Ependymal cells in tissue culture

By

Walter Hild

With 9 Figures in the text

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Introduction

Ependymal cells represent the most primitive elements of the glial series. In certain lower vertebrates the entire glial population of the nervous system consists of these cells, whereas in higher vertebrates they are only present as lining cells of the cavities or ventricles of the nervous system. Here these elements vary considerably in shape and structure according to different areas of the ventricular system (Agduhr 1932). The lateral ventricles and the floor of the IVth ventricle are covered mostly with flat cells; in the choroid plexus the ependyma is generally of cuboidal shape; in the narrow passages of the ventricular system, as in the aqueductus Sylvii, the ependyma generally assumes a columnar form. Columnar ependyma is also found where the ventricular walls form folds or recesses as in the lateral walls in the posterior recess of the IIIrd ventricle where it tapers into the aqueductus cerebri, or (in embryonal and early postnatal stages) in the cerebellar recesses of the IVth ventricle. A remarkable feature commonly found in cuboidal and columnar forms of ependyma is the possession of cilia emerging from the ventricular surface of the cells and extending into the ventricular lumen (Studnička 1900). As to the function of these cilia the opinions of the investigators vary considerably. Ciliary movements have been observed as early as 1836 by Purkinje and Valentín and later by von Kölliker (1864) and Studnička (1900). Ciliary movements have also been observed by Hogue (1947), who found this phenomenon in tissue culture material from cerebellum of a human fetus of 90 mm CRL. On the other hand, von Lenhossek (1891) having studied the ependymal cilia in preparations impregnated by Golgi’s silver method maintains that the cilia do not move. He compares them with rods in olfactory cells, taste cells and hair cells of the inner ear. „Ja, auch die Stäbchen und Zapfen der Netzhaut dürften analoge, nur sehr viel mächtigere Bildungen darstellen.“ Also Benninghoff (1940), in his „Lehrbuch der Anatomie des Menschen“, expresses the opinion that ependymal cilia probably are motionless. Agduhr (1932) writes that at least in some areas the ependymal cells possess stereocilia.

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It is evident that tissue culture should be the method of choice for investigating the activities of ependymal cells since the set up of preparations and the resulting optical conditions are far superior to those obtained from teased preparations of surviving cells.

Material and methods

Brains from newborn to 4 week old kittens, one adult monkey and one human fetus of 7½ months were studied. For convenience in obtaining material, however, kitten material generally was employed especially for perfusion chamber experiments. However, the behavior of cells from all three kinds of explants was similar and comparable. The ependymal linings of the following parts of the ventricular system were used for explantation: lateral ventricles, walls of the IIIrd ventricle anterior and posterior to the massa intermedia, aqueductus Sylvii, floor of the IVth ventricle and choroid plexus. Explants of about 1 to 1½ mm side length were set up on 12 × 50 mm cover slips of number 1 thinness with a clot consisting of equal parts of heparinized rooster plasma and embryonic extract. Two to three hours after explantation the cover slips were placed in roller tubes and 2 ml of fluid nutrient medium were added which consisted of 50% human ascitic fluid, 45% Gey's balanced salt solution (BSS) and 5% embryonic extract. The roller tubes were incubated at 37°C at 8 revolutions per hour for periods from 10 to 120 days. The fluid medium was changed irregularly depending on the general appearance of the cultures, that is, when the rate of ciliary activity became reduced. These intervals varied between 15 and 28 days. No pH indicator was used since the observation of the ciliary activity served as an excellent measure whether the nutrient was exhausted or adequate. At various intervals cover slips were removed from the roller tubes and mounted in perfusion chambers for microscopical observation and cinemographic records using phase contrast and ordinary light optics. The moving picture records were mostly made at 16 frames per second; sometimes 64 frames per second gave excellent “slow motion” effects when projected at a speed of 16 frames per second. This was especially valuable in analyzing details of the ciliary motility. Fixed and stained preparations treated with hematoxylin eosin, iron hematoxylin, azan, Jacobson’s and Bodian’s gave unsatisfactory results since the cilia invariably clumped together during fixation. A total of over 800 explants were used for this study.

Results

Ciliated ependymal cells were maintained in vitro for a period up to 120 days without showing any regressive changes. Since the explants consisted not only of ependymal cells but also contained varying amounts of tissue underlying the ependyma the population of the cultures was consequently mixed. The ependyma, however, did not show as great a migratory activity as oligodendrocytes and the members of the astrocyte family. They kept their contact with each other fairly well and showed a notable degree of migration only when their outgrowth took place in the form of epithelial sheets. The cells were observed to establish two types of outgrowth patterns, the first being in the form of an epithelial sheet (Fig. 1). This mode of emigration was achieved mostly in explants from choroid plexus and in cases where the tissue fragments from other parts of the ventricular linings were explanted in such a way that the original basal ends of the ependymal cells were directed toward the cover slip and in this position fastened by the plasma clot. In such cultures the cells became somewhat flattened attaching to the surface of the cover slip with what seemed to be their original basal end. The free surface bearing the cilia was directed toward the culture medium. In these sheets the cells were in contact apparently influencing each others form. The nuclei were located near the base of the cells. At a particular level of focus all nuclei of a given field came into sharp focus (Fig. 2). However, the nuclei did not show a fixed location with respect