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RABIES ENCEPHALITIS WITH SPECIAL REFERENCE TO THE ULTRASTRUCTURE OF NEGRI BODY

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Pathomorphology of rabies encephalitis has been extensively elaborated. It consists of diffuse inflammatory process, including hematogenic and microglial elements with a characteristic, but not pathognomonic distribution within central nervous system, degenerative changes of neurons and neuronal loss and cytoplasmic inclusion bodies, bearing the names of Negri bodies and lyssa bodies. Non-specific nature of both inflammatory and degenerative processes caused that uptill introduction of immunological and virological diagnostic tests, Negri bodies had been considered as the major pathognomonic indication of rabies infection, although their occurrence was limited to 70 per cent of cases (Dupont, Earle, 1965).

Longlasting discussion concerning the nature of Negri bodies, their relationship with intracellular virus replication as well as identity or distinct character of Negri and lyssa bodies was forejudged by electron microscopic studies on both experimental and human material (Matsumoto, 1962, 1963; Roots, Schultze, 1963; Miyamoto, Matsumoto, 1965; Garcia-Tamayo et al., 1972; De Brito et al., 1973; Joo To Sung et al., 1976).

What inclined us to present our own case were some peculiarities of its clinical and neuropathological picture as well as different ultrastructural appearance of the cytoplasmic inclusion bodies.

CASE REPORT

The case concerned 13 year old country boy, who was entirely healthy, except head injury, which had happened several months prior to the beginning of his final disease. Head trauma had no immediate

clinical consequences. Two months before hospitalization the boy was scratched by a fox. Animal was killed, but not examined. No attention was paid to this accident.

The disease started with fever, headaches, abdominal and back pains and aches of the left hip joint. This was followed by psychomotor excitation and difficulties in drinking. The patient was admitted to the hospital on the fourth day of the disease. Neurological examination revealed nystagmus, anisocoria, and paresis of the left lower extremity with decreased deep tendon reflexes and hypesthesia. Due to worsening general condition, extensive salivation and difficulties in breathing the boy was transferred to the University Pediatric Clinic in Warsaw where he was put on artificial respiration. The patient was stuporous, irritated and showed only very superficial contact with personnel. There was flaccid paralysis of the left inferior extremity, bowel and bladder disturbances, difficulties in deglutition and extensive salivation, requiring constant suction. No meningeal symptoms were present. CSF examination revealed 0.16 mg% of protein and cytosis 11/1 ml. Eeg showed no focal abnormality, although the basic bioelectric activity was very low. Ocular fundi were normal. Due to the presence of the right-sided skull fraction and appearance of left sided neurological symptoms, bilateral drill hole exploration was performed. Right-sided hydroma was found and 100 ml of yellowish fluid were evacuated. Despite that the boy was downgoing. Due to the information concerning the accident of fox bite the possibility of rabies infection was taken into consideration. Fluorescent antibody test of the brain tissue samples taken through the trepanation hole showed a strongly positive reaction. The boy died in deep coma and areflexia, with bradycardia and hypothermia on the 20th day of his illness.

General autopsy findings were irrelevant, except bilateral pneumonia. Brain autopsy revealed features of edema and generalized hyperemia.

Light microscopy of the central nervous system showed moderate mostly lymphocytic infiltration of leptomeninges, being most intense in the vicinity of small pial vessels. Within the brain tissue rich perivascular infiltrations, composed mostly of lymphocytes with some admixture of plasma cells were present in all gray and white structures (Fig. 1). The intensity of perivascular infiltration ranged from single layer of hematogenous cells, to thick multilayer cuffs. In most instances perivascular accumulation of lymphocytes was surrounded by perivascular microglial proliferation (Fig. 2). Diffuse spread of perivascular microglial proliferation was a common feature. In many areas diffuse microglial proliferation, with numerous rod-cells involved a great pro-

portion of a given gray structure. Numerous microglial nodules, laying either on the background of otherwise unchanged tissue or diffuse microglial proliferation were seen (Fig. 3). The intensity of inflammatory process showed remarkable regional differences. In the cerebral cortex frontal, insular and occipital areas were most severely involved. Among subcortical structures thalamus was most affected. In the brain stem substantia nigra, locus coeruleus and inferior olives were the sites of intense inflammatory process (Fig. 4). The gray matter of the spinal cord was also severely involved.

The inflammatory process was accompanied by profuse non-specific degeneration of neurons and neuronal loss. They were present in the cerebral cortex, mostly in frontal temporal and occipital areas. Intense lesions of melanin-bearing neurons were worth noting (Fig. 5). There was an almost total loss of Purkinje cells, accompanied by diffuse proliferations of Bergmann's glia (Fig. 6). The spinal cord gray matter was characterized by great loss of motorneurons, with numerous neuronophagic nodules (Fig. 7). Dorsal root ganglia showed both perivascular and diffuse lymphocytic infiltration, loss of ganglion cells and nodular proliferation of amphicytes (Fig. 8). Spinal roots and peripheral nerves showed lymphocytic infiltration of varying intensity.

Great number of nerve cells in all parts of the central nervous system contained in their cytoplasm small, homogenous, eosinophilic inclusions (Fig. 9). Inclusions, as a rule, did not show any internal structure, although in some of them fine basophilic granules could be noticed. The number of inclusions varied; most often they were single, however, in some neurons they occurred as multiple structures (Fig. 10). They were located in various portions of the cytoplasm, including axonal and dendritic conuses. They were most numerous in bipyramidal cells of Ammon's horn, in numerous cells of inferior olives and dorsal root ganglia. The second in range as far as their frequency is concerned, were cortical neurons, those of globus pallidus, lenticular nucleus and tegmental nuclei of the brain stem. They were rare in thalamus and pontine nuclei.

The electron microscopic studies were performed on tissue samples taken either from paraffin blocks, prepared for EM examination according to Morecki and Becker techniques (1968) or from formalin fixed material. Due to formalin fixation and preceding embedding in paraffin the fine structure of tissue was severely damaged. Therefore the only element studied were intracytoplasmic inclusions.

On the ultrastructural level the intracytoplasmic inclusions varied a great deal in shape and size. Most of them were round or oval. They

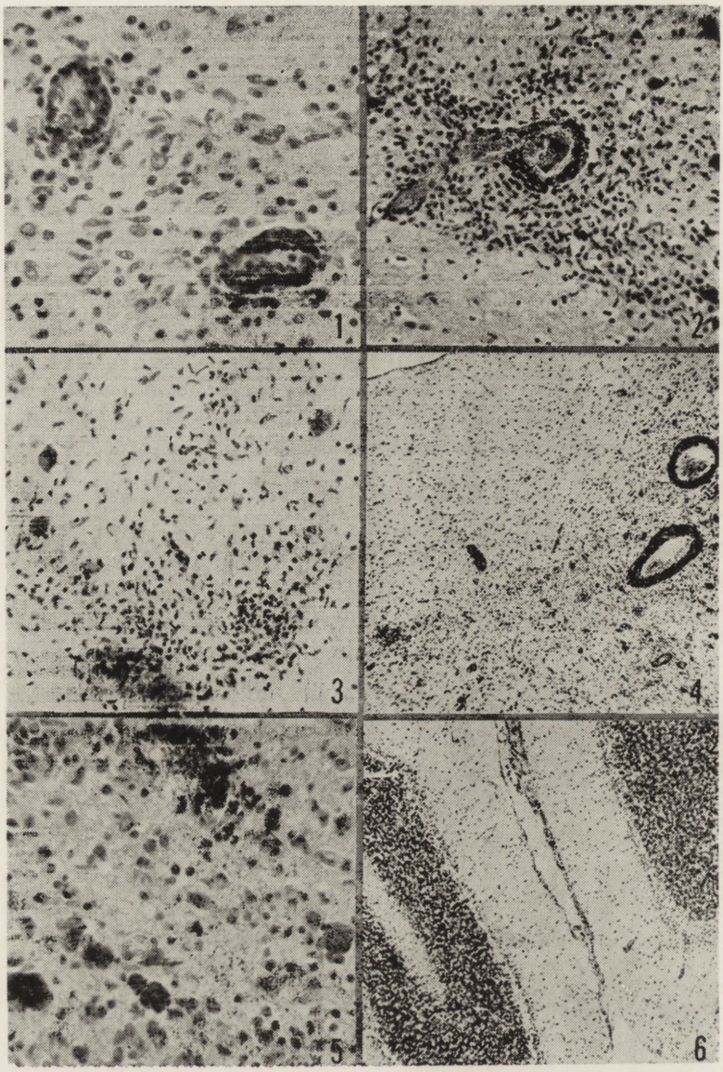

Fig. 1. Perivascular lymphocytic infiltration in the area of diffuse proliferation of microglia. Midbrain. Cresyl violet. × 400.

Ryc. 1. Okołonaczyniowe nacieki limfocytarne w obszarze rozlanego rozplemu mikrogleju. Śródmózgowie. Fiolet krezylu. Pow. 400 ×.

Fig. 2. Lymphocytic-microglial perivascular infiltration in basal pons. H-E. × 200.

Ryc. 2. Limfocytarno-mikroglejowy naciek okołonaczyniowy w podstawnej części mostu. H-E. Pow. 200 ×.

Fig. 3. Microglial nodules in the inferior olive. Cresil violet. × 200.

Ryc. 3. Grudki mikroglejowe w oliwie dolnej. Fiolet krezylu. Pow. 200 ×.

Fig. 4. Massive inflammatory process involving locus coeruleus. Cresyl violet. × 60.

Ryc. 4. Masywne zmiany zapalne w jądrze miejsca sinawego mostu. Fiolet krezylu. Pow. 60 ×.

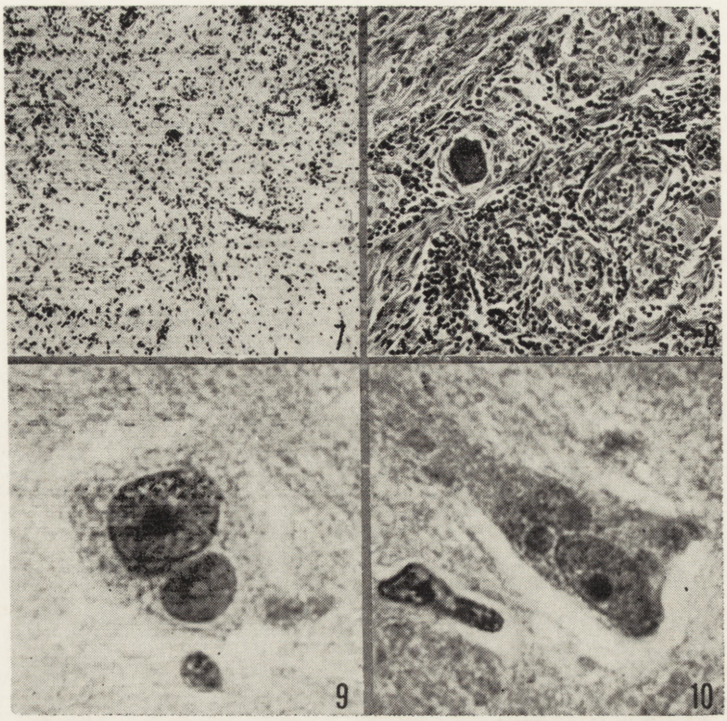

Fig. 7. Diffuse inflammatory process involving anterior horn of the cervical spinal cord. Neuronal loss and neurophagic nodules. Cresyl violet. × 100.

Ryc. 7. Rozległe zmiany zapalne w rogu przednim rdzenia kręgowego. Ubytek komórek ruchowych, grudki neurofagiczne. Fiolet krezylu. Pow. 100 ×.

Fig. 8. Dorsal root ganglion — neuronal loss with nodular proliferation of amphicytes, degeneration of the remaining neurons and interstitial lymphocytic infiltration. H-E. × 200.

Ryc. 8. Ubytek komórek nerwowych z grudkowym rozplemem amficytów, zwyrodnienie zachowanych neuronów oraz śródmiąższowe nacieki limfocytarne w zwoju międzykręgowym. H-E. Pow. 200 ×.

Fig. 9. Negri body in the cytoplasm of Ammon's horn neuron. H-E. × 1 000.

Ryc. 9. Ciałko Negriego w cytoplazmie komórki piramidowej rogu Amona. H-E. Pow. 1.000 ×

Fig. 10. Several eosinophilic inclusions in the cytoplasm of Ammon's horn neuron. H-E. × 900.

Ryc. 10. Kilka kwasochłonnych ciał wtrętowych w cytoplazmie komórki nerwowej rogu Amona. H-E. Pow. 900 ×.

Fig. 5. Loose melanine deposits in locus coeruleus. H-E. × 400.

Ryc. 5. Luźno położone ziarna melaniny w jądrze miejsca sinawego mostu. H-E. Pow. 400 ×.

Fig. 6. Loss of Purkinje cells with proliferation of Bergmann's glia. Slight lymphocytic infiltration in leptomeninges. Cresyl violet. × 60.

Ryc. 6. Zanik komórek Purkinjego oraz towarzyszący rozplem gleju Bergmanna. Dyskretny naciek zapalny w oponach. Fiolet krezylu. Pow. 60 ×.

were not membrane bound. All of them had a similar fine structure. They consisted of matrix of a moderate electron density and coarse granules of a higher density (Fig. 11). The distribution of granular osmophilic material was either equal throughout the whole inclusion body, or showed areas of a greater condensation. In some instances they were densely packed on the inclusion periphery, in others their accumulation was greater in the central part. In another group, central portion of the inclusion body was rarefied, leading to its annular appearance (Fig. 12). In no case we were able to find features of virus replications within inclusion bodies. Neither we found the places of virus replication outside the inclusions as described by Morecki and Zimmerman (1969). In some cells we were able to notice single profiles, which with all reservations could be reminiscent of virion structures. However, due to their rarity and poor preservation of the ultrastructural organization, we do not feel autorized to comment on this phenomenon. The other feature had attracted our attention. This was the relationship of the inclusion bodies with neuronal nuclei (Fig. 13). In great number of instances inclusion bodies, as seen in electron microscopy, were located in the direct vicinity of the neuronal nuclei. In some cases nuclear membrane seemed to be discontinuous at the site adjacent to the inclusion (Fig. 14).

DISCUSSION

From the clinical point of view our case is characterized by a relatively long clinical course, lasting 19 days. Similar or even longer disease duration was observed by Dupont and Earle (1965) in those cases in which during the incubation period full antirabies vaccination was given. In our case vaccination was not applied due to neglection of fox bite accident. The second distinguishing element of our case is the presence of posttraumatic hydroma which complicated the clinical picture.

Both these factors could influence the neuropathology of the case, characterized by extensive cortical degenerative changes and neuronal loss, almost total disappearance of Purkinje cells, extensiveness of inflammatory process and neuronal cytoplasmic inclusion bodies.

Homogenously eosinophilic cytoplasmic inclusion bodies, could be classified in their great majority as lyssa bodies. Only small proportion of them contained, difficult of visualize, minute basophilic granules typical for actual Negri bodies. However, it is worth to mention that Matsumoto (1970), Leech (1971) and Joo Ho Sung et al. (1976) on the ground of electron microscope studies consider Negri bodies and lyssa

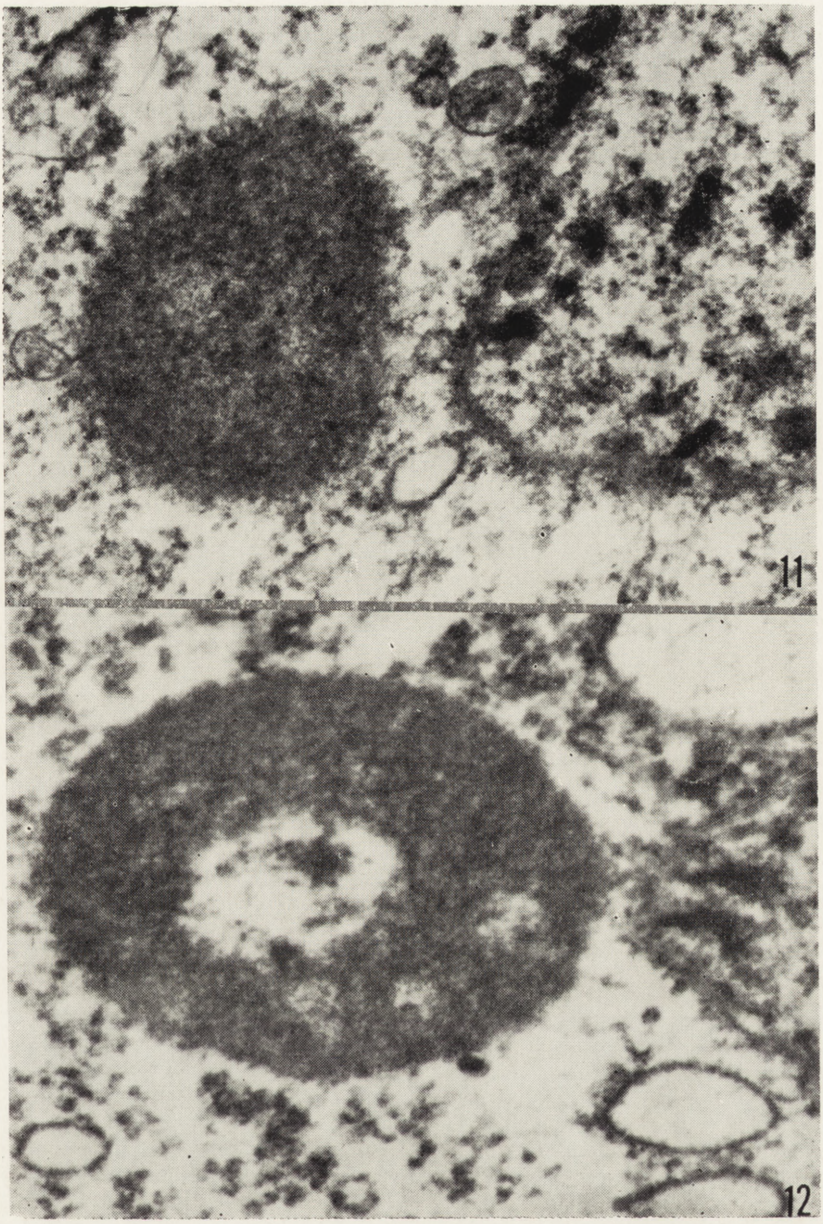

Fig. 11. Intracytoplasmic inclusion body with an equal distribution of osmophilic granular material. × 9.000.

Ryc. 11. Śródplazmatyczne ciało wtrętowe o równomiernym rozkładzie materiału osmofilnego. Pow. 9.000 ×.

Fig. 12. Intracytoplasmic inclusion body with a large central rarefaction of its structure. × 16.000.

Ryc. 12. Śródplazmatyczne ciało wtrętowe z rozległym centralnym rozrzedzeniem materiału osmofilnego. Pow. 16.000 ×.

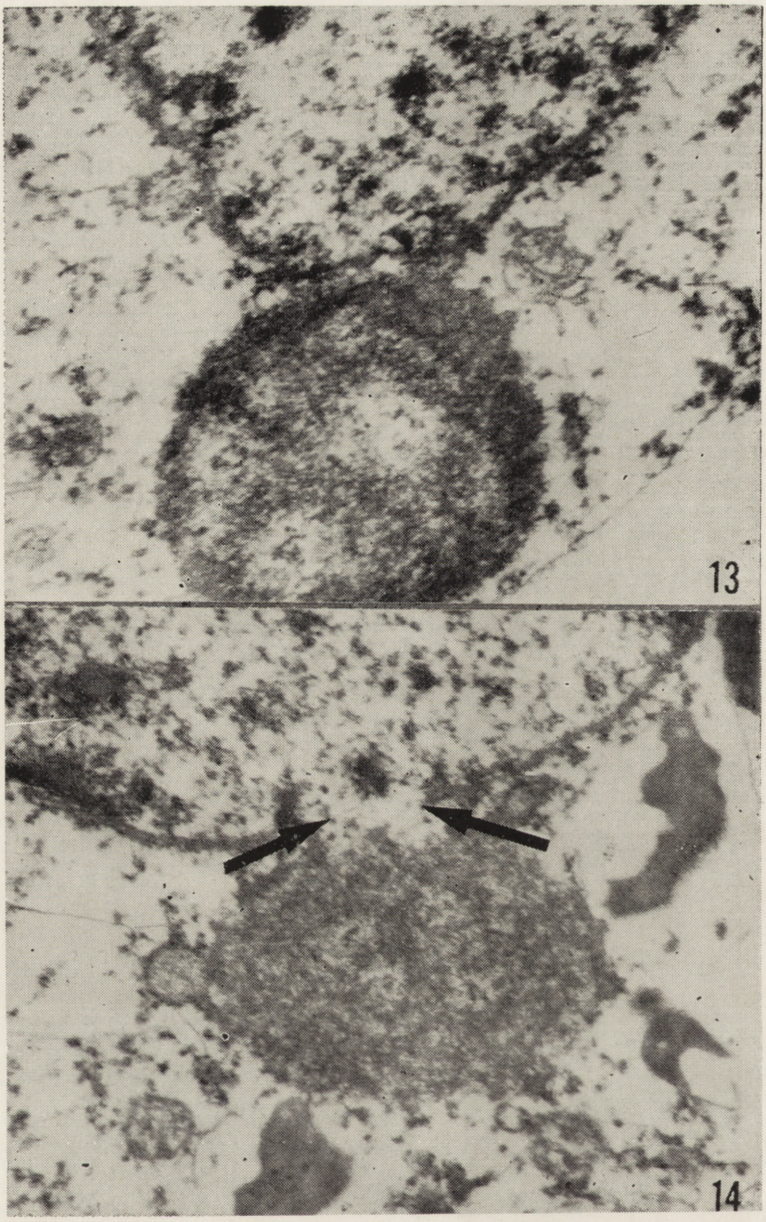

Fig. 13. Perinuclear localization of inclusion body with unequal distribution of granular osmophilic material. × 7.000.

Ryc. 13. Okołojądrowe położenie ciała wtrętowego z nierównomiernym rozkładem ziarnistego materiału osmofilnego. Pow. 7.000 ×.

Fig. 14. Nuclear membrane disrupted (arrows) in the immediate vicinity of the neighbouring intracytoplasmic inclusion body. × 5.000.

Ryc. 14. Uszkodzenie otoczki jądrowej (strzałki) w bezpośrednim sąsiedztwie ciała wtrętowego. Pow. 5.000 ×.

bodies as principally identical structures, the only difference consisting in the amount of viral particles. The structure responsible for the presence of cytoplasmic inclusions according to Matsumoto (1970) is fibrillary matrix, being the site of virus replication. Typical Negri body is composed of matrix aggregations within neuronal cytoplasm, on the background of which there occur typical buller-shaped virions and/or tubular structures opened on both ends toward the matrix (Miyamoto, Matsumoto, 1965; Garcia-Tamayo et al., 1972; De Brito et al., 1973; Joo Ho Sung et al., 1976). In addition, Leech (1971) described within the structure of Negri body, filamentous rod-like and microtubule-like profiles, which he considered to correspond to viral nucleoprotein, analogues to that found by Hummler et al. (1968) in their virological study.

Structures found in our case distinguish from those described above by their inner structure and lack of viral particles. They are identical in their nature with the structures observed in experimental material by Hottle et al. (1951) and Lépine and Croissant (1951) as well as in human case by Morecki and Zimmerman (1969). They are also similar, except the lack of evident viral particles, to those presented by Leech (1970). It is worth of being noted, that in all those cases, the material for electron microscope studies was originally fixed in formalin and in the majority of instances embedded in paraffin. Therefore it would seem acceptable to consider structures found in our case as Negri or lyssa bodies, altered due to unproper fixation and processing. Such an interpretation is weakened by simultaneous occurrence of structures identical with ours with typical matrix aggregations containing viral particles found in the case of Morecki and Zimmerman (1969). Moreover, the authors insisted that only the former structures correspond to Negri bodies seen in the light microscope, the latter being not visualized. They support their view point by emphasizing the lack of Negri bodies in experimental animals infected with fixed strain of virus.

In the light of these discrepancies the question arises as to the nature of structures seen in our case. They were found (similar as in a case of Morecki and Zimmerman, 1969) in a case with immunologically confirmed rabies infection, and with no doubt they correspond to cytoplasmic inclusions, seen in the light microscope. Several possibilities are to be taken into consideration. They may represent structures entirely independent of Negri and lyssa bodies, they can correspond to lyssa bodies being different in nature from Negri bodies, or finally they may be considered as altered Negri bodies due to unproper fixation and processing. The latter possibility seems to be supported by their general similarity to the structures described by Leech (1971), as accurate ultra-

structure of virions in his material is difficult for interpretation. The possibility that they represent a certain stage in Negri body evolution cannot be excluded. This concept finds its support in a prolonged disease duration in our case, as compared with all those human cases in which fine structure of inclusions was identical with that observed in experimental animals.

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PRZYPADEK WŚCIEKLIZNY. OBRAZ MIKROSKOPOWO-ELEKTRONOWY CIAŁEK NEGRIEGO

Streszczenie

Przedstawiono przypadek wścieklizny, rozpoznanej przyżyciowo na podstawie badań immunopatologicznych i wirusologicznych biopsyjnego materiału mózgowego.

Przypadek wyróżniał się 20-dniowym przebiegiem klinicznym, a w obrazie patomorfologicznym rozległością procesu zapalnego obejmującego praktycznie wszystkie struktury ośrodkowego układu nerwowego, zwoje międzykręgowe i nerwy obwodowe. Cechą znamienną przypadku była niezwykła obfitość pojedynczych i mnogich ciał wtrętowych, występujących w neuronach kory mózgu, pnia mózgowego, rdzenia kręgowego i zwojów międzykręgowych. Ciała te na podstawie cech histologicznych uznano za typowe ciałka Negriego i ciałka wścieklizny.

W obrazie mikroskopowo-elektronowym składały się one z macierzy o umiarkowanej gęstości elektronowooptycznej i gęstych ziarnistości osmofilnych. Ciała te nie miały błony otaczającej, a ich strukturę wewnętrzną charakteryzowało znaczne zróżnicowanie rozkładu ziarnistego materiału osmofilnego, prowadzące niekiedy do występowania struktur obrączkowatych. Zwracał uwagę ścisły kontakt ciał wtrętowych z jądrami komórkowymi. Zarówno w obrębie ciał wtrętowych jak i poza nimi nie spostrzegano obecności wirionów.

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СЛУЧАЙ БЕШЕНСТВА. ЭЛЕКТРОН-МИКРОСКОПИЧЕСКАЯ КАРТИНА ТЕЛЕЦ НЕГРИ

Резюме

Представлен случай бешенства, установленный прижизненно на основании иммунопатологических и вирусологических исследований биопсийного материала мозга.

Случай отличался 20-дневным клиническим течением, а в патоморфологическом плане — обширностью воспалительного процесса охватывающего практически все структуры центральной нервной системы, межпозвоночные ганглия и периферические нервы. Характерным свойством случая было необычайное изобилие отдельных и многочисленных включений, находящихся в нейронах коры мозга, ствола мозга, спинного мозга и межпозвоночных ганглиев. Эти

тельца, на основании гистологических свойств, оказались типичными тельцами Негри и тельцами бешенства.

В электрон-микроскопической картине они представляли матрикс с умеренной электрон-оптической плотностью и густой осмофильной грануляцией. Эти тельца не содержали окружающей оболочки, а их внутренняя структура характеризовалась значительной дифференциацией распределения зернистого осмофильного материала, ведущей иногда к образованию перстневидных структур. Обращал внимание плотной контакт телец включений с клеточными ядрами. Как в составе телец включений так и вне их не наблюдали наличия вирионов.

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