be correlated with the level of AChE inhibition. Protein determinations were performed with the Bradford assay procedure[13].

Results. A single dose of soman produced marked effects on brain CAT activity. As shown in the table, CAT activity was decreased in almost all the brain regions examined. These decreases were well below the control levels. The only areas which showed no reduction in CAT were frontal cortex and mesencephalon. In all the other areas, CAT enzyme activity was inhibited by 20–50%. An examination of AChE activity revealed that this soman dosage produced greater than 85% inhibition of the enzyme in all ten brain regions (data not shown). No real differential sensitivity of AChE to soman was detected in any of the brain areas.

Alterations in CAT activity as a consequence of AChE inhibition by organophosphates has not been previously reported. Therefore, at this point the mechanism leading to changes in CAT enzyme activity is unknown. In an effort to elucidate a possible interaction between CAT activity and AChE inhibition, CAT activity was monitored at different time intervals after soman injection. As shown in the figure, CAT activity exhibited an overall pattern of decrease with time in basal forebrain and cerebellum. By 3 h after soman administration, there was little or no CAT activity remaining in these two regions. While AChE activity was almost totally inhibited in basal forebrain and cerebellum at 30 min postinjection, an overall pattern of slow recovery of AChE activity was observed over the ensuing 2–3 h. By 3 h after injection, AChE activity in the basal forebrain had achieved a value of 6.4% of the previously determined control level in this region.

Discussion. The results of this study indicate an effect of soman on ACh synthesis via reduction in the level of CAT enzyme activity. While the exact mechanism underlying the effect on CAT activity is uncertain, it seems quite likely that this may represent a secondary effect of AChE inhibition. The initial alteration in CAT activity occurs almost simultaneously with AChE inhibition. One plausible explanation for the present findings is that soman causes a severe depletion of intraterminal ACh. This occurs as ACh is not hydrolyzed in the synaptic cleft due to AChE inhibition. Thus, no choline is provided for further ACh synthesis in the terminal and since ACh itself cannot be reclaimed, there is a depletion of ACh within the terminal. The relatively complete loss of ACh causes a loss of functionality and/or death of the terminal. The death of the terminal produces the decrease or loss of CAT activity. This mechanism would be consistent with the loss of cholinergic terminals suggested in the delayed neuropathy described by other investigators[6], although the timeframe is certainly different. While this hypothetical mechanism provides a reasonable explanation for the results obtained in this study, at present it certainly represents speculation.

In conclusion, an inhibition of CAT activity by the organophosphate soman has been determined. This effect is considered to be a presynaptic secondary effect of AChE inhibition; however, the mechanism is unknown.

Soman-mediated inhibition of CAT in different brain regions[a]

Brain region	Control[b]	Soman-treated[c]	% inhibition
Frontal cortex	23.3 ± 2.2	35.3 ± 4.2	0.0
Occipital cortex	37.6 ± 7.2	19.9 ± 3.6	47.1
Parietal cortex	28.0 ± 6.4	12.9 ± 2.3	53.9
Temporal cortex	31.9 ± 2.3	24.0 ± 1.9	24.8
Hippocampus	50.2 ± 7.2	37.2 ± 3.2	25.9
Basal forebrain	100	48.3 ± 8.1	51.7
Cerebellum	5.3 ± 2.4	4.1 ± 0.6	22.6
Mesencephalon	44.5 ± 8.1	45.2 ± 6.3	0.0
Thalamus/hypothalamus	31.7 ± 0.7	22.0 ± 2.1	30.6
Corpus striatum	95.3 ± 4.7	58.8 ± 8.3	38.3

[a] All values for CAT activity are expressed as a percentage of the level in the basal forebrain control, the level of activity in the basal forebrain control equals 100% by definition. [b] All values are the mean ± SD of triplicate determinations. [c] The soman dose ranged from 94 to 120 µg/kg. All animals were sacrificed 30 min after injection.

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Distribution of (Na^++K^+)-ATPase in the hindgut of *Glossina morsitans morsitans* Westwood

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Summary. (Na^++K^+)-ATPase activity was higher in preparations from the ileum of *Glossina mortisans* than in those from the rectum. This result suggests that the ileum, as well as the rectum, may play a role in osmoregulation in the tsetse fly.

Key words. Glossina morsitans; hindgut; (Na^++K^+)-ATPase.

The distribution of (Na^++K^+)-ATPase activity in the alimentary canal of *Glossina morsitans morsitans* Westwood has been described previously[1] and it was shown that (Na^++K^+)-ATPase activity was highest in the anterior midgut and lowest in the posterior region of this organ. The (Na^++K^+)-ATPase activity of the hindgut (ileum and rectum combined) was intermediate. The present paper describes the distribution of (Na^++K^+)-ATPase activity in the hindgut of *G.m.morsitans* and shows

ATPase activity of preparations from the ileum and rectum of fed and unfed adult *G. morsitans*

Condition	Tissue	n	ATPase activity nmoles Pi liberated/mg protein/min		
			4 mM Mg^{2+} 20 mM K^+ 100 mM Na^+	4 mM Mg^{2+} 20 mM K^+ 100 mM Na^+ plus 1 mM ouabain	Activity in the presence of Mg^{2+} K^+, Na^+ minus activity in the presence of Mg^{2+}, K^+, Na^+ and ouabain
Unfed	Ileum	8	288.0 ± 7.8	48.8 ± 3.8	239.2 ± 6.7
	Rectum	8	162.3 ± 20.2	38.3 ± 3.0	124.0 ± 17.9
Fed saline meal	Ileum	5	277.0 ± 18.7	63.5 ± 7.4	213.5 ± 12.0
	Rectum	5	199.1 ± 12.3	24.6 ± 4.8	174.3 ± 8.6

Saline meal contained 75 mM NaCl and 0.5 mM ATP. ATPase activity is expressed as the mean of either five or eight determinations ± one SE. All salts as chlorides.

that, in contrast to other insects which have so far been studied[1-3], this enzyme's activity was highest in ileal rather than rectal preparations.

Materials and methods. 17-day-old male *G.m.morsitans* were used throughout the study. Flies were obtained from Langford, Bristol, U.K. They were maintained as described previously[4]. Prior to experiments, 10-day-old flies were starved for three days before being offered a blood meal on a guinea pig; any flies that refused to feed were discarded. The fed flies were then starved for a further three days. This procedure had the combined effect of bringing all flies to the same stage of the hunger cycle and removing remnants of the blood meal from the gut.

Two types of experiment were carried out. In the first, the flies underwent the feeding regime outlined above and were then sacrificed. In the second, the flies underwent the feeding procedure outlined above but were then fed a saline meal containing 75 mM NaCl and 0.5 mM ATP. The flies were sacrificed immediately the residue from the previous meal had been discharged from the rectum (discharge of the residue usually occurred within two min of feeding commencing). This procedure was adopted for the following reason. Although every effort was made to remove the contents of the rectum during dissection, the residue that remains from the previous meal has a high nitrogen content[5]. Since $(Na^+ + K^+)$-ATPase activity was expressed per mg protein, the incomplete removal of this residue could result in depressed $(Na^+ + K^+)$-ATPase activity values for the rectum when compared with the ileum. At the time of sacrifice, in both types of experiment, the head, wings and legs were removed from the flies. The ileum and rectum were then rapidly dissected out under ice cold (0–4 °C) deionized water and the contents of the organs were, as far as possible, removed. The tissues were then transferred to a cold (0–4 °C) tube and rapidly frozen in liquid air and vacuum dried overnight in a Virtis freeze drier. The lyophilate was reconstituted in an appropriate volume of cold (0–4 °C) deionized water and sonicated for 5 s using a Dawe soniprobe (type 1130 A) at an intensity setting of 6. The sonicated lyophilate had a final concentration of between 50 and 100 µg protein ml^{-1}. It was stored on ice until required.

Assays of ATPase activity were carried out under conditions which numerous studies (see review by Anstee and Bowler[6] for references) of insect tissues have shown to be optimal for the demonstration of a classical $(Na^+ + K^+)$-ATPase as described by Skou[7]. The reaction media used were as follows:

1) 4 mM Mg^{2+}, 100 mM Na^+, plus 20 mM K^+;

2) 4 mM Mg^{2+}, 100 mM Na^+, 20 mM K^+, plus 1 mM ouabain.

All media were buffered in 50 mM imidazole, pH 7.3 (HCl) and contained 10 mM sodium azide in addition to 0.1 mM EDTA. ATP (Tris salt) was added to a final concentration of 3 mM and the final volume was 0.8 ml. Reaction media were thermoequilibrated for 10 min at 30 °C and the reaction started by the addition of 0.2 ml of enzyme homogenate. Incubations were carried out for 30 min at 30 °C and the reaction terminated by the addition of 1.6 ml of a 1:1 (v/v) mixture of 1% ammonium molybdate in 0.9 M H_2SO_4 and 1% lubrol. The yellow color produced was allowed to develop at room temperature for 10 min prior to reading at 390 nm in a spectrophotometer. The intensity of the yellow color is proportional to the amount of inorganic phosphate present[8]. $(Na^+ + K^+ +)$-ATPase activity was calculated as the difference in activity between media containing Mg^{2+}, Na^+ and K^+ together and media containing all three cations plus 1 mM ouabain.

Protein was determined by the method of Lowry et al.[9] using bovine serum albumin Fraction V as standard. All solutions were made up in glass distilled water. All inorganic salts were present as chlorides and were AnalaR grades. Imidazole, ATP, BSA, and ouabain were obtained from Sigma Chemical Co. Lubrol was a gift from I.C.I. Dyestuffs Division.

Results. From the table, it will be seen that for both unfed and saline fed flies, the ouabain sensitive ATPase activity i.e. $(Na^+ + K^+)$-ATPase activity, was higher in preparations from the ileum than in those from the rectum. The difference between the $(Na^+ + K^+)$-ATPase activity of these preparations was highly significant ($p < 0.001$) for unfed and barely significant ($p < 0.05$) for saline fed flies.

For ileal preparations, the difference between the ouabain sensitive ATPase activity of fed and unfed flies was not significant ($p > 0.05$) whilst for rectal preparations the difference was barely significant ($p < 0.05$).

Discussion. From the results it is evident that in both fed and unfed flies, the ouabain sensitive ATPase activity i.e. $(Na^+ + K^+)$-ATPase, of the ileum was significantly higher than the corresponding value for the rectum. The significance of this observation lies in the fact that in many insect epithelia which possess high levels of $(Na^+ + K^+)$-ATPase activity there also occurs transport of ions and water against considerable ionic and osmotic gradients. For example, the rectum of a variety of terrestrial insects including *Sarcophaga nodosa*[1], *Locusta migratoria*[2] and *Periplaneta americana*[3] as well as the Malpighian tubules of *L. migratoria*[10] have all been shown to possess high $(Na^+ + K^+)$-ATPase activity when compared with other regions of the alimentary canal. Similarly, in *G.m.morsitans* high levels of $(Na^+ + K^+)$-ATPase activity have been demonstrated in the anterior midgut which itself has been identified as the site of ion and water absorption from the blood meal[1,11]. Consequently, the studies above have led to the suggestion that $(Na^+ + K^+)$-ATPase was somehow involved in either the absorptive or secretory processes of these epithelia[2,3]. Indeed there is some evidence to support this suggestion since fluid secretion by the Malpighian tubules of *L. migratoria*[10] and diuresis in *G.m.morsitans*[4] are both inhibited by the cardiac glycoside, ouabain, a specific inhibitor of $(Na^+ + K^+)$-ATPase. Thus it is not unreasonable to suggest that the high $(Na^+ + K^+)$-ATPase activity of the ileum may reflect the role this organ plays in osmoregulation. $(Na^+ + K^+)$-ATPase, however, is not the only type of ATPase involved in ion transport across insect epithelia. In some tissues

e.g. lepidopteran midgut[12], the movement of ions, particularly K^+, is thought to involve a K^+ modulated Mg^{2+} ATPase. Although the existence and nature of the ion transport across the hindgut of *G.m.morsitans* remains to be established, it would nevertheless be of interest to determine whether other types of ATPases are present in these tissues.

Finally, the barely significant difference ($p < 0.05$) in rectal (Na^++K^+)-ATPase activity between fed and unfed flies probably indicates that in unfed flies, the remnants of the previous blood meal may have affected, the determination of enzyme activity adversely by interfering with the protein assay. Thus the (Na^++K^+)-ATPase activity of unfed flies was depressed.

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Studies of total and protein-bound plasma Mg^{++} in wild and hatchery-reared coho salmon smolts in freshwater and in seawater

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Summary. Total plasma Mg^{++} and Ca^{++}, Mg^{++} in erythrocytes as well as protein-bound plasma Mg^{++} were investigated in wild and hatchery-reared smolts. The proportion of plasma Mg^{++} which was bound to plasma protein did not change significantly during entry into seawater, even though the in vitro addition of exogenous Mg^{++} to the plasma showed that additional binding was possible.
Key words. Plasma Mg^{++}; RBC Mg^{++}; coho salmon; ion binding; smolting; seawater entry.

Magnesium ion is a necessary activator of about 300 enzymes which are widely involved in the metabolism of proteins, carbohydrates, lipids, and nucleic acids of higher vertebrates[1,7]. In fish, Mg^{++} is also essential, but is not well understood. In hatchery-reared coho salmon, migrating into seawater has been associated with moderate to heavy mortality rates, sometimes as high as 80%. Problems with Mg^{++} regulation have been suspected because dying fish are often lethargic and die in a state of flaccid paralysis (L. Smith, personal observation). Suggestions have been made that plasma Mg^{++} levels greater than 7–10 meq/l should be lethal to coho salmon (Gary Wedemeyer, USFWS, Seattle, WA 98115, in preparation), but the exact toxicity limits and Mg^{++} toxicity mechanisms are largely unknown. However, the lower vertebrates typically show some temporary increases in plasma Mg^{++} when exposed to a high Mg^{++} environment such as seawater[2].

This project originated from observations of hatchery-reared coho smolts in which fish in apparently good health after 20–30 h in seawater had plasma Mg^{++} levels which we would have expected to be toxic. We wondered whether part of their plasma Mg^{++} had somehow been inactivated. The objective of this project was to test whether the high level of plasma Mg^{++} found in hatchery coho smolts after transfer into seawater was not harmful because of binding the excess amount of Mg^{++} to plasma proteins in a way not found in wild coho smolts.

Materials and methods. Plasma from four stocks of coho salmon were tested for Mg^{++} binding: 1) Wild yearling coho smolts (10–12 g) were captured during their downstream migration in late May and early June 1984 at a weir just above the estuary of Big Beef Creek which empties into Hood Canal about 25 km west of Seattle, WA. The fish were transported in oxygenated fresh water to our University of Washington lab. This is the same stock of fish investigated by Miles and Smith[5] several years earlier.

2) Hatchery-reared yearling coho smolts (11–18 g) were obtained in freshwater in June 1984 from the NMFS facility adjacent to the UW campus and were transported briefly in freshwater to our UW lab. This stock was originally from the Issaquah hatchery about 50 km east of Seattle.

Both groups of fish were adapted to laboratory conditions in freshwater for one week before being put into natural seawater in a recirculating system of about 30,000 liters capacity which was kept at a temperature of about 15 °C. Blood data from these two groups of fish were statistically comparable.

3) The stock of coho salmon in which we observed the high levels of plasma Mg^{++} in 1982 was available to us only in early July 1984 after the yearling smolts had been in seawater for about one month. We therefore took blood samples at their estuarine rearing site near Coos Bay, Oregon, and transported them to Seattle on dry ice.

4) We obtained blood samples from regular production of zero-age smolts in freshwater in early July from Oregon Aqua Foods, Springfield, Oregon.

For taking blood samples, we first anesthetized fish with tricaine methane sulfonate (MS-222) at a concentration of 100 mg/l. Blood was drawn from the caudal vein behind the anal fin using a small syringe which had been rinsed in citrate. Blood was centrifuged for 5 min at 13,000 × g, the plasma decanted immediately and frozen at −20 °C. Erythrocytes which were to be analyzed for total Mg^{++} were washed and centrifuged three times with equal volumes of cold 0.9% NaCl solution, after which they were frozen at −20 °C for assay on the next day. A plasma ultrafiltrate was prepared according to the method described by D'Costa and Cheng[3], using the Amicon microparti-