Trehalase from the salivary glands and midgut of *S. inferens* showed optimum activity at 50 and 60 °C, respectively (Figure 2) and more than half activity at temperature 35–60 and 45–70 °C, respectively. As the larvae were reared at 32 °C at this temperature, the activity of gut trehalase will be only about 40% of the optimum. Trehalase from *Trinervitermes trinervoides*[18] and *Hodotermes mossambicus*[21] showed optimum activity at 52 °C.

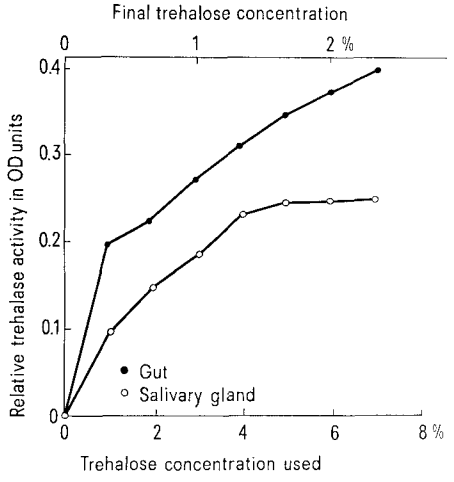

Fig 5. The trehalose concentration-trehalase activity curves of the larvae of *S. inferens*.

With the increase of incubation period, the quantity of end-product continued increasing in case of salivary gland trehalase, but in case of midgut trehalase there was almost no increase after about 100 min (Figure 3). Rate of hydrolysis of trehalose increased with the increase of concentration of trehalase from the gut and the salivary glands of *S. inferens* (Figure 4).

The activity of the salivary glands trehalase increased as the concentration of trehalose increased, but after 5.0% trehalose concentration its activity became almost constant; however, in case of midgut trehalase the activity continued increasing even after 5.0% trehalose concentration (Figure 5).

Dialysis did not affect the activity of the midgut trehalase. Tryptophan accelerated while all other amino acids used inhibited the activity of trehalase (Table II). The activity of trehalase was inhibited even when the concentration of glucose was as low as 0.5%.

It may be concluded that the activity of trehalase was very low at the temperature at which the larvae of *S. inferens* were reared and at the pH of their gut, and its activity was lowered even by as low concentration of glucose as 0.5%. It may be added that presence of an enzyme in abundance does not mean that it functions effectively.

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Changes of the Phosphatidylcholine Content and the Number of Synaptic Vesicles in Relation to the Neurohumoral Transmission in Sympathetic Ganglia

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Summary. In sympathetic ganglia stimulated in the presence of HC-3, the reduction in number of synaptic vesicles was observed to be accompanied by a significant decrease of the ganglionic phosphatidylcholine content.

The quantal release of transmitter at nerve endings is well established physiologically[2], and there is a great deal of suggestive evidence that its morphological basis involves the packaging of the neurotransmitter in the vesicles seen in the electron micrographs of nerve terminals[3,4]. Concerning the mechanism of the actual release, current hypotheses favour a process of exocytosis and presume some sort of recycling process, whereby the vesicle membrane is transiently incorporated into the plasma membrane and is subsequently made available again for the manufacture of more intracellular vesicles[5,6]. In such a system, the materials composing the vesicle membrane would not be lost from the nerve ending. A useful approach for proving this would therefore be to measure some constituent substance of the vesicle membrane in the nerve endings at time when their number was diminished. Although there is no easily measured membrane component which is specific for vesicles, it is known that the synaptic vesicle wall is especially rich in phosphatidylcholine (PC)[7]. We have therefore designed experiments to measure the PC in sympathetic ganglia, during conditions when there is a dramatic fall in the vesicle content. Our experiments involve the use of hemicholinium (HC-3) to interfere with the choline uptake[8], coupled with repeated stimulation of the nerve; this readily produces a depletion of vesicle number[9] in the superior cervical ganglion (SCG) of the cat.

Cats of both sexes (weighing 1.5–3 kg) were anaesthetized with 40 mg/kg pentobarbital, and ganglia of either side were experimented on in a variety of ways. In the 1st group of animals, the ganglion on one side was simply stimulated through its preganglionic nerve trunk for 5 min; in a 2nd group the stimulation was identical, but the animals had been previously given 10 mg/kg

[1] Acknowledgment: We thank Prof. J. DIAMOND for the helpful suggestions and the critical reading of the manuscript.

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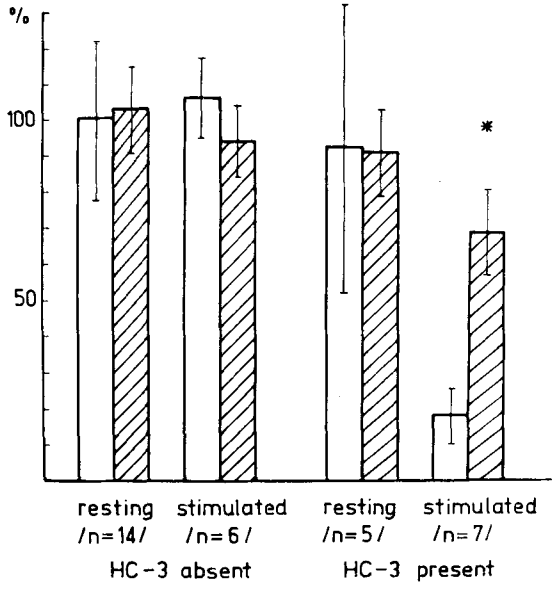
HC-3 i.v. In a 3rd group, HC-3 was administered, but there was no stimulation of the preganglionic trunk. Parameters of stimulation were: frequency = 20/sec, duration = 1 msec, amplitude = 6–8 V. Contractions of the nictitating membrane were recorded on a kymograph. Details of the electron microscopic processing have been described elsewhere[9]. The lipids of the ganglia were extracted according to the method of FOLCH et al.[10], and separated by two-dimensional thin-layer chromatography on silica gel[11]. The spots were charred with 50% sulfuric acid and inorganic phosphorus was determined by the method of KAHOVCOVA and ODAVIC[12]. Results were expressed as μg P/10 mg wet weight. The PC content and the number of synaptic vesicles agree to within a few percent in the left and right ganglia of the same cat; therefore the changes in the experimental ganglia (left side) are expressed as the percentage of the untreated (right side) control. Statistical analysis was performed by using the double *t*-test. Results of phosphatidylcholine determinations in relation to the changes of synaptic vesicles are summarized in the Figure. Electric stimulation alone did not cause significant changes either in the transmission capacity of intraganglionic synapses or in the structure of mitochondria and the number of synaptic vesicles (106 ± 11% of control value). No significant changes were found in the PC content of stimulated ganglia (94 ± 10%). Heterogenity in size and shape of synaptic vesicles however was readily established. Following HC-3 treatment the structure of mitochondria remained normal and the number of synaptic vesicles vary close to those of control side (92 ± 40%) while PC content decreased slightly (91 ± 12%). When the preganglionic trunk was stimulated in the presence of HC-3 the transmission deteriorated rapidly and ceased completely within 1–2 min. Mitochondria became to a large extent swollen and considerable decrease similar to those found earlier[9] was observed in the number of synaptic vesicles (18 ± 8%). The analysis of the ganglionic phospholipids showed a significant decrease in phosphatidyl choline content (69 ± 12%). No significant changes were found in the content of other phospholipids including lysophosphatidylcholine. It should be noted that membranes other than that of synaptic vesicles contain PC and therefore its decrease although slighter proportionately than that of the synaptic vesicles number, would seem to correlate with that change. The fact that apart from the above-mentioned changes in the presynaptic terminals, there was no other membrane alteration observed in stimulated and HC-3 treated ganglia, further supports this correlation.



Correlation between the number of synaptic vesicles and the phosphatidylcholine content of superior cervical ganglia. Each column represents an average value ± SE of the content of synaptic vesicles and phosphatidylcholine in the percentage of control. The number of independent experiments with different animals is given in parentheses. Open columns: synaptic vesicles; hatched columns: phosphatidylcholine. *The number of synaptic vesicles and the PC content in ganglia stimulated in the presence of HC-3 differs significantly from any of the other groups ($p<0{,}02$).

In this experiment, the decrease in number of synaptic vesicles and in the ganglionic PC content was observed in a physiological state, when the uptake of the precursor for the increased acetylcholine (ACh) synthesis was blocked by HC-3. As the degradation of PC is shown[13] to lead, among other products, to the formation of choline, it is conceivable that in such circumstances the choline derived from the intracellularly available PC may be used for supporting the ACh synthesis. The recent observations of MANN[14] have provided evidence in favour of our assumption as to the existence of a PC-choline-ACh pathway. It was shown that the enzyme glycerophosphorylcholine diesterase, which releases choline from glycerophosphorylcholine, can make a substantial contribution to the pool of free choline in rat brain. As its subcellular distribution was associated with the nerve endings, similarly to that of choline acetyltransferase, the choline released was suggested to be utilized for the ACh synthesis in cholinergic neurons. As the synaptic vesicle wall is known to be rich in PC, the decrease in number and structural alteration of synaptic vesicles in active synapses may be the result of the structural changes which take place in the vesicles following the fall of its important membrane constituent. The exact mechanism whereby choline deficiency triggers the decomposition of PC from structures in presynaptic nerve terminals is unknown at present, but the effect of phospholipase A on the structure of synaptic vesicle wall and the ACh release has been already evidenced[15]. It is possible that by mobilizing choline from its esterified form, ACh is produced and stored, as suggested earlier[16] on the surface of synaptic vesicles.

On the other hand, the importance in normal transmission of the vesicular 'bound' ACh pool has just been published[17]. Decomposition of the wall of synaptic vesicles, observed in our in vivo experiments, may provide higher permeability for the acetylcholine to be exchanged from 'bound' to 'free' pool[18] upon depolarization. It is temping to assume that during neurohumoral transmission the vesicular ACh may be released as a first step into the cytoplasm by a similar mechanism involving the above-mentioned permeability change.

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