is obtained. (At the end of this procedure, screw No. 2 also serves to hold the section stretcher in place, as mentioned above). This adjustment by means of screw No. 2 is made possible by the fact that the axis of the holder (No. 4) is arranged in such a way that triangle B (the section stretcher) is positioned slightly to the right prior to fastening. Throughout the initial procedure, triangle B is guided by the feather (No. 5). Technical details of the apparatus are given in Figure 3.

Semithin and ultrathin sections with and without the synthetic coating described by BOLL et al.[8] have been produced with this section stretching apparatus.

Advantages of the apparatus described are that it provides an ideally fitting section stretcher, allowing for good observation of the cutting process, and that even sections can be obtained without the use of a floating medium. The removal of the frozen sections, which usually adhere to the glass knife (A in Figures 1 and 2), can follow without the use of liquids by any of the known methods[2,3,6,7,9–11]. In any case, it is advantageous that the sections are protected from drying until the opening of the section stretcher at the desired moment. Our experience has been, furthermore, that static electricity causes less disturbance with the use of this method.

Zusammenfassung. Es wird ein Schnittstreck-Gerät für die Ultramikrotomie, besonders für die trockene Gewinnung und Abnahme von semidünnen und ultradünnen Gefrierschnitten beschrieben. Das Prinzip des Gerätes besteht darin, von den bei der Herstellung (Brechen) von Glasmessern aus Vierecken erhaltenen beiden komplementären Dreiecken je eines als Messer, das zweite als Schnittstrecker zu verwenden.

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An Autoradiographic Method for the Demonstration of Vagal Afferent Nerve Fibres in the Lower Respiratory Tract of the Chicken (*Gallus gallus domesticus*)

The innervation of the lungs and extrapulmonary airways of the chicken have been extensively studied[1–4]. In these and other investigations of the innervation of viscera, one of the most difficult problems has been to distinguish between afferent and efferent fibres. We believe that we have overcome this problem by utilizing axonally transported ^{3}H-leucine. Autoradiographic techniques have previously been used to study nerve pathways within the CNS[5], central connections of dorsal root ganglia[6], the rate of axonal transport[7], and the optic pathways of several species[8–10]. The present study was undertaken to determine the feasibility of using this method to demonstrate the distribution of afferent nerve fibres to the lower respiratory tract of the chicken.

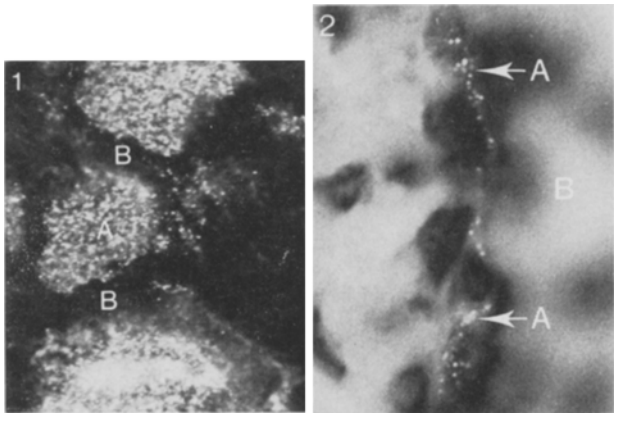

Fig. 1. Nodose ganglion from the treated side. The cell bodies (A) have taken up the ^{3}H-leucine but there is little activity present between the cells (B). ×550.

Fig. 2. Syrinx. A labelled nerve (A) can be seen lying beneath the epithelium (B) of the air sac wall. The epithelium has shrunk more than the other tissue and is out of focus. ×550.

Materials and methods. The nodose ganglia of 2 adult hens each weighing 1.5 kg were treated with ^{3}H-leucine. A slow i.v. infusion of a solution of 400 mg/ml of urethane in avian Ringers solution was given until a constant plane of anaesthesia was obtained, at a dose of about 1.5 g/kg body weight. This gave deep anaesthesia throughout the duration of the experiment (12–18 h). The right nodose ganglion which lies in the thoracic inlet was exposed and carefully dissected free, the bird maintained by artificial respiration. A small sheet of dental wax was placed beneath the ganglion to form a shallow bath and the ganglion was bathed for 30 min in a 1% solution of hyaluronidase (Koch-Light Labs; Cat. No. 3137 t) in normal saline. The ganglion was then surrounded by small pieces of gelatin foam soaked in ^{3}H-leucine solution (Radiochemical Centre, Amersham, Bucks; Cat. No. TRK 70) which were maintained in position by wrapping the dental wax carefully around the ganglion. A specific activity of 5 mCi/ml was obtained by freeze-drying the ^{3}H-leucine and then redissolving in the appropriate volume of normal saline. It was assumed that the rate of rapid transport was about 400 mm/day, therefore the 10 or more h which the bird remained alive should have been sufficient to permit the rapid transport of the amino acid to the receptor endings. The hens were finally killed by intracardiac perfusion with 10% formalin. The nodose ganglion, the vagus just distal to the ganglion, the

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Reflectance measurements demonstrating labelling of nervous structures

Region	No. of fields measured	Photometer readings (arbitrary units)	
		Range	Mean
A) Experimental nodose ganglion			
a) Interstitial tissue	4	2–3	2.25
b) Cell bodies	4	140–175	159.50
Control nodose ganglion			
a) Interstitial tissue	4	2–3	2.50
b) Cell bodies	4	4–5	4.50
B) Experimental vagus just distal to the nodose ganglion			
a) Background	4	4–6	5.25
b) Transverse section of the nerve	4	19–25	21.50
Control vagus just distal to the nodose ganglion			
a) Background	4	2–3	2.5
b) Transverse section of the nerve	4	4–6	4.75
C) Experimental syrinx			
a) Syrinx wall	4	7–11	8.25
b) Outer plexus	4	28–33	30.25
c) Inner plexus	4	27–30	28.00
d) Epithelium	4	7–9	7.75
Control syrinx			
a) syrinx wall	4	10–12	10.75
b) region of outer plexus	4	11–13	11.50
c) region of inner plexus	4	11–13	11.50
d) epithelium	4	9–11	9.75
D) Experimental lung			
a) Background	4	12–15	13.25
b) labelled structures	4	58–65	61.5
Control lung			
a) Background	4	11–13	11.75
b) labelled structures	No labelled structures seen		

All readings were taken using a green filter (580 nm) in the path of the reflected light. Field sizes within groups A, B, C, D, were the same.

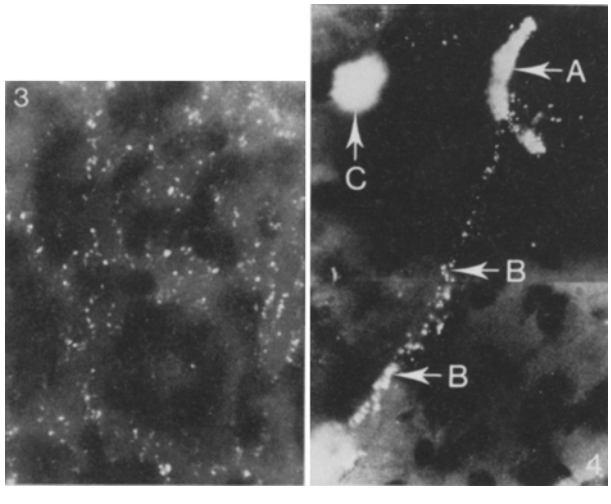

Fig. 3. An example of a fine meshed nerve plexus found in the exchange area of lung. Note that the radioactivity is almost entirely in the interstitial tissue and not within the cell bodies. × 550.

Fig. 4. The heavily labelled structure (A) is probably a bundle of nerve fibres with a small branch (B) leaving the bundle. Artifacts such as C would be distinguished from labelled structures such as A and B the intensity and organisation of the reflecting area. × 550.

trachea, the syrinx, primary bronchus and lung from the treated and untreated sides were taken for histological examination. Lung from an untreated hen was taken as a control. Serial sections were made and every 10th section was mounted on a 'subbed slide'. The slides were coated in the dark with Ilford K_2 nuclear emulsion diluted 1:1 with a 1% solution of glycerol. The slides were placed on a cold plate for a few min, then left to dry for 4–5 h. They were packed in light-tight boxes containing anhydrous calcium sulphate as a desiccant. At the same time, slides prepared from the untreated side and from the untreated hen were processed in the same way to act as controls against positive and negative chemography. All slides were exposed for 4–6 weeks at 4 °C and were subsequently developed in the usual way using Phen-X (Ilford) diluted 1:1 with distilled water. The sections of nodose ganglion were counterstained with cresyl violet and those of syrinx and lung with haematoxylin and eosin. The slides were viewed by means of direct and reflected light using a Vickers M74 autoradiographic microscope. The reflectivity of various parts of the sections was measured by means of a photomultiplier (EMI 9592B) linked to a digital microphotometer (Vickers M720050). The reflectivity has been shown to be linearly related to the number of silver grains[11,12], and is a convenient method of comparing the radioactivity of different parts of the slide.

Results. Nodose ganglion. 3 slides, each containing 4 sections of the nodose ganglion, were examined in detail, 1 slide from the proximal part of the ganglion, 1 from the middle and 1 from the distal part. These sections were chosen randomly and others were found to be similar. In the treated ganglion the ^{3}H-leucine was taken up into the cell bodies within the ganglion and very little was seen in the interstitial tissue (Figure 1). Reflectance measurements (see Table) confirms this affinity of the neurones for ^{3}H-leucine. Marked differences in photometer readings for background and nerve cell bodies were seen in sections from the treated side, but were not evident in the contralateral nodose ganglion nor in the untreated control. It is thus possible to rule out positive and negative chemographic artifact and to conclude that the ^{3}H-leucine was selectively taken up by neurones with their cell bodies in the ganglion. It has been shown previously[5] that axons do not take up ^{3}H-leucine and thus only those neurones with their cell bodies in the nodose ganglion were labelled.

Vagus distal to the nodose ganglion. 3 slides each containing 4 sections of vagus just distal to the nodose ganglion were examined in detail. Silver grains were scattered throughout the transverse section of the vagus (see Table). They were not seen in the contralateral vagus or the untreated vagus, indicating that the ^{3}H-leucine had been transported peripherally along the distal vagus.

Syrinx. 2 distinct bands of radioactivity were found in the wall of the treated syrinx (Figure 2) and not in the control. These bands' positions correspond to the locations of the inner and outer nerve plexuses.

Lung. 9 sections were examined in detail, 3 each from the treated side, the contralateral side and the untreated hen. 3 areas of exchange tissue from each of the sections were examined and the number of positively labelled structures counted. In order that the interpretations of the results should be as unequivocal as possible, only those structures which produced a photometer reading of at least twice the background were counted as being positively labelled (see Table). The structures so labelled

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varied from nerve-like threads to complex plexuses in which individual fibres could be seen (Figures 3 and 4). In the 3 areas from each of the experimental sections at least 4 labelled structures were found, typical photometer readings being as shown in the Table. No such structures were found in lung tissue from the contralateral side or the untreated control hen.

Conclusion. We conclude that this technique can be used to demonstrate the peripheral distribution of vagal afferent nerve fibres which have their cell bodies in the nodose ganglion and this work is now in progress.

Résumé. Par marquage du ganglion nodosum avec la ^{3}H-leucine nous avons étudié la repartition des afférences vagales au niveau de la partie inférieure du système respiratoire du poulet par des techniques d'autoradiographie.

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Sampling of the Rodent Dorsal Lateral Geniculate Nucleus for Electron Microscopy

In our laboratory we have been concerned with the electron microscopy of retinal axon termination and distribution of the neurons of the dorsal lateral geniculate nucleus (DLGN) in the rat. In order that correlation could be made with other anatomical and physiological data it was necessary to develop an accurate sampling technique to insure precise localization within the DLGN of information obtained in ultrastructural studies.

The brains of laboratory rats were perfused through the aorta with a mixture of 4% paraformaldehyde and 0.5% glutaraldehyde with 4% sucrose in 0.1 *M* phosphate buffer (pH 7.3). The perfusate was used at room temperature. Sufficient pressure for gravity perfusion was achieved by maintaining the perfusate reservoir at a height of 3.5 feet (78.5 mm Hg) above the animals. The perfusions continued for a total of 20 min.

Immediately after perfusion each animal was decapitated by first severing the soft tissue structures around the neck region. This was followed by a laminectomy to expose the upper cervical cord. The cervical spinal cord was transected with a sharp razor blade. This step is performed to avoid stretching artifact of the brainstem. The calvarium was removed with a small pair of 'rongeurs'.

After removing the brain from the cranial cavity, the brain was sliced sagittaly. Each half of the brain was trimmed so that the entire desired nuclear region was contained in one tissue block. The tissue block was immersed for two hours in cold 4% glutaraldehyde in the same buffer as the perfusate. The block was transferred to a rinse solution of 0.1 *M* phosphate buffer and 4% sucrose. The block of brain tissue was further trimmed under a dissecting microscope to the smallest dimensions that still contained the entire nuclear group of choice. With the aid of a Smith-Farquhar tissue sectioner this tissue block was cut into serial slices 250 µm thick. Integrity of the slice series was maintained by the agar media used to support the block. The slices were placed serially in partitioned plastic cases that contained the rinse solution. Sections were rinsed for an additional 10 min. Prior to post-fixation each slice was transferred to a small petri dish that contained a solution of 0.5% osmium-tetroxide and 4% sucrose in 0.1 *M* phosphate buffer. After 1 to 2 min in this solution the surface of the slice acquired a light brown color. Each slice was next transferred to a pool of phosphate-buffered sucrose on a clear plastic disk on the transilluminating stage of a dissecting microscope (Figure 1). The osmicated myelinated

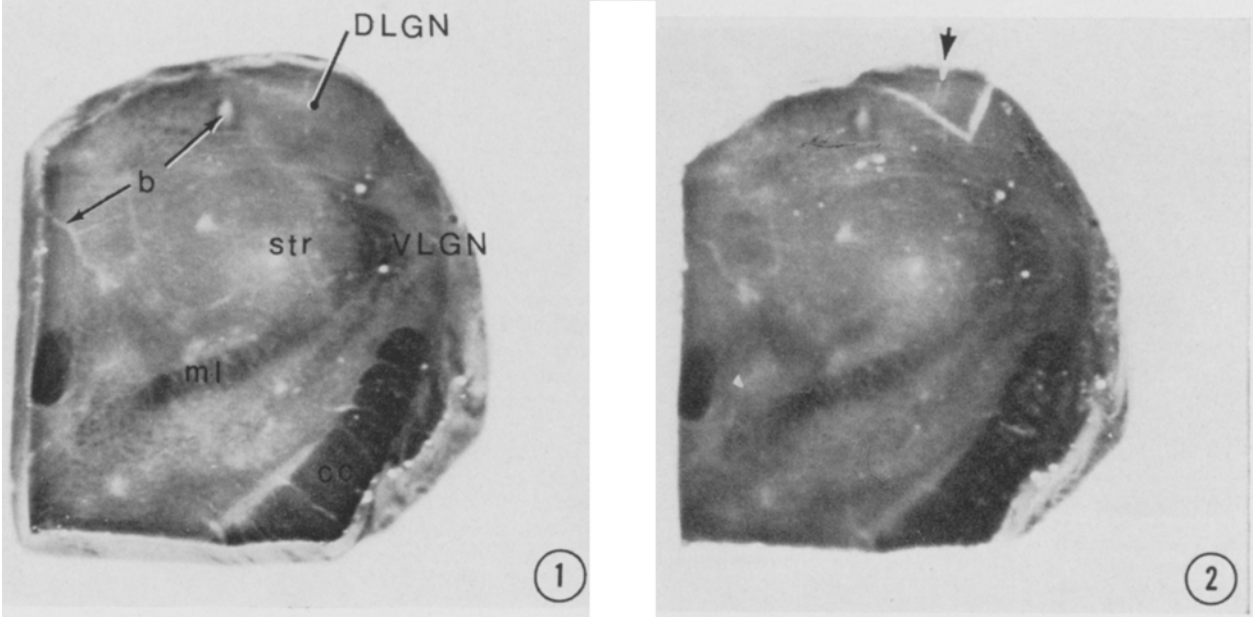

Fig. 1 and 2. Transverse slices through the diencephalon of the rat. The slice has been lightly osmicated. Note the prominent landmarks of myelinated fibre tracts and blood vessels. An arrow in Figure 2 indicates sample taken for electron microscopy. Dorsal and ventral lateral geniculate nuclei (DLGN, VLGN); superior thalamic radiation (str); crus cerebri (cc); blood vessels (b). ×12.