Subacute spongiform encephalopathy

Electron microscopic studies

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SUMMARY Electron microscopic changes in brain biopsies from two cases of subacute spongiform encephalopathy are described. Large vacuoles seen in astrocytic and neuronal processes were surrounded by one or more concentric membranes. Dense-core vesicles were found in axonal endings and in nerve cell perikarya; these particles could represent catecholamine-containing vesicles.

Subacute spongiform encephalopathy is a relatively rare disease. Although the clinical and electroencephalographical features are well defined (Goldhammer, Bubis, Sarova-Pinhas, and Braham, 1972), a definitive diagnosis during life can be made only by cerebral biopsy. During the last few years the main changes have been well described in several papers dealing with the electron microscopical appearance of brain obtained by biopsy. Vernon, Horta-Barbosa, Fuccillo, Sever, Baringer, and Birnbaum (1970) claimed to have found virus-like particles in the brains of two patients suffering from this disease. In two of our own cases studied in this way similar electron microscopic findings were noted, but we believe that at this stage it may be misleading to use the term 'virus-like'.

CASE 1

This case has been described in detail as case no. 6 by Goldhammer *et al.* (1972). B.C., a 63 year old man, had had a head injury without loss of consciousness 10 months before admission for investigation for headaches, giddiness, and difficulty in walking. Weakness of the left limbs, choreiform movements of the left arm, generalized hyperreflexia with equivocal plantar responses, poor coordination, and dysarthria were noted. Cortical atrophy with normal sized ventricles was revealed by pneumoencephalography. Blood, urine, and cerebrospinal fluid (CSF) laboratory findings were normal. The electroencephalogram (EEG), however, showed a characteristic sharp wave pattern.

General deterioration ensued with increased ataxia, aggravation of the choreiform movements on the left, confusion and myoclonic jerking, and the patient became bedridden. Repeat EEGs showed progressive augmentation of the abnormal elements, with synchronization of myoclonus and sharp waves. Biopsy was performed in the right parietal area. A trial of idoxuridine intravenously failed to halt the deterioration in his condition, which led to a state of immobility punctuated only by myoclonic jerks, and in which he died six weeks after admission.

CASE 2

The patient was a 62 year old Russian-born widow with a history of hypertension for the past 12 years. There had been admission to mental hospitals in 1959, 1965, and 1970 for depressive and hypochondriacal symptoms but she had been able to work as a clerk during the intervals. In December 1970, because of a suspected tumour in the apex of the left lung, bronchoscopy was performed under general anaesthesia; no tumour was found. Anti-tuberculosis therapy was decided upon and she returned to the psychiatric hospital because of increasing depression. At the end of January 1971 she became confused. Because of suspected toxic psychosis all treatment was withdrawn but without improvement. On admission to this department on 15 February 1971, she was noted to be mumbling to herself and no contact was possible. Myoclonic jerks were observed, especially of the shoulders and neck; the musculature was generally rigid. Within a few days she sank into a state of stupor and lay in a decorticate posture. The myoclonus increased in frequency, and nuchal rigidity was extreme. Occas-

FIG. 1. Case 1. An irregular 6-7 Hz background is discernible. High voltage diphasic sharp waves recur throughout the record at intervals of about one second. They are seen on both sides but are somewhat more evident on the right. Irregular delta activity appears bilaterally.

ional nystagmoid movements of the eyes to the left were observed. Tendon reflexes were brisk but plantar responses were flexor. A snout reflex was obtainable.

LABORATORY FINDINGS Radiography of the chest showed fibrosis of the lungs and an old tuberculous lesion of the left apex. Skull films were normal. In the initial EEG delta waves were seen in the frontal areas. A repeat record 10 days later showed typical features of Jacob-Creutzfeldt disease with sharp waves appearing at intervals of about one second (Fig. 1). Routine blood and urine tests were normal. The cerebrospinal fluid was under normal pressure and contained 60 mg protein, 8 mg glutamine, and 87 mg glucose per 100 ml. and no cells.

On 1 March 1971 cortical biopsy of the right frontal lobe was performed; the brain was atrophic and arachnoid cysts were noted. The patient died several hours after the brain biopsy. On necropsy no striking changes of internal organs were noticed, no

evidence of active tuberculosis or tumour were seen in the lungs. Section of the brain revealed a large intracerebral haematoma which had destroyed the right frontal lobe.

METHODS

Under general anaesthesia burr holes were drilled in the right parietal area in case 1, and in the right frontal area in case 2. Cerebral cortex was obtained using a sharp curette. Half of each specimen was processed for light microscopy and the other half fixed in glutaraldehyde, post-fixed in osmic acid, embedded in Epon, and examined under electron microscope.

RESULTS

In case 1 no abnormalities were seen on light microscopy of the brain biopsy, except for a

FIG. 2. *Case 1. Microphotograph of brain biopsy. The neurones appear normal; now and then a tiny vacuolar space is seen in the neuropil. H and E, $\times 320$.*

FIG. 3. *Case 2. Electron micrograph of brain biopsy. Neuropil apparently normal except for the large vacuole which has two involving membranes. $\times 10,500$.*

FIG. 4. Case 1. Electron micrograph. Notice the vacuole surrounded by three membranes, the two outermost closely apposed. L: lipofuscin. $\times 14,000$.

FIG. 5. Case 2. Electron micrograph. Club-shaped axonal ending containing several vacuoles, distorted neurotubules and a dense core vesicle. $\times 49,000$.

decreased number of neurones in some areas (Fig. 2).

In case 2 light microscopy of the biopsy revealed a slight decrease in number of neurones, and an increased number of astrocytes and microspangiosis, particularly of the deeper layers of the cortex. The appearance of blood vessels present was within normal limits.

The Epon embedded material examined under light microscope showed abnormalities which in case 1 had been only faintly discerned in paraffin embedded material. Microspangiosis was seen in both cases. Astrocyte nuclei were large and pale. The neurones showed numerous pigment granules not seen in the HE stained material. Axons and myelin sheaths were normal.

FIG. 6. Case 1. Electron micrograph. Part of perikaryon of nerve cell (N) with its Golgi apparatus (G). Dense core vesicles (arrows) are seen in axon terminals. Similar vesicles are also present in the perikaryon. $\times 28,000$.

ELECTRON MICROSCOPIC OBSERVATIONS Typical microspangiosis was seen as large empty spaces invariably surrounded by a definite membrane, some by two or even three concentric membranes, the outer one being perhaps the cell membrane of the process where the large vacuoles were situated. The vacuoles were mainly seen in post-synaptic areas, a few in axon terminals or in glial processes (Figs 3 and 4).

Gliosis with an increase in astrocytic processes which contained thick bundles of microfilaments was noted. Nerve cells were normal except for

the presence of numerous inclusions morphologically similar to lipofuscin. These inclusions were usually large, of bizarre shape with intermingled electronlucent and electronopaque areas. Axons and myelin sheaths were normal but for the presence of artefactual separation of myelin lamellae. Synapses were also normal. The presynaptic areas contained numerous clearcored synaptic vesicles. In many of the presynaptic areas one or two vesicles were seen and these were larger than the synaptic vesicles. They measured from 80–100 nm and contained

a central dense core separated by a thin halo from the limiting unit membrane (Fig. 5). Here and there the dense-core vesicles were numerous and appeared in processes devoid of clear synaptic vesicles. No such dense-core vesicles were seen in postsynaptic areas but did appear in some nerve cell perikarya (Fig. 6).

DISCUSSION

Clinical and electroencephalographic diagnosis was made in both patients as outlined in our previous paper (Goldhammer *et al.*, 1972). As we pointed out, a possible precipitating factor could be found in four out of six cases. Similarly in case 2 of the present paper a precipitating cause could be postulated—namely, general anaesthesia for bronchoscopy six weeks before the beginning of the neurological picture. The previous long psychiatric history of this patient seems to be irrelevant.

The minimal changes in the light microscopy of the biopsy in case 1, in which, however, a characteristic electron microscopic picture was seen, illustrates the difficulty in making a pathological diagnosis. This has led in the past to the separation of a group with so-called 'clinical-anatomical dissociation' (Jacob, 1967; Macchi and Lechi, 1967; Terzian, Rizzuto, Patarnello, and Martin, 1967). The electron microscopic picture in both our cases had the characteristics outlined by several authors (Foncin, Gaches, and Le Beau, 1964; Marin and Vial, 1964; Gonatas, Terry, and Weiss, 1965; Foncin, 1967; Kidd, 1967; Sluga and Seitelberger, 1967; Bignami and Forno, 1970). The large vacuoles, seen in nerve and glial processes, were similar to those described by Gonatas *et al.* (1965), Kidd (1967) and Bignami and Forno (1970). Lampert, Gajdusek, and Gibbs (1971) described the vacuolar changes also in nerve and glial processes in the brain of chimpanzees inoculated intracerebrally with brain suspensions from patients suffering from the disease.

The double or triple membranes which envelop some of the vacuoles have not been previously described, but Figs 12 and 13 of Lampert's work do, in fact, show vacuoles limited by a double membrane inside glial cells. In our cases the double membrane vacuoles are also seen in

nerve endings. The significance of this finding is unknown.

Our own studies confirm the findings of 'particles' with morphological characters as described by Vernon *et al.* (1970). However, we are of the opinion that at present there is insufficient evidence to designate them as being virus or of viral origin. Similar vesicles are seen in the nervous system and are considered to be collections of biogenic amines (Budd and Salpeter, 1969; Hökfelt, Jonsson, and Linbrink, 1970; Iraldi and de Robertis, 1970; Kuhar, Green, Snyder, and Gfeller, 1970). Loss of mono-amino-oxidase (MAO) was observed in Creutzfeldt-Jakob disease by Robinson (1969), particularly in substantia nigra and cortical pyramidal cells. It is possible, then, that diminished activity of MAO in the cerebral cortex could lead to accumulation of biogenic amines and therefore increase in the number of dense core vesicles. Furthermore, we found no particles in glial cells, in which they have been reported in two cases of Creutzfeldt-Jakob disease (Bots, Man, and Verjaal, 1971) and in other diseases by Gonatas, Martin, and Evangelista (1967). The last mentioned authors emphasized that no conclusions could be drawn of the nature of the particles from morphological evidence alone; the mentioned lipid droplets or secretory granules as alternative explanations.

Nevertheless, since the cause of the disorder has been shown to be a transmissible agent (Gibbs, Gajdusek, Asher, Alpers, Beck, Daniel, and Matthews, 1968; Beck, Daniel, Matthews, Stevens, Alpers, Asher, Gajdusek, and Gibbs, 1969; Lampert *et al.*, 1971), it is tempting to believe that the particles referred to are indeed of viral nature, a proposition which has led to therapeutic trials with idoxuridine in case 1, and with amantadine in another case (Braham, 1971).

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