MYELINOTOXIC ACTIVITY OF SERA FROM ANIMALS WITH EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN ORGANOTYPICAL CULTURES OF THE RAT CEREBELLAR CORTEX AT DIFFERENT STAGES OF DIFFERENTIATION

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Organotypical cultures of the neonatal cerebellar cortex are used to investigate differentiation of neurons and neuroglia and also to study the role of serum myelinotoxic factors concerned in the pathogenesis of experimental allergic encephalomyelitis (EAE) and demyelinating diseases of the human nervous system [1].

Explants of the neonatal cerebrellae cortex taken for culture do not yet contain myelinated nerve fibers and the whole process of formation of myelin sheaths takes place *in vitro* [4].

Demyelination in organotypical cultures arising under the influence of myelinotoxic properties of sera usually appears after 3-4 weeks of culture, for by that time the process of myelination is complete [2].

Myelination and demyelination are most frequently assessed by means of morphological methods, which yield mainly qualitative or only semiquantitative results [3].

For a quantitative evaluation of myelination and demyelination the writer has developed a radiometric method, based on determination of incorporation of ^{3}H-cholesterol and ^{14}C-thymidine into cells of a cerebellar culture.

The object of the work was a quantitative study of the myelinotoxic action of sera from animals with EAE on an organotypical culture of the cerebellum at different stages of differentiation.

EXPERIMENTAL METHOD

Pieces of cerebellum (0.5 mm in diameter) from newborn rats were used for culture. Two explants were placed on each coverslip, covered with collagen. The flying coverslip technique was used.

The culture medium contained serum (placental, 35%), Eagle's medium (40%), Hanks' solution (20%), chick embryonic extract (5%), glucose (660 mg/100 ml medium), and insulin (3 units/ml).

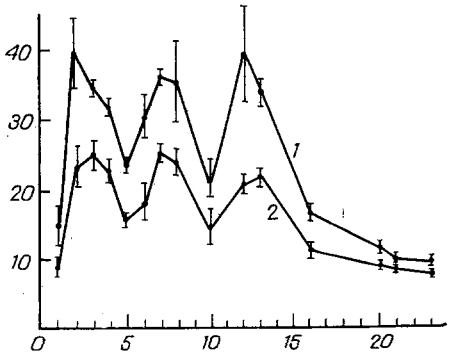

Fig. 1. Incorporation of ^{3}H-cholesterol (1) and ^{14}C-thymidine (2) into cells of organotypical cultures of the neonatal rat cerebellum at different stages of culture. Abscissa, days of incubation; ordinate, radioactivity of tissue in cpm × 10^{3}.

KEY WORDS: tissue culture; cerebellum; allergic encephalitis; cholesterol; differentiation.

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TABLE 1. Sensitivity of Long-Living Cultures of Rat Cerebellum at Different Stages of Culture to Serum Antibodies of Guinea Pig with EAE

Time of culture, days	Incorporation of ^{3}H-cholesterol, cpm			Incorporation of ^{14}C-thymidine, cpm		
	control	experiment	percent of control	control	experiment	percent of control
6	33 040±1 471 (n = 12)	17 511±1192 (n = 8)	47 P<0,01	—	—	—
10	26 512±2 572 (n = 13)	14 582±2 061 (n = 6)	45 P<0,05	16 247±22 (n = 13)	15 435±30 (n = 6)	5 P>0,05
13	23 530±365 (n = 16)	11 294±1 091 (n = 5)	52 P<0,01	17 581±520 (n = 16)	2 636±63 (n = 5)	85 P<0,001
16	16 015±234 (n = 14)	7 886±393 (n = 7)	51 P<0,001	12 223±631 (n = 14)	6 471±285 (n = 5)	47 P<0,01
20	8 844±1 561 (n = 8)	1 829±384 (n = 4)	79 P<0,05	2 665±162 (n = 8)	240±63 (n = 4)	91 P<0,05
25	9 832±488 (n = 16)	1 543±108 (n = 8)	84 P<0,001	2 305±150 (n = 16)	461±45 (n = 8)	80 P<0,001

Legend. n) Number of cultures used in the given experiment or control; control value shown is mean of three control experiments (see Experimental Method).

To induce EAE in guinea pigs, brain homogenate with Freund's complete adjuvant was injected. Blood was obtained on the 14th day after injection of the antigen. The sera were heated to 56°C for 30 min.

Sera of animals with EAE (25%) and complement (10%) were added simultaneously to the cultures. Fresh guinea pig serum was added as complement. The following cultures served as the control: 1) incubated in normal culture medium; 2) with the addition of serum from animals with EAE only; 3) with the addition of complement only.

Incorporation of ^{3}H-cholesterol and ^{14}C-thymidine (the double label method) was investigated at different times of culture. The isotopes were added to the medium for 12 h in a concentration of 2 mCi/ml. Radioactivity of the acid-insoluble precipitates of the culture was measured in a scintillation counter.

To estimate myelinotoxic activity of the serum, labeled cholesterol and thymidine were added to both experimental and control cultures for 48 h; the isotopes were added to the experimental cultures simultaneously with the serum of animals with EAE.

EXPERIMENTAL RESULTS

As Fig. 1 shows, curves of incorporation of ^{14}C-thymidine and ^{3}H-cholesterol into cells of the nerve tissue culture practically coincided. At the end of the 1st day after explantation incorporation of these isotopes was at a low level, but by the 2nd or 3rd day of culture it rose sharply to reach a maximum. During the first 2 weeks of culture three peaks of maximal incorporation were observed: on the 2nd, 7th-8th, and 12th-13th days.

At the beginning of the 3rd week of growth of the cultures DNA synthesis and cholesterol utilization by the nerve tissue cells decreased, and toward the end of that week incorporation of the isotopes into acid-insoluble precipitates of the cells stabilized at levels of 13,000 cpm for cholesterol and 6000 cpm for thymidine. Incorporation of the isotopes into cells of tissue cultures with signs of degeneration did not exceed 30-40 cpm, corresponding to the background level.

The results of estimation of the action of the myelinotoxic sera on the cerebellar cultures are given in Table 1. They show that the action of the sera varied depending on the stage of culture and, consequently, on the degree of differentiation of the cells in culture. For instance, in the early period of culture no significant differences were found between experiment and control as regards incorporation of ^{14}C-thymidine into nerve cells. By contrast, incorporation of ^{3}H-cholesterol into these same cultures on the 6th-10th day after explantation decreased significantly under the influence of the myelinotoxic serum, to reach 47% of the control. Toward the end of the 2nd week of culture the animals' serum significantly inhibited incorporation not only of cholesterol, but also of thymidine. By the beginning of the 4th week of culture inhibition of incorporation of the isotopes was increased compared with the control, and on the 25th day it amounted to 84% for cholesterol and 80% for thymidine.

The investigations showed that cells in a cerebellar tissue culture undergo three surges of activity in the course of their differentiation; curves characterizing DNA synthesis and cholesterol utilization coincided. Each of these surges probably characterizes differentiation of the cells in the explant in a different way. The first surge of incorporation of isotopes can be explained by activation of DNA synthesis in the glial cells [5] and macrophages, which proliferate actively at that time and phagocytose products of cell disintegration [6], and also by increased utilization of cholesterol by glial cells. The second surge is evidently connected with oligodendrocytes, for the period of the 8th-10th day of culture precedes myelination of the axons [2]. A significant increase in the number of oligodendrocytes synthesizing DNA in spinal cord cultures also has been demonstrated at this stage of differentiation [7]. The third surge is probably connected with myelination processes, for at this period, as electron microscopy has shown, the first lamellae of myelin appear [7]. Myelination of nerve fibers is a metabolic process, as has been shown by experiments on detection of tritium-labeled lipids of the myelin sheath. Activation of DNA synthesis at these times of culture may perhaps take place in astrocytes [7].

On the basis of these data on the degree of the pathogenic action of serum of animals with EAE on nerve tissue at different stages of culture it can be concluded that the myelinotoxic serum acts on the early stages of differentiation of the cultures before the appearance of myelinated fibers in them and that inhibition of incorporation of labeled cholesterol takes place on account of injury to the neuroglial cells (possibly oligodendrocytes). The myelinotoxic serum has no effect on DNA synthesis in the early stages of culture. In the later stages of explantation of nerve tissue DNA synthesis was inhibited, so that the level of synthesis remained virtually unchanged. This suggests that in this stage of differentiation depression of DNA synthesis is limited to one cell population — the astrocytes.

Quantitative evaluation of demyelination shows that during differentiation of the CNS and with the appearance of myelinated fibers, the nerve tissue culture becomes more sensitive to the pathogenic action of myelinotoxic serum.

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