Table 2 *Proline accumulation in Azospirillum isolates*

	5 Days' growth		10 Days' growth	
Isolate	30 C	50°C	30 C	50°C
AZ Ht 1	64.0	104 0	89 6	136 0
AZ Ht.2	32 0	192.0	69.1	284.0
Sp.7	68.0	78 0	74.2	91.2
Mean for 15 HTR isolates	61.8	96.8	74.6	112 8

Proline in μg per mg dry weight of cells. Each value is mean of three determinations.

organism at times of stress. PBHB content of HTR isolates AZ.Ht.1 and AZ.Ht.2 was higher than that of the standard mesophilic strain Sp.7. Also, PBHB content of the HTR strains was higher when the cells were grown at 50°C, while that of strain Sp.7 tended to decrease, presumably owing to the utilization of PBHB as a source of energy during the prolonged incubation. In general the 15 HTR isolates showed a spectacular increase in PBHB content at elevated temperature.

The HTR strains AZ.Ht.1 and AZ.Ht.2 strains also recorded sharp increase in proline content after growth at 50°C. In Sp.7 strain there was a slight increase in proline content at 50°C. All the 15 HTR isolates tended to accumulate proline at high temperature. The results suggest the role of PBHB and proline in temperature resistance of *Azospirillum* spp.

The problem of temperature sensitivity has been realized in the case of the root nodule bacterium *Rhizobium*. Eaglesham and Ayanaba[8] have indicated the occurrence of several strains of *R. trifolii* and *R. japonicum* which exhibited temperature tolerance up to 70–80°C. They have also stressed the need for developing HTR strains in *Rhizobium*. The occurrence of higher ratios of high-melting-point, saturated fatty acids to branched-chain fatty acids was reported to confer temperature tolerance to bacteria[9,10]. Also, intracellular accumulation of amino acids like proline and hydroxyproline[11], presence of thermostable proteins, and synthesis of heat shock proteins[12] have been suggested as possible mechanisms of high-temperature tolerance in microorganisms.

One of the authors (KV) thanks M/s Aspee Foundation, Bombay, for financial support.

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IN VITRO EFFECTS OF THREE ORGANOPHOSPHORUS INSECTICIDES ON KINETIC CONSTANTS OF ACETYLCHOLINESTERASE IN A FRESHWATER TELEOST, *CLARIAS BATRACHUS* (LINN.)

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It is well known that acetylcholinesterase (AChE, E.C. 3.1.1.7) is the target enzyme of both organophosphorus (OP) and carbamate pesticides[1,2]. The inhibition of AChE disrupts the transmission of the nerve impulse in the central and peripheral nervous system in vertebrates[3–5]. Several attempts were made to correlate the toxic action of OP pesticides with AChE inhibition and thereby to estimate their anticholinesterase activity[6–8]. OP and carbamate compounds inhibit cholinesterases by binding cova-

lently to a serine residue in the active site of the enzyme[9]. Fish brain AChE activity can serve as a monitor of pollution by OP insecticides[10,11]. The *in vitro* inhibition kinetics of AChE by trichlorfon, malathion and methylparathion (technical grade OP compounds) in brain and muscle tissues of the

Table 1 *Effect of trichlorfon, malathion and methylparathion on kinetic parameters of Clarias batrachus acetylcholinesterase*

Kinetic parameter	Tissue	Control	Trichlorfon	Malathion	Methyl parathion
V_{max} (μmoles of ACh hydrolysed/ mg protein/h	Brain	5.9	5.2 (−11.86)	5.5 (−6.78)	5.7 (−3.39)
	Muscle	3.7	3.0 (−18.92)	3.2 (−13.51)	3.5 (−5.41)
K_m	Brain	3.3	6.8 (+106.06)	5.0 (+51.52)	3.8 (+15.15)
(μM)	Muscle	2.9	5.1 (+75.86)	4.3 (+48.28)	3.7 (+27.59)

Values in parentheses indicate per cent change over control.

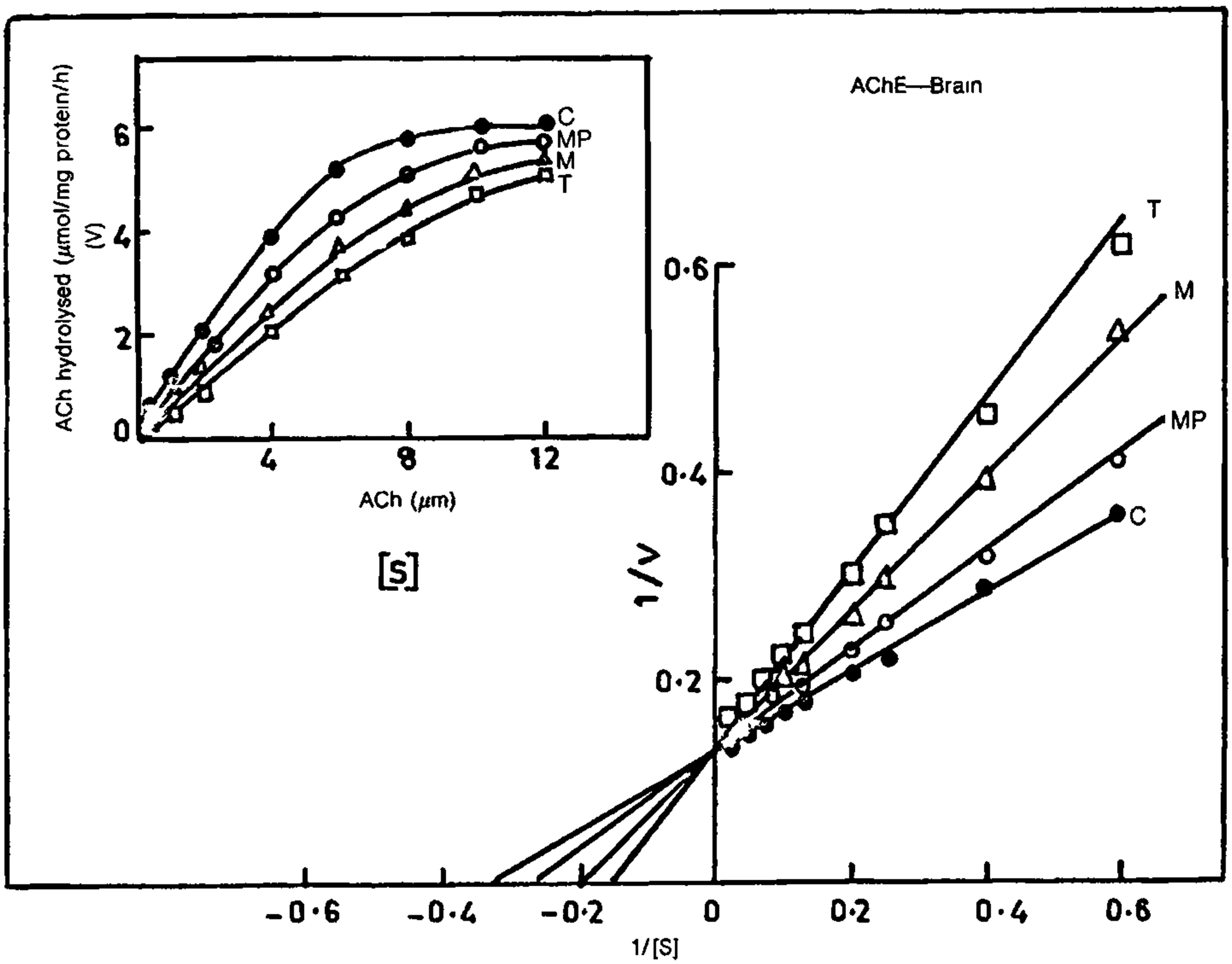

Figure 1. Lineweaver–Burk plots for *C. batrachus* brain AChE assayed in absence (●——●) and in presence of trichlorfon (□——□), malathion (△——△) and methylparathion (○——○). Inset, substrate concentration vs reaction velocity curves.

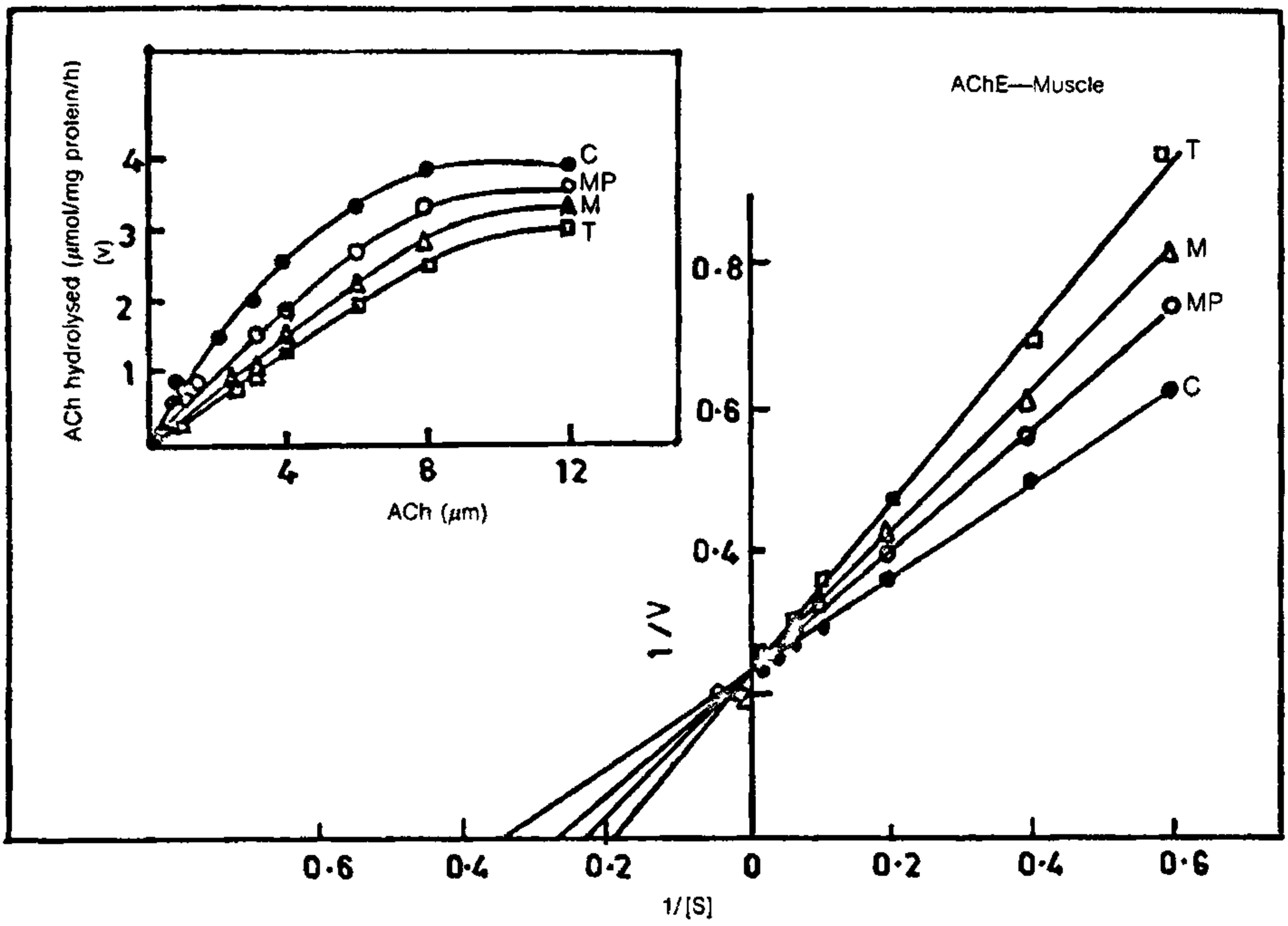

Figure 2. Lineweaver–Burk Plots for *C. batrachus* muscle AChE. See legend to figure 1 for details.

freshwater edible fish *Clarias batrachus* (Linn.) have been studied.

C. batrachus weighing 30–40 g and 20 cm in length were collected from the local market and acclimatized to laboratory conditions for about two weeks. They were fed *ad libitum* with groundnut cake and deprived of food before the day of experiment[12]. AChE was assayed by the method of Metcalf[3] in the presence of different concentrations (2–20 µmol) of trichlorfon, malathion and methylparathion individually to determine the IC_{50} values. Enzyme activity was expressed as µmoles of ACh hydrolysed/mg protein/h. Protein was determined according to Lowry *et al.*[13] The double reciprocal plots were prepared[14], and V_{max} and K_m values were calculated.

Fifty per cent inhibitory concentrations (IC_{50}) for trichlorfon, malathion and methylparathion on brain AChE were 4, 10 and 12 µM; in the case of muscle AChE the IC_{50} values were 8, 12 and 15 µM respectively. The effects of the pesticides on V_{max} and K_m of brain and muscle AChE are presented in table 1 and figures 1 and 2. In all cases there was significant increase in K_m values but no significant change in V_{max} values. Increase in K_m suggests decreased affinity of the enzyme for the substrate and also decreased rate of breakdown of the enzyme–substrate complex. The three OP compounds studied exert a competitive type of inhibition on AChE. Competitive inhibitors[15,16] are known to compete for the same active site with the substrate, resulting in the competitive type of inhibition. Most of the OP insecticides have structural analogy with the ester part of acetylcholine and bind to the esterase part of AChE[17].

One of the authors (VJS) thanks CSIR, New Delhi, for a fellowship.

8 August 1988

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ANNOUNCEMENT

EVOLUTIONARY BIOLOGY WORKSHOP

A one-week workshop titled 'Topics in Evolutionary Biology' will be held at the Indian Institute of Science from 11 to 15 December (both days inclusive) this year. The subjects to be covered, at a level aimed at students as well as research workers, will be varied and will include optimization strategies, sexual selection, the evolution of insect sociality and the evolution of complexity. Intending participants should write to V. Nanjundiah, Centre for Theoretical Studies, Indian Institute of Science, Bangalore 560 012, with their CV and a brief but specific statement of purpose indicating their interest. The **last date** for receipt of applications is 7 **November 1989**. Those selected will be paid return second class train fare to Bangalore and back and a daily allowance sufficient to cover their stay at the IISc campus.