MORPHOLOGY AND PATHOMORPHOLOGY

Effect of Amniotic Fluid from Patients with Fetal Pathology (Anencephaly) on Cell Differentiation in Nervous Tissue Culture

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The addition of amniotic fluid from pregnant women with anencephaly to neuronal and organotypic cultures initiated from germinal brain cortex of 8-12-week-old human embryos has no appreciable effect on growth and differentiation of neurons. However, it stimulates the formation of endothelial channels and affects cell-to-cell interactions. This is assumed to be a factor associated with an abnormal development of the CNS in anencephaly.

Key Words: *anencephaly; amniotic fluid; neuronal culture; organotypic culture*

Elucidation of the mechanisms underlying abnormal prenatal development of the central nervous system (CNS), search for new approaches to the investigation of cause-and-effect processes in CNS pathologies, and development of new test systems for the diagnostics of hypo- and anencephaly are urgent problems of theoretical and practical medicine. Previous studies showed that nervous tissue cultures can serve as a sensitive test system to analyze blood factors in Parkinsonism [4], and amniotic fluid (AF) obtained in fetal pathology (the babies have developed infantile cerebral paralysis) has a pronounced cytotoxic effect on neurons in a culture of human spinal cord ganglia [3].

Here we report the results of a comparative morphological study of the effect of AF from patients with fetal abnormality (anencephaly) and with normally developing fetuses.

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MATERIALS AND METHODS

Neuronal and organotypic cultures were initiated from germinal brain cortex of 8-12-week-old human embryos. Isolation and culturing procedures were described elsewhere [1,2]. Amniotic fluid sampled on the 21st-24th week of pregnancy was added to the growth medium to a final concentration of 15% of the total volume immediately after the attachment of neuronal aggregates and organotypic explants to substrate (collagen). The viability and specific features of the cell or tissue culture development were analyzed every day by invert microscopy. The ultrastructure of neuronal aggregates was studied on day 16 of culturing, and organotypic cultures were analyzed on day 19. The cultures were fixed with 2.5% glutaraldehyde on phosphate buffer (pH 7.2) at 4°C for 24 h, washed with the same buffer, and postfixed with 2% osmic acid. The preparations were embedded in Araldite by the standard method. Semithin and ultrathin sections were cut in an LKB-III ultratome. Ultrathin sections were rou-

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tinely contrasted with uranyl acetate and lead citrate and studied under an electron microscope.

RESULTS

Lifetime comparative analysis of cell development in neuronal cultures revealed no differences in the formation of cell aggregates and in neuritic growth after the addition of "pathological" (anencephaly) or control AF. In both cases differentiating neurons had round nuclei, sometimes with noticeable invaginations of the nuclear membrane. Chromatin of intermediate electron density was evenly distributed over the nucleoplasm. The nuclei and aggregated

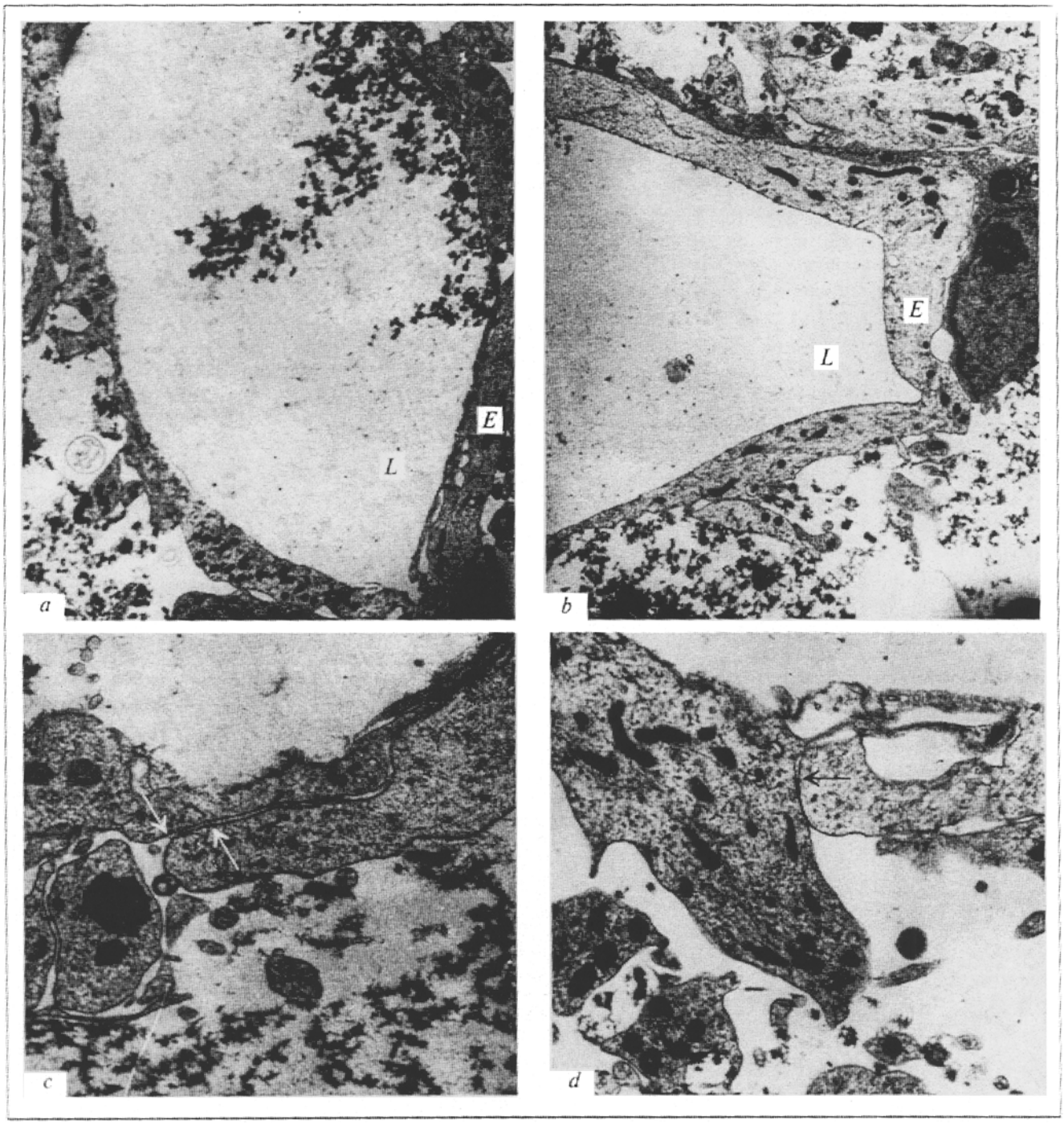

Fig. 1. Endothelial channels in organotypic culture grown in the presence of "pathological" AF. *a*) endothelial capillary, × 7100; *b*) fragment of endothelial capillary, endothelial cells exhibit a very low endocytotic activity, × 12,400; *c*) "simple" contact between endothelial cells formed as mutually corrected invaginations (arrows), × 23,300; *d*) specific contact between endothelial cells, × 20,100. *L*: lumen; *E*: endothelium.

heterochromatin of high electron density were seen. Few cisternae of the endoplasmic reticulum and Golgi complex as well as occasional lysosomes and ribosomes were present in the cytoplasm of differentiating neurons.

Lifetime analysis of organotypic cultures revealed neither cytotoxic effect nor modification of neuritic growth after the addition of "pathological" or control AF. Study of organotypic cultures growing in the presence of "pathological" or control AF showed that the implants had equally good viability and formed outgrowth zones consisting of neurites and poorly-differentiated glial cells, which gradually differentiated predominantly into astrocytes with distinct glial filaments.

Study of neuronal and glial elements in the central zone of explants growing in the presence of "pathological" AF revealed no substantial damage to cells or slowing of their differentiation in comparison with cells grown in the presence of normal AF. Neurons in the same phase of development with analogous structure and equally developed neuropil were seen. Occasional damaged cells were found in the central area of the explant, where activation of the myoglia occurred. However, the number of these cells did not differ in control cultures and cultures grown in the presence of "pathological" AF.

Organotypic cultures grown in the presence of "pathological" AF were characterized by well-developed endothelial cells forming channels within the central part of the culture (Fig. 1). Endothelial cells which formed structures similar to capillaries were connected to each other by nonspecific and specific contacts (Fig. 1, *c*, *d*). In some explants growing in the presence of "pathological" AF, a fairly developed capillary network was formed.

Our main goal was to use nerve tissue culture as a test system for early diagnostics of abnormal brain development in human embryos and fetuses, specifically, to reveal hypo- and anencephaly. Cell culture initiated from human embryonic brain was assumed to be the most sensitive and adequate model for screening "pathological" AF, since these AF might contain some factors with cytotoxic activity. However, this was not confirmed by the present study: AF from anencephalic fetuses elicited no cytotoxic effect and did not slow down the differentiation of neurons and glial cells of human embryonic brain cortex. Meanwhile, our findings show that abnormal development of the brain (specifically, hypoencephaly) is determined by disorders in cell-to-cell interactions at the tissue level. At the same time, "pathological" AF contains a factor facilitating the differentiation of endothelioblasts. It cannot be ruled out that the early differentiation of endothelial cell precursors is an important factor associated with impairment of cell-to-cell interactions in a developing embryo (fetus). In cell culture, differentiating endothelial cells have no effect on neurons; however, in a growing organism prematurely developing cells of endothelial origin and primary capillaries may hamper normal migration of neuroblasts along the radial glia, thus affecting the formation of the cortical and "layer" structures.

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