lar weight compared with controls (table 1). Similar deficits in body and brain weights in previously undernourished rats have been described previously[11].

There were no statistically significant differences in the numerical densities of neurons, synapses, nor in the synapse-to-neuron ratios between control and previously undernourished rats in any of the brain regions studied (table 2). Our measurements do not preclude the possible existence of persisting deficits in the synapse-to-neuron ratio, which although less than the current limits of experimental error, could nevertheless be of biological importance.

Turning to a comparison of the present data with that in our previous paper[6], a much larger number of synapses per neuron appears to have been developed in the frontal cortex by 30 days of age[6] than persists into adult life, when only about half the number formerly present are found.

The present experiments do not give any information on the total number of neurons or synapses in the brain regions studied. It may be possible to have significant deficits in the total number of these components and yet have similar synapse-to-neuron ratios. Although it would be desirable to obtain estimates of the total nerve cell number (and/or total synapse number) in whole brains or whole brain regions, dependable and simple histological methods for doing so do not as yet exist, due to the brain's extreme structural heterogeneity.

In conclusion, early undernutrition produces a well documented series of distortions of brain structure and function, including large deficits in the number of synapses per neuron in certain layers of the frontal, visual and cerebellar cortex. Many of these effects are permanent; however the present experiments show that the deficits in the synapse-to-neuron ratio are at least partially reversible. Whether this was primarily due to the nutritional rehabilitation, or whether it would still have occurred in the presence of continuing undernutrition remains uncertain but it may well be that a return to normal numbers may demand the provision of an optimum nutritional environment, as occurred in these experiments. The functional consequences of the initial deficit and the subsequent catch-up must, however remain an open question.

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Effects of intracellular or extracellular application of tetraethylammonium on the action potential in cultured chick embryonic heart muscle cell

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Summary. The effects of tetraethylammonium (TEA) on the action potential in cultured chick embryonic heart muscle cells were investigated. The onset of prolongation of the action potential occurred within 10 min following intracellular iontophoretic application of TEA, but after more than 50 min following extracellular application. These facts suggest that the major site of action of TEA is on the inner surface of the membrane in these cells.

It is well known that tetraethylammonium (TEA) prolongs the action potential in various excitable cells by decreasing the potassium outward current (squid giant axon[1], Ranvier node[2], barnacle muscle[3] and smooth muscle[4]). In squid giant axon, TEA prolongs the action potential when it is injected into the axon, whereas external application has no effect. In the Ranvier node of the myelinated nerve, however, the site of action of TEA is both on the inside and on the outside of the membrane. In myocardial cells, Ochi and Nishie have successfully applied TEA to the inside of the membrane from a cut-end of ventricular muscle and have shown that the site of action of TEA is on the inside of the membrane[5]. Considering the difference in the site of action of TEA in different cells, we examined the effect of TEA applied internally or externally in cultured chick embryonic heart muscle cells.

Material and methods. The tissue culture techniques used in the present experiments were similar to those reported previously by many workers[6,7]. In brief, hearts of 5–7-day-old chick embryos were dispersed by 0.2% trypsin in a Ca-Mg free solution. The incubation medium consisted of Eagle's solution containing 10% embryo extract and 10% horse serum. The dispersed cells usually assembled into monolayer groups, the rosette-like aggregates of cells growing to 200–300 μm in diameter in the course of 5–10 days. During the experiment, the culture medium was replaced by a bathing solution having an ionic composition of (in mM): 149.3 Na^+, 2.7 K^+, 1.8 Ca^{++}, 1.0 Mg^{++}, 145.3 Cl^-, 11.9 HCO_3^-, and 0.42 $H_2PO_4^-$. The pH of the ungassed solution was 7.9 and was constant in each experiment lasting a few hours. The bathing solution was stagnant and less than 2 mm deep. A hot water perfusion bath was used to maintain the temperature of the culture dish (Corning tissue culture dish) at 36 °C. These conditions were essentially the same as those reported by Sperelakis and Lehmkuhl[8]. Under these conditions, muscle cells contracted rhythmically, and the rate of firing and also the shape of the action potentials remained unchanged during several hours. Transmembrane potentials were recorded through a conventional glass microelectrode with a resistance of

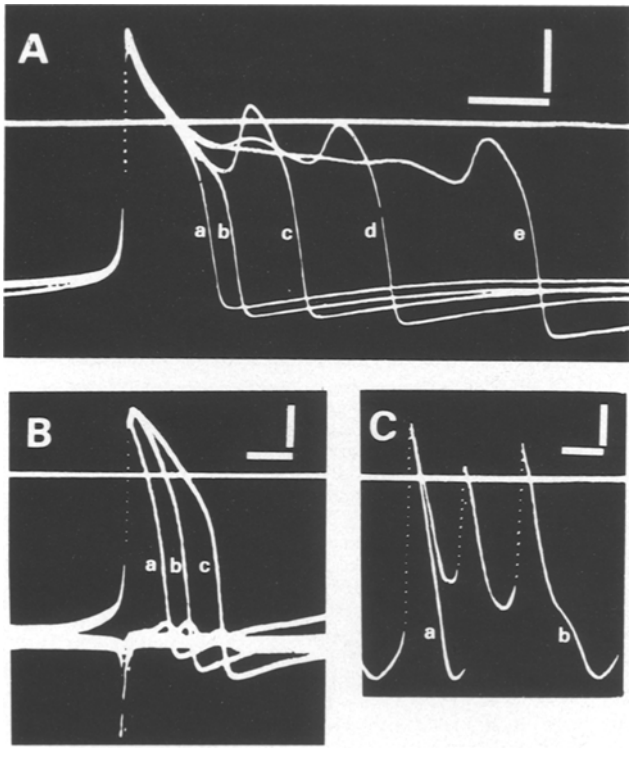

Fig. 1. Effects of external and internal application of TEA on the spontaneous action potential. Action potentials were superimposed photographically. *A* External application of TEA (20 mM). *a*, Control; *b*, *c*, *d* and *e*, after 60 min, 95 min, 100 min, 120 min, respectively. *B* and *C* Internal application of TEA (injected into the cell iontophoretically by a steady current of 1 nA). *B* and *C* show prolongation of action potentials with (C) without (B) accompanied action potential upstrokes. The lower trace in B indicates the time derivative (dv/dt) of the membrane potential. *B*, *a*, Control; *b* and *c*, 6 min and 20 min after injection, respectively. *C*, *a*, Control; *b*, 8 min after injection. Calibration: vertical bars, 20 mV, 2.5 V/sec; horizontal bars, 200 msec. Upper horizontal lines indicate the zero membrane potential levels.

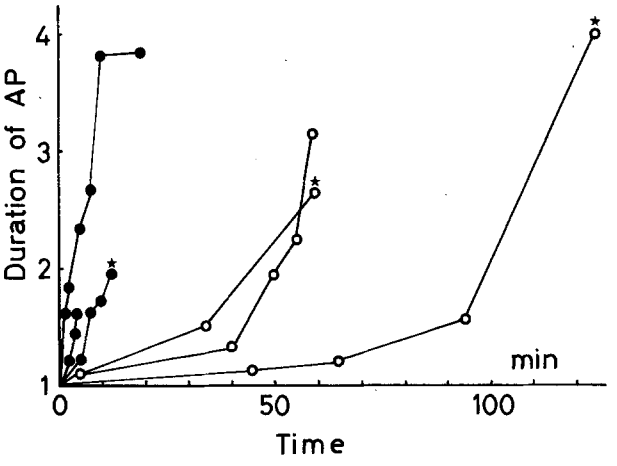

Fig. 2. Time course of the prolongation effect of TEA. Ordinate: duration of 50% repolarization of the action potential after TEA application relative to that before application. External application of TEA (20 mM); internal application. Stars indicate the appearance of an oscillatory potential or action potential upstroke in the repolarization phase.

40–70 MΩ. Electrodes were filled with 3 M KCl (KCl electrode) or, in the case of intracellular application of TEA, with 2.5 M KCl and 500 mM TEA (TEA electrode).

In order to inject TEA into the cell, the TEA electrode was connected to a Model 707 bridge amplifier (W-P Instrument, Inc.) and a steady 1 nA cationic current was passed for a period of 2–20 min, by the method reported previously[9,10]. In the case of external application of TEA, after recording during a control period, a small volume of TEA-Cl solution was introduced into the test dish with a syringe, and the mixture was adjusted to a final concentration of 20 mM.

Results and discussion. The spontaneous action potentials recorded before application of TEA are shown in figure 1 (A-a, B-a, C-a). The mean duration of the action potential at a 50% repolarization level was 115.8±48.0 msec (n=30) with a range of 66–190 msec. Figure 1,A, shows the changes of the action potential in a bathing solution containing 20 mM TEA. Exposure to TEA did not bring about any detectable change in the action potentials within 30 min. After 60 min exposure, the duration of the action potentials increased progressively (figure 1,A-b). Further exposure resulted in the appearance of an oscillatory potential in the prolonged repolarization phase (figure 1,A-c, -d, -e). In some cells, similar effects were observed at low concentrations of TEA (6 mM). When TEA was injected into the cell by a steady cationic current of 1 nA through a TEA electrode, the duration of the action potential increased within 6–20 min (figure 1,B-b, -c). In some cells, the prolonged repolarization was accompanied with a few action potential upstrokes (figure 1,C-b). No significant changes in action potentials were elicited by electrical current applied through a KCl electrode, with a strength and duration sufficient to cause prolongation of the action potential when a TEA electrode was used. In figure 2, the prolongation effects of TEA are plotted against time. When TEA was applied internally, marked prolongation of the action potential at 50% repolarization was observed within 10 min. However, when TEA was applied externally, more than 50 min were required to develop a similar prolongation.

The differences in the time course of TEA effects between internal and external application are explicable if it is assumed that in cultured chick embryonic heart muscle cells, the site of action of TEA is on the inner surface of the cell membrane, as in squid giant axon[1], barnacle muscle[3], and mammalian ventricular muscle[5]. Furthermore it seems likely, in the light of the results of external application, that TEA can permeate the cell membrane in these cells. In conclusion, we have found that the major site of action of TEA in cultured chick embryonic heart muscle cells is on the inner surface of the cell membrane.

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