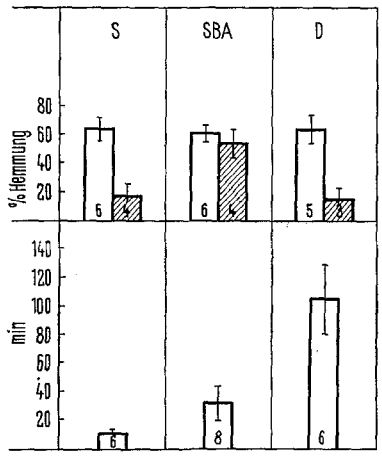
effektiven Konzentrationen der drei untersuchten Substanzen für beide Systeme verschieden. Für die inotrope Wirkung ergibt sich eine Relation für Digitoxin:Strophanthidin:Strophanthidin-3-bromazetat wie 1:10:20, für die Hemmwirkung auf die ATPase dagegen wie 1:2:1. Diese Beobachtungen lassen eine gewisse Vorsicht beim Vergleich kardiotoner Effekte mit der Wirkung auf isolierte Membran-ATPase angebracht erscheinen, entweder weil gewisse Eigenschaften der Membran-ATPase verschiedener Organherkunft verschieden sind (trotz prinzipiell gleichartiger Hemmwirkung der Herzglykoside), oder weil die Wirkung auf isolierte ATPase nicht vergleichbar ist mit der Wirkung auf die Funktion des intakten Organs[14].



Oben. Prozentuale Hemmung der Aktivität des Na^+-K^+-stimulierten Anteils einer mikrosomalen ATPase aus dem Meerschweinchenhirn durch Strophanthidin (S) $2 \times 10^{-4} M$, Strophanthidin-3-bromazetat (SBA) $10^{-4} M$ und Digitoxin (D) $10^{-4} M$ (Dreifachbestimmung). Helle Säulen: Hemmung nach 20 min Einwirkung dieser Substanzen. Dunkle Säulen: Hemmung nach 2stündigem Auswaschen der Substanzen.
Unten. Zeitdauer des Auswaschens von 90% des maximalen positiv inotropen Effektes am Papillarmuskel des Meerschweinchens. Einzelheiten siehe Text.
Dargestellt sind Mittelwerte ± Standardabweichung der Mittelwerte aus der jeweils in den Säulen angeführten Zahl von Versuchen.

Summary. The reversibility of the inhibitory action of strophanthidin, strophanthidin-3-bromoacetate, and digitoxin on the Na^+-K^+-ATPase activity (microsomal fraction, guinea-pig brain) was compared with the rate of decline of their positive inotropic effects (papillary muscle, guinea-pig heart) after exposure to drug-free solution. The actions of strophanthidin and digitoxin were easily reduced on both systems. Strophanthidin-3-bromoacetate, however, was found to have an irreversible blocking effect on the transport ATPase, whereas its inotropic action could be rapidly abolished.

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[14] Mit Unterstützung der Deutschen Forschungsgemeinschaft.
[15] Teilergebnisse der Dissertation von U. Fricke, Mainz.

Proteolytic Enzymes in the Salivary Gland of *Chironomus tentans*

The larval salivary glands of *Chironomus* and other Dipteran insects undergo autolysis early in metamorphosis. This is accompanied by specific alterations of the pattern of chromosomal puff activity, thus providing a unique system for studying nucleocytoplasmic interactions during a characteristic step of insect development[1]. We found that a change in the pattern of proteolytic enzymes, including an increase in the activity of an acid protease, is among the cytoplasmic events preceding glandular regression in *Ch. tentans*. A decline in glandular proteolytic activity[2], or no proteolytic activity at all[3] had been found by previous investigators.

Methods. Ch. tentans larvae were raised in the laboratory and staged as described previously[4]. Glands were collected in $0.1 M$ ice-cold NaCl solution and disrupted by treatment on ice for 1 min with a Branson sonifier (special microtip) at setting 2. Proteolytic enzyme activity was assayed using a modification of Anson's method with urea-denatured hemoglobin as substrate[5,6]. Product released by the enzyme was estimated using the method of Lowry et al.[7]. The assay was performed in a total volume of 1 ml, containing 0.1 ml sonicate, 0.5 ml hemoglobin solution, and 0.4 ml buffer; incubation time was 3 h. Under these conditions measured activities increased linearly with enzyme concentration and duration of incubation for a period of 6 h. In controls either $0.1 M$ NaCl solution was substituted for the sonicate, or the addition of sonicate was immediately followed by the addition of TCA.

Results and discussion. Proteolytic enzyme activity is present in glands of larvae of all developmental stages tested. The pH optimum of the proteolytic activity differs, however, at different developmental stages (Figure 1). In old prepupae protease activity has its maximum at pH 3.5. In glands of intermolt larvae, on the other hand, the optimum is at pH 5.5, with only a slight shoulder at pH 3.5. Mixtures of prepupal and larval sonicates display pH profiles with distinct peaks at pH 3.5 and at pH 5.5.

Since it had been suggested that the salivary gland takes up some of its enzymes from the hemolymph rather than synthetizing them itself (cf. [2]), protease activity was also assayed on hemolymph samples from larvae and prepupae. The assay was performed in a total volume of 0.2 ml, containing 0.005 ml hemolymph, 0.095 ml buffer and 0.1 ml hemoglobin solution. Larval hemolymph shows no indication that the larval glandular protease

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is present in the hemolymph (Figure 1). In prepupal hemolymph, activity is slightly higher in the lower pH range, possibly reflecting the fact that many larval tissues are in a state of regression at this stage.

From day 5 to day 15 of the last instar the average activity of the pH 5.5 protease increases about twofold (Figure 2). This difference is significant in the Student's t-test ($\alpha = 5\%$). During the same period of time the average protein content per gland increases from about 7.5 µg to about 16 µg, indicating that the increase in enzyme activity essentially follows the growth of the gland. The activity of the pH 5.5 protease drops sharply and significantly towards the end of the pupal molt.

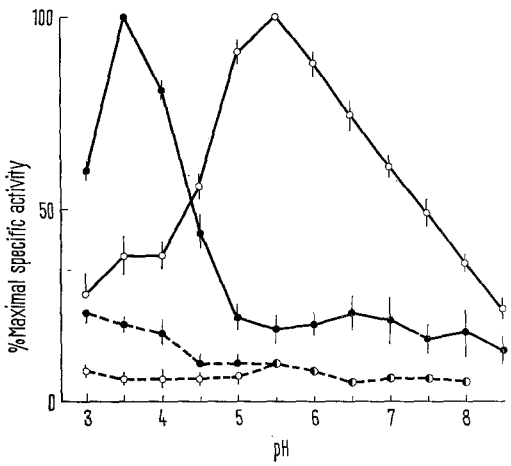

Fig. 1. pH dependence of proteolytic activity (µM tyrosine released in 3 h/µg protein) in 10-day-old last instar larvae (open circles) and in prepupae (filled circles). Solid lines, salivary gland sonicates; broken lines, hemolymph. Specific activities of hemolymph are compared with glandular activity at pH 3.5 (= 100%) for prepupae, and at pH 5.5 (= 100%) for larvae. Points are averages from 4–10 determinations for the glands and from 15–25 determinations for hemolymph. Bars give standard error of mean. 0.1 M phosphate-citrate buffer was used between pH 3.0 and 6.5, and a 0.1 M *tris*-maleate NaOH-buffer for pH 6.0–8.5.

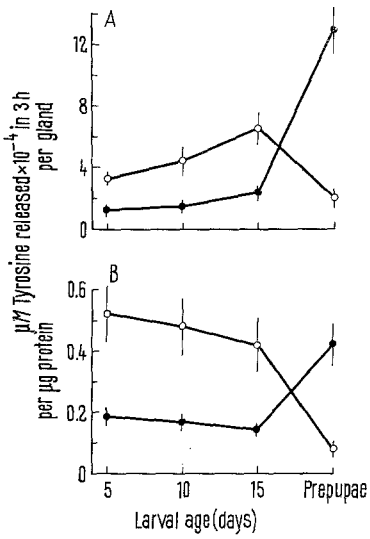

Fig. 2. Total (A) and specific (B) proteolytic activity at pH 3.5 (filled circles) and pH 5.5 (open circles) in salivary glands from larvae of various stages in the last larval instar. Points are averages of 7–9 experiments, bars indicate standard error of mean.

The activity per gland of the pH 3.5 protease (Figure 2a) showed only a slight and statistically not significant increase with larval growth (day 5 to day 15). On the other hand, since the slight decrease in specific activity (Figure 2b) is also statistically not significant, it appears that factors different from glandular growth greatly contribute to the variation of this enzyme in glands of intermolt larvae. The activity of the pH 3.5 protease increases sharply, and significantly during the pupal molt. The increase per gland is more than fivefold, the increase in specific activity about threefold (Figure 2). However, about 70% of total protein in prepupal glands is contributed by secretory proteins contained in the glandular lumen, in contrast to only about 40% of total protein in intermolt larvae. Assuming that all the pH 3.5 protease is intracellular (see below), one may recalculate the specific activities of Figure 2 on the basis of cytoplasmic protein to be 1.26 for prepupae, and 0.23 for the 15 day larvae. The increase in specific activity thus would equal the increase in total glandular activity.

Glandular protein and RNA syntheses are inhibited for 95% or more if cycloheximide (10 µg/ml) or Actinomycin D (2 µg/ml) respectively, are added to larval cultures[8]. In 10-day-old larvae the activity of the pH 5.5 protease declines to about 50% of their controls within 24 h after stopping protein synthesis and within 48 h after stopping RNA synthesis. In contrast, neither of the 2 drugs seems to affect the activity of the pH 3.5 protease at this stage.

The pH optimum of the prepupal protease corresponds to that of cathepsin D, a lysosomal protease characteristic of degrading tissues (cf. [9–13]). Indeed, the preparation for gland regression and changes of the glandular lysosome population coincide with the increase of the pH 3.5 protease activity[14]. The pH 5.5 protease, resembling in this respect the protease found in *Ch. thummi*[2], is maximally active in functioning glands and becomes inactive before glandular regression begins. Conceivably, it is released as part of the secretion[15].

Zusammenfassung. Die in den larvalen Speicheldrüsen von *Chironomus tentans* vorkommende Protease mit einem pH-Optimum bei 5.5 wird vor Beginn des Zellzerfalls in der Puppenhäutung durch eine Protease mit einem pH-Optimum bei 3.5 ersetzt. Diese Enzymveränderungen stehen in zeitlicher Übereinstimmung mit Veränderungen des Puffmusters und der Lysosomenpopulation.

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