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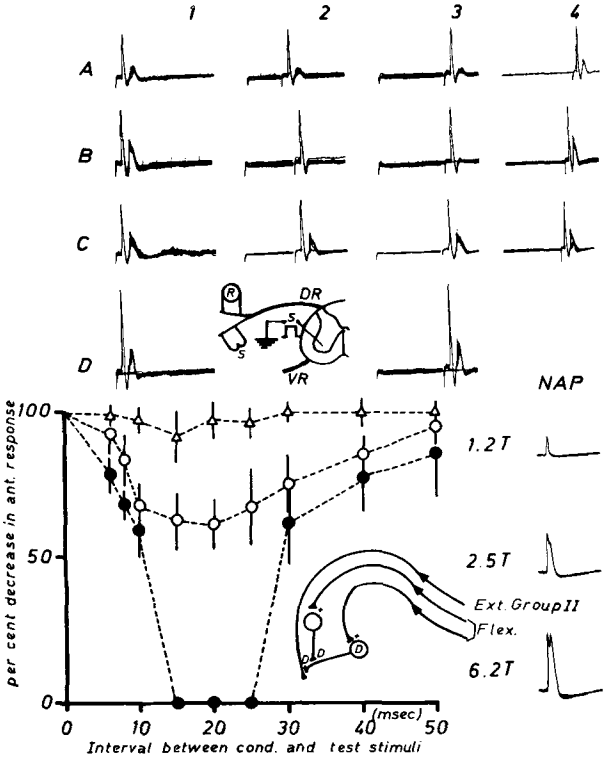
Summary. The excitability of the extensor secondary afferent terminals is decreased by volleys applied to the flexor group II afferents. This presynaptic excitability decrease was completely abolished after bicuculline, indicating GABA may act as transmitter in this circuit.

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-

leys in the antagonistic flexor reflex afferents, indicating facilitation versus inhibition.

Materials and methods. The experiments were performed on 6 decerebrate unanesthetized cats, which are also spinalized at the first lumbar level. After laminectomy extending from L_5 to S_1, the lumbar roots L_6, L_7 and S_1 were cut on the left side. The spinal cord was covered with warmed paraffin oil at 37 °C. The ipsilateral hind leg was denervated. The gastrocnemius (GS) and the peroneal nerves (PDP) were prepared for recording and stimulating, respectively. The animals were immobilized by i. v. injection of 3 mg/kg gallamine triethiodide. Respiration was maintained by a variable-stroke respirator. The arterial blood pressure was monitored continuously and was always above 70 mm Hg.

The excitability of the central terminals of the GS afferents was tested by using the Wall's method[3] (see figure, D_2): a stainless steel microelectrode having a tip diameter of 2 μm was inserted into the ventral horn of L_7 near the GS motor nucleus where the largest antidromic group I action potential could be recorded from the peripheral ends of the GS nerves during intraspinal stimulation with 0.2 msec current pulses every 5 sec. Group II antidromic GS action potentials were produced by increasing the intraspinal stimulus intensity up to the supramaximal values for the group I GS afferents. A conditioning single shock stimulus was applied to the PDP nerve in order to reveal excitability changes in the GS afferent terminals stimulated antidromically by the microelectrode (test shock). The incoming conditioning volley was recorded as a PDP afferent nerve action potential from a thin dorsal root filament. The intensity of the test stimulus was kept constant throughout the experiment.



Presynaptic excitability decrease in GS group II afferent terminals. D_2: experimental arrangement; DR, dorsal root; VR, ventral root L_7; R, recording electrode on GS nerve; S, stimulating electrode on PDP nerve; S (⊓), stimulating microelectrode. Original records (10 traces superposed): Group I and II GS action potentials recorded antidromically. The conditioning-test intervals 0, 15, 22 and 50 msec in 1, 2, 3 and 4. The conditioning stimulus intensity is 1.2 T in D, 2.5 T in A, and 6.2 T in B. C: as in B but 6 min after injection of 4 mg/kg bicuculline. Time: 5 msec in records 1, 2 and 3; 10 msec in 4 (squares). NAP: afferent nerve action potentials recorded from PDP-dorsal root filament; time 4 msec for each square. The diagram shows the per cent excitability decrease in the antidromic test response. The intensities of the conditioning stimuli: 2.5 T (○), 6.0 T (●). (△) 6 min after bicuculline. Vertical lines plus minus standard deviations. Inset: the proposed neuronal connections; +, excitation; D, flexor interneuron depolarizing extensor group II axon terminal; solid interneuron excited by flexor afferents depolarizes the axon terminal of the internuncial D-cell; D indicates axo-axonal depolarization.

Results and discussion. In the figure, the intraspinal test shock produced group I and II action potentials recorded from the GS nerves (D_1). The condition velocity of the group I fibres was 93.3 m/sec, and that of the group II fibres 61.2 m/sec. When the test shock was preceeded by a single conditioning stimulus of 1.2 threshold (T) applied to the PDP nerve at intervals of 0, 15, 22 and 50 msec, the excitability of the group I and II afferent terminals remained unchanged (D_1: 0 msec; D_3: 22 msec delay). As intensity of the conditioning stimulus was made 2.5 T, the group I component of the PDP action potential increased and smaller afferents were recruited as seen in neurogram (NAP: 1.2 T and 2.5 T). When these afferents were stimulated, the conditioning stimulus caused a decrease in the area of the antidromic group II action potential. This effect began at 5 msec conditioning-test interval (A_1: 0 msec), increased at 15 and 22 msec intervals ($A_{2,3}$) and disappeared at 50 msec interval (A_4). The diminution of the antidromic group II action potential became most prominent, as the intensity of the conditioning stimulus was made 6.2 T (NAP: 6.2 T). This stage of the experiment is shown in B. In the control, the conditioning and antidromic test stimuli were given simultaneously (B_1). At 15 and 22 msec conditioning-test intervals ($B_{2,3}$), the group II test response disappeared completely. At 50 msec delay the test response was identical with the control (B_4). As seen in C, the i.v. injection of 4 mg/kg bicuculline abolished the effect of the conditioning stimulus applied to the PDP nerve at 6.2 T.

The diagram in the figure summarizes the results of 6 experiments. The area of the group II test responses was measured planimetrically, and that at 0 msec conditioning-test interval was taken as 100 (ordinate). When the medium threshold (○: 2.5 T) or the high threshold (●: 6.0 T) PDP afferents were stimulated, the test response usually began to decrease at about 5 msec conditioning-test interval, became smallest and even disappeared at 15–35 msec intervals, and approached gradually to its control value at about 50 msec interval. The i.v. injection of 4 mg/kg bicuculline abolished completely the depressing effect of the conditioning stimulus on the test response (△).

As seen above, the high threshold PDP afferents may decrease the excitability of the group II GS afferent terminals. This phenomenon may have been produced by a presynaptic mechanism, since the latency for the excitability decrease of the GS secondary afferents was 5 msec, and the excitability decrease became most effective at 15–35 msec conditioning-test intervals. These are well-

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known properties of presynaptic inhibition. Mendell[4] reported the occurrence of positive dorsal root potentials induced by stimulation of group III muscle afferents and suggested that positive dorsal root potentials may be the result of inhibition of a tonic depolarizing pathway. Similarly one can propose that the excitability decrease in the GS secondaries may result from the inhibition of the internuncial D-cells depolarizing the intraspinal terminals of the GS group II afferents. GABA may act as neurotransmitter in this presynaptic disinhibitory circuit, since the GABA-antagonist bicuculline[5] antagonized the depressing effect of the conditioning PDP volleys on the GS secondary afferent excitability.

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Adrenal responses to hypoxia and hypercapnia in the young calf[1]

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Summary. Quantitative analysis of adrenal responses to moderate hypoxia and hypercapnia in the conscious calf shows that the sensitivity of the adrenal cortical response far exceeds that of the adrenal medulla.

Adrenal responses to hypoxia and hypercapnia have been quantified in conscious calves, 3–5 weeks after birth, using the 'adrenal clamp' technique[3] to collect the effluent venous blood from the right adrenal gland. After recovery from surgery the animals were habituated to wear a light, transparent 'helmet' through which air was perfused at a rate of 15 l/min. Hypoxia or hypercapnia was induced by infusing a mixture of gases through the 'helmet' for 30 min as follows:

		Air (l/min)	Nitrogen (l/min)	Carbon dioxide (l/min)
Hypoxia	Grade I (n = 7)	7.5	7.5	–
	Grade II (n = 4)	11.5	3.5	–
Hypercapnia	Grade I (n = 7)	13.5	–	1.5
	Grade II (n = 6)	14.25	–	0.75

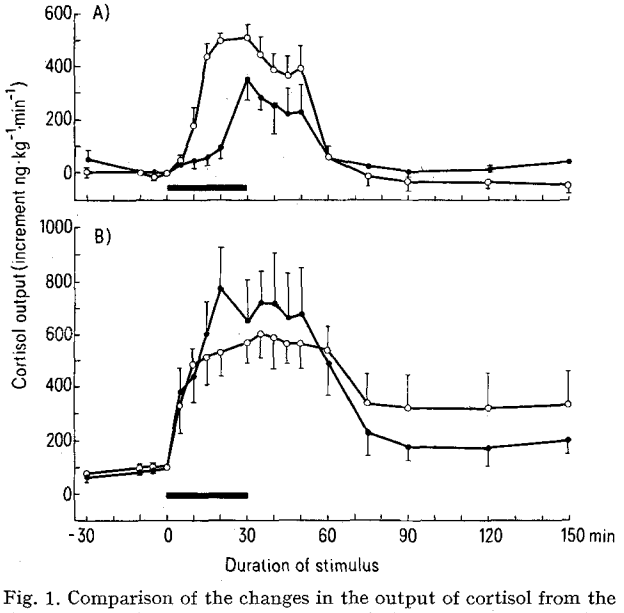

Fig. 1. Comparison of the changes in the output of cortisol from the right adrenal gland in response to hypoxia (A) and hypercapnia (B). Open circles: Grade I stimuli. Closed circles: Grade II stimuli. Horizontal bars: duration of stimulus. Vertical bars: SE of each mean value.

Cortisol and corticosterone were measured by competitive protein binding[4] and catecholamines by a modification of the trihydroxyindole method[5]. Adrenal blood flow was determined gravimetrically during each sample to allow calculation of hormone output.

During grade I hypoxia the P_{O_2} of the arterial blood fell to less than half the initial value within 5 min. Thereafter it declined more slowly to between 20 and 25 mm Hg during the last 10 min. Grade II hypoxia constituted a less severe stimulus; arterial P_{O_2} was reduced by c60% but did not fall below 30 mm Hg at any stage. Grade I hypercapnia caused a progressive increase in arterial P_{CO_2} from c40 mm Hg to maximum values of between

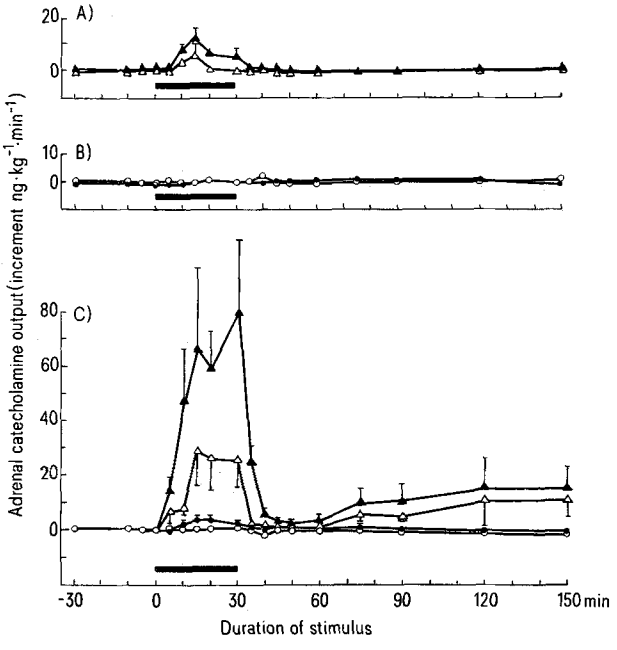

Fig. 2. Comparison of the changes in catecholamine output from the right adrenal gland in response to hypoxia or hypercapnia.
A Grade I hypoxia. ▲ = noradrenaline; △ = adrenaline. *B* Grade II hypoxia. ● = noradrenaline; ○ = adrenaline. *C* Hypercapnia. ▲ = noradrenaline, grade I; △ = adrenaline, grade I; ● = noradrenaline, grade II; ○ = adrenaline, grade II.
Horizontal bars: duration of stimulus. Vertical bars: SE of each mean value.