CHOLINERGIC DEVELOPMENT IN CHICK BRAIN REAGGREGATED CELL CULTURES

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Reaggregated cell cultures from dissociated 7-day-old chick embryo whole brains were prepared, and the developmental profiles of acetylcholinesterase and choline acetyltransferase, in the aggregates, determined over a 30-day period. Enzyme activities in vitro, at different times of culture, typically lie between 30 and 60% of the values obtained for embryos or chicks of the same developmental age, up to day-10 posthatching. The increase in acetylcholinesterase activity over a 24-day period of culture/incubation is fourfold in the aggregates vs. sixfold for embryos, while the choline acetyltransferase values increase, during the same period of time, 32-fold in the aggregates vs. 17-fold in vivo. Choline acetyltransferase activity seems to be more dependent on good cell-to-cell contact than acetylcholinesterase activity. On the other hand, morphological studies on the aggregates with light and electron microscopy reveal a number of structural features characteristic of well-developed nervous tissue. It is suggested that aggregate cultures of chick brain cells are an adequate model system that is especially useful in analyzing developmental phenomena requiring free tridimensional interaction.

INTRODUCTION

The search for a convenient system to study the temporal correlation between different parameters currently used to measure brain development and differentiation has led neurobiologists to examine a number of in vitro preparations in which a more or less faithful imitation of the main maturation events, as they are seen in vivo, is associated with a

Abbreviations: ACHE, acetylcholinesterase; ChAT, choline acetyltransferase; BW284 C51 dibromide, 1,5-bis-(4-allyldimethylammoniumphenyl)pentan-3-one dibromide; ACh, acetylcholine.
variable ease of maintenance, long-term survival, and accessibility to manipulation (1). Of these model systems, reaggregating cultures seem to fulfill many of the necessary requirements (2).

The aim of the present and future studies is to carry out a reassessment of the possibilities of reaggregated cell cultures and, at the same time, to gather more data on some critical aspects of nervous tissue development. Although the mouse has been the experimental animal of choice in a number of previous reports (2), the chick was chosen for these studies for a number of reasons: the possibility of starting the developmental process at a precise and predetermined time, with the associated advantages of synchronization and reproducibility for a large amount of embryos; the abundance of data on normal embryonic development in vivo through a series of well-defined developmental stages; and the widespread use of some chick embryo neural structures (i.e., optic tectum and retina) in recent studies on cellular recognition and interaction (3–6). Very recently, Schmidt (7) has used this system to study sulfatide synthesis during myelination.

EXPERIMENTAL PROCEDURE

Preparation of Reaggregating Chick Brain Cell Cultures

White-Leghorn fertile eggs from a single local source were used throughout this study. They were incubated in a Brower-Humidaire Model 50 incubator, with forced draft and automatic turning, at 37.5°C and 86% relative humidity. Eggs were withdrawn from the incubator after 6.5–7 days of incubation [stages 30–32 of Hamilton (8)] and processed essentially as described (9). The brains were quickly removed, washed in sterile basal Eagle's medium, supplemented with 0.4% glucose, and cut into pieces that were as small as possible. The tissue was dissociated in 0.25% trypsin (Difco) in saline, at 37°C, for 20 min, and pipette-triturated at 5-min intervals. The resulting cell suspension was passed through a nylon screen (180 µm), and inoculated into 25-ml Erlenmeyer flasks containing 3 ml of basal Eagle's medium (Gibco), supplemented with 0.4% glucose and 10% fetal calf serum, at a final cell concentration of (2–5) × 10^6 cells/ml. More than 95% of the cells were able to exclude trypan blue at this stage. The flasks were gassed with CO₂-air (5 : 95) and incubated in a gyrotory bath shaker (New Brunswick) at 37°C/70 rpm. The next day the aggregates were transferred to 50-ml Erlenmeyer flasks, increasing the volume of medium to 8–12 ml. Half the volume of medium was changed every other day.

Enzymatic Assays

Acetylcholinesterase (AChE) and choline acetyltransferase (ChAT) were assayed as described (9). The aggregates were washed with saline and homogenized in 0.05 M potassium phosphate buffer, pH 6.8, containing 1 mM EDTA (K⁺) and 0.5% Triton X-100. The AChE assay measured the amount of [¹⁴C]acetate produced by incubation of the tissues with [¹⁴C]acetylcholine chloride (The Radiochemical Centre, Amersham, Bucks,