is of the greatest importance. This is the major contribution of modern genetics to biological evolution.

Gould's emphasis on regulation of gene activity in no way minimises the origin of new characters. Indeed, he recognises that evolution is under the influence of two major events: (*a*) introduction of new characters accounted for by structural genes, and (*b*) changes in the characters already present brought about by changes in developmental timing, due to regulatory genes. The relative importance of these two in the process of evolution has been Gould's major concern. Indeed 'Ontogeny and Phylogeny', is a critical exposition of genetic regulation and not merely a reassessment of Haeckel's Recapitulation theory. It is a profoundly important discussion on the concepts of evolution in the light of modern knowledge, not merely a re-examination of the relationship between ontogeny and phylogeny. It is also a historical assessment of the Biogenetic Law, a judicious examination of what can be salvaged from the original formulation of Haeckel and what needs to be altered and conditioned in the light of modern ideas in biology. A major book.

A COMPARATIVE STUDY ON ACETYLCHOLINE AND ACETYLCHOLINESTERASE DURING DEVELOPMENT OF THE LEPIDOPTERAN *PHILOSAMIA RICINI* AND THE DIPTERAN *SARCOPHAGA RUFICORNIS*

RADHA PANT, SUMAN KUMAR AND RATAN DUBEY

Department of Biochemistry, The University, Allahabad 211 002

ABSTRACT

Acetycholinesterase activity and acetycholine content in the larvae, pupae and adults of both sexes of *P. ricini* and *S. ruficornis* have been studied and their physiological role in cholinergic system discussed.

INTRODUCTION

DETECTION of several insect neurosecretory substances distinct from those connected with growth and metamorphosis has recently been made. The most well known among them being acetylcholine and acetylcholinesterase.

Acetylcholine is considered as a neurotransmitter having a role in adrenergic transmission[1] and in general oxonic transmission[2]. The latter role, however, has been challenged or modified by others[3].

Insect neurosecretory compounds have been the topic of several reviews[4,5] and recently Smallman and Mansingh[6] summarized the existing data on the distribution of acetylcholine in various insect tissues and at different stages of development. However, lack of information on the neurotoxicology of the lepidopteran *P. ricini* and the dipteran *S. ruficornis*, both of considerable importance, prompted the study on the two neurosecretory compounds during development of these insects.

MATERIALS AND METHODS

Rearing of *P. ricini* and *S. ruficornis* were carried out as described by Pant and Agrawal[7] and Pant and Kumar[8] respectively.

6 hour starved larvae, pupae and adults were randomly selected from colonies of known age and homogenized to 10% (w/v) tissue concentration as described by Pant and Morris[9].

Acetylcholinesterase activity and acetylcholine content were assayed by the method of Hestrin[10] as modified by Metcalf[11].

All assays were made in duplicates on three individual homogenates prepared from 10 insects each and the average values were calculated.

RESULTS AND DISCUSSION

Acetylcholinesterase activity in the lepidopteran *P. ricini*, during fifth instar development, gradually declined but on commencement of spinning increased three-fold. During larval–pupal transformation from day 2 onwards till day 5, 90% activity of the enzyme was lost thereafter, it maintained a very low activity all through the quiescent pupal development (Fig. 1).

Acetylcholine, on the other hand, maintained nearly a constant concentration till spinning commenced but gradually got depleted till day 5 and remained steady in the metamorphosing pupa till 15th day (Fig. 1). In the newly emerged both acetylcholinesterase activity and acetylcholine concentrations increased significantly. All along development, acetylcholine maintained a marked higher level than the esterase.

In the dipteran *S. ruficornis* both acetylcholine and acetylcholinesterase activity (Fig. 2) increase during larval growth. On the onset of pupation, while acetylcholine gradually decreases till the emergence, acetylcholinesterase activity with a significant depletion in the white pupa increases during pupal–adult development with intermittent fluctuations. Curiously, the esterase activity in this insect, all through development, stood at a markedly higher level than acetylcholine unlike in the lepidopteran *P. ricini* where the position is just the reverse.

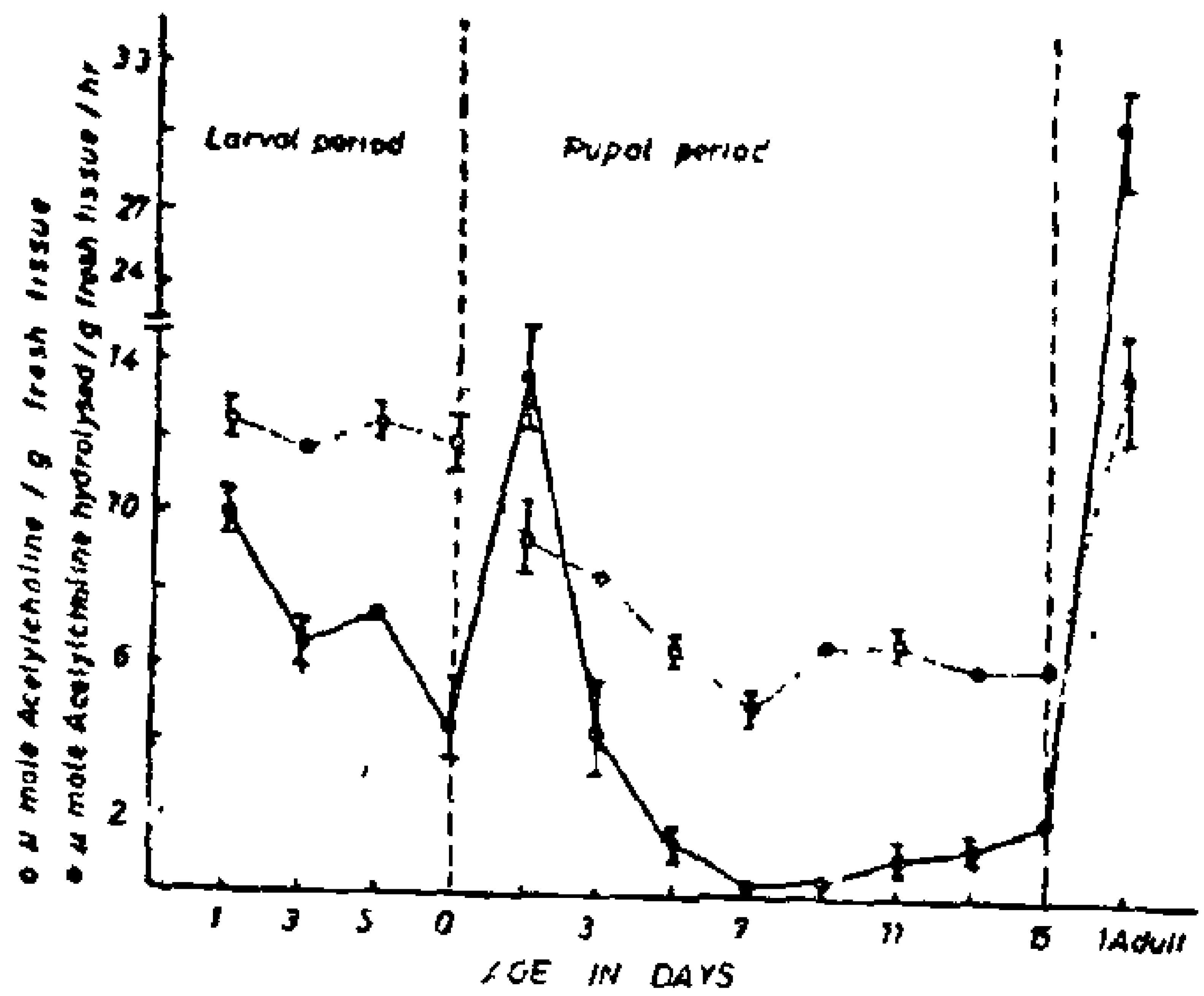

FIG. 1. Variation in acetylcholine content and acetylcholinesterase activity during development of *Philosamia ricini.*

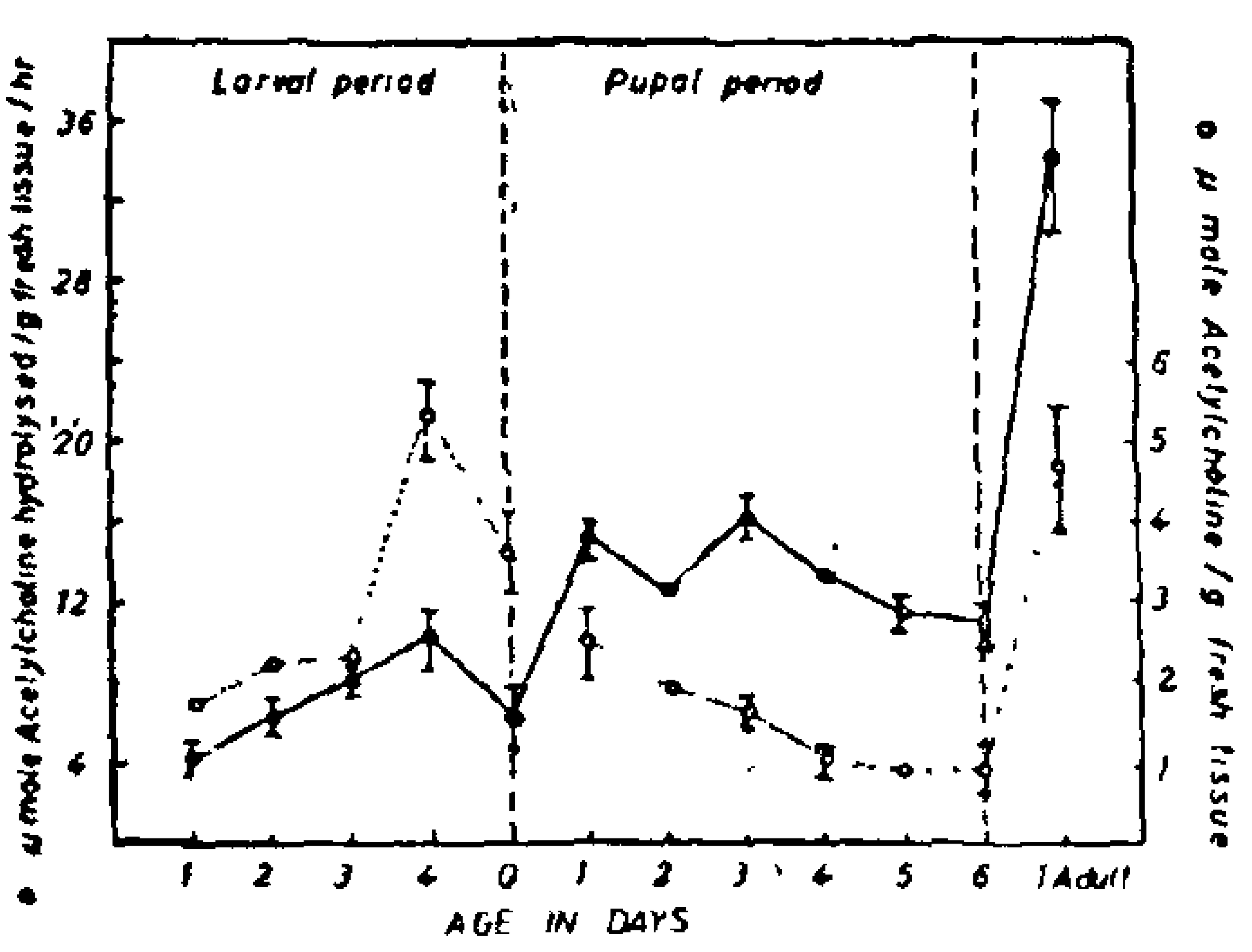

FIG. 2. Variation in acetylcholine content and acetylcholinesterase activity during development of *Sarcophaga ruficornis.*

The significant increase in both acetylcholine and acetylcholinesterase activity during adult development could be associated with the general process of metamorphosis evincing growth of brain and ganglia. Van der Kloot[12] and Shappirio *et al*[13] noted similar observations in *H. cecropia* and *A. polyphemus* during larval–pupal–adult transformation which prompt one to conclude that the increase in the cholinergic enzyme reflects simply on the overall increase in structural and functional proteins associated with growth and elaboration of the adult nervous system.

Nearly 75% of the total tissue acetylcholinesterase activity was found in the head part of the insect and varied in a similar manner as the total activity studied during larval and spinning period of *P. ricini* (Fig. 3).

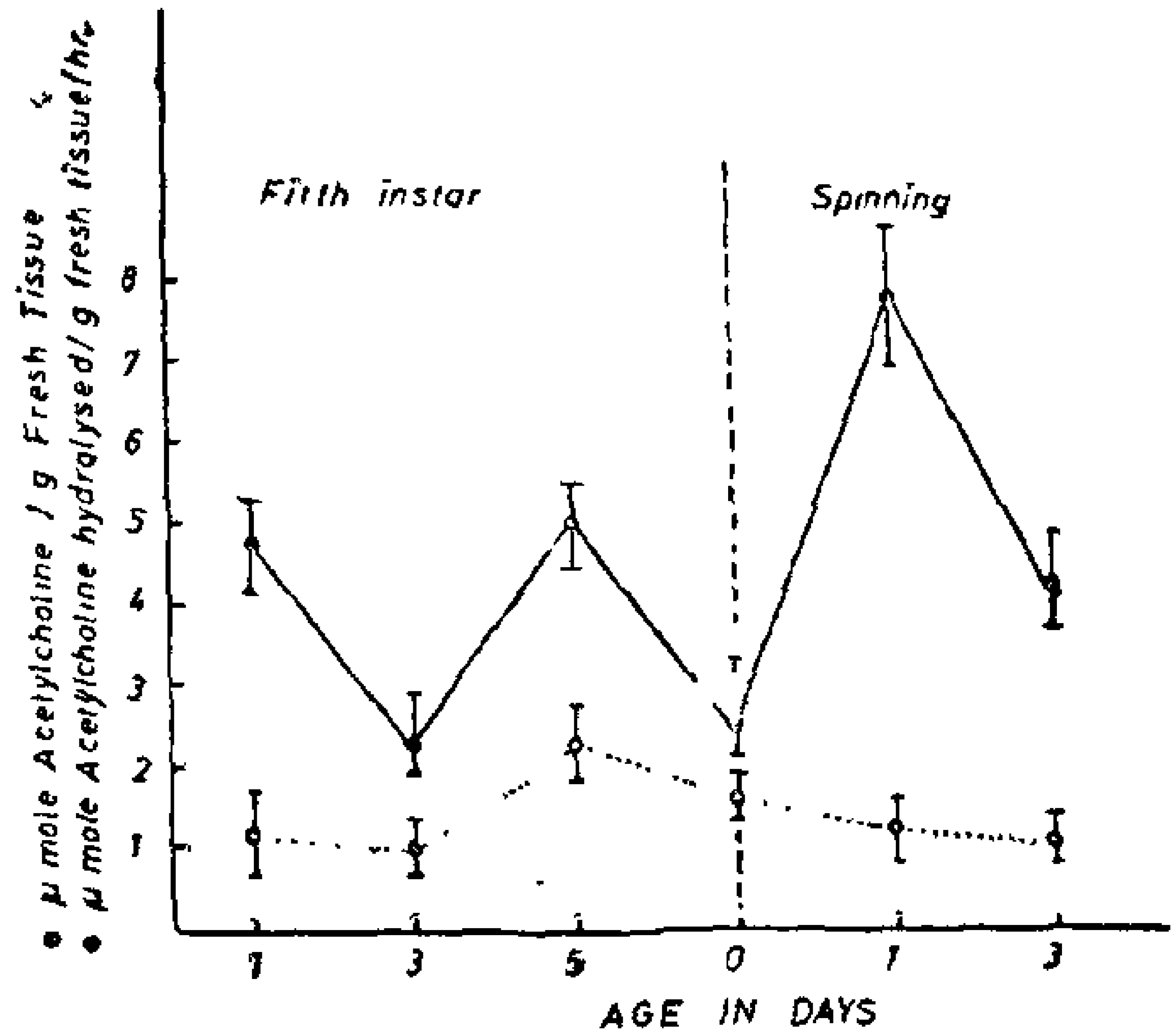

FIG. 3. Variation in acetylcholine content and acetylcholinesterase activity in the head part (First segment) of *Philosamia ricini* during larval development and spinning period.

The remaining 25% activity in the beheaded part gradually declined during fifth instar larval development but the developing pupa regained it (Fig. 4). High acetylcholinesterase activity detected in the head part is suggestive of its participation in the cholinergic function of the brain.

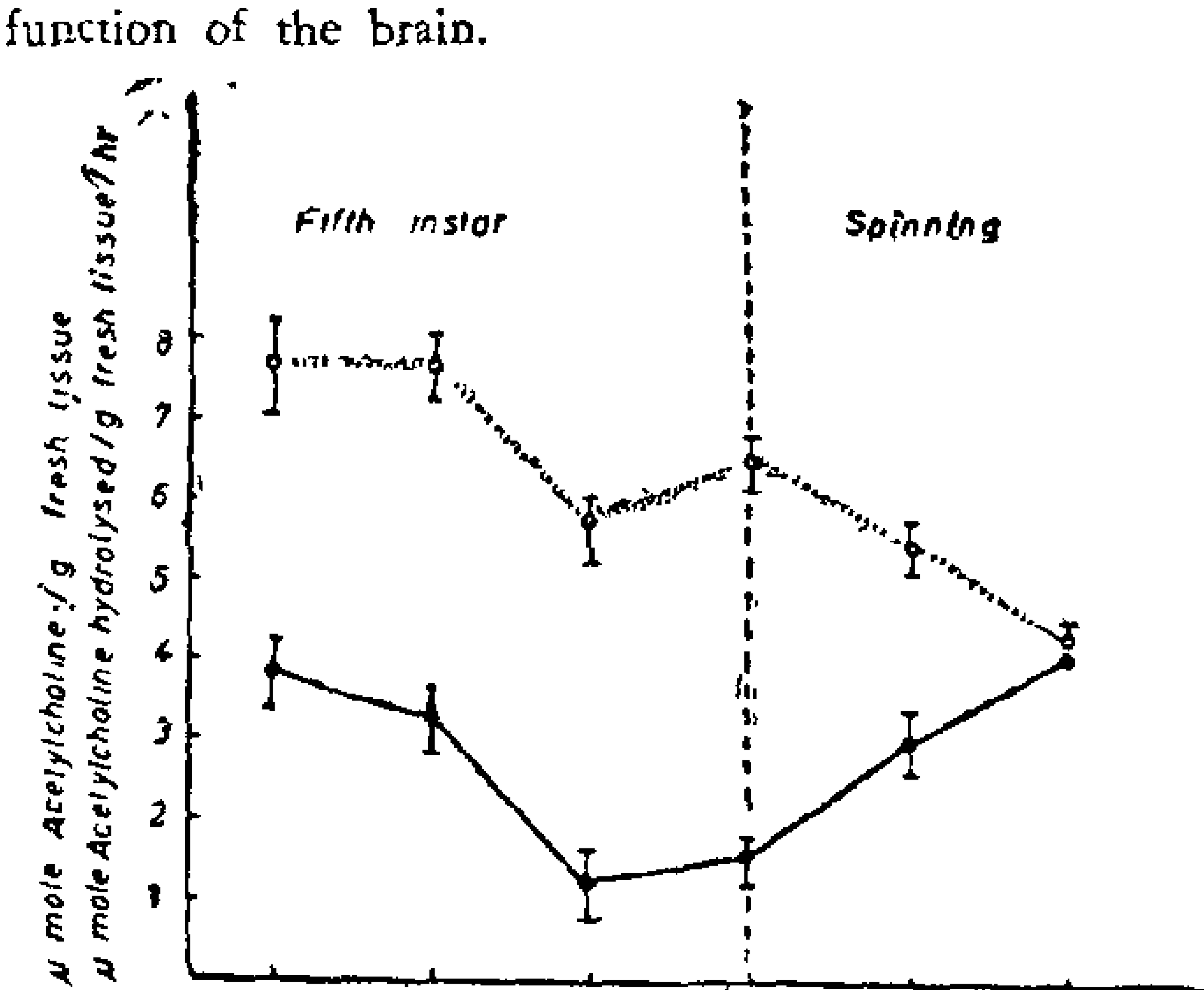

FIG. 4. Variation in acetylcholine content and acetylcholinesterase activity in the beheaded part of *Philosamia ricini* during larval development and spinning period.

The head part of *P. ricini* larva concentrated slightly lower percentage of acetylcholine (40%) whereas the beheaded part held the remaining 60% of the total. The head acetylcholine after a slight increase before commencement of spinning, gradually declined through spinning period till pupation (Fig. 3) while the beheaded part exhibited a decrease prior to spinning and, after an increase in the spinning larva on day 0, declined in line with its counterpart (Fig. 4).

The typical U-shaped variation patterns of acetylcholine content and acetylcholinesterase activity during larva–pupa–adult development of *P. ricini* indicate that the larval and adult stages of insects being vigorously active require high enzymic activity and acetylcholine concentration for cholinergic function of the brain and transmission of nerve impulses. However, *S. ruficornis* even during its pupal development reveals high enzymic activity.

Mansingh and Smallman[14] obtained characteristic patterns in four lepidopteran insects revealing a relatively low acetylcholinesterase activity in the fourth instar larva which subsequently increased in the fifth. On pupation, the enzymic activity after a slight decline increased 22-fold during the next eight days followed by further increase, prior to adult emergence. These results compel one to assume that this type of increase in the components of the cholinergic system during pupation and adult development is a general characteristic of lepidopteran insects.

In both the insects, the male adults manifest slightly higher activity of acetylcholinesterase in comparison to the female (Table 1). The enzymic activity is mainly and maximally confined to the head part followed by the thorax and abdomen in the order mentioned. The male adbomen, however, reveals high enzymic activity with low acetylcholine content. In the female adult it is just the reverse.

Cholinergic system in the dipteran *Musca domestica* has extensively been studied[15] and high concentration of acetylcholine, acetylcholinesterase and choline acetylase has been detected in the adult head tissue, the system being largely concentrated in the brain. The same components have been observed in the vertebrate brain as well. High concentration of cholinesterase was detected in the region of optic lobes of insects[16] accompanied with high concentration of synaptic structures associated with optical reception[17]. High acetylcholinesterase activity in the head part of both the sexes indicates its rôle in the cholinergic function of the brain.

Presence of acetylcholine and cholinesterase activity in the thorax of adult moths (male moth revealing higher concentration) indicates their involvement in the neuromuscular activity during flight.

Curiously, the female abdomen in both the insects reveals a high concentration of acetylcholine and low enzymic activity while the male abdomen reveals the opposite. This has been attributed to the eggs in the females which are reported to be rich in acetylcholine content[18]. Presence of acetylcholine in reproductive tissues of insects is perhaps parallel to the occurrence of choline acetylase and acetylcholine in human placenta. This induced Bull *et al*[10] to ascribe a role to acetylcholine in controlling the diffusion of ions.

TABLE I

Acetylcholine and acetylcholinesterase activity in head, thorax and abdominal tissues of the male and female adults of P. ricini *and* S. ruficornis.

Insect	Acetylcholine*		Acetylcholinesterase activity**	
	Male	Female	Male	Female
Philosamia ricini				
Head	20·17±1·561	15·12±1·492	60·21±3·563	46·91±4·331
Thorax	3·45±0·182	5·23±0·231	12·17±0·894	7·42±0·652
Abdomen	3·36±0·133	11·33±0·556	11·16±0·787	3·96±0·191
Sarcophaga ruficornis				
Head and Thorax	2·82±0·121	2·26±0·111	23·15±1·645	20·13±1·774
Abdomen	2·51±0·109	3·19±0·187	10·17±0·581	11·82±1·009

*Expressed in μ moles/g fresh tissue.

**Expressed in μ moles acetylcholine hydrolysed/g fresh tissue.

Presence of high concentration of acetylcholine—a neurosecretory substance—in the abdomen of insects is rather intriguing. Grzelak and co-workers[20] assigned an alternative metabolic pathway to this compound although no "reasonable hypothesis" was forwarded for this. According to them both the products of acetylcholine, formed by the enzymic degradation in the abdomen, are capable of being utilized not only in carbohydrate and lipid metabolism but in the nervous system as well.

In the female abdomen where intensive enzymic breakdown of the acetylcholine occurs, a considerable pool of the breakdown products accumulates. Eggs located in the female abdomen naturally have more demand for high energy providing carbohydrates and lipids than the males. Thus one could conclude that the presence of acetylcholine and acetylcholinesterase activity in the abdominal tissue are sex dependent and do not involve the central nervous system.

ACKNOWLEDGEMENTS

This research has been financed in part by a grant made by the United States Department of Agriculture, Agricultural Research Service, under PL 480.

1. Burn, J. H. and Rand, M. J., *A. Rev. Pharmac.*, 1965, **5**, 163.
2. Nachmansohn, D., *Chemical and Molecular Basis of Nerve Activity*, Acad. Press New York, 1959.
3. Ehrenpreis, S., *Nature*, London, 1964, **201**, 887.
4. Treherne, J. E., *The Neurochemistry of Arthropods*, 1963, p. 93.
5. Colhoun, E. H., *Advances in Insect Physiology*, 1963, **1**, 1.
6. Smallman, B. N. and Mansingh, A., *A. Rev. Ent.*, 1969, **14**, 387.
7. Pant, R. and Agrawal, H. C., *Biochem. J.*, 1965, **96**, 824.
8. — and Kumar, S., *Ibid.*, 1978, **174**, 341.
9. — and Morris, I. D., *J. Biochem*, Tokyo, 1969, **66**, 29.
10. Hestrin, S., *J. Biol. Chem.*, 1949, **180**, 249.
11. Metcalf, R. L., *J. Econ. Entomol.*, 1951, **44**, 883.
12. Van der Kloot, W. G., *Biol. Bull.*, 1955, **109**, 276.
13. Shappirio, D. G., Eichenbaom, D. M. and Lockie, B. R., *Am. Zool.*, 1965, **5**, 698.
14. Mansingh, A. and Smallman, B. N., *J. Insect Physiol.*, 1967, **13**, 447.
15. Mehrotra, K. N., *Ibid.*, 1961, **6**, 215.
16. Smallman, B. N., *J. Physiol.*, 1956, **132**, 343.
17. Bullock, T. H. and Horridge, G. A., *Structure and Function in the Nervous System of Invertebrates* (Ed. Freeman, W. H., San Francisco), 1955, **2**, 1079.
18. Yushima, T., *Jap. J. Appl. Ent. Zool.*, 1958, **1**, 38.
19. Bull, G., Hebb., C. and Ratkovic., D., *Nature*, London, 1961, **190**, 1202.
20. Grzelak, K., Lassota, Z. and Wronszlwska, A., *J. Insect Physiol.*, 1970, **16**, 1405.

POLAROGRAPHIC DETERMINATION OF THE STABILITY CONSTANT OF COMPLEXES FORMED BY LEAD(II) AND CADMIUM(II) WITH ACETYLENE DICARBOXYLIC ACID

A. L. J. RAO AND MAKHAN SINGH

Department of Chemistry, Punjabi University, Patiala (Punjab), India

ABSTRACT

The composition and formation constants of the complexes formed by lead and cadmium with acetylene dicarboxylic acid have been determined polarographically. The reductions are reversible and diffusion controlled. Lead forms two complexes, *viz.*, lead (acetylene-dicarboxylic acid) and lead (acetylene-dicarboxylic acid)$_2^{2-}$ having β_1 and β_2 values 60 and 11,100. But cadmium forms a soluble complex in the ratio 1:1 with acetylene dicarboxylic acid. The value of the stability constant of the complex at an ionic strength 1 is $1 \cdot 678 \times 10^{-2}$. The percentage distribution of various complex species present at each (ligand) in the lead system has been calculated.

THE polarographic behaviour of the complexes of lead and cadmium with various acids, *e.g.* Oxalic acid[1], salicylic acid[2], glycollic acid[3], formic acid[4], tartaric acid[5], malonic acid, succinic acid, glutaric acid and adipic acid[6], valeric acid[7], maleic acid[8] and acetate[9] has been investigated. The present paper deals with the determination of the stability constants of the complexes formed by lead and cadmium with acetylene dicarboxylic acid.

EXPERIMENTAL

All the chemicals used were of reagent grade purity. Potassium nitrate was used to keep constant ionic strength. Gelatin was used as maximum suppressor. Acetylene dicarboxylic acid was used as the complexing agent. A Toshniwal polarograph of CLO_2 type was used. The pH was adjusted with sodium hydroxide and all the polarograms were recorded at pH 6 at 25° ± 0·1° C. The dropping