ACETYLCHOLINESTERASE HETEROGENEITY IN THE BRAIN OF THE FROG *RANA CYANOPHLICTIS*

ACETYLCHOLINESTERASE (E.C. 3.1.1.7) has been found in the innervated tissues of all the vertebrates and invertebrates that have been studied[1]. The Significance of this enzyme has been demonstrated by the prolonged duration of ACh effects seen after administration of esterase inhibitors[2]. Baldwin and Hochachka[3] have studied the isoenzymes of AChE in the brain of rainbow trout, as a function of temperature acclimation. However no information regarding the isozyme pattern of acetylcholinesterase in the brain of frog is available. Since frog is the common experimental animal in biological research and in view of the importance of AChE in neuronal activity, it was felt desirable to initiate the present study. An attempt has been made here to resolve the different isozymal forms of the enzyme in the cortical region of the brain of frog.

Specimens of *R. cyanophlictis* of medium size (22–27 g) were maintained in the laboratory aquaria at $23 \pm 2°$ C. The animals were fed on the leg muscle of frog once in three days. They were killed by decapitation and the brain was dissected and removed at 0° C. The cortex of the brain was isolated in amphibian Ringer[4] at 0° C and immediately used for analysis.

The isozymic pattern of acetylcholinesterase was studied by polyacrylamide gel electrophoresis (disc electrophoresis) developed by Davis and Ornstein[5,6]. The gel was formed from two organic monomers, acrylamide and bisacrylamide, the latter being the cross-linking agent. The catalyst used to initiate polymerization cross-linking reaction was tetramethyl-ethylenediamine (TEMED).

The matrix was prepared in small glass tubes with three gel layers. The lower gel (consisting of acrylamide. bisacrylamide. potassium fericyanide riboflavin, TEMED and tris) was overlaid with 2 mm of distilled water and allowed to polymerize for 40 minutes under a fluorescent lamp after which the water layer was removed and gently washed with upper gel which consisted of tris, phosphoric acid, acrylamide, bisacrylamide and riboflavin. The upper gel was then laid similarly with 2 mm of distilled water and allowed to polymerize for 40 minutes after which the water layer was gently removed.

A 10% homogenate of the cortical matter of the brain of frog was prepared in phosphate buffer pH 7, and 0·1 ml of it was thoroughly mixed with 0·1 ml of upper gel solution and laid as the third gel layer and allowed to polymerize for 40 minutes. The tubes were run at 6 milliamps per tube (DC power supply model CM 07/02 Sr. No. 111, Toshniwal) for an hour in tris-glycine buffer at pH 9·5.

After the electrophoretic run, the tubes were removed and the gel was gently isolated from the glass tube. It was divided into two vertical halves. One half was further cut into 2·5 mm bits and processed for quantitative estimation of AChE activity by the colorimetric method of Hestrin[7]. The activity of AChE was expressed as micromoles of Ach hydrolysed per mg protein per minute. The other half of the gel was similarly processed for proteins by microbiuret method[8].

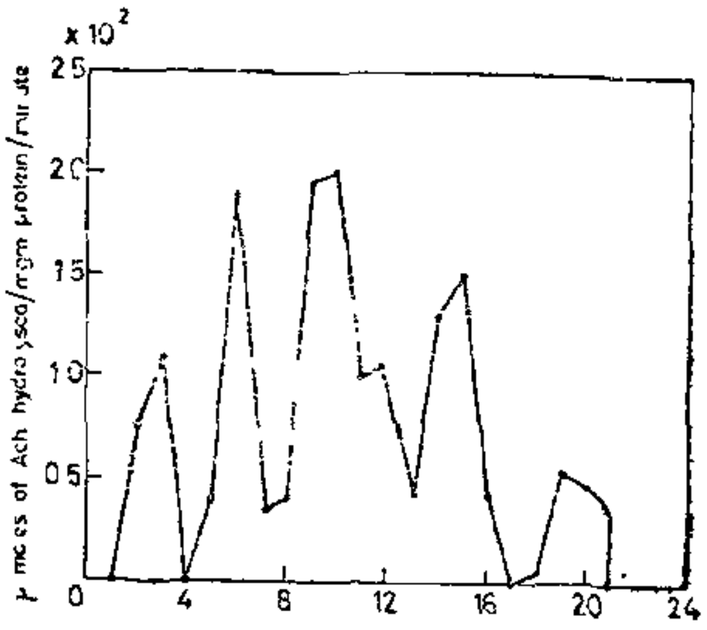

FIG. 1. Profile of acetylcholinesterase (AChE) in the cerebrum of frog. 'X' axis represents section numbers of gel bits processed for the activity level of AChE. Peaks (starting from section No. 1) are designated as A_1, A_2, A_3, A_4 & A_5.

It is clear from Fig. 1 and Table I that by polyacrylamide gel electrophoresis, cortical acetylcholin-

TABLE I

Brain acetylcholinestrase heterogeneity in frog, Rana cyanophlictis

Body wt. (g)	Brain wt. (mg)	Isozymal forms				
		A_1	A_2	A_3	A_4	A_5
$26·4 \pm 3·1$	80 ± 14	*110 ± 27	197 ± 29	202 ± 56	152 ± 20	55 ± 15
% change		58·8	97·5	100	75·2	27·2

* Activity expressed as micromoles of Ach hydrolysed/mg protein/minute. Each value represents mean $\pm$ SD of 2 observations.

For each observation brain (cortical matter) from 3 animals was pooled.

% change is calculated with reference to the activity level of peal A, which is considered 100.

esterase could be resolved into 5 isozymal forms designated as A_1, A_2, A_3, A_4 and A_5. All the five isozymes exhibited anodal migration (so distinguished because of the disc electrophoresis run in Tris-Glycine-buffer at pH 9·5 in which the flow of current was unidirectional, from cathode to anode). It is also clear (Fig. 1) that the second and the third isozymal forms (A_2 and A_3) exhibited comparatively higher activity. Of these, the form, designated A_3, exhibited the highest activity. Of the two comparatively fast moving forms, A_5 exhibited the least activity and the activity level of slowest moving isozyme (A_1) was approximately double that of A_5. Thus from the isozymic pattern of AChE in the cortical matter of the frog, *R. cyanophlictis*, it appears that in general the animal depends more on the slow moving anodal forms for its metabolic activity under normal physiological conditions, compared to the fast moving ones, since the activity of the former is higher.

We are thankful to Dr. A. R. Kasturi Bai for offering facilities to carry out this study.

Department of Zoology, U. P. NALINI.
Bangalore University, NAYEEMUNNISA.
Bangalore 560 001, *March 24*, 1976.

1. Nachmansohn, D., *Handbook of Experimental Pharmacology*, (G. B. Koelle, ed.), Springer-Verlag, Berlin, 1963, **15**, 701.
2. Katz, B., The Sherrington Lectures as in *Handbook of Neurochemistry*, 4th ed. by A. Lajtha, 1969, p. 263.
3. Baldwin, J. and Hochachka, P. W., *Biochem. J.*, 1970, **116**, 883.
4. Cavanaugh, *Formulae and Methods* (Marine Biological Laboratory, Woods Hole, Mass., 1956).
5. Davis, B. J., *Ann. N.Y. Acad. Sci.*, 1964, **121**, 404.
6. Ornstein, L., *Ibid.*, 1964, **121**, 321.
7. Hestrin, S., *J. Biol. Chem.*, 1949, **180**, 249.
8. Itzhaki, R. T. and Glick D. M., *Analyt. Biochem.*, 1964, **9**, 401.

THE EFFECT OF "PAPER FACTOR" ON THE FECUNDITY AND HATCHABILITY IN *DYSDERCUS CINGULATUS* (HETEROPTERA: PYRRHOCORIDAE)

THE "Paper Factor" (PF), an inhibitor of embryonic development, was earlier reported to be effective only on *Pyrrhocoris apterus*[1]. Following the report of its morphogenetic effect on *Dysdercus koenigii*[2], the authors studied its ovicidal effect on *D. cingulatus*. The bugs were regularly reared in our laboratory at 28 ± 1° C, 70 ± 5% RH and 12 hour photoperiod. They were fed on soaked cotton seeds. The 'PF' was extracted from paper using petroleum ether as solvent and after evaporation, the residue was redissolved in acetone. The female bugs were topically treated with 1 µl (30 µg) of 'PF'. Each pair was kept in a separate petridish. 150 pairs were used in this study. The eggs laid by each female and their hatchability were recorded. Acetone treated controls and normal untreated insects had no differences. Normal bugs laid 70 eggs on an average in each batch and almost all hatched. The experimental insects were treated with 'PF' at different stages of imaginal life. In group A, 32 freshly ecdysed females were treated. Of them 18 failed to mate and did not oviposit. Among those that mated and oviposited, the hatchability was 49% and 63% in the first two batches respectively. In group B, the 'PF' was applied to the females on third day after adult ecdysis; in group C, one day prior to first oviposition; and in group D, it was applied for three days continuously after first oviposition. The oviposition and percentage of hatchability is shown in Table I.

TABLE I

No. of eggs/percentage of hatchability

	A	B	C	D
I Batch	65/49	61/52	63/25	62/19
II Batch	67/63	52/77	51/69	51/6

Figures indicated are averages of minimum 20 insects except in A.

Irrespective of when the 'PF' was applied during the gonadal cycle, there has been a reduction in the number of eggs laid and in the number of larvae hatched. However, the mature eggs (C) appear to be more sensitive for the treatment and inhibition was found less in second batch except in group D, where the low rate in the second batch of eggs may be due to the large amount of active substance applied to the insect. Slama, *et al.*[3] stated that in *Pyrrhocoris apterus*, the treatment of normal females just after adult ecdysis with large doses of juvenoids had no profound influence on the course of its reproductive cycle and that they deposited perfect eggs. But in the present investigation, it has been observed that when freshly ecdysed females of *Dysdercus cingulatus* were treated with 'PF', some of the bugs did not even mate or oviposit and in those which mated and oviposited there has been a considerable inhibition.

When the treated bugs were dissected prior to oviposition, some ovarioles had only one developed erg, some had two, while in several others the ovaries were in a degenerated condition. In few cases when they were dissected after oviposition, some of the eggs were found attached to the