Average (± SD) number of impulses in the first sec of counting (50 msec after onset of stimulation) generated by a receptor cell in S. exempta larvae. Stimulation with 4 purine compounds (0.03 M) and 3 sugars (0.1 M)

Lateral sensilla (N = 6)			
Adenosine	Adenine	Guanosine	Hypoxanthin
84.2 ± 39.4	50.6 ± 24.4	0.0 ± 0.0	0.0 ± 0.0
Medial sensilla (N = 12)			
D-ribose	Sucrose	m-Inositol	
36.9 ± 17.0	21.2 ± 14.3	35.5 ± 16.3	

instead stimulated a neuron in the medial sensilla (figure 5). This cell was also very sensitive to sucrose and meso-inositol (table). However, in contrast to sucrose, neither D-ribose nor meso-inositol or any other sugar acts as a strong phagostimulant for S. exempta[3,5]. A possible explanation for this apparent discrepancy is that the larval feeding reactions to sugars are not primarily governed by signals from this particular receptor type, but rather by inputs from other sugar-sensitive neurons. This argument is supported by the observation that another type of sugar-sensitive receptor showing a specific sensitivity towards sucrose is located in the lateral sensilla (figures 1 and 5). It remains, possible that the sugar-sensitive neuron in the medial sensilla participates in the total gustation coding via a subtle across-sensillum patterning, which, however, remains undetectable with the currently employed behaviour methods[3,5]. Experiments comparing the response patterns of the lateral sensilla to sucrose and adenosine showed that either 1 or 2 cells fired depending on whether the compounds were applied singly or in a mixture (figure 1). Thus, 2 separate receptor types are inferred, one sensitive to sucrose and the other to adenosine. Both receptors are sufficiently sensitive to monitor the amounts of sucrose and adenosine as they occur in the natural food[3]. In fact, the aqueous extract of fresh maize leaves, from which adenosine and sucrose were isolated at 2 and 6 mM/1000 g (fresh wt) respectively[3], elicited vigorous response patterns in both the medial and lateral sensilla (figures 4 and 5). If one considers the limited number of 4 chemosensory neurons in each sensillum[5] it seems reasonable to suggest that part if not most of the recorded impulse activity originated in the adenosine- and sugar-sensitive neurons.

Experiments with S. frugiperda, S. littoralis and S. litura have shown that other species of the genus Spodoptera possess a similar arrangement of chemoreceptor cells as described above for S. exempta. Although S. exempta is the only species with an oligophagous behaviour, it is interesting to note that grasses have been recorded as part of the food plant range for all these Noctuids[4].

A comparative study of the differentiation of dissociated nerve cells under different culture conditions

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Summary. In vitro differentiation of chick embryo brain cells was compared under several culture conditions. Morphological observations and acetylcholinesterase histochemical staining revealed that the development was similar in all conditions tested if cells have been derived from 7 days embryos. Considering the cultures from 11 days embryos, the cell dissociation by trypsin and the plastic surface proved to be the most favourable conditions in contrast to mechanical dissection and collagen surface.

Several studies have suggested that primary cultures of dissociated nervous tissues are very good model systems to investigate morphological and biochemical aspects of nerve and glial cell differentiation [3-6]. However, culture conditions can modify the pattern of the differentiation process[7-9]. Therefore, it was considered to be worthwhile to study the stages of differentiation as a function of 1. the age of the embryo yielding the brain cells, 2. the dissociation method of the brain tissue and 3. the surface substrate on which the cells were cultivated. In the present investigation, we have undertaken such a comparative study of the morphological differentiation, combined with the presence of acetylcholinesterase (AChE) activities in chick embryonic brain cells.

Materials and methods. Chick embryos of either 7 or 11 days of age were used for cell preparation. The cells were prepared according to one of the 2 following methods. In the first group of experiments, the cerebral hemispheres were passed through a nylon sieve of 48 µm pore size to dissociate cells, as was described before[8,10]. These cultures were compared to those obtained by stepwise trypsin digestion using 0.05% of trypsin (1 : 250) dissolved in Ca-Mg free BSS (balanced salt solution) supplemented with 0.3% of glucose. 3 times 5 min of incubation at 37 °C combined with gentle pipetting was enough to dissociate the tissue pieces. All the steps were stopped by adding ice-cold serum to the samples. The cells were collected by 5 min centrifugation at 250 g ×g.

Each experimental group was then subdivided again for using one half on a plastic surface and the other half on a surface previously coated by 1% collagen[11]. The different

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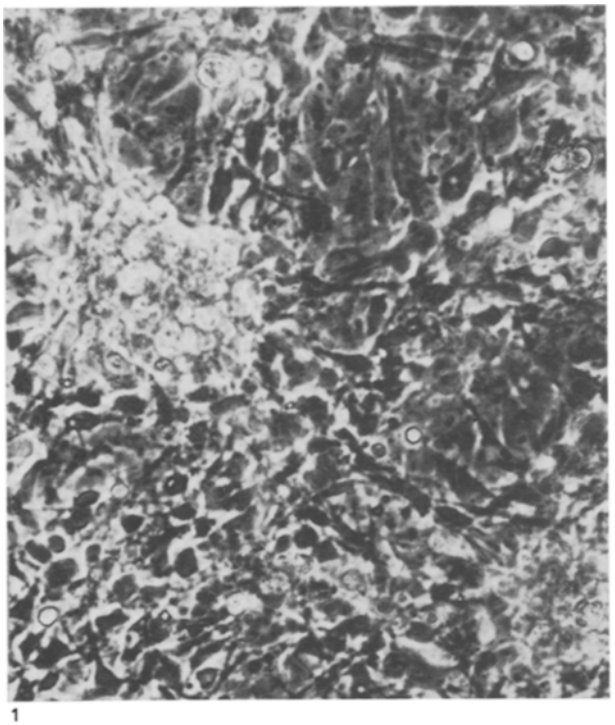
Age of embryo (days)	Dissociation methods	Cultivation surfaces
7	Mechanical	Plastic
		collagen
	Enzymatic	Plastic
		collagen
11	Mechanical	Plastic
		collagen
	Enzymatic	Plastic
		collagen



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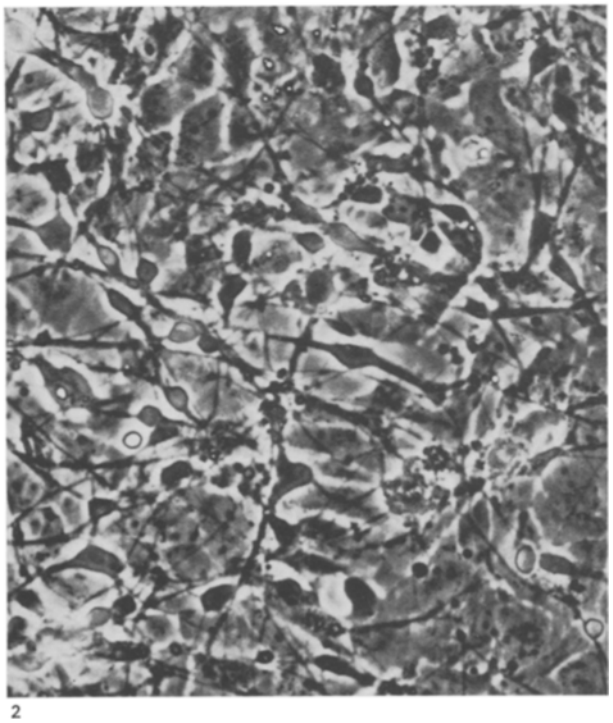

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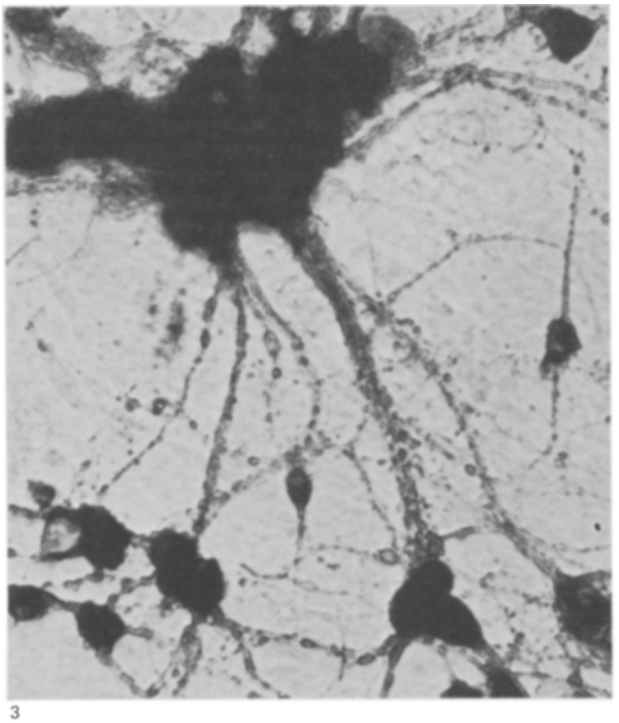

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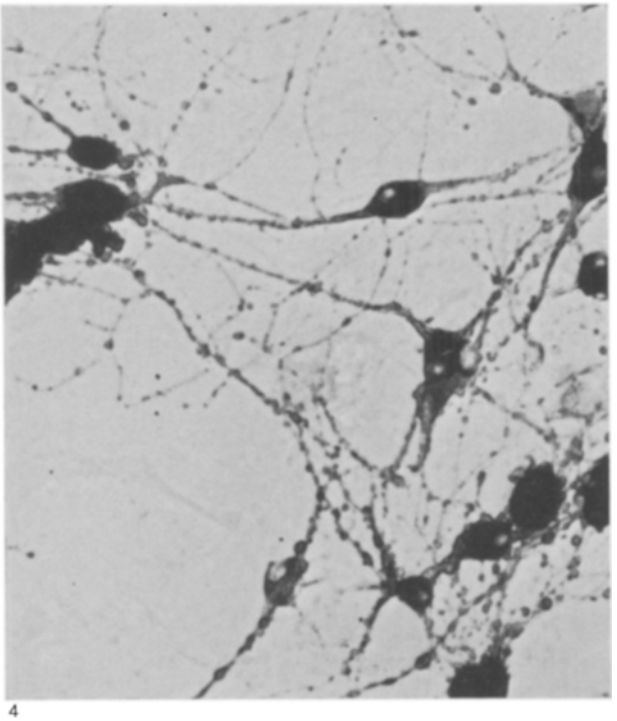

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Fig. 1–4. Cultures from 11-day-old chicken embryo hemispheres. *1* Dissociated by trypsin digestion, cultivated on collagen surface. 4 days in culture. *2* Dissociated by passing through a nylon sieve, cultivated on collagen. 4 days in culture. Phase contrast × 400. *3* Dissociated by trypsin digestion, cultivated on plastic surface. 14 days in culture. *4* Dissociated by passing through a nylon sieve, cultivated on plastic surface. 14 days in culture. Acetylcholinesterase staining × 500.

experimental groups are summarized in the table. The cells were seeded at a starting population density of ca. 3×10^5 cell/cm^2 and cultivated in Eagle minimum essential medium (Institut Pasteur, Paris) plus 15% of fetal calf serum (Gibco) in Falcon petri dishes.

The morphological differentiation of the cells was followed by phase contrast microscopic observation on living preparations. To determine the stage of differentiation of neuronal cells, we established the following criteria: the size of the cell body; the length, thickness and number of processes; whether the processes are forming bundles or not; the number of the cells remaining in clumps without processes or spreading out from the clumps and developing.

After 2, 3 or 4 weeks of cultivation some cultures were fixed and stained for AChE by the method of Karnovky and Roots[12]. The semiquantitative AChE activity in the cell body and processes was measured because this enzyme is known to characterize the neuronal differentiation[13–17].

Results and discussion. Taking the criteria described above, we could not find essential differences in the growth pattern of the cultures derived from 7-day-old chick embryos comparing the 2 methods of dissociation. The development of the neuronal cells appeared very similar. Comparing the 2 cultivation surfaces, it was revealed that the attachment of the cells was quicker on collagen than on a plastic surface and the cell development was accelerated. This confirmed previously reported results[10,11]. Within 5 days of cultivation, the neuronal cells on collagen reached a differentiated state similar to that reached on plastic within 16 days, and this was independent of the dissociation method used. However, after longer cultivation time (4 weeks), as the AChE staining showed, the starting differences caused by the cultivation surfaces were eliminated, and the neuronal cells were present in similar density, and the same stage of differentiation was reached in all type of cultures studied.

In the experiments in which cells were derived from 11 days chick embryos, significant differences were evoked by the 2 dissociation methods. It appeared from the morphological examination of relatively young cultures that the trypsin-dissociated cells attach better to the surface, they multiply during a longer period of time, and the neuronal cells were less differentiated than those derived by mechanical dissection. It can be seen from figures 1 and 2 that the trypsinized cell population after 4 days in culture contains many round-shaped cells in clumps, while in the mechanically dissociated cultures more bipolar and multipolar neurons with well-developed processes were present. The pictures were similar on collagen and on plastic.

From these results, it can be suggested that the dissociation by trypsin causes a relative dedifferentiation of the cells similar to what was observed for heart muscle by Mc Lean et al.[18]. This effect seemed to change the ability of the cells to grow and differentiate in vitro compared to the mechanically dissociated cultures. Indeed, after a longer cultivation period, the trypsinized cell populations contained much more neuronal cells than the mechanically dissociated population.

Considering the results of the AChE histochemistry presented in figures 3 and 4, the nerve cells of the trypsinized cultures were heavily stained, and the fibres were assembled into fibre bundles. In the mechanically dissociated population, there were less neurons and their cell body, as well as their fibres, were less stained. The most striking differences could be seen on the plastic surface. These neurons were even more differentiated than the cells cultivated in similar conditions but derived from 7-day-old embryos.

From all our observations, it can be concluded that the ages of the embryo from which the brain cells originated, the dissociation procedures used, as well as the cultivation surfaces used, evoke differences in growth and differentiation of neuronal cells in vitro. Concerning the central nervous system of young embryos, the trypsinization technique gives results as good as the mechanical dissection, but for older embryos the enzymatic digestion seems to be better. On the contrary, for the peripheral nervous systems, the mechanical dissociation method has proved to be better[19]. Therefore, by using such culture systems, morphological studies in parallel to biochemical investigations seem to be of great importance.

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Presynaptic excitability decrease in the extensor group II afferent terminations

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Summary. The excitability of the extensor secondary afferent terminals is decreased by volleys applied to the flexor group II afferents. This presynaptic excitability decrease was completely abolished after bicuculline, indicating GABA may act as transmitter in this circuit.

The excitability of the presynaptic axon terminals of the primary afferent fibres is modulated by a presynaptic control mechanism. A presynaptic depolarizing action reduces the excitatory effectiveness of the action potential propagating towards the axon terminals. The resulting decrease in synaptic efficacy has been called presynaptic inhibition, which was subjected to a detailed analysis by Schmidt[1]. The principal findings are that group Ia and Ib terminals are depolarized by volleys in group I muscle afferents, and cutaneous afferents are generally depolarized by other cutaneous afferents. Concerning the high threshold muscle afferents, Eccles et al.[2] found that group II muscle afferents are generally depolarized by flexor reflex afferents. However, as will be seen in the present work, the presynaptic depolarization of the high threshold extensor afferent terminals may also be blocked by vol-