

## ACKNOWLEDGEMENT

One of us (RS) acknowledges financial assistance from the University Grants Commission, New Delhi, India.

1. Aravamudan, G. and Venkappayya, D., *Talanta*, 1968, 15, 704.
2. Mahadevappa, D. S. and Naidu, H. M. K., *Ibid.*, 1973, 20, 349.
3. Srivatsva, A. and Bose, S., *J. Indian Chem. Soc.*, 1975, 52, 214.
4. Yathirajan, H.S., Rangaswamy and Mahadevappa, D. S., *Ibid.*, 1979, 56, 421.

5. Katgeri, S. N., Mahadevappa, D. S. and Naidu, H. M. K., *Talanta*, 1979, 26, 420.
6. Rangaswamy, Yathirajan, H. S. and Mahadevappa, D. S., *Indian J Chem.* (in press).
7. Farooq, O. and Ahmad, N., *Acta Chim.* (Budapest), 1975, 85, 395.
8. Barratt, M F., Mc Donald, T. R. R. and Topp, N. E., *J. Inorg. Nucl. Chem.*, 1964, 26, 931.
9. Morris, J. C., Salazar, J. A. and Winemann, M. A., *J. Am. Chem. Soc.*, 1948, 70, 2036.
10. Chrzasczczewska, A., *Chem. Abstr.*, 1955, 49, 212.
11. Soloway, S. and Lipschietz, A., *Analyt. Chem.*, 1952, 13, 976.

# SEX-SPECIFIC DIFFERENCES IN TOTAL ESTERASE, ACETYLCHOLINE AND ACETYLCHOLINESTERASE OF HEADS OF *PHILOSAMIA RICINI* ADULT MOTHS

RADHA PANT AND DWIJENDRA K. GUPTA

Biochemistry Department, The University, Allahabad 211 002

## ABSTRACT

Some sex-specific differences with regard to the activity of esterases (total and acetylcholine) and acetylcholine content have been studied in the head part of *Philosamia ricini* virgin moths. The cholinergic system of females seems to be relatively more active than that of males.

## INTRODUCTION

ACETYLCHOLINE is present in high concentrations in insects when compared with most other invertebrates and vertebrates. In vertebrates, it plays a vital role in preganglionic transmission of the autonomic nervous system, at the nerve endings of the parasympathetic system, skeletal neuromuscular junctions<sup>1-3</sup> and in the brain<sup>4</sup>. The distribution of acetylcholinesterase, whose role in nerve transmission has been established, has been sought by biochemical and histochemical techniques. Histochemical results show that acetylcholinesterase occurs in the nervous tissue, muscle, digestive organs, reproductive organs and haemolymph of *Periplaneta*<sup>5,6</sup>. Although the presence of acetylcholine, acetylcholinesterase and choline-acetylase has been detected in many insects<sup>7</sup>, the highest activity has been demonstrated in the most active insects such as *Musca*, *Apis* and *Periplaneta*<sup>8</sup>. In addition to the basic requirement for normal functioning of the nervous system, some insects may need more acetylcholine necessary for more specialized purposes<sup>9</sup>.

The present study was undertaken to see if any sex-specific differences occurred in the lepidopteran, *Philosamia ricini*.

## MATERIALS AND METHODS

Virgin male and female moths were sorted immediately after emergence. Moths of known age were chilled briefly in a refrigerator and their heads clipped off. A 1% (w/v) homogenate each of the male and female heads during adult development was prepared in glass-distilled water, employing 10 heads in each case.

All assays were made in duplicate sets on two individual homogenates and the mean values have been calculated. Acetylcholine was assayed by the method of Hestrin<sup>10</sup> as described by Metcalf<sup>11</sup> with modification. Esterases (total and acetylcholin-) were assayed by the method of Ellman *et al.*<sup>12</sup>. The method involves the colorimetric determination of sulfhydryl groups produced by enzymic hydrolysis of acetylthiocholine. The reaction mixture consisting of sodium phosphate (3.5 ml, 0.1 M, pH 8.0), colour reagent [0.2 ml, 0.0063 M 5.5'-dithiobis-2-nitrobenzoic acid (Sigma Chemical Company) in 0.065 M sodium phosphate (pH 7.0) containing sodium bicarbonate, 1 mg/ml], substrate (0.2 ml, 0.0095 M acetylthiocholine iodide, stability 3 days) and tissue homogenate (0.6 ml, 1% w/v) was incubated both in presence and absence of eserine solution (0.01 ml, 1.1 mg/ml) for 30 minutes.

at 30° C, together with reagent blanks and controls. Following incubation, the optical density was measured in a colorimeter at 412 m $\mu$ . The activity of both total esterases and acetylcholinesterase ( eserine sensitive) has been represented as  $\mu$  mole acetylthiocholine iodide hydrolysed for 30 minutes per mg head protein.

## RESULTS AND DISCUSSION

Pant *et al.*<sup>13</sup> observed a 90% decrease in acetylcholinesterase activity during pupal development of *P. ricini*, while in a dipteran *Sarcophaga ruficornis*, a significant decrease in the enzyme activity was noticed. They further reported that 75% of the total tissue acetylcholinesterase activity was confined to the head part only and varied in a similar manner as the total activity studied during larval and spinning period of *P. ricini*<sup>13,14</sup>. Likewise, the cholinergic system was found to be largely concentrated in the head tissues of adult Diptera<sup>7,15</sup>.

A perusal of the data (Fig. 1) reveals that heads of female adults manifest higher activity of total esterase and acetylcholinesterase than the male heads, suggesting a more active cholinergic system of females. Pant *et al.*<sup>13</sup> also observed a slightly higher activity of acetylcholinesterase of female abdomens compared to males.

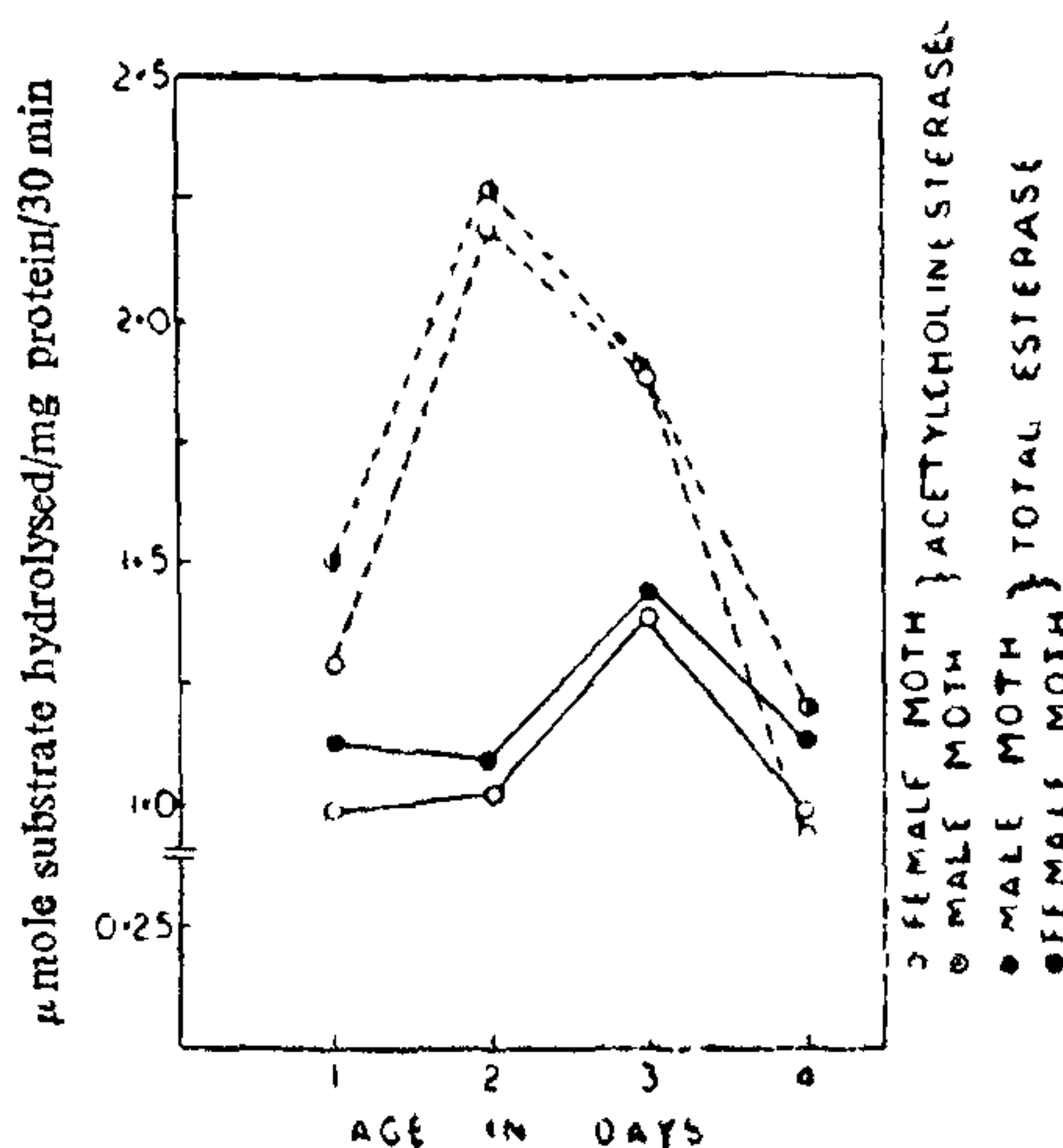


FIG. 1. Variation in total esterase and acetylcholinesterase activity in the head part of adult moth of *P. ricini*.

The content of acetylcholine in heads was very low in newly emerged male adult (Fig. 2) and increased sevenfold on day 2 (12.19 mg/g fresh weight). The content further increased significantly on day 3 (31.25 mg/g fresh weight) but decreased on the last day of its life span.

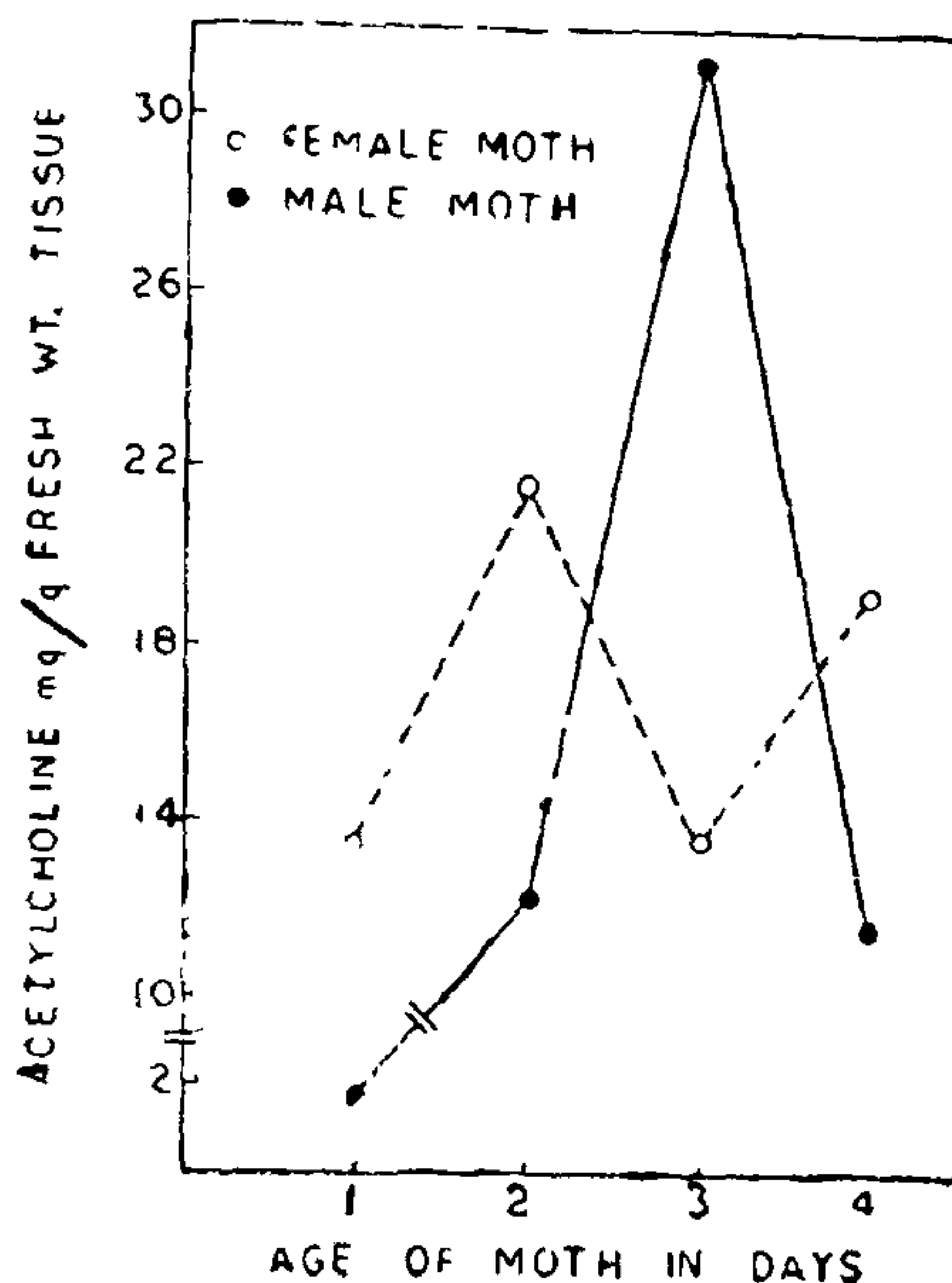


FIG. 2. Variation in acetylcholine in the head part of adult moth of *P. ricini*.

Adult female heads of *P. ricini*, conversely show an eightfold increase in the acetylcholine content on day 1 over that in males (Fig. 2). Acetylcholine increases significantly on day 2 and with a decrease on day 3, it records once again a sharp increase on day 4.

The significant increase in both acetylcholine concentration and acetylcholinesterase activity could be attributed to the general increase in structural and functional proteins associated with growth and elaboration of adult nervous system.

It is concluded that the presence of the cholinergic system of head tissue is sex-dependent in addition to involving central nervous system. The presence acetylcholine and acetylcholinesterase activity in the abdominal tissue of *P. ricini* was also found to be sex-dependent but not involving the central nervous system<sup>13</sup>.



## ACKNOWLEDGEMENT

One of us (DKG) is grateful to the UGC, New Delhi, for a research fellowship.

1. Katz, B., *Proc. Roy. Soc.*, 1962, 155, 455.
2. Thesleff, S. and Quastel, O. M. J., *A. Rev. Pharmac.*, 1965, 5, 263.
3. Triggle, D. J., *Ibid.*, 1965.
4. Gaddum, J. H., *Nature, London*, 1963, 197, 741.
5. Iyatomi, K. and Kanehisa, K., *Jap. J. Appl. Ent. Zool.*, 1958, 2, 1.
6. Kanehisa, K., *Bull. No. 2, Laboratory Appl. Ent. Faculty of Agriculture, Nagoya University, Anyo, Japan*, 1961.
7. Smallman, B. N., *J. Physiol.*, 1956, 132, 343.
8. Metcalf, R. L., March, R. B. and Mexan, M. G., *Ann. Ent. Soc. Amer.*, 1955, 48, 222.
9. Grzelak, K., Lassota, Z. and Wronszkivska, A., *J. Insect Physiol.*, 1970, 16, 1405.
10. Hestrin, S., *J. Biol. Chem.*, 1949, 180, 249.
11. Metcalf, R. L., *J. Econ. Entowd.*, 1951, 44, 883.
12. Ellman, G. L., Courtney, K. D., Andres, V. and Featherstone, R. M., *Biochem. Pharmac.*, 1961, 7, 88.
13. Pant, Radha, Kumar, S. and Dubey Ratan, *Curr. Sci.*, 1978, 47 (13), 445.
14. Pant, R., Gupta, D. K. and Sharma, B., *Indian J. Exp. Biol.*, 1978, 16, 706.
15. Mehrotra, K. N., *J. Insect Physiol.*, 1961, 6, 215.

## FOSSIL RHINOCEROS FROM TAMILNADU

S. C. JAYAKARAN

*Regional Hydrogeologist, Water, P.O. Box 164, Morogoro, Tanzania, East Africa*

## ABSTRACT

Fossil rhinoceros is being reported from the most southern part of India. The fossil skull indicates that the geographic distribution of fossil rhinoceroses was wider than it was known earlier.

## INTRODUCTION

THIS notes records the occurrence of a fossil rhinoceros from the district of Thirunelveli. The nearest living rhinoceroses are confined to Burma, Malaya, Sumatra, Java, Nepal and Assam in India. Fossil rhinoceroses have been documented from extra-peninsular India mainly from the Siwalik system and from peninsular India from the alluvium of Krishna valley and from the ossiferous caves of Karnool. Fossil rhinoceroses from the Pleistocene beds of Sri Lanka had earlier been recorded. This find, from the most southern part of India would contribute to the knowledge of their earlier distribution.

## DESCRIPTION OF THE SITE

The fragmented skull of a fossil rhinoceros was found in a well cutting in Sathankulam—Lat.  $8^{\circ} 27' N$ —Long.  $77^{\circ} 55' E$  (Fig. 1). The site is about 21 m above the mean sea level and about 30 m south of the southern bank of Karamanyar river.

The fossiliferous layer was about 2 m thick and was composed of semi-consolidated sandy materials overlying a layer of calcareous sandstone. The calcareous sandstone in turn overlay the smoothly-

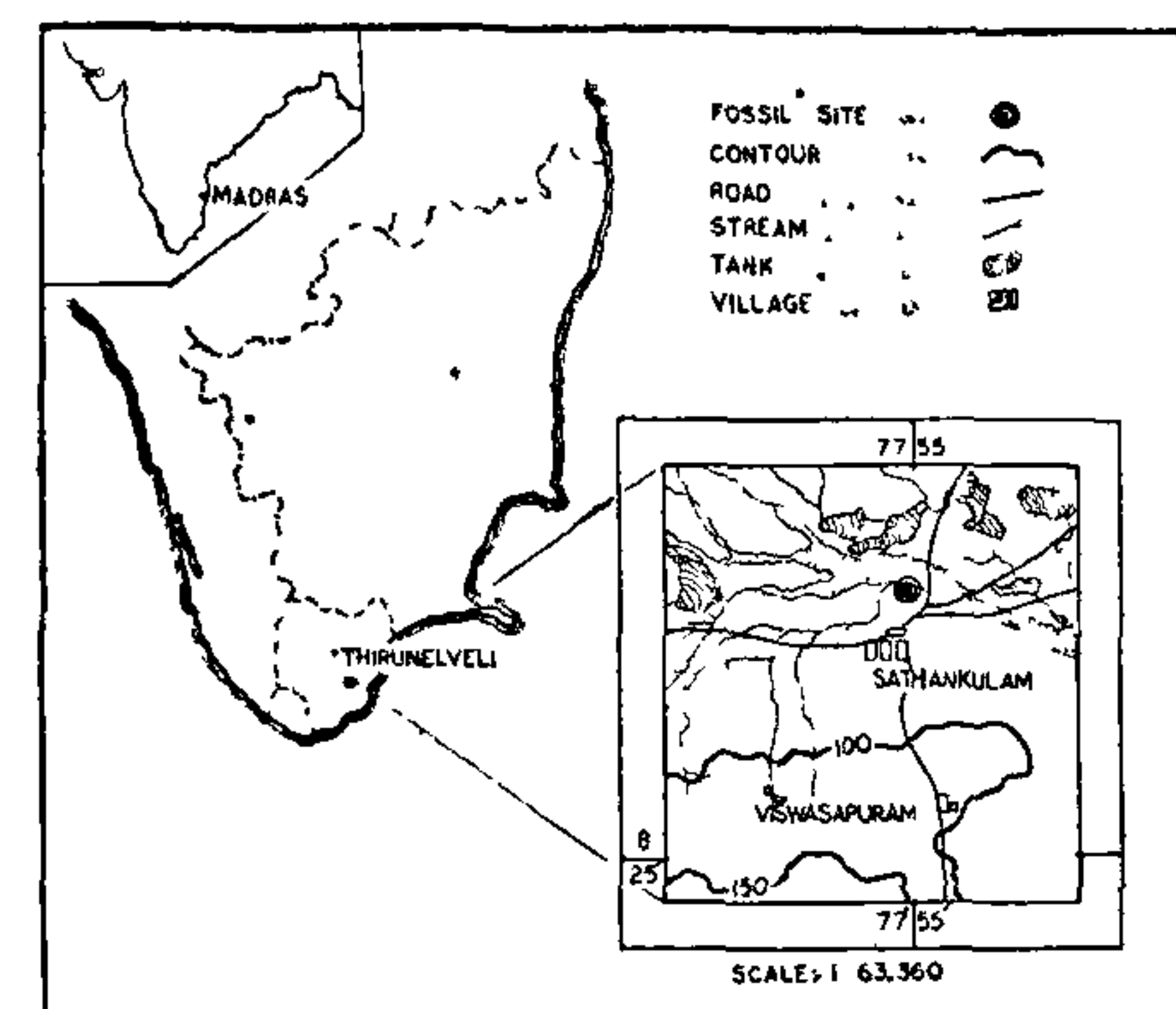


FIG. 1

eroded pre-cambrians at a depth of about 8.5 m below ground level. Over the fossiliferous layer was a tuffaceous calcareous formation of five metres thickness. The fossiliferous layer could only be traced to a limited extent of about twenty acres south of the river and west of the road as could be observed in the nearby well cuttings,