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GLIAL FIBRILLARY ACIDIC PROTEIN AND S100 PROTEIN IN ABNORMAL ASTROCYTES IN WILSON'S DISEASE

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Wilson's disease, alongside with hepatogenic encephalopathies resulting from acquired liver damage and/or abnormal portal circulation are neuropathologically characterized by a dominant alteration of astrocytes. Therefore, both these clinical conditions are considered as primary gliopathies (Mossakowski 1966; Seitelberger 1970; Diemer 1978; Norenberg 1981). The leading feature of brain pathology in these cases are progressive astrocytic changes, taking the form of generalized proliferation and hypertrophy concomitant with regressive alterations expressed as non-specific degeneration of astrocytes. These non-specific changes are accompanied by special forms of abnormal astrocytes, which are considered as typical for both genetically-conditioned and acquired forms of hepatocerebral degeneration. These are Alzheimer cells type I and II and Opalski cells. Hitherto Alzheimer cells, type I were described exclusively in Wilson's disease while Opalski cells were observed both in hepatolenticular degeneration and in non-specific hepatogenic encephalopathies. Least specific are Alzheimer cells, type II. They were described not only in both forms of hepatocerebral degeneration but also in a number of other pathological conditions among them in severe cerebral ischemia (Maślińska, Oniszczyk 1970) and in acute infectious diseases of childhood (Opalski 1936).

The abundance and great variability of pathological astrocytic forms in both genetical and acquired hepato-cerebral degeneration, offer an unique opportunity to study their immunomorphological properties. Contrary to the profuse literature, concerning antigenic properties of astrocytes in normal conditions and in neoplastic proliferation (Bignami et al. 1972; Bignami, Dahl 1973, 1974; Antanitus et al. 1975; Bock et al. 1975, 1980; Weinrauder, Lach 1975, 1977; Martinez-Hernandez et al. 1977; Eng, Rubinstein 1978; De Armond et al. 1980 and others), data con-

cerning this aspect of hepatocerebral degeneration are scarse. The publications of Sobal et al. (1981) and Kimura and Budka (1984) are limited to problems of acquired hepatogenic encephalopathy, while Norenberg (1981) deals with experimental hepatic encephalopathy. The studies of Mossakowski and Weinrauder (1984) were performed under model conditions in tissue culture.

This inclined us to perform immunomorphological studies in a case of clinically, biochemically and neuropathologically verified Wilson's disease. Rabbit antisera to glial fibrillary acidic protein (GFAP) and S100 protein were used.

MATERIAL AND METHODS

Studies were carried out on paraffin sections from a cerebral hemisphere, including frontal cortex, white matter and the anterior part of basal ganglia from a case of Wilson's disease, previously published by one of us (Mossakowski et al. 1964). The reason for publication was the atypical localization of tissue abnormalities, consisting in severe diffuse spongiosis of subcortical white matter with relatively slight involvement of basal ganglia. The surroundings of the large spongiotic focus contained numerous pathological glial forms, such as hypertrophied gemistocyte-like astrocytes, Alzheimer cells, type I and Opalski cells. They were found also in other grey structures, such as cerebral cortex and basal ganglia, and Opalski cells additionally in the white matter. In most grey structures abundant Alzheimer type II cells were present, alongside with hypertrophied and degenerated astrocytes, showing considerable fragmentation of their processes.

Immunohistochemical staining was performed according to the procedure described by Hsu and Raine (1981) and Hsu et al. (1981). Microtome sections, taken from 20-year-old paraffin blocks, were deparaffinized in toluene and absolute ethanol and then transferred through graded alcohol solutions to distilled water. Endogeneous peroxidase was blocked by 5-min immersion in 3% hydrogen peroxide in phosphate buffer, pH 7.2. After 5 min rinsing in the same buffer, the sections were subjected to 30-min incubation in normal goat serum, diluted 1:50. Both this and all other incubations were carried out at room temperature.

Antiserum to GFAP was diluted 1:100, and that against S100 protein — 1:200. Incubation in the respective sera lasted 60 min. Then the sections were rinsed for 10 min in phosphate buffer, pH 7.2. The next step consisted in incubation of sections in biotinylated goat anti-rabbit antiserum (1:200) (biotinylated goat antiserum, avidin and biotinylated peroxidase produced by Vector Laboratories, Burlingame, USA). Incubation time was 30 min. After short rinsing in phosphate buffer the sections were transferred for the next 30 min to the avidin-biotinylated

peroxidase complex, diluted 1:100. Rinsing in phosphate buffer preceded transfer of sections to TRIS-HCl buffer, pH 7.6 and 10-min incubation in 0.05% solution of diamine benzidine (in TRIS-HCl buffer) with addition of 0.01% H_2O_2.

After several washings in distilled water, the sections were shortly counterstained in hematoxylin (10 sec) and, after rinsing with a tap water, they were dipped for 3 sec in a concentrated solution of lithium carbonate ($LiCO_3$) and again washed in water. The last step consisted in their dehydration in graded ethanol solutions and toluene and mounting with use of Eukitt medium.

RESULTS

The results obtained with the use of both immune sera were essentially similar, however, some differences in the immunomorphological picture justify their separate presentation.

Anti-GFAP serum

The most striking feature of the general picture of the cerebral cortex consisted in a concentration of strongly positive astrocytes in the molecular layer with a much less abundant astrocytic population in the intermediate ones. Numerous astrocytes appeared also in deep cortical layers (Fig. 1). Subpial astrocytes were hypertrophied, their perikarya and processes were densely filled with products of immunocytochemical reaction (Fig. 2). Astrocytes in deep cortical layers, some of them with evident features of hypertrophy, were characterized by considerable fragmentation of their processes. In many cases only their perikarya were stained. As a rule, perivascular rings of astrocytic sucker feet were visualized (Fig. 3). Astrocytes of edematous white matter were stained moderately. In most of them reaction products visualized only cellular perikarya with short residual processes (Fig. 4). Gemistocytes surrounding a large spongiotic focus within the subcortical white matter revealed a strong immunochemical reaction (Fig. 5). The most intense reaction characterized Opalski cells (Fig. 6). It was diffuse in nature and showed no relation with fibrillary cytoplasmic structures. The same was typical of what is called intermediate cells, which differ from Opalski cells by their smaller diameter and presence of residual processes. Alzheimer cells, type I were stained intensively (Fig. 7). Typical of them was uneven distribution of the reaction products in their cytoplasm. Most of the Alzheimer cells, type II did not show any reaction products around their sharply delineated nuclei (Fig. 8A). In some of them, however, a thin rim of brown cytoplasm was present (Fig. 8B).

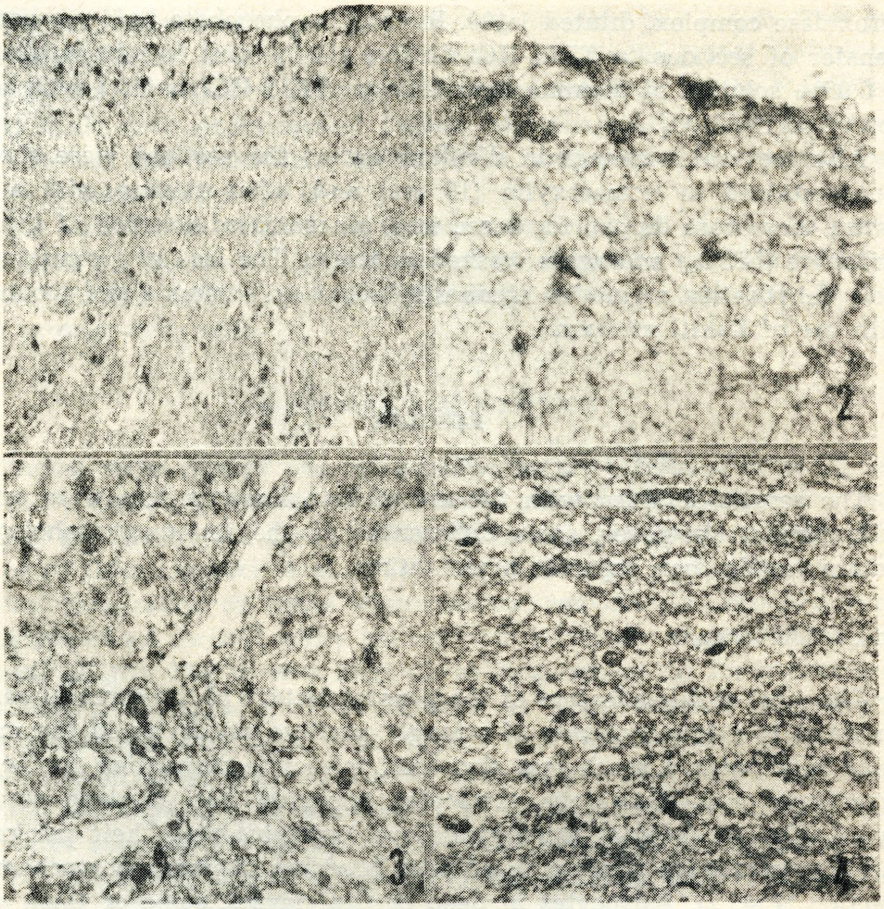

Fig. 1. GFAP. Cerebral cortex. Numerous positively stained astrocytes in the molecular layer and very scanty in the deeper ones. × 100

Ryc. 1. GFAP. Kora mózgu. Widoczne liczne żywo dodatnie astrocyty w warstwie drobinowej i pojedyncze w warstwach głębokich. Pow. 100 ×

Fig. 2. GFAP. Hypertrophied astrocytes in the molecular cortical layer with positive immunohistochemical reaction in perikarya and processes. × 400

Ryc. 2. GFAP. Przerosłe astrocyty warstwy drobinowej kory z dodatnim odczynem immunohistochemicznym w perykarionach i wypustkach. Pow. 400 ×

Fig. 3. GFAP. Astrocytes from deep cortical layer with features of klasmatodendrosis. Note intensive staining of perivascular processes. × 200

Ryc. 3. GFAP. Astrocyty z cechami klazmatodendrozy w głębokiej warstwie kory mózgu. Zwraca uwagę zagęszczenie dodatnich wypustek okołonaczyniowych. Pow. 200 ×

Fig. 4. GFAP. Scanty astrocytes of the white matter with fragmented processes displaying moderate immunohistochemical reaction. × 200

Ryc. 4. GFAP. Skąpe astrocyty istoty białej mózgu z niewyraźnie zaznaczonymi wypustkami, wykazujące umiarkowany odczyn immunohistochemiczny. Pow. 200 ×

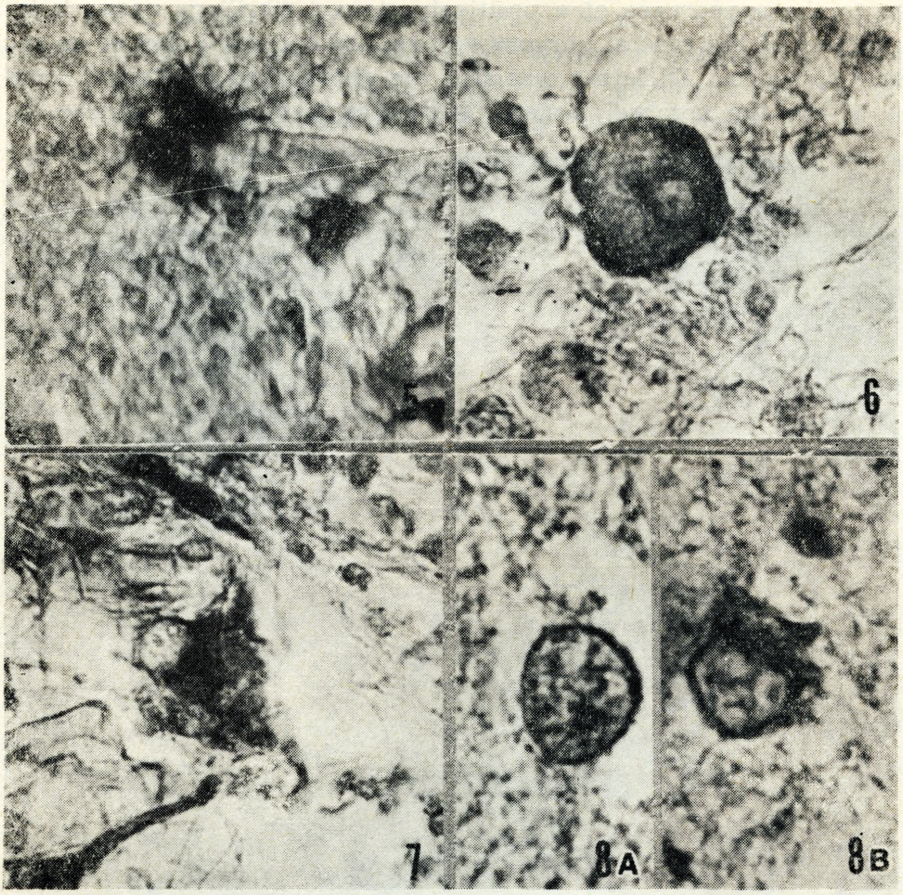

Fig. 5. GFAP. Gemistocytes with intense immunohistochemical reaction in the vicinity of spongiotic focus. × 400

Ryc. 5. GFAP. Gemistocyty z intensywnym odczynem immunohistochemicznym w otoczeniu ogniska zgąbczenia tkanki. Pow. 400 ×

Fig. 6. GFAP. Opalski cells with strong immunohistochemical reaction. × 400

Ryc. 6. GFAP. Komórka Opalskiego z silnym odczynem immunohistochemicznym. Pow. 400 ×

Fig. 7. GFAP. Alzheimer cell, type I. Note uneven distribution of the end product of immunohistochemical reaction. × 400

Ryc. 7. GFAP. Komórka Alzheimera typu I. Zwraca uwagę nierównomierny rozkład produktu odczynu immunohistochemicznego. Pow. 400 ×

Fig. 8. GFAP. Alzheimer cells, type II. × 1000. A. Negative immunohistochemical reaction; B. Showing narrow rim of positively stained cytoplasm

Ryc. 8. GFAP. Komórki Alzheimera, typ II. Pow. 1000 ×. A. Ujemny odczyn immunohistochemiczny; B. Wykazująca wąski rąbek zabarwionej cytoplazmy

Anti — S100 protein serum

A general impression of more intense immunohistochemical reaction was typical of sections, processed with anti-S100 protein serum, as compared with those for which anti-GFAP serum was used. This was mostly

due to the appearance of a positively stained fibrillary network in all structures of the central nervous system, not excluding foci of extensive tissue spongiosis. Different was also the distribution of stained astrocytes within the cerebral cortex. Astrocytes were evenly spread in all

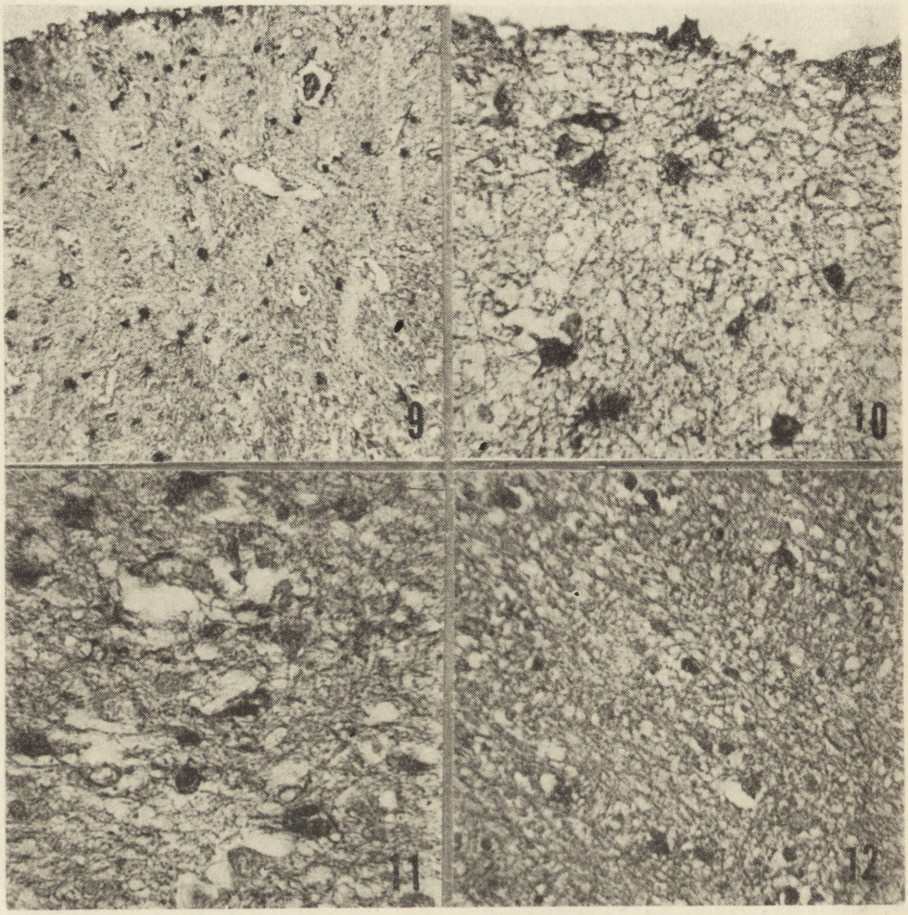

Fig. 9. S100. Cerebral cortex with evenly distributed astrocytes revealing positive immunostaining. × 100

Ryc. 9. S100. Kora mózgu z równomiernie rozproszonymi astrocytami wykazującymi dodatni odczyn immunohistochemiczny. Pow. 100 ×

Fig. 10. S100. Hypertrophied astrocytes of the molecular cortical layer, revealing strong immunohistochemical reaction in perikarya and processes. × 400

Ryc. 10. S100. Przerośnięte astrocyty warstwy drobinowej kory z silnym odczynem immunohistochemicznym w cytoplazmie i wypustkach. Pow. 400 ×

Fig. 11. S100. Astrocytes from deep cortical layer revealing features of klasmatodendrosis. × 400

Ryc. 11. S100. Dodatnio wybarwione astrocyty głębokiej warstwy kory wykazujące cechy klazmatodendrozy. Pow. 400 ×

Fig. 12. S100. White matter astrocytes with features of klasmatodendrosis revealing strong immunostaining. × 200

Ryc. 12. S100. Astrocyty istoty białej mózgu z cechami fragmentacji wypustek. Pow. 200 ×

cortical layers, without their condensation in the molecular layer (Fig. 9). Similarly as in the case of anti-GFAP serum, hypertrophied subpial astrocytes in the molecular layer accumulated abundant reaction products in their perikarya and processes (Fig. 10). Processes of damaged astrocytes in the remaining cortical layers were relatively well visuali-

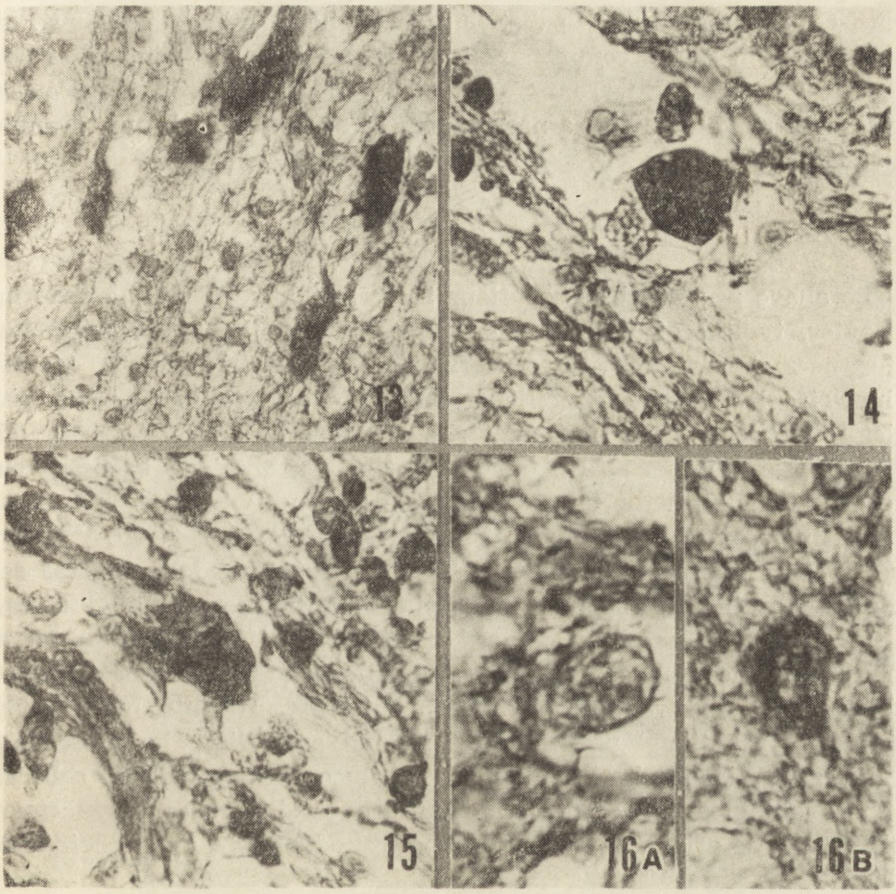

Fig. 13. S100. Gemistocytes from the surroundings of extensive tissue spongiosis, revealing strong immunohistochemical reaction. × 400

Ryc. 13. S100. Gemistocyty z otoczenia ogniska zgąbczenia z bardzo silnym odczynem immunohistochemicznym. Pow. 400 ×

Fig. 14. S100. Opalski cell with strong immunohistochemical reaction. × 400

Ryc. 14. S100. Komórka Opalskiego z silnym odczynem immunohistochemicznym. Pow. 400 ×

Fig. 15. S100. Alzheimer cell, type I with uneven distribution of end product of immunohistochemical reaction. × 400

Ryc. 15. S100. Komórka Alzheimera typu I z nierównomiernym rozkładem produktu końcowego reakcji immunohistochemicznej. Pow. 400 ×

Fig. 16. S100. Alzheimer cells, type II. × 1000. A. Negative immunohistochemical reaction; B. Surrounded by a rim of positively stained cytoplasm

Ryc. 16. S100. Komórka Alzheimera typu II. Pow. 1000 ×. A. Ujemny odczyn immunohistochemiczny; B. Otoczona pierścieniem dodatnio zabarwionej cytoplazmy

zed (Fig. 11). So were astrocytes in the edematous white matter, most of them showing features of advanced klasmatodendrosis (Fig. 12). Gemistocytic forms of astrocytes in the neighbourhood of focal spongiosis were deeply stained (Fig. 13). So were Opalski cells, localized both here and in the remaining areas of the brain (Fig. 14). Staining of Alzheimer cells, type I was less intense than that of Opalski cells. Like in the case of anti-GFAP serum, uneven distribution of the reaction products within their cytoplasm was a common feature (Fig. 15). In most instances Alzheimer cells, type II were negative (Fig. 16A). However, some naked nuclei were surrounded by a narrow rim of accumulated brown granules (Fig. 16B).

DISCUSSION

The above presented observations indicate that most of the abnormal astrocytes, typical of Wilsonian gliopathy, retain the antigenic properties of normal astroglia. This concerns both astrocytes revealing features of hypertrophy and non-specific degeneration as well as their morphologically modified forms such as Alzheimer type I and Opalski cells. The present observations confirm our previous data, obtained in tissue culture conditions with the use of sera from patients with Wilson's disease and the hyperammonemic form of hepatic coma (Mossakowski, Weinrauder 1984). In those studies anti-GFAP sera were used as well as sera containing antibodies against glutamine synthetase and glial antigen common for oligo- and astrocytes, described by Weinrauder and Lach (1975). The concordance of these results allows to assume that damaged and transformed astrocytes may retain not only antigenic, but also metabolic and functional properties of normal astroglia. The presence of specific astroglial markers both in naturally occurring Opalski cells and in those obtained in model tissue culture conditions confirms clearly their astrocytic origin, despite of some discussion concerning their histogenesis (Greenfield 1972).

The immunomorphological properties of Alzheimer cells, type II require a separate discussion. Despite their constant appearance in all cases of hepatocerebral pathology, both naturally occurring and evoked experimentally, Alzheimer cells, type II are also present in other types of cerebral lesions. In the examined case of Wilson's disease most Alzheimer type II cells did not show the presence of either GFAP and S100 protein. This is concordant with the observations of Sobel et al. (1981) and Kimura and Budka (1984). The constant lack of reaction with immune sera against GFAP inclined Sobel et al. (1981) to consider that transformation of protoplasmic astrocytes into Alzheimer cells, type II is connected with a reduced production of glial fibrillary acidic protein. In our material, however, in a small proportion of naked nuclei, a posi-

tive reaction with both specific astroglial antigens was found. It does not seem possible to explain this divergence by the fact that the studies of Sobel et al. (1981) were performed on material of acquired hepatic encephalopathy, while ours in a case of typical Wilson's disease. In our previous studies carried out in tissue culture conditions with the use of sera from patients both with Wilson's disease and hepatic coma, Alzheimer cells, type II consistently revealed a positive immunocytochemical reaction for specific astrocytic antigens. This took the form of a narrow rim of bright fluorescence surrounding enlarged cellular nuclei. The width of the latter ranged from hardly visible to a well defined ring of fluorescence.

The discordance of immunomorphological results can find explanation in the ultrastructure of Alzheimer cells, type II. As shown in electron microscope studies, carried out in cases of Wilson's disease (Anzil et al. 1974) and in natural (Martinez 1968; Foncin, Nicolaides 1970) and experimentally induced hepatic encephalopathy (Norenberg, Lapham 1974; Ostenda et al. 1976), Alzheimer cells type II correspond to enlarged astrocytic nuclei, surrounded by a narrow rim of cytoplasm, iompletely or almost completely devoid of any organelles. Transformation of normal or hypertrophied astroiytes to naked nuclei is a progressing process, different phases of which can be observed both in light microscopic and immunomorphological studies.

Another noteworthy question in our material is the immunomorphological picture of the white matter astrocytes, especially that with the use of anti-GFAP serum. According to the observations of Rubinstein et al. (1962) brain edema provokes a rapid fibrous astrocytic reaction. In this context a strong positive reaction with GFAP-antiserum could be expected in the edematous white matter. This was not the case in our material, in which fibrous white matter astrocytes, showing greatly advanced klasmatodendrosis gave a rather weak immunohistochemical reaction. This phenomenon may be possibly connected with astrocytic damage due to the primary pathological process. A reduced ability of fibre production by astrocytes was observed both in Wilson's disease (Mossakowski 1966) and in experimental hepatic encephalopathy (Diemer 1978; Norenberg 1981). Another possible explanation can be related with the chronic nature of brain edema in a case of Wilson's disease with extensive spongiosis of the white matter. It is well known that prolonged brain edema results in astrocytic lesions, expressed first of all by klasmatodendrosis (Klatzo 1967). This was the most common type of astrocytic pathology observed in our case. Concomitance of both mechanisms cannot be excluded.

A worth mentioning additional observation concerns the differences in distribution of cortical astrocytes when stained with both immunological sera. Anti-GFAP serum visualized mostly astrocytes in the mole-

cular and deeper cortical layers, while in the intermediate cortical layers the astrocytic population was hardly visible. In the reaction with S100 protein antiserum astrocytes were evenly distributed in all cortical layers. This corresponds to the differences observed in the cerebral cortex of normal experimental animals (Ludwin et al. 1976) and is probably connected with the fact that GFAP, although appearing in all types of astrocytes, is above all a specific marker of fibrillary ones (Eng et al. 1971; Bignami et al. 1972; Antanitus et al. 1975; Bock et al. 1975, 1980), while S100 protein is a more universal astrocytic antigen (Ludwin et al. 1976).

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KWAŚNE WŁÓKIENKOWE BIAŁKO GLEJOWE ORAZ BIAŁKO S100 W PATOLOGICZNYCH POSTACIACH ASTROCYTÓW W CHOROBIE WILSONA

Streszczenie

Przeprowadzono badania immunomorfologiczne dotyczące zawartości kwaśnego białka włókienkowego (GFAP) i białka S100 w patologicznych postaciach astrocytów występujących w ośrodkowym układzie nerwowym w chorobie Wilsona. Wykazano, że zarówno hypertroficzne postaci astrocytów, jak i komórki z cechami zwyrodnienia zawierają swoiste antygeny glejowe. Różnią się jedynie intensywnością odczynu immunochemicznego. Szczególnie znaczne nasilenie osiąga on w gemistocytach otaczających ogniska rozpadu tkankowego, w komórkach Opalskiego, komórkach Alzheimera typu I oraz w przerosłych astrocytach molekularnej warstwy kory mózgu. Cechą charakterystyczną komórek Alzheimera typu I było nierównomierne rozmieszczenie produktu reakcji immunohistochemicznej w cytoplazmie. W większości komórek Alzheimera typu II odczyn był negatywny zarówno w przypadku surowic odpornościowych anty-GFAP, jak i anty-S100. Tylko nieznaczna część ich populacji wykazywała dodatni odczyn przeciwko antygenom, ujawniający się w postaci wąskiego rąbka otaczającego nagie jądra. Różnice te zależą prawdopodobnie od stopnia uszkodzenia struktur cytoplazmatycznych komórek Alzheimera typu II.

КИСЛЫЙ ФИБРИЛЛЯРНЫЙ БЕЛОК ГЛИИ И БЕЛОК S100 В ПАТОЛОГИЧЕСКИХ ФОРМАХ АСТРОЦИТОВ В БОЛЕЗНИ ВИЛЬСОНА

Резюме

Проведены иммуноморфологические исследования содержания кислого фибриллярного белка (GFAP) и белка S100 в патологических формах астроцитов, выступающих в центральной нервной системе в болезни Вильсона. Показано, что как гипертрофические формы астроцитов, так и клетки с признаками дегенерации, содержат специфические антигены глии.

Они различаются только интенсивностью иммунологической реакции. Она особенно сильна в гемистоцитах окружающих очаги тканевого распада, в клетках Опальского, в клетках Альцгеймера типа I и в гипертрофических астроцитах молекулярного слоя мозговой коры. Характерной чертой клеток Альцгеймера типа I было неравномерное размещение продукта иммуногистохимической реакции в цитоплазме. В большинстве клеток Альцгеймера типа II не было реакции, как с сыворотками иммунитета против GFAP так и против S100. Незначительная только часть этих клеток проявляла положительную реакцию против антигенов, которая обнаруживалась в виде узкого рубчика, окружающего голые ядра. Эти различия зависят, вероятно, от степени нарушения цитоплазматических структур клеток Альцгеймера типа II.

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