NEURONAL CEROID-LIPOFUSCINOSIS WITH PROMINENT CHOREA AND WITHOUT VISUAL MANIFESTATIONS

A CASE REPORT

LUCIANO DE SOUZA QUEIROZ *
JOAQUIM NOGUEIRA DA CRUZ NETO **

Biochemical, histochemical and ultrastructural investigations during the 1960's lead to the subdivision of amaurotic familial idiocy (AFI) into two distinct groups of nosological entities: the gangliosidoses and the neuronal ceroid-lipofuscinoses[28].

In the *gangliosidoses,* the stored lipid is a ganglioside and its accumulation depends on well characterized enzymatic defects in the catabolism of sphingolipids [2,16]. This group includes the infantile form of AFI or Tay-Sachs disease.

The cases of AFI with no detectable sphingolipid abnormality have been placed by Zeman and Dyken [31], and Zeman et al.[30] under the generic heading of *neuronal ceroid-lipofuscinosis* (NCF). The common denominator in these patitnts is the accumulation of lipofuscinlike autofluorescent substances in neurons, glial and mesodermal cells of the central nrevous system and, to a variable extent, also in peripheral nerves, muscle, skin and viscera. Although the clinical picture is multiform in the NCL, three variants can now be recognized [4]:

a) The infantile type [7,15,22] has its onset from 6 months to 2 years of age [19], most frequently with progressive psychomotor deterioration, motor disorders [19] and visual loss. Microcephaly, ataxia, hypotonia and myoclonic jerks also occur [22]. After a quiescent stage death supervenes at an age of 6 1/2 years on the average [22].

b) The late infantile or Jansky-Bielschowsky type usually starts between 2 1/2 and 5 years [19] although it may begin at any age [30]. It is characterized by rapidly progressive seizures, dementia, blindness, cerebellar signs and myoclonus, ending in a decorticate state 2 to 6 years after the onset of symptoms [30].

From the Department of Pathology (Chairman — Prof. Dr. J. Lopes de Faria), Faculdade de Ciências Médicas da Universidade Estadual de Campinas (UNICAMP), and the Instituto de Neurologia Clínica e Neurocirurgia de Limeira, SP. Presented at the XII Brazilian Congress of Pathology, Campinas, July 1977: *Pathologist; **Neurologist.

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c) The juvenile or Batten-Spielmeyer-Vogt type begins at 5 to 10 years of age and is heralded by deterioration [9]. Dementia, pyramidal syndrome and convulsions occur later and evolution lasts from 6 to 11 years [19].

A few heterogeneous cases, however, fail to fit into the above established types and should be provisorily accomodated into a fourth group, in which the adult patients (Kufs' disease) may be included [4]. Clinical hallmarks in these cases are cerebellar and/or extrapyramidal signs, as Kell as the absence of retinal degeneration.

Our personal experience with the neuronal ceroidlipofuscinoses presently amounts to eight patients, of which four have been published [18]. The following report is concerned with an atypical case of NCL, in whom choreic movements were prominent but visual impairment was absent. The correct diagnosis could only be drawn on anatomical grounds.

CASE REPORT

An 11-year-old white girl was admitted to the hospital because of progressive dementia and severe choreic movements. Her parents were third cousins and of no Jewish ancestry. The patients was the first daughter of the couple and two younger sisters aged 8 and 4 years were examined and found to be normal. She was born after an uneventful gestation and her neuromotor development was normal up to the age of 4 years, when her mother noticed some difficulty in the pronunciation of words, loss of interest in her toys and some clumsiness of fine hand movements. Urinary incontinence also developed by this time. This picture progressed slowly and at age 7 she was unable to talk; her mother was the only relative she could recognize. Some unsteadiness of gait started to be noticed at the age of 8. Physical rehabilitation therapy at another service was attempted at this time, with no results. One year prior to admission choreic movements developed symmetrically in both upper extremities and gradually involved the trunk, face and lower limbs. Six months before examination she became totally unable to walk. Visual deterioration, seizures and myoclonic jerks have not been referred.

Examination — The patient was an afebrile, severely emaciated pale child, with diffuse muscle wasting. Occipital frontal head circumference was 49 cm. Physical findings were not otherwise remarkable. Neurological examination revealed severe dementia and decorticate rigidity. Prominent involuntary movements of the choreic type were evident in the face, trunk and limbs, most intense in the upper limbs. Complete anarthria and impossibility to walk were also noticed. Deep tendon reflexes of the limbs were universally abolished, but those of the face were brisk. Grasping, groping and suction reflexes weres present but the Babinski sign and clonus were not obtained. Optic fundi were normal, with no signs of atrophy or pigmentary changes. The retinal blood vessels showed normal features. Nystagmus was absent bilaterally and swallowing was normal. Cerebrospinal fluid examination was normal. Vacuolated lymphocytes were found in the peripheral blood. EEG under barbituric sleep disclosed burst of diffuse and synchronous slow waves and occasional spike-wave complexes. Pneumoencephalogram showed diffuse atrophy of the cerebral

hemispheres especially marked in the frontal and occipital lobes. An occipital cerebral biopsy was taken. During hospitalization an unsuccessful therapeutic trial with haloperidol and diazepam was made, in an attempt to control the involuntary movements.

The patient was lost to follow-up, but the parents informed that the involuntary movements disappeared some months after her discharge from the hospital. Her general condition progressively deteriorated and she died at home. No necropsy was performed.

Pathological examination — Occipital cerebral cortex obtained at surgery was fixed in unbuffered 10% formalin for light microscopy. Frozen sections were stained with PAS and Sudan Black B[20]. Paraffin sections were stained with hematoxylin and eosin, PAS and Sudan Black B. Both frozen and paraffin sections were mounted unstained in glycerol and examined in ultraviolet light. For electron microscopy, small fragments of cerebral cortex were fixed in PFG (paraformaldehyde, glutaraldehyde and picric acid)[23] for four hours, washed overnight in phosphate buffer, dehydrated in ethanol, cleared with propilene oxide and embedded in Spurr's low viscosity resin. Sections 1μ thick were stained with toluidin blue for the selection of blocks. Ultrathin sections contrasted with Reynold's lead citrate were examined in a Hitachi HU-12 electron microscope.

A) Light microscopy — Neurons in the cerebral cortex presented intracytoplasmic accumulation of granules, in two different degrees:

a) In layer III, and to a lesser extent in layer V, there were many severely ballooned neuronal perikaria (Fig. 1), in which a pyknotic, eccentric nucleus was seen only occasionally. The aspect recalled the neurons in Tay-Sachs disease. These markedly distended neurons were filled up with pale eosinophilic granules which, in frozen sections, stained dark gray by Sudan Black B and magenta with PAS, but remained unstained by both techniques in paraffin sections. They were not autofluorescent, even in frozen sections.

b) The majority of nerve cells in all cortical layers contained a smaller quantity of stored material which did not cause neuronal ballooning. The nucleus was often not displaced and kept its normal appearance (Fig. 2), but the initial segment of the axon was sometimes enlarged by accumulated material (axon torpedo). The intracytoplasmic granules in these less distended cells were slightly yellowish, displayed a greenish autofluorescence under ultraviolet light (Fig. 3) and stained with PAS and Sudan Black B in both frozen and paraffin sections (Fig. 2). The intensity of staining with Sudan Black B increased in paraffin material, when compared to frozen sections.

A few neurons looked normal. Gliosis was absent. Macrophages filled with sudanophil granules were observed around a blood vessel in the white matter in a frozen section. PAS-positive or sudanophil granules were not apparent elsewhere in the white matter.

B) Electron microscopy — The severely ballooned neurons described above contained in the cytoplasm abundant rounded corpuscles measuring 0.5 to 1.0μ in diameter, formed by membranes arranged concentrically in a onion bulb fashion (Fig. 4). They resembled the membranous cytoplasmic bodies (MCB) of Tay-Sachs disease[25]

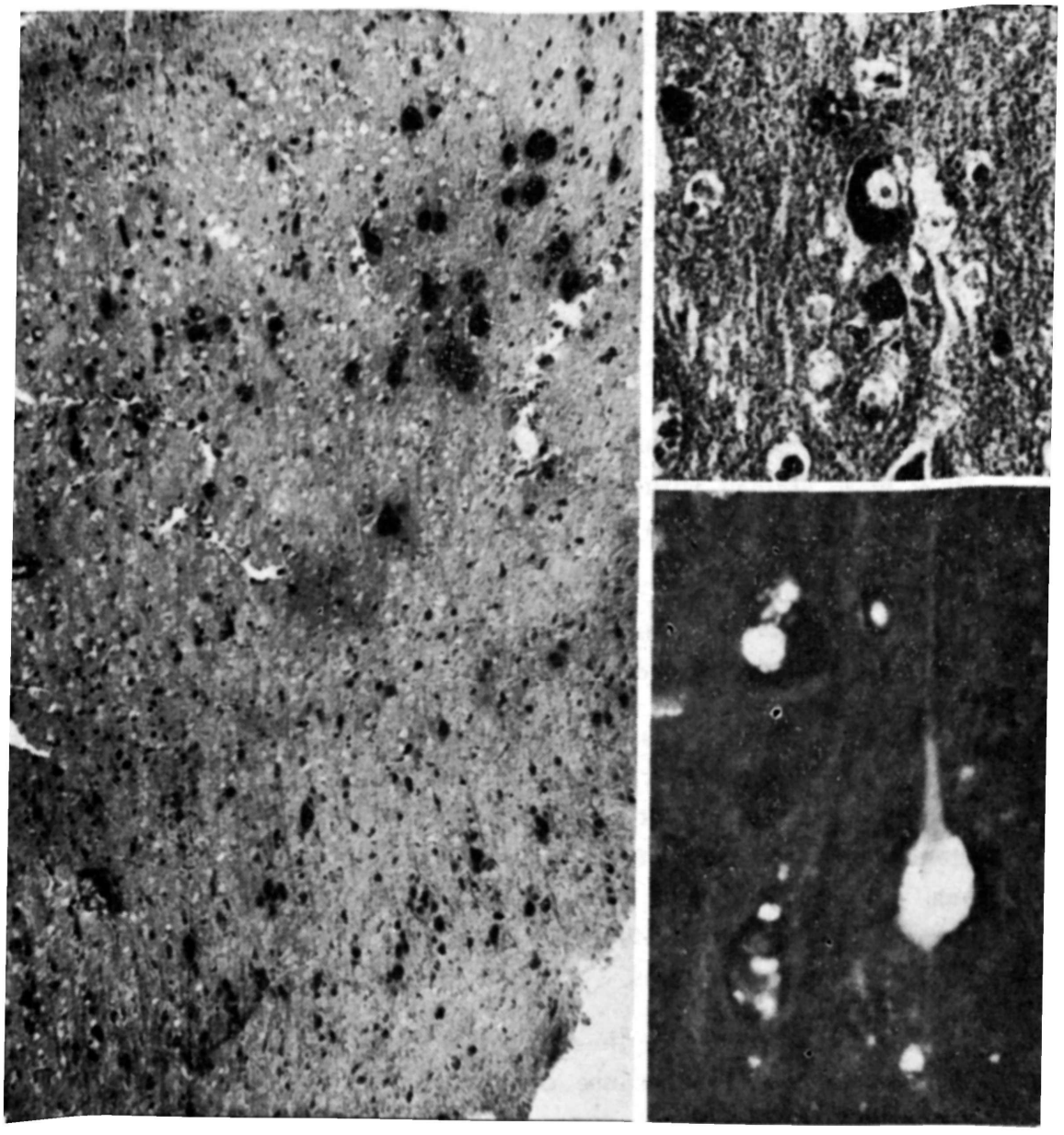

Fig. 1 — (left). Cerebral cortex. Markedly distended nerve cells in layer III, filled with PAS-positive material. Less severely involved neurons in all layers. Frozen section, PAS, x 40.

Fig. 2 — (top right). Sudanophil material in cytoplasm of a pyramidal cell. Note normal nucleus. Parraffin section, Sudan Black B, x 330.

Fig. 3 — (bottom right). Fluorescent substance in the cytoplasm of a pyramidal cell. Unstained paraffin section, ultraviolet light, x 330.

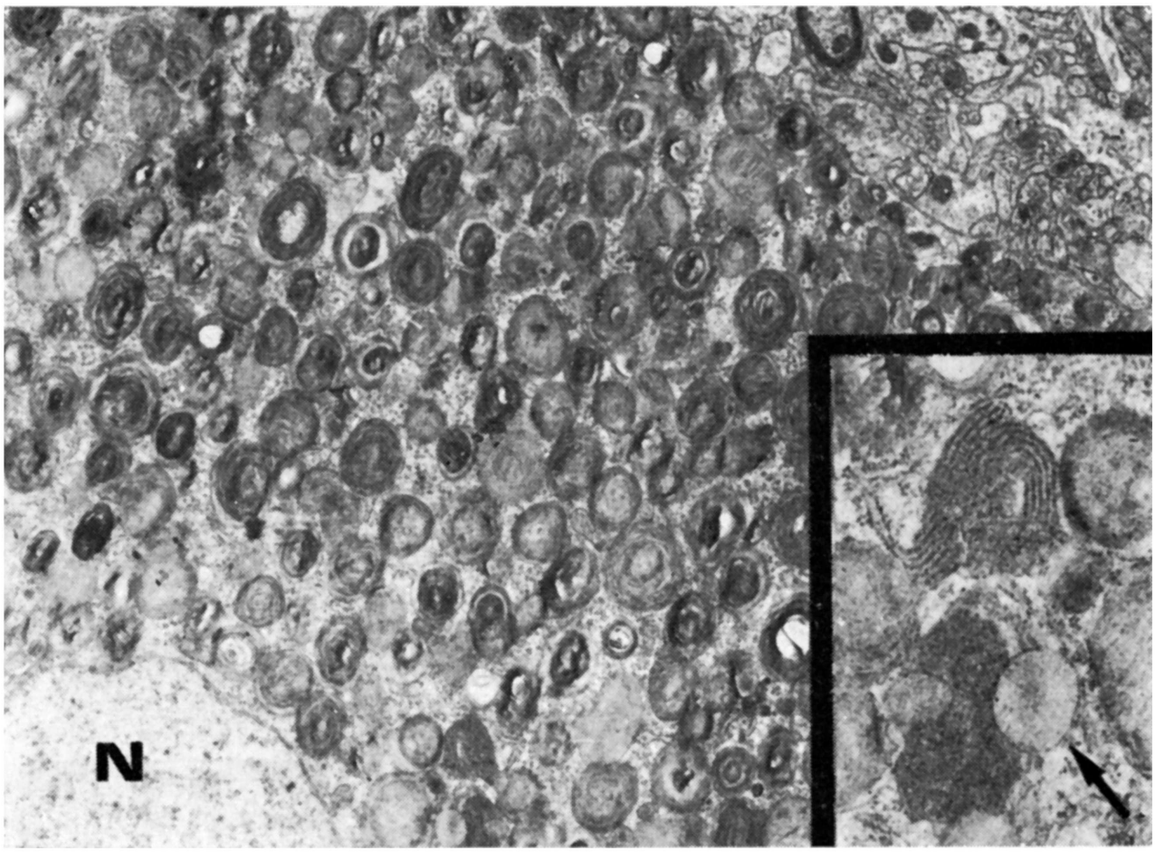

Fig. 4 — Cytoplasm of ballooned neuron showing numerous onion-bulb-like corpuscles (similar to the so-called membranous cytoplasmic bodies). N = nucleus. x 8,300. Inset: same neuron to show a fingerprint-like inclusion (above) and a lipopigment body (below) with electron-lucent vacuoles (arrow). x 21,600

(Fig. 5). These cytosomes coexisted with others of similar size formed by stacks of parallel membranes surrounded by a single membrane, which recalled the zebra bodies described in gargoylism [1] (Fig. 6). A few cytosomes had the features of lipofuscin granules and rare others showed fingerprint profiles (Fig. 4, inset).

In the neurons in which the stored material was autofluorescent, PAS-positive and sudanophil in paraffin sections the intracytoplasmic bodies revealed an ultrastructure similar to that of lipofuscin [21] (Fig. 7). They measured 0.5 to 1.0μ on the average, had an irregular contour and were constituted by a granular osmiophilic matrix with variable number of electron-lucent vacuoles, 120 to 360 mμ in size. Sometimes the osmiophilic component predominated and vacuoles became rare. Occasional zebra bodies were observed among the lipofuscin-like corpuscles.

Lipopigment granules were the most frequent type of stored cytosome in cortical neurons considered as a whole. Axonal torpedoes showed rounded dense bodies measuring 0.6 to 1.0μ, with occasional vacuoles, recalling the aspects described by Elfenbein [6]. Similar bodies were sometimes detected in astrocytes and endothelial cells.

DISCUSSION

The patient's clinical picture deviated from the usual findings in the neuronal ceroid-lipofuscinoses by prominent choreic movements and absence of visual manifestations. Extrapyramidal signs such as stiffness, akinesia and dystonic

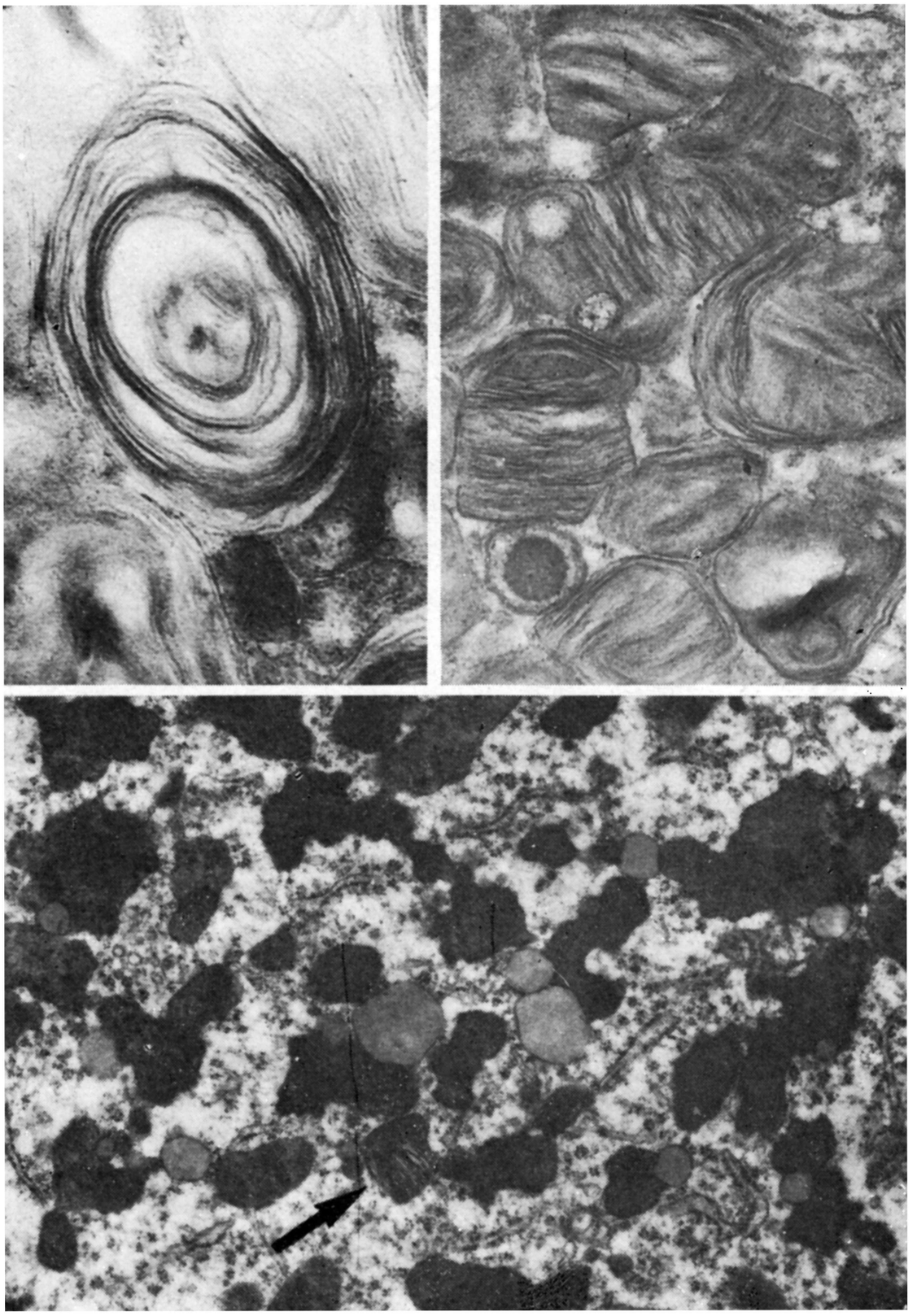

Fig. 5 — (top left). Detail of one onion-bulb-like corpuscle seen in Fig. 4. x 62,000.

Fig. 6 — (top right). Another kind of intracytoplasmic bodies, similar to the so-called zebra bodies, formed by parallel membranes, observed in the same neuron of Fig. 5. x 34,500.

Fig. 7 — (bottom). Lipopigment bodies interspersed among the rough endoplasmic reticulum of a cortical neuron. Arrow indicates a zebra body. x 20,700.

postures occur frequently and early in the NCL[30], but involuntary movements have been reported in only a few [4,12,14,27]. Visual loss, although a hallmark of the NCL, is not considered sine qua non for the diagnosis [30] and cases with normal vision and/or absence of fundoscopic abnormalities have also been on record [4,5,6,10,13,14,27].

It has been a matter of debate whether the fine structure of the stored lipopigments is important for classification of the NCL[29,30]. Nowadays, a more or less well defined ultrastructural pattern has been found in each of the three clinical categories. Thus, granular osmiophilic deposits are a feature of the infantile form [22]. Curvilinear bodies and fingerprint profiles are described are described respectively in the late infantile and juvenile types [8], although some overlapping of the ultrastructural findings in these varieties is often referred to [24,26]. Nevertheless, many more intracytoplasmic inclusions have been recorded, quite different from those described above [11], and Zeman [29] correctly points out that if strict morphologic features were the sole criterion for classification of the NCL, each patient would present his own exclusive variety.

The ultrastructural findings in our case cannot be accomodated into any of the three accepted variants. Lipofuscin-like granules were the most frequent storage material in neurons, but this pigment has seldom been observed in large quantities in cases of NCL[17,26]. A smaller number of neurons was greatly distended by cytosomes similar to the membranous cytoplasmic bodies (MCB) of Tay-Sachs disease [25], and also contained zebra bodies reminescent of those described in gargoylism [1]. Despite several differences, these ultrastructural features still recall those of Elfebein's case [6], who also had normal optic fundi but lacked involuntary movements. However, the patient of Dal Canto et al.[4], which clinically was the most similar to ours, presented a notably distinct fine structural morphology. It may be concluded that the present case is unique if the clinical and pathological aspects are considered together.

The ultrastructural findings in our patient are similar to an instance of canine lipidosis [3]. The dog lacked visual disturbances and fundoscopic changes, and the neurons contained typical lipofuscin granules, zebra bodies and MCBs, in addition to other types of inclusion.

It is unfortunate that neurochemical analyses could not be performed in present material. Many nerve cells harboured non-autofluorescent corpuscles, soluble in organic solvents, which ultrastructurally resembled the MCBs of Tay-Sachs disease. This fact suggests that the patient might present a disturbance of sphingolipid metabolism. Nevertheless, since the majority of neurons contained autofluorescent lipopigments, our case should be tentatively placed among the NCL. As suggested by Dal Canto et al.[4], many more reports of atypical cases of non-glycolipid neuronal storage disorders will be needed before a sound classification of these disease is accomplished.

SUMMARY

A case of neuronal ceroid-lipofuscinosis (NCL) is reported in a 11-year-old girl, whose main symptoms were progressive dementia since the age of 4 years and choreic movements since age 10. Seizures, myoclonus and visual

deterioration were absent and optic fundi were normal. A cerebral biopsy disclosed two basic types of stored substance in the cytoplasm of neurons: *a*) severely balloned nerve cells in cortical layers III and V contained a non-autofluorescent material, which stained with PAS and Sudan Black B in frozen, but not in paraffin sections; ultrastructurally, these neurons showed abundant corpuscles similar to the membranous cytoplasmic bodies of Tay-Sachs disease and, in smaller amounts, also zebra bodies; *b*) slightly distended or non-distended neurons in all layers contained lipopigment granules, which were autofluorescent, PAS-positive and sudanophil in both frozen and paraffin sections; their ultrastructure was closely comparable to that of lipofuscin. Similar bodies were found in the swollen segments of axons and in a few astrocytes and endothelial cells.

The histochemical and ultrastructural demonstration of large amounts of lipopigments allows a presumptive classification of the case as NCL. However, the presence of involuntary movements, the absence of visual disturbances and the unusual ultrastructural features place the patient into a small heterogeneous group within the NCL. A better classification of such unique instances of the disease must await elucidation of the basic enzymatic defects.

RESUMO

Ceróide-lipofuscinose neurônica: estudo histoquímico e ultraestrutural de um caso com movimentos coreicos e sem distúrbios visuais.

É relatado um caso de ceróide-lipofuscinose neurônica, excepcional por suas características clínicas e ultraestruturais. Trata-se de menina de 11 anos, cujos principais sintomas foram demência progressiva de início aos 4 anos e movimentos coreicos na face e membros a partir dos 10 anos. Convulsões, mioclonias, deficiência visual e anormalidades fundoscópicas não foram observadas. Uma biópsia cerebral revelou basicamente: *a*) em neurônios de aspecto abalonado nas camadas corticais III e V, acúmulo de material não-fluorescente, que era PAS-positivo e Sudan Black B — positivo em cortes de congelação, porém que perdia estas propriedades após inclusão em parafina; ao microscópio eletrônico, tais neurônios revelaram corpúsculos intracitoplasmáticos semelhantes aos "membranous cytoplasmic bodies" da doença de Tay-Sachs e alguns "zebra bodies"; *b*) neurônios pouco ou não distendidos em todas as camadas corticais continham grânulos PAS-positivos, Sudan Black B-positivos e autofluorescentes tanto em cortes de parafina como de congelação, cuja ultraestrutura era semelhante à da lipofuscina. Corpúsculos análogos foram observados em alguns axônios, astrócitos e células endoteliais.

A demonstração histoquímica e ultramicroscópica de grande quantidade de lipopigmentos no córtex cerebral permite-nos classificar o caso como ceróide-lipofuscinose neurônica (CLN). Sua características ultraestruturais incomuns e peculiaridades clínicas como os movimentos coreicos e a falta de distúrbios visuais justificam porém situá-lo em um pequeno grupo especial nas CLN. Uma correta correta classificação só será possível após a descoberta dos defeitos enzimáticos responsáveis pela doença.

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Departamento de Anatomia Patológica — Faculdade de Ciências Médicas da UNICAMP — Caixa Postal 1170 — *13100 Campinas, SP — Brasil.*