would have to be less stable to hypo-osmotic conditions than to iso-osmotic conditions and be sensitive to acid and Ca\(^{2+}\). The dual-population hypothesis demands therefore that exactly opposite properties be ascribed to the two proposed populations of vesicles. The opposite sensitivities to differing osmolarities are particularly contradictory, and we find the dual-population hypothesis implausible for these reasons.

If the loosely bound acetylcholine is not in a different population of vesicles it must reside within the same vesicles as does the tightly bound acetylcholine but be bound in a different way. It could be bound near to the surface of the vesicle, its lability being simply a reflection of how near the surface it is. Alternatively it could be disposed throughout the vesicle but not be as tightly bound as the less-recently synthesized acetylcholine. In either case the coupling of the exchanges can be readily explained by the necessity for tightly bound acetylcholine to be lost in order to vacate sites for occupation by the loosely bound acetylcholine.

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**The Role of 4-Aminobutyrate in the Lower Auditory System of the Guinea Pig**

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The lower auditory system of the mammal has an abundance of inhibitory processes (Whitfield, 1967). These include centrifugal inhibition as well as several different types of centripetal inhibition, e.g. tonal inhibition of spontaneous activity, two-tone inhibition and mutual inhibition (Galambos & Davis, 1944; Katsuki *et al.*, 1959; Whitfield, 1955). The identity of the chemical mediators of these inhibitory processes, particularly the centripetal ones, remains completely unknown, either in the cochlear nucleus or in the nuclei of the higher brain stem. 4-Aminobutyrate when applied to single neurons has a powerful inhibitory effect on cells in the cochlear nucleus (Whitfield & Comis, 1966). With this in mind, the role of 4-aminobutyrate in the lower auditory system, and in particular the cochlear nucleus, was investigated by making lesions centrally and peripherally to the cochlear nucleus followed by histochemical analyses for 4-aminobutyrate transaminase activity 60–113 days later.

Lesions peripheral to the cochlear nucleus were made in the guinea pig by unilateral fracture of the cochlea soon after birth. At various times (60–113 days) after surgery the animals were killed under urethane anaesthesia by perfusion with a saline-glucose mixture and the brain stem of each was rapidly removed and plunged into a light petroleum–solid CO\(_2\) mixture. Frozen sections (8–15 \(\mu\)m) were cut and stained for 4-aminobutyrate transaminase activity by the method of Van Gelder (1965). The method is based on the 4-aminobutyrate-dependent reduction of a tetrazolium salt to insoluble formazan.
Central lesions were made in the superior olivary complex under Nembutal anaesthesia. The lesions were made unilaterally by drilling a small hole 3 mm deep in the basi-sphenoid, 2.5 mm rostral and 2.0 mm lateral to the midline of the ventral edge of the foramen magnum. The animals were killed after a similar length of time to the animals with peripheral lesions and the 4-aminobutyrate transaminase activity was observed in the brain-stem area.

Histochemical demonstration of 4-aminobutyrate transaminase in the normal guinea-pig cochlear nucleus shows two distinct kinds of activities, one diffusely distributed all over the nucleus and one concentrated in certain neurons, the ventral cochlear nucleus being particularly rich in such neurons. Of the nine types of neurons described by Osen (1969) in the cat cochlear nucleus, those that were identified as having appreciable 4-aminobutyrate transaminase activity included the giant cells in the posterior ventral cochlear nucleus and the globular and multipolar cells of the anterior ventral cochlear nucleus. The pyramidal cells of the dorsal cochlear nucleus on the other hand appeared to be particularly poor in 4-aminobutyrate transaminase activity. The deeply staining neurons of the ventral cochlear nucleus, although smaller, are very similar in appearance to cells of Deiter's nucleus (often seen in the same sections). The latter are known to have an inhibitory input from the cerebellar Purkinje cells (Ito et al., 1966), and, further, 4-aminobutyrate has been strongly implicated to be the inhibitory transmitter at this site (Obata et al., 1967).

These two distinct types of 4-aminobutyrate transaminase activity, one diffusely distributed all over the tissue, the other associated with particular neurons, could possibly represent, respectively, transaminase activity functional in the mitochondrial 4-aminobutyrate shunt and transaminase activity associated with neurons receiving an inhibitory input of 4-aminobutyrate. These different transaminase activities could also represent different isoenzymes known to be present in brain (Salganicoff & De Robertis, 1965).

In the guinea pigs with peripheral lesions there was an almost complete disappearance of neurons staining for 4-aminobutyrate transaminase from the cochlear nucleus on the operated side. As the transaminase activity of these neurons is found in the perikarya of the cells, its disappearance is almost certainly a trans-synaptic phenomenon. Whether its disappearance is accompanied by complete degeneration of the cells or not is as yet uncertain. Guinea pigs with lesions of the superior olivary nuclei, however, showed no comparable change in the appearance of neurons staining for 4-aminobutyrate transaminase in the two cochlear nuclei.

These findings are consistent with the idea that, if 4-aminobutyrate is functional as an inhibitory transmitter in the cochlear nucleus, then it is more likely to act as a mediator of activity arising from the cochlea than of centrifugal activity arising from or passing through the superior olive. This does not, however, preclude it from being a transmitter in centrifugal pathways returning to the cochlear nucleus from other higher centres. This is not to suggest that 4-aminobutyrate is a primary transmitter of axon endings derived directly from the spiral ganglion cells of the cochlea. Rather it is suggestive that 4-aminobutyrate is functional in the local, inter-neuron-mediated, inhibition of the cochlear nucleus. Indeed, preliminary measurements of the activities of cochlear-nucleus glutamate decarboxylase after death of the spiral ganglion suggest that very few, if any, ascending fibres synapsing in the cochlear nucleus that use 4-aminobutyrate as their primary synaptic transmitter.

The suggestion that 4-aminobutyrate is functional only as the transmitter of short axon inhibitory loops exerting only local action may also explain the inconsistent reports on the ability of picotoxin and bicuculline to block two-tone inhibition in the cat cochlear nucleus (Watanabe, 1971; Whitfield & Comis, 1966; W. E. Davies & S. D. Comis, unpublished work). The involvement of 4-aminobutyrate in local inhibition in the cochlear nucleus may be comparable with such a system as that described by Storm-Mathisen (1972), who showed that deafferentiation of the hippocampus led to no apparent change in the activity of glutamate decarboxylase, and suggested that the latter was a component mainly of the intrinsic basket cells.
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Development of Respiration in Fresh and Cultivated Brain-Cortex Cells

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Previous studies have shown that cultivated dissociated cells from the cerebral cortex differentiate into neurons. Morphological as well as histochemical differentiation were enhanced by the presence of brain extract (Sensenbrenner et al., 1972). The present report is a study of the O2 consumption of cells from the cerebral cortex of the chick embryo during maturation in vivo and in culture.

Fresh cells were obtained from 7- or 13-day-old embryos. Preliminary experiments indicated no difference in the rate of O2 uptake by sieved cells (pore size 50 μm) and clumps of cells. Accordingly clumps of cells were used for most of the experiments.

 Cultures were prepared from 6-day-old embryos. The cerebral hemispheres were dissociated and the cells were grown on collagen without plasma in Rose (1954) chambers as described by Sensenbrenner et al. (1971a) in a slightly modified (Booher et al., 1971a) Eagle’s (1959) minimum essential medium supplemented with 20% (v/v) of foetal calf serum (Flow Laboratories, Irvine, Ayrshire, U.K.). The medium was changed at intervals of 1–2 days. In some cultures (marked ⋄ in Fig. 1) an extract prepared from the brains of 10-day-old chick embryos (Sensenbrenner et al., 1971b; Springer et al., 1971) were added to the medium (10%, v/v) from the fourth day of culture and onwards. After various cultivation periods the chambers were opened and the cell clumps were carefully loosened under a phase-contrast microscope (Leitz Laborlux) and introduced into an ampulla-type Cartesian diver (Zeuthen, 1953) as described by Booher et al. (1971b). The incubation medium in the diver contained 150 mm-NaCl, 3.0 mm-KCl, 8.0 mm-Na2HPO4, 1.7 mm-KH2PO4, 1.0 mm-CaCl2, 0.6 mm-MgCl2 and 7 mm-glucose, pH 7.2–7.4, and the temperature was 37°C.

The clumps of both fresh and cultivated cells contained in general 30–100 cells, which on the one hand gave a suitable high rate of O2 consumption/diver and on the other permitted relatively accurate and easy counting of the number of cells. Cells were counted before and after the metabolic experiment (4 h), and in general no significant changes in cell appearance or cell number were noticed. In some cases the cells did, however, show a markedly deteriorated morphology after the measurement (Nissen et al., 1973), and the results of such experiments were discarded.

The cells were found to respire at an almost constant rate during the 4 h of the measurement. Because of the increased instability of Cartesian divers during the first