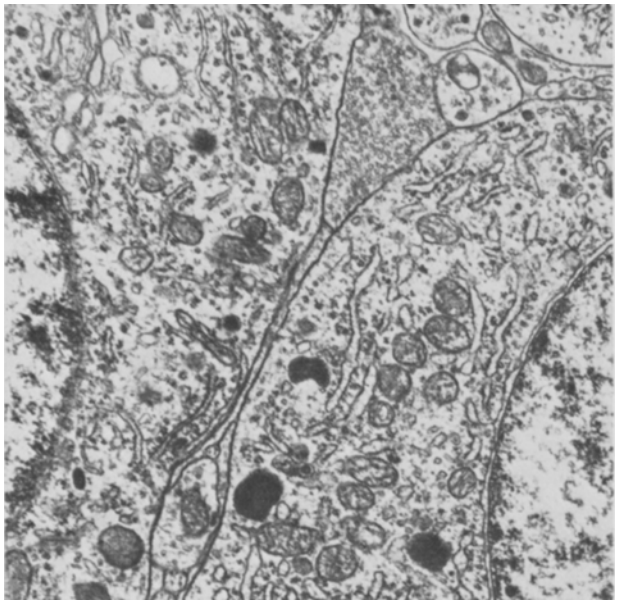

Fig. 2. A synaptic, bouton containing cholinergic synaptic vesicles, and establishing synapses with 2 neurosecretory neurons. × 12.000.

number and distribution of the cellular organoids of the perikaryon in diverse zones of this nucleus. Nervertheless, this asynchrony is preferentially zonal, and within the same zone the neurosecretory neurons show ultrastructural characteristics which are fairly constant.

In our ultrastructural observations we have frequently found wide zones of apposition of the neuronal somas without the interposition of a glial barrier (Figure 1). In these zones the membranes of the neuronal somas establish intimate contact or are separated by a narrow interstitium of about 200 Å. The neurons interconnected by these appositional zones of their somas present identical morphological characteristics in both quantity and distribution of their organoids, suggesting that they may be in the same functional state.

The other morphological aspect which we wish to emphasize is the presence of cholinergic axo-somatic synapses, with synaptic boutons which are shared by 2 neural somas (Figure 2), i.e., the same synaptic bouton forms a synapse with the somas of 2 juxtapositioned neurosecretory neurons.

Discussion. Extensive contact zones between the neural somas, without the interpositioned glial barrier, have been observed in other cerebral locations, especially during fetal and postnatal development[9]. In the supraoptic nucleus of the adult rat these zones are frequently seen. Although the presence of 'tight junctions' has not been observed, it is very possible that some type of interneuronal communication exists in these narrow appositional zones of the neuronal membranes. This communication could function in a manner analogous to the 'gap junctions' seen in other localities of nervous tissue[10,11]. This hypothesis seems to be strengthened by the fact that these interconnected neurons present identical morphological characteristics.

We feel that the appositional zones of the neuronal somas, as well as the presence of synaptic boutons shared by 2 neurons, are morphological structures which may permit an interneuronal coordination and a synchronized discharge. In this sense, the groups of interconnected neurons, within each zone of the supraoptic nucleus, would behave as functional units composed of various synchronized neurons. The asynchrony of the secretory activity of the supraoptic nucleus neurons would be zonal, i.e., the diverse zones of the supraoptic nucleus may present different functional states which could be cyclically modified[4]. Within the same zone, the neurons maintain similar morphofunctional characteristics. The zonal asynchrony is interpreted as a mechanism which permits the maintenance of a high activity state in the supraoptic nucleus during long periods of time without fatigue[6].

Resumen. El estudio ultraestructural de las neuronas del núcleo supraóptico de la rata demuestra amplias zonas de aposición de los somas neuronales y botones sinapticos que son compartidos por dos somas. Los autores postulan que estas estructuras morfológicas pueden ser mecanismos de coordinación y sincronización interneuronal.

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Membrane Filters do not Prevent Cell Contacts

Experimental embryologists studying induction systems have made extensive use of membrane filters to separate two cell populations[1,2]. The filters were originally employed in an attempt to distinguish between a chemical inducer[3] and induction due to cellular contacts[4]. The majority of these studies have utilized Millipore filters (Millipore Ltd.) to separate the two cell groups. Several studies using these filters have reported the presence of cellular extensions along the borders of the filter[5,6]. However, some authors still hope that thicker filters might prevent cellular contact[6].

The present paper describes the results obtained when thick Millipore filters were used in a primary neural induction system in the chick. Preliminary studies however showed that where filters were sterilized with 70% ethanol/water as recommended[7] the filter structure

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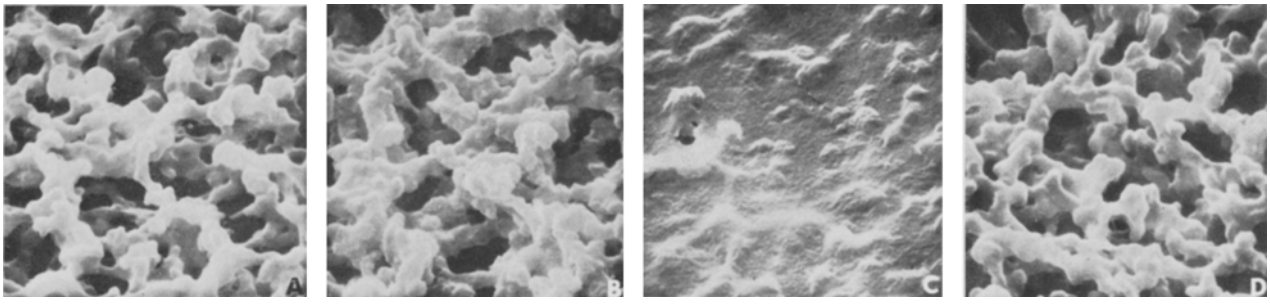

Fig. 1. Millipore filters examined by stereoscanning electron microscopy. In Figure A the structure of the untreated GS 0.22 μm filter is shown (×15,600). When the filter is immersed in 70% ethanol/water for 30 min the fibres become swollen and partially dissolved (Figure B, ×24,400). Absolute alcohol rendered the GS 0.22 μm filter completely unstable in the electron beam and no photographs could be obtained. The effect of 100% alcohol is shown instead on the HA 0.45 μm filter (Figure C, ×10,400). It can be seen that the structure of the filter has been completely removed. The GS Millipore filter after autoclaving in saline (Figure D). The structure of the filter remains unaffected (× 25,000).

became swollen and distorted and in higher concentrations of alcohol the filter actually dissolved[8]. The filters were, therefore, sterilized by autoclaving which does not affect their structure[9] (Figure 1).

In the induction studies chick embryos stage $3^3/_4$ and 4 were mounted by NEW culture[10]. A microincision was made in the area opaca of the stage $3^3/_4$ embryos and a 'pocket' was formed by separating the host ectoderm and host endoderm. Millipore filters approximately 1.2×1.2 mm types HA (0.45 μm ± 0.02 μm pore size, 150 μm ± 10 μm thick) or GS (0.22 μm ± 0.2 μm pore size, 135 μm ± 10 μm thick) were inserted into the 'pocket'. Hensen's node from a stage 4 embryo was then excised and transferred by pipette to a stage $3^3/_4$ embryo and placed in the 'pocket' between the host endoderm and the Millipore filter with the node endoderm adjacent to the filter. The culture system was reincubated at 37.5 °C and examined by light and transmission electron microscopy.

In the electron microscope cellular debris was seen at the edges of the newly-excised Hensen's node (Figure 2). Throughout the filters the pores are clearly outlined and small droplets of cytoplasm are present. After 3 h reincubation there are no signs of the cellular debris and damage adjacent to the node. The cytoplasmic droplets

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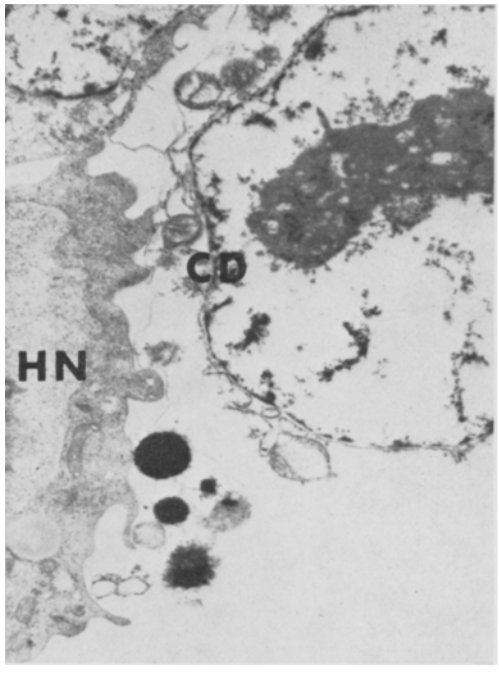

Fig. 2. The appearance of the newly-cut edge of Hensen's node (HN) examined by transmission electron microscopy immediately after grafting into the host embryo (×6,000). Cellular debris (CD) can be seen adjacent to the edge of the node.

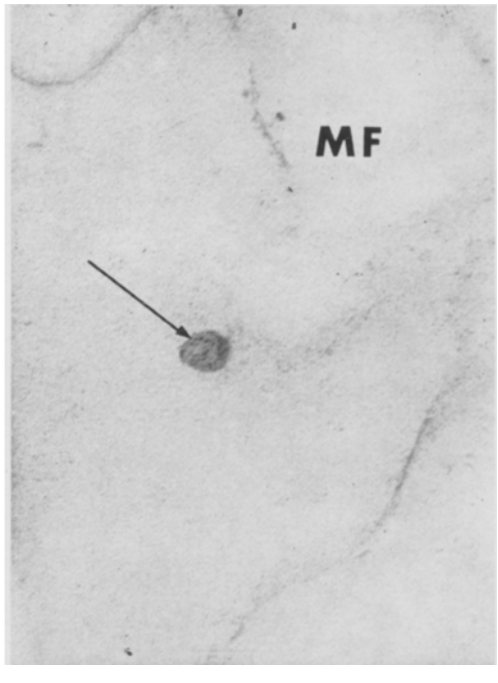

Fig. 3. Cellular debris (arrow) in a HA 0.45 μm Millipore filter (MF) after 24 h incubation in the embryonic induction system. The structure of the filter is outlined by fluid in the pores. (×180,000).

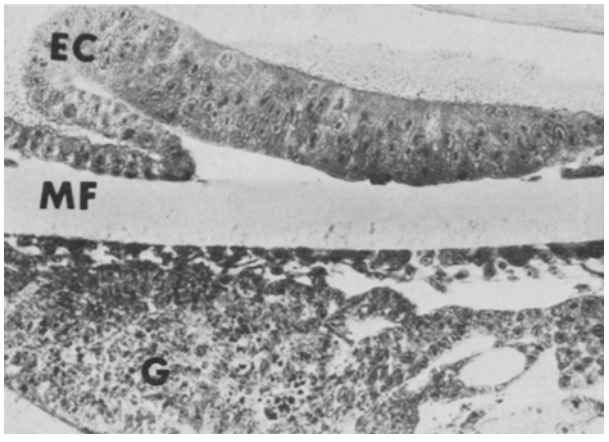

Fig. 4. Cellular processes extending into a Millipore filter (MF) incubated for 24 h in a chick embryo between a graft of Hensen's node (G) and the host ectoderm (EC). Note that more processes emerge from the graft towards the host ectoderm than vice versa, and that neural induction has occurred.

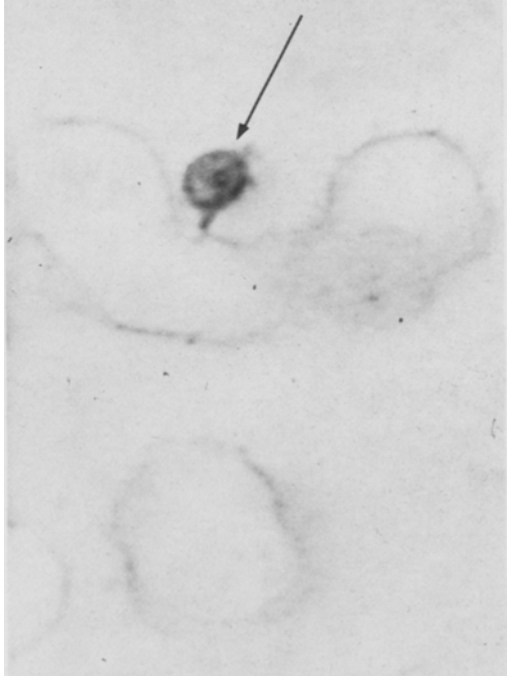

Fig. 5. A Millipore filter following immersion in a solution of saline and Hensen's nodes cut into smaller pieces. The filter is filled with cytoplasmic droplets (arrow). ×185,000.

remain in the filter, however, and are present even after 16–24 h incubation (Figure 3).

Many cellular processes can be seen by light microscopy in both of the filter types incubated 24 h in the chick embryos. Usually more processes emerge from Hensen's node toward the host ectoderm than vice versa. Several of the processes appear to project into the filter to about half of the thickness of the filter (Figure 4). The remainder of the filter appears to have darkened contents.

In the electron microscope the pores of the filters in the chick embryos are clearly outlined and cellular processes are present in the pores adjacent to the node and host ectoderm. Throughout these filters small droplets of cytoplasm were present.

In addition, stage 4 Hensen's nodes were excised and minced in saline. Millipore filters were added to this preparation and incubated at 37.5 °C for 10 min. The pores of the filters were outlined and cytoplasmic droplets similar in appearance to those in Figure 3 were present throughout the filter (Figure 5).

Microsurgery causes damaged cells and cell debris. This cell debris appears as cytoplasmic droplets and readily passes into the filters. Cellular processes can also extend from cell populations on either side of the filter and contact this debris without penetrating very far into the intervening filter. Thick membrane filters do not, therefore, prevent cellular contact[11].

Résumé. La microchirurgie cause des dommages aux cellules. Les débris cellulaires ont l'apparence de gouttelettes cytoplasmiques qui pénètrent dans les filtres Millipore. Dans un système d'induction neurale primaire, les polpulations de cellules d'un côté ou de l'autre du filtre étendent des processus cellulaires qui prennent contact avec ces débris sans pénétrer très loin dans le filtre. Par conséquent même les filtres épais n'empêchent pas le contact cellulaire.

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Some Ultrastructural Observations on the Cytotoxicity of an Alcoholic Extract of *Suberites inconstans* on HeLa Cells

Some marine sponges are known to produce antitumour substances[1]. In our studies of local marine sponges, we have shown that the species, *Suberites inconstans*, contained an alcohol-soluble principle which was cytotoxic to HeLa cells[2]. This alcoholic extract, at a concentration of 60 µg/ml and above, irreversibly inhibited the growth of HeLa cells; and by means of phase contrast microscopy, it was shown that cell death was caused by the rupture of both nuclear and cell membranes. This paper describes further studies of the cytotoxic effect of this sponge extract on HeLa cells at the ultrastructural level and attempts to correlate the electron microscope observations with those of our previous results.

Materials and Methods. An alcoholic extract was made of the sponge, *Suberites inconstans*, as described previ-

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