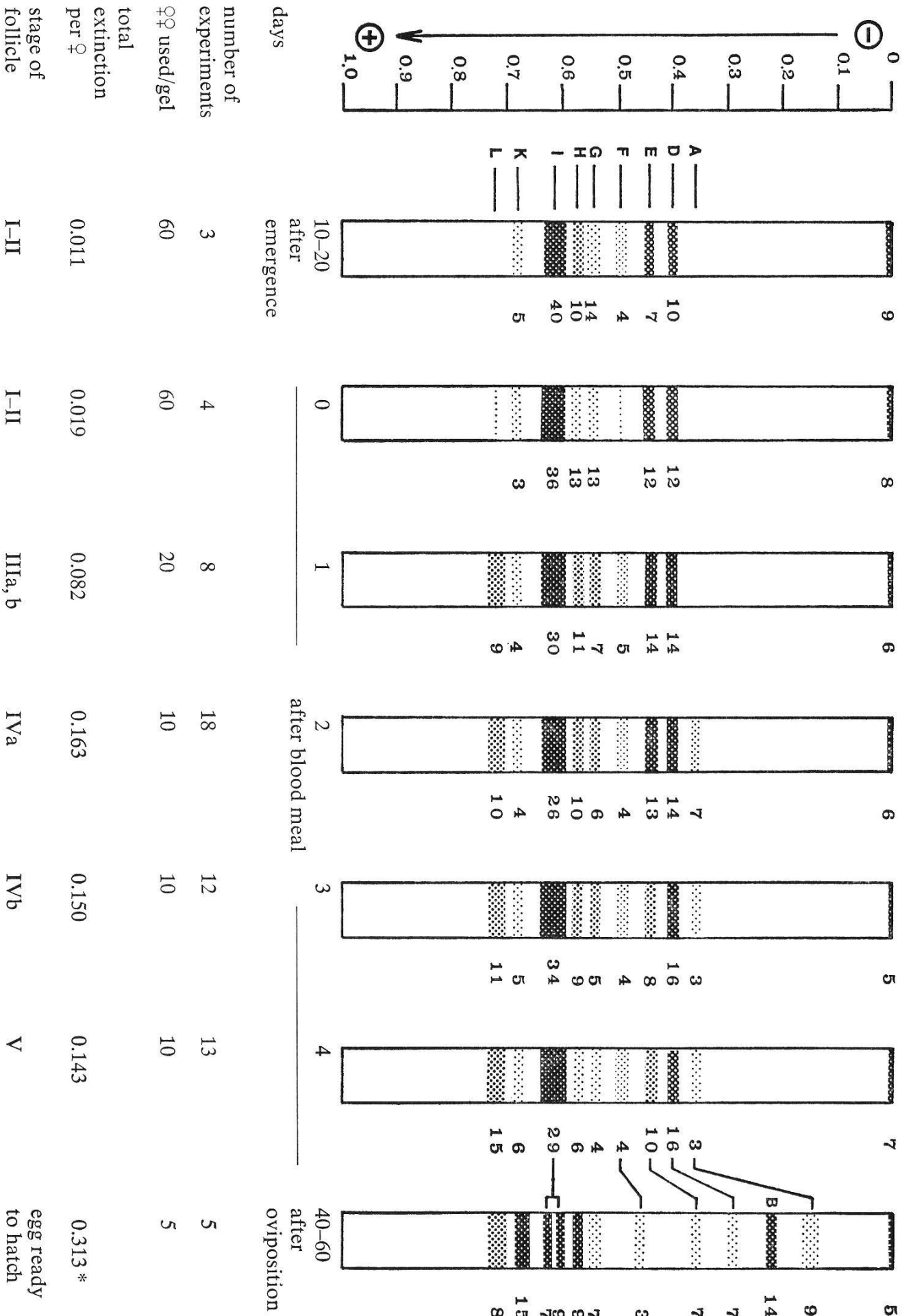
### *The esterase pattern of the ovary during a gonotrophic cycle*

Fig. 2 shows the esterase pattern of the ovary before and during a gonotrophic cycle. No drastic changes in the esterase pattern can be observed. Including a comparatively low esterase activity at the origin ten fractions at the most could be demonstrated. Throughout all the stages, the main zone of activity with 26–40% of the total extinction is represented in band I.

The total esterase activity in the ovary of 10–20 days old sugar fed females is extremely low (0.011 per female). The bands A and L are not detectable. Eight hours after a blood meal the esterase activity has already increased (0.019 per female). Using 90 females per gel all ten esterase bands could be demonstrated in this stage. With 60 females per gel the bands A, F and L were very faint and seen only occasionally. One day after a blood meal the total esterase activity is further increased (0.082 per female). The fractions F and L appear as distinct bands. Two days after a blood uptake the total esterase activity reaches the highest level in the course of the gonotrophic cycle showing an almost fifteen-fold increase (0.163 per female) compared to the sugar fed mosquitoes. The band A, too, occurs now with a considerable activity. Within the next 48 hours the total esterase activity decreases slightly. Band E becomes fainter but the esterase pattern is essentially unchanged.

### *The esterase pattern of fully embryonated eggs*

Considerable alterations in the esterase pattern could be observed in the egg ready to hatch (Fig. 2). The *rm*-values of the bands A–F are comparable to the homologous bands of the head. An additional band B



is detectable, band I is split into two fractions and the activity of band K shows a marked increase. The other bands are faint, showing an activity of less than 10% each. The total extinction calculated per female is further increased (0.313 per female).

### Discussion

In the present study, no marked changes could be detected in the esterase patterns of the ovary during a gonotrophic cycle. Quantitative changes are restricted to a few esterase fractions such as bands L and A, which show an increase during the time of egg development. These results are in agreement with the findings of CHO et al. (1972), who did not observe any changes in the electrophoretic pattern of the *Oncopeltus* esterases during the course of oogenesis. The considerable alterations in the zymogram of the ovary of *Aedes aegypti* as described by BRIEGEL & FREYVOGEL (1973) could not be demonstrated. Esterase fractions with  $R_m$ -values below 0.35 occurring 0 and 1 day after a blood meal have never been observed in our study.

However, in agreement with their results, a considerable increase in the total esterase activity per female takes place in the first days after a blood meal reaching a maximum on the 2nd day and remaining around this high level until the day of oviposition. Possible interpretations of these results may involve either an increased synthesis of pre-existing esterases triggered by a blood meal or, else, an accumulation of esterases by the oocytes from the general circulation. In both cases, the esterases may be very stable or they are being subjected to a constant metabolism (CHO et al., 1972), which would explain the fluctuations in the intensities of certain bands.

In any case, no activation of genes as suggested by BRIEGEL & FREYVOGEL (1973) must be postulated, at least until the time of embryogenesis. Fertilized eggs, ready to hatch, show a different esterase pattern including quantitative and qualitative changes. Analogous results were obtained by BRIEGEL & FREYVOGEL (1971) in *Aedes aegypti*, and by SALKED (1965), CHO et al. (1972), GUSS & KRYSAN (1972) and GADALLAH & MAREI (1973) in other insects. Thus, a gene activation occurs only after fertilization and oviposition (SALKED, 1965) leading

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*Fig. 2.* Esterase patterns of the ovary before and during a gonotrophic cycle and of fully embryonated eggs. Numbers to the right of the gel strips = relative extinction of the bands expressed as percentage of the total extinction. \* Calculated by assuming an average of 150 eggs per female.

to an increased protein synthesis, including enzyme production, as an expression of new metabolic activities (GADALLAH & MAREI, 1972).

The changes of the esterase pattern in the ovary 1 day after a blood meal and the reduced number of fractions as described by BRIEGEL & FREYVOGEL (1973) during oogenesis may not be attributed to the use of merthiolate in the electrode buffer. In agreement with the findings of GEERING (1973) in fat body homogenates, the fungicide inhibited the same esterase bands in the ovaries. It is more probable that the discrepancy in the results partly originates from factors such as incomplete separation eventually being conditioned by variable homogenization.

This interpretation is further supported by the fact that the esterase bands A to F show markedly increased  $R_m$ -values in the ovary compared with other organs and with the fertilized egg. Mixture of different organs with the ovaries causes the same phenomenon. This suggests the presence of a factor localized in the ovary which influences the mobility of certain esterase fractions and possibly also the occasional appearance of incomplete bands. The same effect was observed in the midgut of *Aedes aegypti* (GEERING & FREYVOGEL, 1974) where the esterases of the vertebrate blood showed increasing electrophoretic mobilities during their digestion. Systematic studies on the alterations of enzymes and their variable electrophoretic behaviour revealed influences by ultrasonic treatment and substances like urea and enzymes (AUGUSTINSSON, 1973) and by binding of charged ligands (RESSLER, 1973) and carbohydrates (MUNJAL & ROSE, 1972). These factors were not taken into consideration by BRIEGEL & FREYVOGEL (1973) so that their nomenclature of the esterases cannot be considered adequate. The six esterase bands described by the above authors probably correspond to the following fractions: G of their study to A/D/E, H to F/G/H, I to I, K to K and L to L.

Only a few suggestions can be made as to the function of esterases during oogenesis and embryogenesis. HOOPER & WAN (1969) proposed that aliesterases were associated with the production of ootheca and with vitellogenesis in *Musca domestica*. It will be necessary to specify the esterases for further clarification of their physiological role by the use of natural substrates such as acetylthiocholine for the identification of acetylcholinesterases and triglycerides for lipases. The latter enzyme was detected by GUSS & KRYSAN (1972) in eggs of *Diabrotica undecimpunctata* as well as in the ovary of *Aedes aegypti* (GEERING, unpublished data).

In *Aedes aegypti*, cholinesterases (AchE) were found in all organs studied (BRIEGEL & FREYVOGEL, 1973; GEERING & FREYVOGEL, 1974). GADALLAH & MAREI (1973) observed a significant increase in AchE levels of fertilized compared with unfertilized eggs; this corresponds to



our results on the occurrence of AchE in ovaries and in eggs ready to hatch. AchE are essential for the control of acetylcholine (Ach) which was found in the reproductive organs of Lepidoptera and, at high concentrations, in eggs of various insects (BRIDGES, 1972). A large increase in Ach was reported in eggs of some insects just prior to hatching (BRIDGES, 1972). The appearance and increase of AchE in the eggs during embryogenesis is most probably associated with neuroblast development (BRIDGES, 1972; GADALLAH & MAREI, 1973).

Further studies on the substrate specificity of the esterases and on the phenomenon of the increased *rm*-values of certain esterases are necessary for more insight into their function.

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### Zusammenfassung

Die Esterasen von *Aedes aegypti* wurden mit Hilfe der Diskelektrophorese vor und während eines gonotrophen Zyklus im Ovar wie auch in schlüpfreifen Eiern untersucht. Während der Oogenese können keine bedeutenden Veränderungen beobachtet werden außer einer beträchtlichen Erhöhung der totalen Esterasenaktivität. Ein unterschiedliches Esterasemuster wird in den Eiern nach der Oogenese gefunden. Im Vergleich zu anderen Organen ist die elektrophoretische Mobilität einiger Esterasenbänder im Ovar stark erhöht. Eine Esterasenfraktion im Ovar und zwei in den befruchteten Eiern wurden als Acetylcholinesterasen identifiziert. Alle andern Enzyme sind Carboxylesterasen. Die Resultate werden mit denjenigen anderer Autoren verglichen und im Hinblick auf mögliche Funktionen der Esterasen während der Reproduktion diskutiert.

### Résumé

Les estérases d'*Aedes aegypti* ont été étudiées par discélectrophorèse dans les ovaires avant et pendant le cycle gonotrophique, ainsi que dans les œufs embryonnés. Pendant l'oogenèse aucune modification significative n'a été observée à l'exception d'une augmentation de l'activité estérasique globale. Un aspect différent des estérases est trouvé dans les œufs après l'embryogenèse. La mobilité électrophorétique de quelques bandes d'estérase est considérablement augmentée dans les ovaires en comparaison avec les autres organes. Une fraction d'estérase dans les ovaires et deux dans les œufs fertilisés ont été identifiées comme des acétylcholinestérases. Tous les autres enzymes sont des carboxylestérases. Ces résultats sont comparés à ceux d'autres auteurs et la fonction possible des estérases durant la reproduction est discutée.