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<sup>11</sup>.

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<sup>1</sup>

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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<sup>2-5</sup>

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<sup>6-10</sup>.

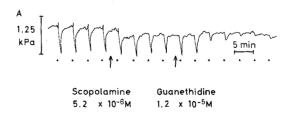
Material and methods. 21 guinea-pigs of either sex weighing 600-1000 g were stunned and bled. The trachea was dissected out together with the cervical vagus nerves and/or the stellate ganglia. The trachea was then mounted according to Farmer and Coleman<sup>3</sup> and Coleman and Farmer<sup>11</sup> 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<sub>2</sub> in O<sub>2</sub> 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  $(1-60\times10^{-7} \text{ M})$  within 15 min.

Guanethidine  $(2-20\times10^{-6} \text{ M})$  markedly decreased the inhibitory response in 30-60 min. However, a small inhibitory response still persisted (figure 1), even after addition of sotalol  $(2-6\times10^{-6} \text{ M})$ . It was, however, blocked by lidocaine  $(2-4\times10^{-5} \text{ M})$  and also by hexamethonium  $(2-4\times10^{-3} \text{ M})$ . 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  $(1-60\times 10^{-7}\ M)$ . The inhibitory response to stellate ganglion stimulation was abolished by guanethidine  $(2-20\times 10^{-6}\ M)$  (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 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



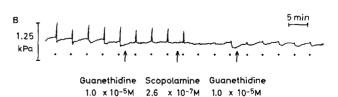


Fig. 1. Intraluminal pressure registration of isolated guinea-pig trachea. A Stimulation of right vagus nerve (25 Hz, 30 V, 1 msec) at dots. The excitatory response is abolished by scopolamine. Guanethidine markedly reduces the inhibitory response, but a small inhibitory response persists. B Record from 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.

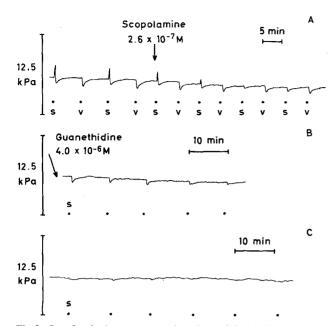


Fig. 2. Intraluminal pressure registration of isolated guinea-pig trachea. A Stellate ganglion stimulation (s), by comparison, alternated with vagus nerve stimulation (v) (parameters 25 Hz, 30 V, 1 msec). In this preparation, vagus nerve stimulation resulted in relaxation only. Scopolamine abolishes the excitatory sympathetic response. In B and C, which are continuous records, the sympathetic inhibitory response is blocked by guanethidine in a low concentration.

levels between the vagal trunk and the sympathetic chain are well documented<sup>7,10,12</sup>. Our denervation experiments suggest that the adrenergic fibres which reach the trachea via the cervical vagus nerve derive from anastomoses close to the origin of the vagus nerve.

The nonadrenergic, noncholinergic inhibitory nerves<sup>4,5</sup> of the trachea seem to have a preganglionic vagal supply. This would be in accordance with findings on lungs from amphibians and reptiles, and be an analogy to the nonadrenergic, noncholinergic inhibitory innervation of the stomach<sup>13</sup>. In conclusion, the guinea-pig trachea is one more example demonstrating the complexity of the autonomic innervation of different organs.

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## Laser Doppler microscope in an oblique-backward mode and pulsatile blood flow velocity in pulmonary arteriole

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Summary. Blood flow velocity, pulsatile in correspondence to cardiac events, in pulmonary arterioles of anesthetized bullfrogs could be measured on lung surfaces covered with a water-containing plastic disc by means of a laser Doppler microscope arranged in an oblique-backward mode.

Laser Doppler microscope<sup>3,4</sup> was originally designed in a see-through position, i.e. in a dual-beams forward-scatter mode, in which the laser tube and a combination of the microscope and photomultiplier were set in a face-to-face position and the tissue to be studied was placed between them. The Doppler shift of laser beams scattered in the probing area of a micro-vessel was detected by the photomultiplier situating in the opposite side of the laser source. Therefore, the laser Doppler microscope was hitherto used for measuring blood flow velocity in a thin transparent tissue of frog web<sup>5,6</sup>. If it is used in a reflection position, i.e. in an oblique backwared-scatter mode, where the microscope-photomultiplier is placed abreast of the laser tube so as to detect the laser light scattered in the probing area at an acute angle with the direction of the incident laser beams, the applicability of the laser Doppler microscope may be much increased, because it permits the observation of micro-vessel existing just beneath the surface of the organs. However, the well-known difficulty occurring in the optical system having such a mode is the shot noise caused by the light reflected on the tissue surface. In this paper, we study a method of minimizing the disturbance by the shot noise and of quantitatively measuring blood flow velocity in a pulmonary arteriole.

Experimental method. The experimental arrangement is schematically shown in figure 1. An unilateral lung of the anesthetized Bullfrog was exposed by thoracotomy and inflated by means of an intratracheal catheter. The incident dual beams were crossed in an arteriole of the alveolus of the exposed lung. The microscope-photomultiplier was focussed to the probing area of the arteriole on the alveolus. An area of the lung surface under observation was covered with a water-containing plastic disc, Soft 38® (thickness, 0.23 mm, curvature, 8.8 and diameter, 13 mm, Nichicon, Nagoya, Japan), to study the effects of covering

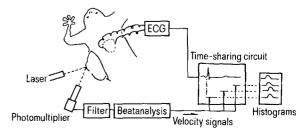


Fig. 1. Schematic illustration of an experimental arrangement.