Statistical analysis of the results was performed for each cat separately with the use of Wilcoxon rank sum test. Changes in the acid output from periods before to during, and from during to after stimulation in the stimulation sessions were compared to changes from the corresponding periods in the control sessions. No significant differences ($p > 0.05$) were found between the control and the stimulation sessions in 6 cats. In only one cat (GH11) a significant decrease ($p < 0.016$) in acid output was observed during stimulation sessions while an increase in acid output occurred during control sessions.

Discussion. This study showed that electrical stimulation within the lateral hypothalamus which had been effective in evoking feeding in satiated cats did not produce any significant increase in gastric acid secretion in the same cats in the hungry state.

These results are not consistent with the observations of the other authors[5–8] mentioned earlier in this paper. This difference in the results might be attributable to the difference in methods and in animal species used in their studies[5–7]. In addition, we cannot exclude a possibility that the sites of stimulation used by these authors were somewhat different than those used in our experiments. The electrolytic lesions within the lateral hypothalamus in studies of KADEKARO et al.[8] produced experimental conditions quite different from those of the present study. Therefore, the data obtained by those authors cannot be directly compared with our results.

On the other hand, the results of our experiments seem to be consistent with the observations of SEN and ANAND[12]. These authors found that electrical stimulation within the mid-lateral hypothalamus did not produce any significant change in gastric acid output in conscious cats. They also found that the sites effective in increasing acid secretion were located in the anterio-medial hypothalamus and preoptic area. In fact, in another study which is now in progress we did find sites within the antero-medial hypothalamus where electrical stimulation produced an increase in acid secretion in cats tested in the same experimental conditions as those of the present study; these effective sites corresponded to the loci indicated by SEN and ANAND[12]. Electrical stimulation of these sites did not, however, produce stimulus-bound feeding in these cats[13].

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[13] This study was supported by USPHS Grants Nos. AM 17328 and MH 13958.

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

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Summary. The pH and temperature for the optimum activity of trypsin from the midgut of *Sarcophaga ruficornis* and *Musca domestica* was 7.5 and 8.0 respectively and 50 °C. The enzymic activity increased with the increase in incubation period and enzyme concentration.

Proteins or their degradation products are essential dietary constituents for most insects[2]. The primary enzymes facilitating breakdown of complex proteins are proteinases or endopeptidases. Insect proteinases are generally active in neutral or alkaline medium, and thus resemble vertebrate trypsin. The present study deals with the nature of the midgut trypsin from the larvae and adults of *S. ruficornis* and *M. domestica*.

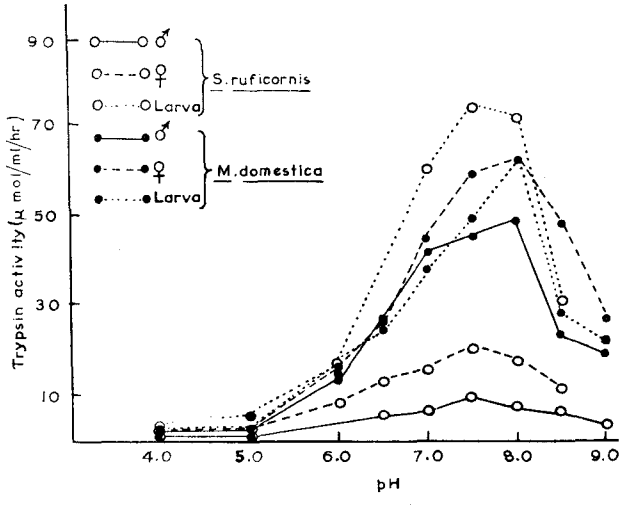

Fig. 1. Effect of pH on the activity of the midgut trypsin.

Materials and method. S. ruficornis was reared on cane-sugar and meat *M. domestica* on cane-sugar and milk[3]. The midgut homogenate was prepared as described earlier[4]. Enzyme homogenate (0.1 ml) was incubated with 0.2 ml of the 0.25 *M* substrate (*p*-tosyl-L-arginine methyl ester HCl) and 0.3 ml of appropriate buffer (Sörensen's phosphate buffer, 0.1 *M*, from pH 5.5–8.0; glycine-NaOH buffer, 0.1 *M*, from pH 8.5–10.5) at 37 °C. The enzyme concentration and incubation period was 1 gut/0.1 ml and 30 min in case of the larvae and 2 guts/0.1 ml and 2 h in case of the adults of *S. ruficornis*; 2 guts/0.1 ml and 1 h in *M. domestica*. After incubation, the enzyme was inactivated by adding 0.5 ml of 10% trichloroacetic acid (TCA), and the mixture was centrifuged at 2500 rpm for 10 min. Trypsin activity was measured by the colorimetric method of YANG and DAVIES[5].

Optimum pH for trypsin activity was determined first and then the effect of temperature, substrate concentration, enzyme concentration and incubation period on enzymic activity was studied at optimum pH.

Results and discussion. Effect of pH. The pH optima for midgut proteinase activity ranged from 7.5[6] to 8.5[7] in

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Optimum pH for trypsin activity and the pH of the midgut

Insect	Optimum pH	pH in the midgut			
		Anterior	Middle	Third	Last
S. ruficornis					
Larva	7.5	7.2–7.6[a]	2.8–3.6	8.4–8.8	–
Adult	7.5	6.2–6.8	3.6–4.2	8.0–8.4[a]	–
M. domestica					
Larva	8.0	7.0–7.2	3.6–4.2	8.0–8.4[a]	7.6–8.0
Adult	8.0	7.0–7.2	3.6–4.2	7.2–7.6[a]	6.8–7.0

[a] The region where maximum activity of trypsin would be anticipated.

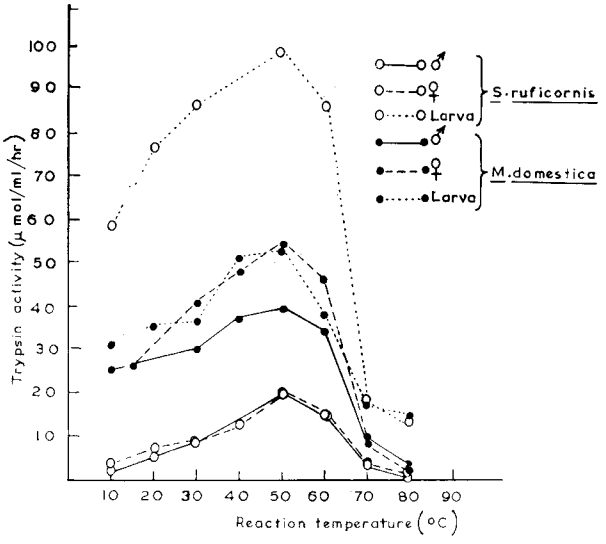

Fig. 2. Effect of temperature on the activity of the midgut trypsin.

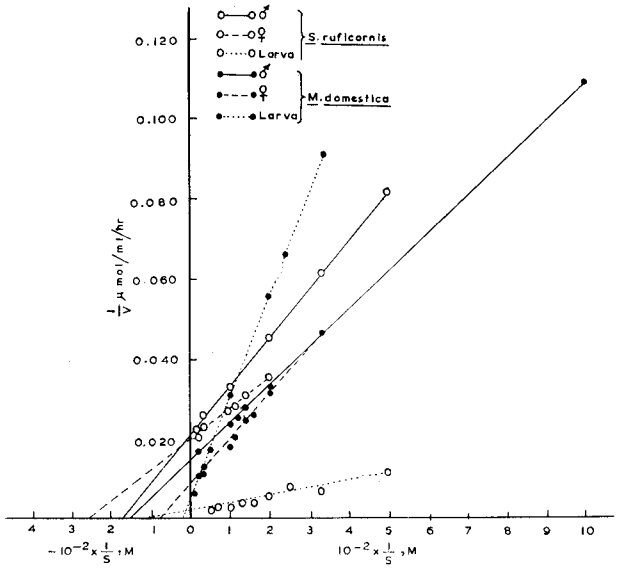

Fig. 3. LINEWEAVER-BURK plot for Michaelis-Menten constant of midgut trypsin.

pipterans. The midgut trypsin from the larvae and adults of *S. ruficornis* and *M. domestica* showed maximum activity at pH 7.5 and 8.0 respectively (Figure 1). The optimum pH value of the enzyme from *S. ruficornis* is similar to that of the enzyme from the larvae of *Lucilia sericata*[8] and *Gastrophilus intestinalis*[6]. POWNING et al.[7] reported the optimum pH of the trypsin-like enzyme from the larvae of *M. domestica* to be 8.5 using gelatin as substrate, while GREENBERG and PARETSKY[9] reported it to be 8.0 using casein as substrate. The difference between the two observations may be due to the difference in the substrate used[6,8,10]. However, the present observations on the enzyme from *M. domestica* are in agreement with those of GREENBERG and PARETSKY[9], inspite of the difference in the substrate used, and the optimum pH value also agrees with that of *Stomoxys calcitrans*[10].

Midgut is the chief site of the digestion of food[11]. Morphologically this region is not subdivided in these flies. However, on the basis of pH values, the midgut of *S. ruficornis* and *M. domestica* is divisible into 3 and 4 zones respectively (Table)[12]. These values indicate that, although trypsin is active throughout the midgut except the midmidgut, the pH of the first zone of the midgut in the larvae of *S. ruficornis*, the last zone in its adults and the third zone in *M. domestica* is most suitable for optimum tryptic activity.

Effect of temperature. The optimum temperature for trypsin activity from the midgut of the 2 flies is 50 °C (Figure 2) and it is similar to that of *Stomoxys calcitrans*[13], *Aedes aegypti* and *Culex fatigans*[14]. LIN and RICHARDS[15] reported that the optimum temperature for the gut proteinase from the larvae of *M. domestica* loses half of its activity when exposed to 70 °C even for 1 min, but the present observations indicate that the enzyme was active even after 1 h incubation at 80 °C. Trypsin from the larvae of both the species of flies is active from 10 °C to 80 °C, but the activity of the enzyme is almost negligible at 80 °C in the case of the adult flies (Figure 2). Hence it may be concluded that the enzyme from the larvae is more heat-stable as compared to that from the adults.

Effect of substrate concentration. Data on the effect of substrate concentration on the catalytic activity of the enzyme, when plotted according to LINEWEAVER and BURK[16], gave a straight line (Figure 3). Michaelis-Menten constant of the enzyme was found to be 7.6×10^{-3} *M* for the larvae, 5.5×10^{-3} *M* for the males and 3.8×10^{-3} *M* for the females of *S. ruficornis* and 3.3×10^{-2} *M*, 6.6×10^{-3} *M* and 1.2×10^{-2} *M* for the larvae, males and females of *M. domestica* respectively.

Effect of incubation period and enzyme concentration. The hydrolysis of the substrate increased linearly with the increase in incubation period and enzyme concentration indicating that the enzymic activity was not affected by the concentration of hydrolytic products of the substrate[17].

Trypsin activity was highest in the larvae and lowest in the male flies (Figures 1 and 2). However, enzyme activity was the same in the two sexes of *Stomoxys calcitrans*[13] and adult simulids[5].

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