Proportion of esterase positive lymphocytes (EPLs)

Untreated lymphocyte suspension	Adherent cell depleted	SRBC rosette forming lymphocytes[a]	Free cells (B-rich fraction)	Following PHA stimulation
12.8 ± 6.1 (26)[b]	14.0 ± 6.1 (8) (NS)	17.7 ± 8.6 (12) ($p < 0.05$)	11.7 ± 4.3 (12) (NS)	17.8 ± 6.3 (12) ($p < 0.03$)

[a] The SRBC rosettes formed a mean (± SD) of 65 ± 5.6% of the untreated lymphocyte suspension. [b] A minimum of 200 lymphocytes or rosettes were counted and the proportion of EPL calculated. Number in brackets signify number of observations. NS, not significant.

batch K6674) for 72 h. Parallel cultures were pulsed with ^{3}H-thymidine at that time to ensure that proliferation has occurred. Cell viability at the end of 72 h was greater than 85%.

Smears prepared from lymphocytes or rosettes were fixed in 25% formaldehyde and stained for non-specific esterase[6].

The Table shows the results obtained. The T lymphocyte fraction shows a higher proportion of esterase positive lymphocytes (EPL) than either the B lymphocyte fraction or unfractionated lymphocytes. This observation was directly confirmed when smears of rosette preparations were made, and it was found that cells forming rosettes had a higher percentage of EPLs than unfractionated lymphocytes. Culture with PHA also increases the proportion of EPLs. Furthermore PHA stimulation causes some lymphocytes to acquire a rim-staining pattern.

Our results make it unlikely that the mononuclear cells showing discrete staining for non-specific esterase are monocytes with lymphocyte-like morphology. The proportion of EPLs remains unchanged following depletion of adherent cells, and following such treatment there tends to be a greater number of EPLs which form SRBC rosettes. Monocytes do not form rosettes with untreated SRBC[7].

While accumulating evidence points to the fact that PHA induces proliferation of both T and B lymphocytes as measured by ^{3}H-thymidine uptake in the human[8–10], work by GREAVES et al.[11] and JONDAL[12] indicate that the overwhelming majority of blast cells developing in response to PHA carry surface markers specific to T lymphocytes. We have studied the effect of PHA-induced proliferation to investigate the possibility that the appearance of esterase staining is associated with an increase in lymphocyte metabolic activity. Our findings of PHA-induced increase in EPLs is in keeping with the previously reported observation that immunoflorescent staining for cholinestrase increases following PHA stimulation[13].

It is significant that esterase activity is represented more on cells of the T lymphocyte differentiation axis, in view of the recent observations that anticholinestrase agents inhibit SRBC rosette formation[14]. The effect of these pharmacologic agents on B lymphocyte receptors is still unknown. Our preliminary results show that cholinestrase inhibition by physostigmine sulphate and depletion by echothiophate iodide significantly reduces the proportion of EPLs[15]. The biological significance of the presence of cholinestrase activity on lymphocyte membranes is not established; however it has been suggested that it may play an essential role in the stabilization of membrane structure[14] and in the process of lymphocyte activation[13].

[6] L. T. YAM, C. Y. LI and W. H. CROSBY, Am. J. clin. Path. *55*, 283 (1971).
[7] F. AUITI, J.-C. CEROTTINI, R. R. A. COOMBS, M. COOPER, H. B. DICKLER, S. S. FRØLAND, H. H. FUDENBERG, M. F. GREAVES, H. M. GREY, H. G. KUNKEL, J. B. NATVIG, J.-L. PREUD'HOMME, E. RABELLINO, R. E. RITTS, D. S. ROWE, M. SELIGMANN, F. P. SIEGAL, J. STJERNSWÄRD, W. D. TERRY and J. WYBRAN, Scand. J. Immun. *3*, 521 (1974).
[8] B. PHILLIPS and I. M. ROITT, Nature New Biol. *241*, 254 (1973).
[9] H. W. LISCHNER, M. A. VALDES-DAPENA, J. BIGGIN, S. HANN and N. ANONI, in Proceedings of the 7th Leukocyte Culture Conf. (Ed. F. DAGUILARD; Academic Press, New York 1973), p. 547.
[10] L. CHESS, P. R. MCDERMOTT and S. F. SCHLOSSMAN, J. Immun. *113*, 1113 (1974).
[11] M. GREAVES, G. JANOSSY and M. DOENHOFF, J. exp. Med. *140*, 1 (1974).
[12] M. JONDAL, Scand. J. Immun. *3*, 739 (1974).
[13] A. S. COULSON, im Proceedings of the 5th Leukocyte Culture Conf. (Ed. J. E. HARRIS; Academic Press, New York 1907), p. 235.
[14] R. K. CHANDRA and K. M. KUTTY, Experientia *31*, 858 (1975).
[15] N. R. FARID, unpublished data.

Invertase Activity in the Midgut of *Sarcophaga ruficornis* and *Musca domestica* (Diptera: Insecta)

M. SINHA

Department of Zoology, University of Lucknow, Lucknow (India), 17 July 1975.

Summary. The pH for the optimum activity of the midgut invertase was 5.5 in the adults of *S. ruficornis*, 6.0 in its larvae and adults of *M. domestica* and 6.5 in the larvae of the latter fly. The optimum temperature was 50 °C. Enzyme activity was retarded by the addition of glucose and fructose.

Adults of *S. ruficornis* and *M. domestica* survived well on cane-sugar and water, while their larval stages failed to develop on this diet inspite of the fact that both the stages possess the enzyme required for the hydrolysis of cane-sugar, namely, invertase (unpublished observations). Presuming that there might be some difference in the nature of the enzyme of the two stages, the effect of various factors on the invertase activity has been studied.

Materials and methods. S. ruficornis was reared on sugar and meat, and *M. domestica* on sugar and milk[1]. Midgut homogenate was prepared as described by SINHA[2]. The enzyme concentration was 4 guts/ml and $^1/_2$ gut/ml in the case of larvae and adults respectively.

[1] M. SINHA, Curr. Sci. *43*, 320 (1974).
[2] M. SINHA, Entomologia exp. app., in press (1975).

Assay system consisted of 0.2 ml of 1% sucrose solution, 0.2 ml of appropriate buffer (HCl-sodium citrate buffer, 0.1 *M*, for pH 3.0 to 5.0; Sörensen's phosphate buffer, 0.1 *M*, for pH 5.5 to 8.0; Glycine-NaOH buffer, 0.1 *M*, for pH 8.5 to 9.0), and 0.1 ml of enzyme homogenate. The mixture was incubated at 37 °C for 1.5 h in the case of the larvae, and 45 min in the case of adults. After incubation, the reaction was stopped by adding 0.5 ml of 0.1 *N* NaOH to the reaction mixture. The enzyme activity was measured by the method of SUMNER and HOWELL[3].

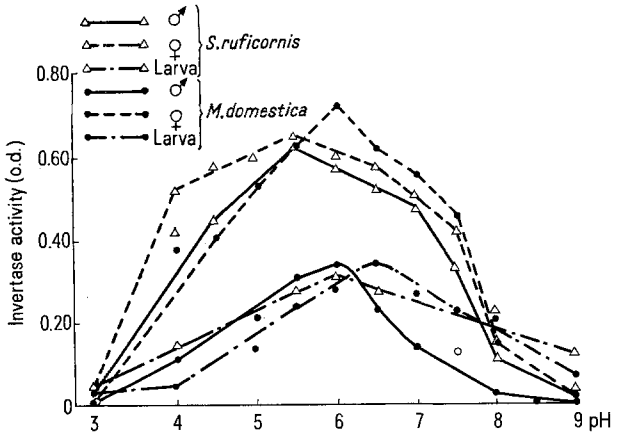

Fig. 1. Effect of pH on the activity of invertase from the midgut of flies.

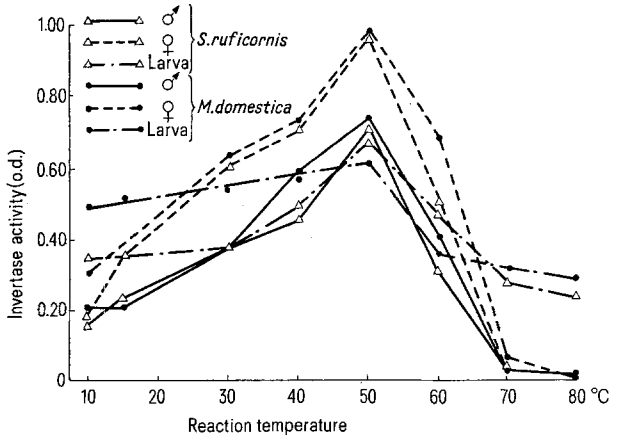

Fig. 2. Effect of temperature on the activity of invertase from the midgut of flies.

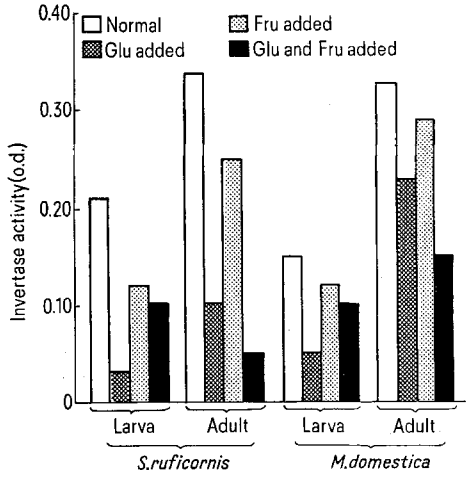

Fig. 3. Effect of glucose and fructose on the activity of invertase from the midgut of flies.

The pH for the optimum activity of invertase was determined first, and then the effect of temperature, incubation period, enzyme concentration and hydrolytic products (glucose and fructose) was determined at optimum pH.

Results and discussion. Invertase of various insect species is known to have different pH for the optimum activity ranging from 5.4[4] to 7.3[5,6]. The optimum pH for the midgut invertase activity was 5.5 in the adults of *S. ruficornis*, 6.0 in its larvae, and in adults of *M. domestica*, and 6.5 in the larvae of the latter fly (Figure 1). Invertase activity was quite appreciable between pH 5.0 and 8.0 in the larvae and between pH 4.5 and 7.5 in the adults (Figure 1). Observations on the pH of the midgut of these flies (Table[7]) indicate that suitable pH for maximum invertase activity is present only in the most anterior part of the midgut in the larvae of both the flies and adults of *S. ruficornis*, while in the adults of *M. domestica*, the pH of the entire midgut except the midmidgut is suitable for invertase activity. Hence the midgut of the adults of *M. domestica* seems to be best adapted for sucrose digestion amongst the two species.

The maximum activity of the midgut invertase from the two flies was at 50 °C (Figure 2), although the enzyme was quite active between 25° and 40 °C. This means that the enzyme is physiologically adapted to high temperatures and appreciable invertase activity occurs at normal temperatures (30° to 37 °C) at which the flies flourish. The optimum temperature for the invertase activity ranges from 25°[8] to 37 °C[9,10] in other insects.

Invertase activity was inhibited by glucose and fructose (Figure 3), as has also been reported by EVANS and PAYNE[11] for glucose and trehalose.

[3] J. B. SUMNER and S. P. HOWELL, J. biol. Chem. *108*, 51 (1935).
[4] P. L. BHATNAGAR, Indian J. Ent. *24*, 19 (1962).
[5] G. SUBBA RAO and S. G. RASTOGI, Proc. zool. Soc., Calcutta *22*, 61 (1967).
[6] O. SHINODA, J. Biochem. *11*, 345 (1930).
[7] M. SINHA, Indian J. exp. Biol. *13*, 88 (1975).
[8] M. F. DAY and R. F. POWNING, Australian J. Sci. Res. B, *2*, 175 (1949).
[9] I. ISHAAYA and E. SWIRSKI, J. Insect Physiol. *16*, 1599 (1970).
[10] K. HORI, Res. Bull. Obihiro Zootech. Univ. Ser. *1*, 666 (1971).
[11] W. A. L. EVANS and D. W. PAYNE, J. Insect Physiol. *10*, 657 (1964).

Optimum pH for invertase activity and the pH of the midgut

Name and stage	Optimum pH	pH in the midgut regions[b]			
		Anterior	Middle	Third	Last
S. ruficornis					
Larva	6.0	7.2–7.6[a]	2.8–3.6	–	8.4–8.8
Adult	5.5	6.2–6.8[a]	3.6–4.2	–	8.0–8.4
M. domestica					
Larva	6.5	7.0–7.2[a]	3.6–4.2	8.0–8.4	7.6–8.0
Adult	6.0	7.0–7.2[a]	3.6–4.2	7.2–7.6	6.8–7.0[a]

[a] The region where maximum invertase activity would be anticipated.
[b] These regions are not morphologically distinct.

The ammount of reducing sugars produced due to invertase activity increased linearly with the incubation period and enzyme concentration up to a certain limit; thereafter the activity of the enzyme was retarded. HORIE[12], KHAN and FORD[13] and SRIVASTAVA and AUCLAIR[14] also noted similar decrease in the rate of reaction of the gut invertase of *Bombyx mori*, *Dysdercus fasciatus* and *Acyrthosiphon pisum* respectively. This drop was due to the inhibiting effect of the end products of sucrose hydrolysis. Usually this does not happen in vivo, since the monosaccharides produced in the system are simultaneously absorbed. But under abnormal conditions, when the flies are forced to feed on sucrose alone, the phenomenon may be an important measure to check hyperglycaemia.

Invertase activity was maximum in the femal flies and minimum in the larvae (Figures 1 and 2). The ability to digest sucrose is very low in the larvae, since they do not need carbohydrates for growth and development[15,16].

[12] Y. HORIE, Bull. seric. Exp. Stn. Japan *15*, 365 (1959).
[13] M. A. KHAN and J. B. FORD, J. Insect Physiol. *8*, 597 (1962).
[14] P. N. SRIVASTAVA and J. L. AUCLAIR, J. Insect Physiol. *8*, 527 (1962).
[15] V. J. BROOKES and G. FRAENKEL, Physiol. Zool. *31*, 208 (1958).
[16] R. H. DADD, *Chemical Zoology* (Eds. M. FLORKIN and B. T. SHEER; Academic Press Inc., London 1970), vol. 5.

Inhibition and Enhancement of Photically Evoked Responses by Different Doses of L-DOPA

I. KADOBAYASHI, M. MIKAMI[1] and N. KATO[2]

Department of Psychiatry, Kyoto Prefectural University of Medicine, Kawaramachi-hirokoji, Kamigyo-ku, Kyoto (Japan), 22 September 1975.

Summary. Administration of small doses of L-DOPA (10 and 20 mg/kg) resulted in reduction in amplitude of photically evoked responses in the primary visual, association, and cerebellar vermal cortices, while large doses (40 and 80 mg/kg) produced enhancement.

In our previous study, modification of visual, auditory, and somatosensory evoked responses by electrical stimulation of the substantia nigra was demonstrated[3]. Since recent histochemical experiments[4] revealed dopaminergic pathways from the substantia nigra to the neostriatum (caudate nucleus and putamen), in this study we investigated effects of several doses of L-DOPA (L-3,4-dihydroxyphenylalanine), precurser of dopamine, on photically evoked responses in the primary visual, association, and cerebellar vermal cortices.

Methods. The experiments were carried out on 20 adult cats. A tracheotomy was performed on each cat under ether anesthesia. The animal was then placed in a stereotaxic frame under artificial respiration and immobilized with gallamine triethiodide. All pressure points and wound edges were infiltrated with 2% procaine. The left pupil was dilated with atropine, and the right eye was shaded with a thick black vinyl wrapper. The cat remained in a semi-dark room, and recording was begun several hours later. Silver ball electrodes were placed on the right primary visual area (lateral gyrus), right association area (middle suprasylvian gyrus) and midline vermis. The indifferent electrode was placed on the frontal sinus. 60 flashes at 0.5 Hz were presented by a xenon flash lamp facing the eye at a distance of 80 cm. Responses of the brain were amplified by an EEG-machine or an oscilloscope, and recorded onto FM tape. For each cat, 5 selected good responses and either 30 or 50 of 60 – excluding those in which basic waves were extremely variable or artifacts appeared – were averaged with a computer. These were virtually the same in waveform. Averaged responses were photographed and read out on an X-Y plotter.

At first 3 control records were obtained before administration of L-DOPA. Injection of the drug was followed by recordings every 5 min for the first 40m in, and then every 10 min for the following 80 min. The 20 cats were divided into 4 groups. To each group of 5 cats

[1] Present address: Department of Clinical Laboratory, Kyoto 2nd Red Cross Hospital, Kamanza-dori, Marutamachi-agaru, Kamigyo-ku, Kyoto, Japan.
[2] Acknowledgment. We thank Prof. K. KURIYAMA and Sr. M. MCCORMICK for their help.
[3] I. KADOBAYASHI and M. NAKAMURA, Experientia *30*, 260 (1974).
[4] N.-E. ANDÉN, A. DAHLSTRÖM, K. FUXE, K. LARSSON, L. OLSON and U. UNGERSTEDT, Acta physiol. scand. *67*, 313 (1966).

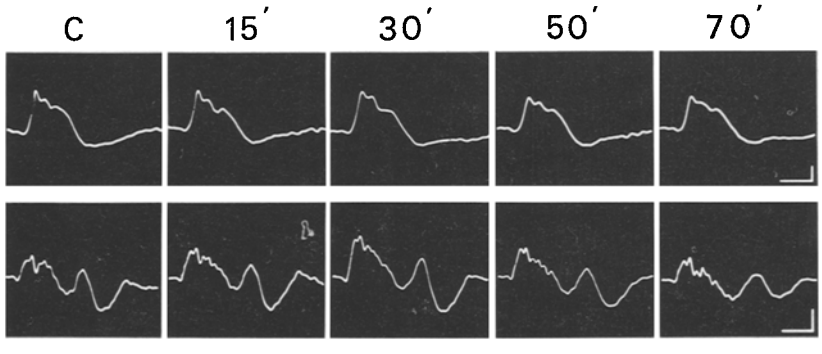

Fig. 1. A) Photically evoked responses in the primary visual cortex. C presents the control record prior to L-DOPA injection. The numbers indicate time in min after i.p. administration of the drug. The upper row shows effects of 20 mg/kg L-DOPA on the average response to 5 flashes, and the lower row effects of 40 mg/kg L-DOPA on the average response to 30 flashes. Flashes were given at the beginning of the sweep. Negativity recorded upwards. Calibrations: 100 μV, 100 msec.