The Qualitative Effect of Anesthetic Gases on Spontaneous Potentials from Explants of Brain Tissue in Culture[1]

The spontaneity, duration, form, and repetitive pattern of the potentials arising spontaneously from adult and embryonic tissue explants of brain tissue in culture[2] are different from the multitude of previous reports of evoked and 'spontaneous' potential from cerebral tissues. These differences raise the question as to the cells of origin of the potentials arising spontaneously from explants of brain tissue in culture. By both light and electron microscopy, the explants giving rise to spontaneous potentials in culture have always contained 'normal' neuronal cells as well as the other elements usually found in nervous tissues. However the mere physical presence of neuronal cells in the explants does not necessarily mean that the potentials arose from these cells. In addition to the histological evidence, other experimental approaches have been used to try to determine the properties and hence the nature of the cells responsible for the spontaneous potentials. The study reported here concerns the general qualitative response of the spontaneous potentials from explants of the telencephalon of 14 day chick embryos to exposure to anesthetic gases.

The explants were made in the usual way onto the upper aspect of a piece of cellulose sponge on a coverglass in such a way as to lie between the sponge and a 36 gauge platinum electrode. The reference electrode (also of 36 gauge platinum wire) was cemented onto the side of a Kahn tube into which the coverglass with the sponge, explant, and electrode were placed. A supernatant made from balanced salt solution TDL1[3] and 0.25% human serum protein at 37°C was added to the Kahn tube in such a way as to come half way up the cellulose sponge and immerse the lower end of the reference electrode but not to touch the explant itself (except insofar as the supernatant permeated the cellulose sponge). The Kahn tube was sealed with a

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[2] A. W. B. Cunningham and B. J. Rylander, J. Neurophysiol. *24*, 141 (1961).

[3] A. W. B. Cunningham, M. Dougherty, and B. J. Rylander, Nature (Lond.) *186*, 477 (1960).

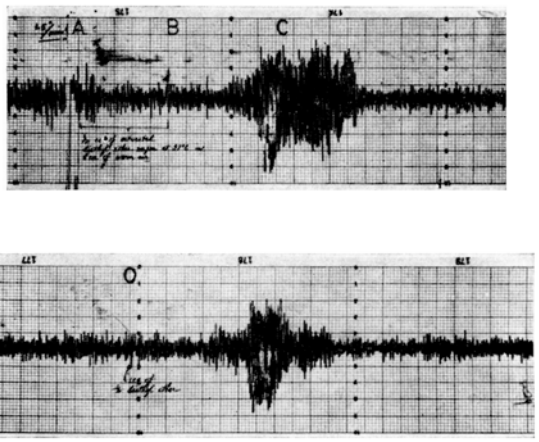

Fig. 1. The excitatory effect of small doses of anesthetic (1/10 cm³ of air saturated with di-ethyl ether vapor in 8 cm³ warm air injected into 7 cm³ Kahn tube) on the spontaneous potentials from explants of chick embryo telencephalon. Lower trace is a direct continuation of the upper one. From A to B – slow injection of anesthetic vapor. C start of excitatory effect. O – second injection of anesthetic vapor ($^1/_2$ cm³ of air saturated with di-ethyl ether vapor injected into 7 cm³ Kahn tube). X axis – each division equals 4 sec. Y axis – eight large divisions equals 30 μV.

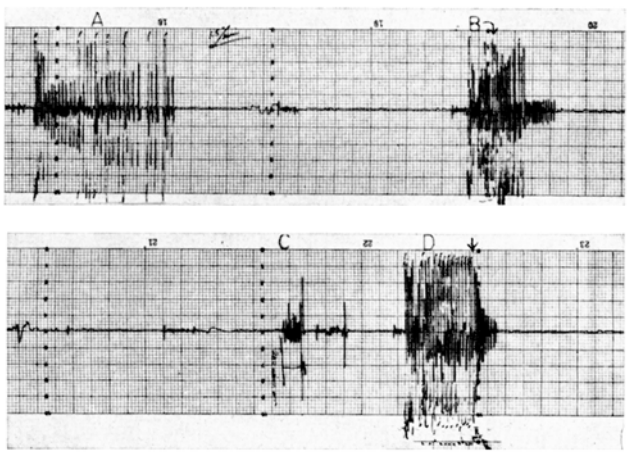

Fig. 3. The effect of di-ethyl ether vapor on the spontaneous potentials from explants of chick embryo telencephalon. Lower trace is the direct continuation of the upper one. A – normal series of potentials, B – 1 cm³ of $^1/_5$ di-ethyl ether vapor (air saturated with anesthetic vapor at 37°C diluted with more air) administered at the arrow of this series, C – point at which di-ethyl ether vapor displaced by warm air, D series of potentials still showing some 'excitement'. At the end of this series (indicated by arrow) 1 cm³ $^1/_2$ di-ethyl ether vapor was administered. X axis – each division equals 4 sec. Y axis – eight large divisions equals 30 μV.

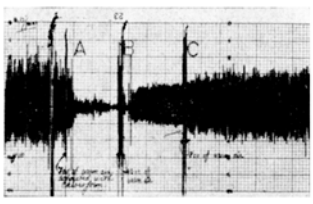

Fig. 2. The effect of chloroform vapor (1 cm³ of air saturated with chloroform vapor at 37°C injected into 7 cm³ Kahn tube) on spontaneous potentials from an explant of chick embryo telencephalon. A – point of injection of chloroform vapor. B and C – points of injection of warm air to 'wash out' chloroform vapor. X axis – each division equals 8 sec. Y axis – eight large divisions equals 30 μV.

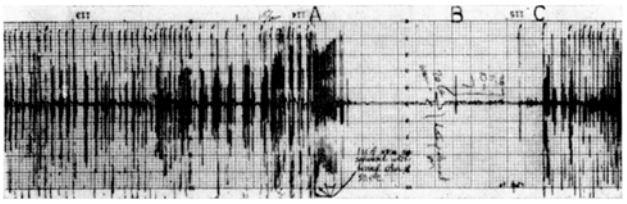

Fig. 4. The effects of di-vinyl ether vapor (1 cm³ of air saturated with di-vinyl ether vapor at 37°C injected into 7 cm³ Kahn tube) on spontaneous potentials from explants of chick telencephalon. A – point of administration of anesthetic vapor, B – start of injection of warm air to displace anesthetic vapor, C – recommencement of spontaneous potentials. X axis – each division equals 4 sec. Y axis – eight large divisions equals 30 μV.

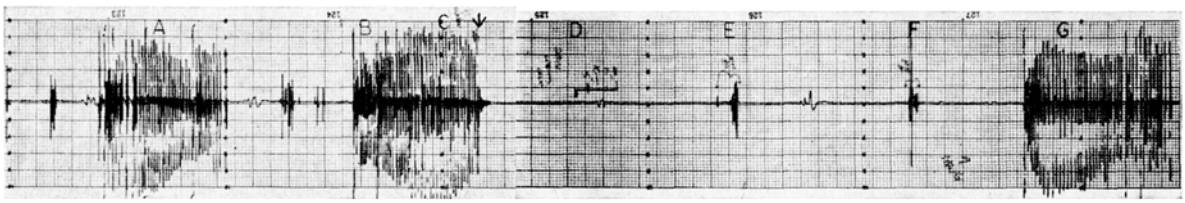

Fig. 5. The effect of halothane vapor on spontaneous potentials from an explant of chick embryo telencephalic tissue in culture. The lower trace is a direct continuation of the upper trace. A and B are normal series of potentials. At the arrow at C, a dose of halothane vapor (1 cm^3 air saturated with halothane vapor at 37°C) is administered and then washed out repeatedly with warm air at D, E, and F. G is the point of return of the spontaneous potentials. X axis — each division equals 1 sec. Y axis — eight large divisions equals 30 μV.

serum stopper containing an air filter (to prevent any change in the pressure inside the Kahn tube) and a length of Teflon tubing to allow the introduction of the anesthetic gases. The stopper was inserted so as to allow the egress of the two platinum electrodes. The Kahn tube was placed in a shielded incubator at 37°C and connected by shielded cables to amplifiers and a paper strip recorder.

An injection of a volume of warm air similar to that already in the Kahn tube caused only a transient incidental electrical disturbance as can be seen at the time of injection in any of the illustrations. The anesthetic gas to be used was firstly carefully warmed to 37°C before injection by syringe into the outer end of a piece of Teflon tubing. The anesthetics used were: Chloroform (Figure 2), di-ethyl ether (Figure 3), divinyl ether (Figure 4), and Halothane (Figure 5). Each of the anesthetics had the same general effect. They caused an initial short period of increased frequency of potential production—a stage of 'excitement' which is best seen in Figure 1 where low concentrations of anesthetics were used. It is also visible in the traces seen after the introduction of some of the anesthetics (Figure 3 and 4). After this excitement phase, the potentials were diminished or suppressed by the presence of anesthetic.

This effect can be reversed if the anesthetic is washed out with warm air or when a low concentration of anesthetic is used the explant will slowly recover activity with the passage of time even if the anesthetic is not physically washed out. High concentrations of anesthetic will cause permanent suppression of the potentials if the anesthetic is not removed rapidly by the immediate and repeated replacement by warm air. After a dose of anesthetic had been washed out with warm air, the explant was then susceptible to further doses of the same or other anesthetics.

The increase in frequency of potential production or 'excitement phase' is of interest. It is notable that in spite of the second dose of anesthetic being five times that of the first (Figure 1) the interval between the end of injection and onset of excitation is 80 sec in each case. Possibly this is because the vapor had to penetrate through the same thickness of explant to reach the active focus in each occasion. Unless an inhibitory effect by some of the cells in the explant is present and removed by anesthetic action, this 'excitement phase' must be due to direct stimulation by the anesthetic. This suggests that the excitement stage encountered in humans and animals during induction with anesthetics may also be due to direct stimulation of cortical cells and not to paralysis of inhibitory action.

Zusammenfassung. Die spontanen Potentiale von Telencephalon-Explantaten *in vivo* werden durch anästhetische Gase nach anfänglichem Potentialanstieg vermindert bzw. gehemmt. Die Potentiale kommen in ihrer ursprünglichen Form nach Entfernung des Betäubungsmittels mit warmer Luft wieder zurück, und die Explantate sind dann wieder durch dasselbe oder andere Betäubungsmittel beeinflussbar.

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The Effect of Puromycin on Protein Metabolism and Cell Division in Fertilized Sea Urchin Eggs[1]

It has recently been shown that puromycin is a specific inhibitor of protein metabolism at the S-RNA-ribosome level[2,3]. Preliminary experiments on the effects of this drug on early sea urchin development indicated that it inhibits the mitotic activity of fertilized eggs in a very characteristic way. When puromycin was added to the eggs some minutes before fertilization at concentrations above $10^{-4}M$, activation and early development proceeded in a seemingly normal way until the 'clear streak' stage, but then development came to a standstill. The 'clear streak' attained a rigid appearance with abnormally sharp borders. The nucleus gradually swelled, but no spindle was observed and no cell divisions occurred. The present report deals with an attempt to correlate this antimitotic effect of puromycin with its inhibitory action on the incorporation of labeled amino acids into protein by the same eggs, *in vivo* and *in vitro*.

Experimental. Puromycin (Lederle Laboratories Division) at varied concentrations was added to unfertilized eggs of the sea urchin *Paracentrotus lividus* (LM), 10 min before fertilization. At various intervals after fertilization, equal egg samples (approximately 10 mg protein) were withdrawn from the egg suspensions. After sedimentation of the eggs, the volume of the medium was reduced to 4 ml, and 75 mμM of ^{14}C-*L*-valine (6.53 mc/mM) was

[1] Supported by a grant from the Swedish Natural Science Research Council. Experiments carried out at the Zoological Station, Naples (Italy).

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[3] A. VON DER DECKEN and T. HULTIN, Biochim. biophys. Acta *45*, 139 (1960).