PHOSPHATASE ACTIVITY IN DIFFERENT LIFE STAGES OF THE STABLE FLY, STOMOXYS CALCITRANS (L.)*

SHAHID H. ASHRAFI

Drugs, Pharmaceuticals and Pesticides Research Division, Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi

AND

FRANK W. FISK, *Ohio State University, Columbus, Ohio, U.S.A.*

(Received March 24, 1961)

Acid phosphatase activity during the study of different life stages of the stable fly, *Stomoxys calcitrans*, was found highest in eggs, and a second peak was noted in pupae. Three-day-old males and females showed the same level of acid phosphatase activity. The lowest activity was found in two-day-old larvae.

Similarly alkaline phosphatase activity was found highest in the egg stage. The lowest activity was found in two-day-old larvae, but this alkaline phosphatase activity was higher than that of acid phosphatase in the same stage. Again, alkaline phosphatase activity was increased in seven-day-old larvae, and attained a second peak in pupae, then it decreased in three-day-old adults.

The varying amounts of acid and alkaline phosphatase activity in different life stages of the stable fly was definitely associated with tissue growth and differentiation.

Introduction

It has been shown by Yao[1] that alkaline phosphatase is associated with growth and differentiation in insect tissues, such as embryo, larva, and pupa of *Drosophila melanogaster* (Meig.), but in contrast, the gradients of acid phosphatase activity were not reported. Barker and Alexander[2] made a detailed study of acid and alkaline phosphatase activity in different stages of the house fly, *Musca domestica*, using β-napthol phosphate as a substrate for biochemical analysis. They associated the acid phosphatase activity with tissue transformation during the development of house fly from egg to adult.

Since the house fly and stable fly both belong to the order Diptera and family Muscidae, and since Barker and Alexander[2] have already reported the activity of acid and alkaline phosphatases in all stages of the house fly, it was decided to study both enzymes in different life stages of the stable fly by using a new substrate, disodium *p*-nitrophenyl phosphate, and other materials at optimum conditions. The acid and alkaline phosphatase activity was determined in one-day-old eggs, two-day-old larvae, seven-day-old larvae, three-day-old pupae, and three-day-old adults. The choice of three-day-old adults was made, because at this stage both sexes showed the same level of acid phosphatase activity. Similar results were also obtained in case of alkaline phosphatase, although its activity was lower than that of acid phosphatase.

*Part of a dissertation presented to the Graduate School of The Ohio State University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Materials and Methods

Stable flies were reared by the method described by Appleby and Fisk,[3] with the exception that the adults were fed on a 10% solution of sucrose. The tests were based on fresh tissues, which were chilled at −15 C. for half an hour before using them to prepare homogenate. The assays were grouped into triplicates of five samples each for acid and alkaline phosphatase activity. Three zero-time readings were taken for each sample.

The methods, reported by Ashrafi and Fisk[4] for acid phosphatase determination and by Ashrafi[5] for alkaline phosphatase determination, were followed with modifications. Disodium *p*-nitrophenyl phosphate solution of 0.0143 M was used as substrate. The citric acid buffer solution of pH 4.4 and of 0.09 M was used for acid phosphatase; and "tris" alkaline buffer solution of pH 7.3 and of 0.1 M was used for alkaline phosphatase determination. The temperature of water bath was kept constant at 40 C. For alkaline phosphatase, half a millimeter of 0.01 M ferrous ions solution was also added to the assaying mixture. According to the general plan of analysis, the volume of five milliliters of the reaction mixture was maintained constant by adding appropriate volume of distilled water.

The activity was expressed as micromoles of *p*-nitrophenol liberated per thirty minutes period of incubation at 40°C. The age of the different stages is shown in days, following the emergence of that particular stage.

Results and Discussion

The results obtained are given in Table 1,

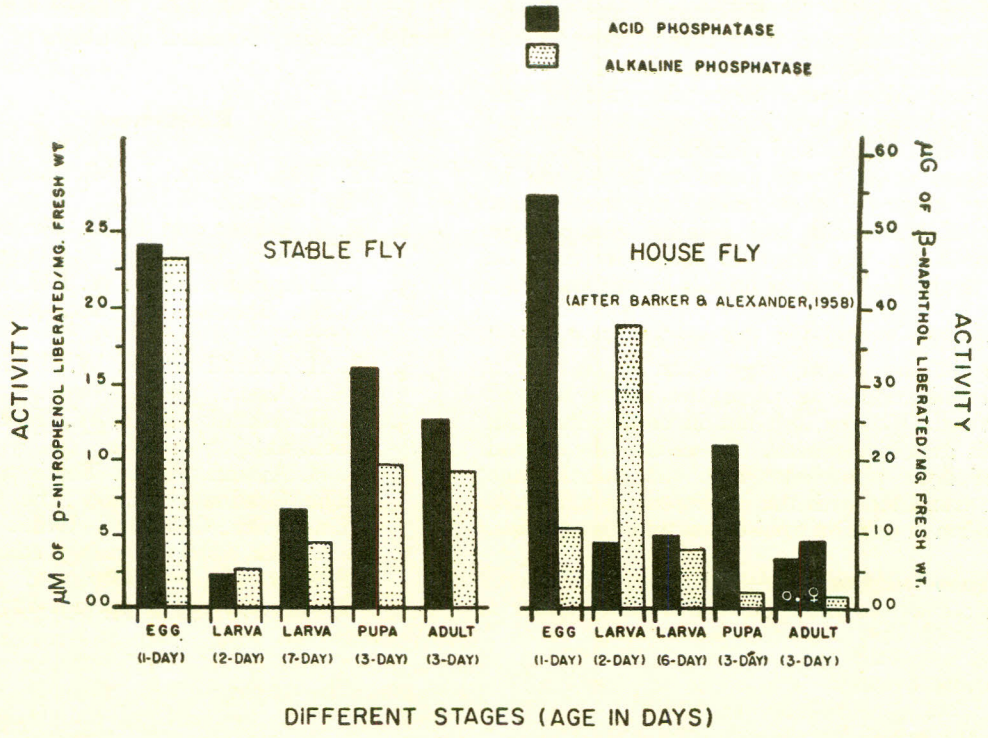

Fig. 1.—Average acid and alkaline phosphatase activity in different spceified stages of stable fly as compared with those of house fly.

TABLE 1.—THE ACTIVITY OF ACID AND ALKALINE PHOSPHATASES IN THE TISSUES OF DIFFERENT LIFE STAGES OF THE STABLE FLIES.

Source of tissue	Acid phosphatase activity	Alkaline phosphatase activity
Egg (1-day-old)	24.0	23.25
Larva (2-day-old)	2.0	2.25
Larva (7-day-old)	6.5	4.25
Pupa (3-day-old)	16.0	9.50
Adult (3-day-old)	12.5	9.00

and are compared with those of similar stages of the house fly, reported by Barker and Alexander[2] in the form of histograms showed in Fig. 1.

The highest acid phosphatase activity was reported in the egg stage of the stable fly; similar results were reported by Barker and Alexander[2] in the house fly eggs. The second peak of activity was noted in the contents of puparia which was 66.6%, when compared with that of egg stage. Barker and Alexander also reported a second peak of activity in house fly pupae. The lowest activity (8.3% that of the egg stage) was found in two-day-old larvae. Three-day-old males and females showed the same level of acid enzyme activity (52.8% that of the egg stage), while Barker and Alexander reported different activities in both sexes of three-day-old house fly adults. Ashrafi and Fisk[6] reported histochemical localization of acid and alkaline phosphatases in three-day-old adult stable flies.

The eggs of the stable fly showed the highest activity of alkaline phosphatase. The lowest activity, when compared with that of the egg, was found to be 9.6% in two-day-old larvae of the stable fly, but this stage like that of the house fly indicated higher alkaline than acid phosphatase activity. In seven-day-old larvae the alkaline phosphatase activity was increased again, reached to a second peak in the pupal stage and then decreased in three-day-old adults. In case of house fly, alkaline phosphatase activity was decreased in six-day-old larvae and did not attain a second peak in the remaining life stages (Fig. 1).

The variable results of acid and alkaline phosphatase activity in different stages of the stable fly may be related to the rate of tissue growth and differentiation as suggested by Yao[1] and Barker and Alexander.[2] Since the rate of cell division is always highest in the egg stage as compared to other stages and since acid and alkaline phosphatase activity was found to be highest in the egg stage of the stable fly, the highest activity of both acid and alkaline phosphatase enzymes in the egg stage of stable fly may be related to the high rate of mitosis in this stage.

According to Buck,[7] the major biochemical changes in the pupal stage seem to involve a marked utilization of carbohydrates, mainly derived from stored glycogen and fat, in connection with histolysis and histogenesis. Therefore, the second peak of both phosphatases in the pupal stage may be associated with the increased carbohydrate metabolism in relation to histolysis and histogenesis.

Acknowledgements.—This investigation was carried out at the Ohio State University under a research grant from the Department of Health, Education, and Welfare, United States Public Health Service, National Institutes of Health.

References

1. T. Yao, Quart. J. Microscop. Sci., **91**, 89 (1950).
2. R. J. Barker and B. H. Alexander, Ann. Ent. Soc. Amer., **51**, 255 (1958).
3. J. E. Appleby and F. W. Fisk, Proc. North Cent. Branch. Ent. Soc. Amer., **14**, 41 (1959).
4. S. H. Ashrafi and F. W. Fisk, Ann. Ent. Soc. Amer. (1961). *in press.*
5. S. H. Ashrafi and F. W. Fisk, Ohio J. Sci., **61**(1), 7 (1961).
6. S. H. Ashrafi, Ph. D. Thesis submitted to The Graduate School, The Ohio State University, Columbus, Ohio.
7. J. P. Buck, *Insect Physiology*, edited by K. D. Roeder (John Willey and Sons, Inc., New York, 1953), pp. 191-227.