tance by hindering ion diffusion from the external environment to the chemosensory dendrites. As regards *Phormia*, opening or closing of the tips of the chemosensory hairs has been described[11], but this mechanism seems to be rather improbable under physiological conditions[12]. Graded changes in the fluid bathing the tips of the dendrites seems more likely in *Phormia* hairs[11] and this fits better with our results for the chemosensory hairs of *Phormia*. Bernays et al.[10] have demonstrated that the mechanism involved in resistance variations is based on hormonal changes in *Locusta*, whereas in *Phormia* humoral intervention on chemoreceptor function has been ruled out by means of parabiosis experiments[13]. Although our data do not provide any information in this respect, it is possible that an increased volume of the alimentary canal may affect the secretory functions of the accessory cells at the hair sockets aspecifically by simply increasing internal body fluid pressures. Because of their anatomical location, it seems likely that tarsal and wing hairs may be influenced more than labellar hairs by a pressure/volume variation mainly involving the thoracic and abdominal regions (where the crop is located). The crop is in fact the portion of the alimentary canal that increases most in volume after feeding. This latter hypothesis may explain the lower resistance increase that we observed in the labellar hairs as compared with tarsal and wing hairs. In conclusion, our data show that an increase in hair resistance, and hence a reduction in ionic fluxes between the external environment and the chemosensory dendrites, follows food ingestion. This may represent a possible further mechanism in feeding control.

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Electrophysiological characteristics of *Bombyx mori* L. ventral nerve cord (effect of sodium and potassium on the membrane potential)

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Summary. Na^+ and K^+ effects on the resting cellular membrane potential of desheathed ganglia of the *Bombyx mori* L. ventral nerve cord have been studied. The cells are depolarized by high concentrations of external potassium ions in the same way as in vertebrates, mollusca and crustacean cells. The possibility that the behaviour of the resting potential is not only influenced by the potassium equilibrium potential, but also by the conductances to other ions, is discussed.

A great number of experimental data has shown that in many animals potassium concentration is higher in the axoplasm than in the serum, while sodium concentration is higher in the serum than in the axoplasm and that the more important ions for the equilibrium across the cellular membrane are sodium, potassium and chloride. Preparations of excised nerve or isolated cells of these animals can maintain their excitability if perfused with Ringer solutions containing a large amount of sodium and a small amount of potassium and minor quantities of calcium and other ions together with the proper anions.

A very different situation has been found in some phytophagous insects whose hemolymph contains a large amount of potassium and a very small amount of sodium.

In these conditions, it seems difficult to reconcile this situation with the conventional membrane theory for the propagation of the action potential, which depends on the presence of a high Na^+ concentration in the fluid bathing the axon surface.

Aim of the present work is to characterize, from an electrophysiological point of view, the nervous system of the phytophagous larva of *Bombyx mori* L. in the last instar. These insects have an hemolymph richer in bivalent (Mg^{++}, Ca^{++}) than in monovalent (Na^+, K^+) cations, where the K/Na ratio is about 27[2,3].

The sodium concentration is very low, only 1.7 mM/l. The present paper reports experiments performed in order to assess the role of potassium and sodium ions in the maintenance of resting potential.

Materials and methods. Experiments were carried out on *Bombyx mori* larvae in their last instar. The animals were pinned ventral surface to a Sylgard 184 layer in a glass chamber. The dorsal integument was opened by a median longitudinal incision, the midgut removed and the exposed

Membrane potentials ± SE (mV)			ΔMP%	P
0 mEq (Na)	1.7 mEq (Na)	17 mEq (Na)		
48.7 ± 0.4 (222)	48.6 ± 0.5 (147)	-	+ 0.1	0.95*
-	40.4 ± 0.4 (279)	28.8 ± 0.4 (289)	−28.7	< 0.01

Mean values of the membrane potential ± SE for different sodium concentrations in the perfusing solution. * No significant difference between the results obtained in Ringer and sodium-free Ringer.

ventral nerve cord washed with normal Ringer solution (see below).

In order to aid the microelectrode penetration, the tissue covering the ventral nerve cord was enzymatically softened by exposure to Pronase, a proteolytic enzyme, in normal Ringer (0.35 mg/ml) for 35–50 min.

The Ringer solution had the following composition (mM): NaCl 1.7, $MgSO_4$ 44.0, $CaCl_2$ 9.0, K_2SO_4 18.0, sucrose 157.3, KH_2PO_4 1.1, K_2HPO_4 4.3, pH 6.9±0.1 (20 °C); osmolarity 290±2.9 mOsm, the same as the hemolymph[4]. Changes in potassium and sodium concentrations were made by increasing or decreasing the concentration by an equivalent amount of sucrose so that the osmolarity remained constant. The potassium used in the Ringer was either K_2SO_4 or KCl, while for sodium NaCl only was used. Intracellular recordings were obtained using glass microelectrodes filled with 3M-KCl and with electrical resistances of 10–20 MΩ . The electrical circuit was of conventional type. Experiments were performed at room temperature (20–23 °C), but in any single experiment the temperature did not vary by more than 0.5 °C.

Results. Preliminary experiments were performed in order to verify whether there is a time decay in the electrical characteristics of the ventral nerve cord neurons perfused with normal Ringer solution. The membrane potentials (MP) of a great number of cells were measured and the mean values and SE as a function of time are reported in figure 1. In the first 2 h, there is no appreciable decay of the membrane potential. During this period, it was possible to change 3 different solutions to test sodium and potassium effect on the membrane potential. In our experiments, MP in normal Ringer ranged from about −40 to −55 mV.

To test the influence of sodium on the membrane potential, 2 sets of experiments were made and the results compared with those in normal Ringer (reference solution). In 1 set, the perfusing solution contained a 10-fold sodium concentration (17 mM), while the other was sodium-free. In all these experiments, the potassium concentration was the normal one.

For each set of experiments, cells from 7 different preparations were used and for each preparation a control recording in normal Ringer solution was made. In the table, the mean values of the membrane potential with their SE in the different experimental conditions are summarized.

The difference in the resting potential between 2 sets of experiments (Na concentration 17 mEq/l and 1.7 mEq/l) is highly significant ($p < 0.01$), while no significant difference was observed between normal and sodium-free Ringer. To test the influence of external potassium, the concentration was varied between 9.6 and 91.6 mM. The resting potentials of different neurons were measured in each solution and referred to the values obtained in reference Ringer solution. The mean values of MP plotted against the logarithm of $[K_0^+]$ are shown in figure 2. The experimental point of $K^+ = 9.6$ mEq/l corresponds to the potassium content of the phosphate buffer of the Ringer. Each point represents the mean of 200–400 microelectrode penetrations.

It can be seen that the reduction of $[K_0^+]$ under 20–25 mM had little effect on the resting potential, whereas higher values of potassium concentration caused a marked depolarization. The slope of the exponential portion of the graph (for all points with $[K_0^+] > 35$ mEq/l) is about 58.4 mV ($r = 0.94$) for a 10-fold change in $[K_0^+]$, not significantly different from Nernst theoretical value at 20 °C.

To assay the effect of anions, the different $[K_0^+]$ were obtained both with K_2SO_4 and KCl salts. The results are shown in figure 2. The slope from the points with $[K_0^+]$ obtained from KCl only was about 44 mV ($r = 0.97$).

Discussion. The results of the experiments described above clearly show that the neurons of *Bombyx mori* ventral nerve cord are affected by potassium ions in a way similar to that of vertebrate and crustacean fibres studied by previous investigators. The suggestion that insects may have evolved a different mechanism to generate muscle and neurons resting potentials[5] can, in part, be ruled out.

The resting potentials plotted against the logarithm of $[K_0^+]$ resulted in 58.4 mV change for a 10-fold change in concentration.

This result agrees with 55.3 mV/decade at high $[K_0^+]$ obtained in muscle fibres of *Ephestia kuehniella*[6] and, therefore, the membrane can be said to behave as a perfect potassium electrode.

The use of KCl salt to increase $[K_0^+]$ gives a lower slope to indicate a deviation from the Nernst law and suggests a different role of SO_4^{--} and Cl^- ions.

Similar behaviour has been observed in preparations of insects characterized by a low K/Na ratio in the hemolymph (42 mV in the giant axons of the cockroach[7], 45 mV in muscle fibres of *Locusta migratoria* migratorioides[8]) as well as in preparations of insects presenting a higher K/Na ratio in the hemolymph as in *Bombyx mori* larvae (37 mV in neurons of *Carausios morosus*[9], 43 mV in nervous fibres of *Manduca sexta*[10].

These low values indicate that the cell membranes have a relatively large permeability to ions other than potassium. So the resting potential is influenced by the potassium equilibrium potential, but the conductances to sodium and other ions presumably contribute to its genesis.

For a 10-fold change in the external sodium concentration, we have obtained a decrease of 29% (about 11–12 mV) in the resting potential. Also in *Manduca sexta* nervous fibres, the resting membrane conductance to sodium ions is rela-

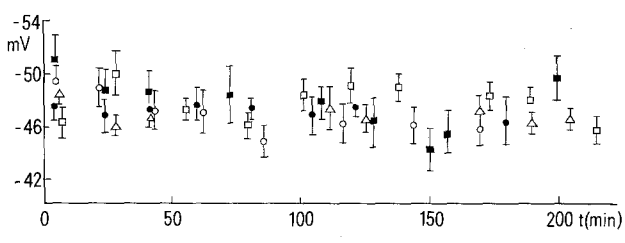

Fig. 1. Membrane potential as a function of time. Each point represents the mean value of the membrane potentials registered in 15–30 cell bodies. Results from 5 preparations.

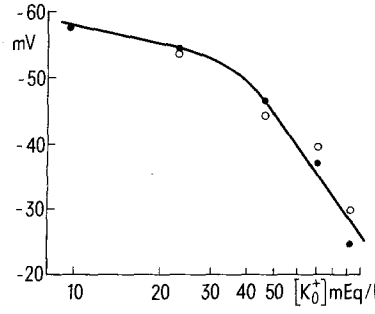

Fig. 2. The relation between the resting potential and the logarithm of the external concentration of potassium in cell bodies of desheathed ganglia. Each point represents the average resting potential from several neurons (200–400 microelectrode penetrations) of different preparations (6–9). SE less than 1 mV. Potassium from: ● K_2SO_4, ○ KCl.

tively large and may be responsible for the deviation from the potassium equilibrium potential. A 15 mV slope for a 10-fold change in external sodium was obtained[10].

It should be noted that doubt has been raised as to whether the hemolymph actually provides the ionic environment for the nervous system of insects, or whether an ion selective nervous sheath isolates a special compartment from the hemolymph space[11].

While in de-sheathed ganglia the effect of sodium and potassium ions on the resting membrane potential is sufficiently clear, the role of calcium and magnesium, present in high concentration in the *Bombyx mori* L. hemolymph, must be investigated.

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Pathways for excitatory and inhibitory innervation to the guinea-pig tracheal smooth muscle[1]

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Summary. The trachea receives excitatory cholinergic innervation from the vagus nerve and the stellate ganglion. Inhibitory adrenergic fibres have the same sources. Those in the vagus nerve probably derive from high vagosympathetic anastomoses. Nonadrenergic inhibitory fibres have a preganglionic vagal supply.

Field stimulation of isolated guinea-pig trachea has shown that tracheal smooth muscle has an excitatory cholinergic innervation and an inhibitory adrenergic innervation, as well as an inhibitory nonadrenergic noncholinergic innervation[2-5].

In this study we have investigated the external pathways of this innervation by stimulating the cervical vagus nerve and the stellate ganglion. Earlier studies centred on bronchial innervation indicate a rather complex innervation pattern of respiratory smooth muscle, thus bronchoconstrictor as well as bronchodilator fibres seem to run in the vagus as well as in the sympathetic nerves[6-10].

Material and methods. 21 guinea-pigs of either sex weighing 600–1000 g were stunned and bled. The trachea was dissected out together with the cervical vagus nerves and/or the stellate ganglia. The trachea was then mounted according to Farmer and Coleman[3] and Coleman and Farmer[11] for registration of intraluminal pressure variations. Pressure was recorded on a Grass Polygraph via a Statham Transducer. The nerves were placed on platinum electrodes and isolated with paraffin oil. Tyrode solution at 37 °C aerated with 6.5% CO_2 in O_2 was used as organ bath solution. The nerves were stimulated with square wave pulses (1–2 msec, 25–30 V, 10–25 Hz) for 10 sec at about 5-min intervals. Drugs used were scopolamine hydrobromide, guanethidine sulphate, sotalol hydrochloride, lidocaine chloride, hexamethonium bromide and barium chloride. In 9 animals, 1 vagus nerve was sectioned about 2 mm below the entrance from the skull 7–10 days before the experiment was undertaken.

Results. Stimulation of the intact cervical vagus nerve resulted in a biphasic response, i.e. contraction followed by relaxation (figure 1, A and B). The excitatory answer was fully blocked with scopolamine ($1\text{–}60\times10^{-7}$ M) within 15 min.

Guanethidine ($2\text{–}20\times10^{-6}$ M) markedly decreased the inhibitory response in 30–60 min. However, a small inhibitory response still persisted (figure 1), even after addition of sotalol ($2\text{–}6\times10^{-6}$ M). It was, however, blocked by lidocaine ($2\text{–}4\times10^{-5}$ M) and also by hexamethonium ($2\text{–}4\times10^{-3}$ M). Neither excitatory nor inhibitory responses could be elicited when previously sectioned vagus was stimulated.

Also stimulation of the stellate ganglion resulted in a biphasic response (figure 2, A). As was the case with vagus nerve stimulation, the excitatory response was abolished by scopolamine ($1\text{–}60\times10^{-7}$ M). The inhibitory response to stellate ganglion stimulation was abolished by guanethidine ($2\text{–}20\times10^{-6}$ M) (figure 2, Band C).

Discussion. Apparently the trachea receives an excitatory cholinergic supply from both the vagus nerve and the stellate ganglion. That the stellate ganglion supplies the guinea-pig trachea with excitatory cholinergic fibres is in line with Hebb's[9] findings on the bronchial smooth muscle of this species. Also the adrenergic inhibitory innervation of the trachea derives from the stellate ganglion as well as from the cervical vagus nerve. Anastomoses at different

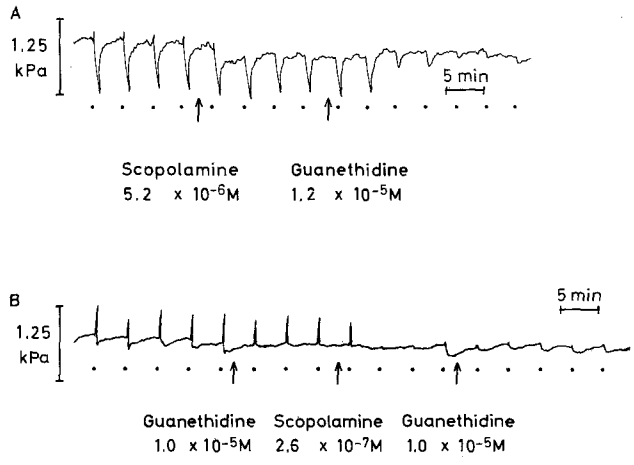

Fig. 1. Intraluminal pressure registration of isolated guinea-pig trachea. *A* Stimulation of right vagus nerve (25 Hz, 30 V, 1 msec) at dots. The excitatory response is abolished by scopolamine. Guanethidine markedly reduces the inhibitory response, but a small inhibitory response persists. *B* Record from another preparation, stimulation parameters as in *A*. Guanethidine diminishes the inhibitory response and scopolamine abolishes the excitatory response, thereby unmasking the inhibitory component not susceptible to guanethidine.