FURTHER OBSERVATIONS ON THE PATHOLOGY OF KURU*

(A Study of the Two Cerebra in Serial Section)

B. A. KAKULAS, M.D.,†**, ANDRE-ROCHE LECOURS, M.D.***
AND D. CARLETON GAJDUSEK, M.D.****

(Boston, Mass. and Bethesda, Md.)

It is now 9 years since the first description of Kuru as a subacute degenerative disease of the central nervous system afflicting the Fore and neighboring peoples of the eastern New Guinea Highlands was published by Zigas and Gajdusek (1957) (12, 29). The etiology and pathogenesis of this disease (1-3, 6-18, 21, 24, 28) remains obscure (8, 9), but the recently reported successful transmission of a kuru-like syndrome to chimpanzees (11) suggests that a microbial, possibly a viral, agent may be involved. The histopathology of the disorder was studied by Klatzo, Gajdusek and Zigas (17, 18) and by other workers (2, 6, 21, 22, 26) in some 40 cases, and the principal anatomic lesions were described as a degeneration and a loss of neurons, microglial proliferation and fibrous astrocytosis, status spongiosus in the cerebral cortex and basal ganglia, variable white matter lesions (loss of myelinated fibers), and cortical cerebellar degeneration. Characteristic plaques were described as an outstanding feature in half of the cases, and moderate peri-vascular cuffing by mononuclear cells was noted. In the present study, based on the examination of 2 specimens in gapless serial section, we can corroborate most of the previous histopathological findings and, in addition, report several features not previously emphasized, such as the very wide distribution of the typical plaques observed in the white matter, as well as in the cerebral and cerebellar cortex and basal ganglia, and note for the first time excessive vulnerability of the limbic and para-limbic system.

MATERIAL AND METHODS

The autopsies on both Case 1 and Case 2 were performed soon after death (about 3 hours in case MU-28-59, and ½ hour in case RPSL-EX-141-59), and the brains were fixed imme-

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† On leave from the Department of Pathology, The University of Western Australia. The work was undertaken during tenure of a C. J. Martin Travelling Fellowship of the National Health and Medical Research Council of Australia, while on leave from the Department of Pathology of the University of Western Australia (B.A.K.) and during tenure of a Medical Research Fellowship of the Medical Research Council, Canada (A.L.) and a Research Fellowship in Neuropathology, Harvard Medical School and Massachusetts General Hospital (B.A.K., A.L.).
** Massachusetts General Hospital and the Warren Anatomical Museum, Harvard Medical School, Boston, Massachusetts.
*** National Institute of Neurological Diseases and Blindness, National Institutes of Health, Bethesda, Maryland.
by immersion in 10 per cent formal saline. They arrived at the National Institutes of Health, Bethesda, Maryland, after 2 months' fixation, and the formalin was renewed. Later the two brains were submitted to the Warren Anatomical Museum of the Harvard Medical School and were treated as follows.

CASE REPORTS

Case 1, MIU-28-59. The brain was embedded in celloidin and cut in gapless serial sections, in a coronal plane, at 35 micra, yielding 4,629 sections. Every twentieth section was stained for myelin by the Loyez method, and the adjoining section was stained with cresyl-violet (C.V.). Similarly sections at regular intervals were stained with hematoxylin and eosin (H&E), periodic acid-Schiff (P.A.S.), phosphotungstic acid hematoxylin (P.T.A.H.), Gres-Bielschowsky silver impregnation, phloxine tartrazine, Mallory's trichrome and Turnbull blue stains.

Case 2, RPSL-EX-141-59. The brain was also embedded in celloidin and cut in the coronal plane, in gapless serial sections at 20 and 35 micra thickness, yielding 3,762 sections. Every twentieth section was stained for myelin by the Loyez method, and the adjoining sections were stained with the cresyl violet. Additional sections were stained, at regular intervals, by the methods listed above for Case 1.

Clinical Data

Clinical data on the patients are scanty. Although they were classical cases of kuru, they were not of the group of intensively studied and repeatedly examined patients.

Case 1. History: XAMUNTO (Amuno), MIU-28-59. This patient was an adult female about 30 years of age; she was a resident of Miarasa village in the South Fore linguistic group. Her mother is said to have died of kuru. Xamunto had 6 healthy children, ranging from a mid-adolescent to an infant of a few months of age at the time of the onset of her disease in 1958. However, by the late stage of her disease she was no longer able to care properly for her infant son and the child was brought to the Kainantu Hospital suffering from gross malnutrition, shortly before the mother's death on February 7, 1959. She had been examined briefly several times in her village by the kuru research team and found to be a typical case of advancing kuru. In August, 1958, she was found to be suffering from extreme motor ataxia, marked tremor, and dysarthria. She was still able to sit upright unaided, but could not walk without bilateral support. Thereafter, her condition deteriorated continuously until she was confined to her house unable to sit, speak or eat.

Case 2. History: KEBANO (Ke'evano), RPSL-EX-141-59. This patient was a middle-aged woman who was studied through much of the latter part of her illness at the Kainantu Hospital, outside of the kuru region where she was brought with her own and her relatives' consent. The onset of her disease had been insidious in the early part of 1959. By August 1959, when she was first examined by medical workers, she had marked kuru tremor and such severe locomotor ataxia that she could not stand without support, although she could sit upright and could support her weight and walk if well supported, bilaterally. She had a moderate dysarthria, but no strabismus or ankle clonus, nor were her deep tendon reflexes abnormal. Lumbar puncture revealed clear, colorless cerebrospinal fluid; pressure 90 mm, with normal dynamics. Examined macroscopically, only one mononuclear leucocyte was seen per mm²; Pandy reaction was negative. The patient was discharged after a brief hospital stay and was returned in mid-October 1959 to the Kainantu Hospital by Landrover as a terminal patient, completely helpless and showing gross wasting, hypoactive deep tendon reflexes, deep decubitus ulcerations lateral to her left knee and over her right hip. She lay mostly in a flexed posture, unable to talk; she had been abandoned to urinate and defecate in her house, and her relatives had ceased trying to feed her.

She died the day following admission to the hospital, on October 20, 1959, and autopsy was performed within a half hour of death. At post mortem the liver was slightly enlarged, but appeared normal otherwise, and there was some evidence of hypostatic pneumonia in the base of the lungs.
Fig. 1. *Case 1.* Shrinkage of the heads of the caudate nuclei, section no. 860, Loyez stain; ×2.

**Neuropathological Findings**

*Case 1, MU-28-59. Gross Findings:* The brain weighed 1005 grams after fixation in 10 per cent formalin. Its external appearance was normal, and there was no evidence of raised intracranial pressure. The leptomeninges were thin and translucent. The cerebral arteries were free of atheroma. The circle of Willis showed no anomaly. The brain was cut in the coronal plane and revealed shrinkage of the heads of both caudate nuclei so that the normal convex ventricular outlines formed by these structures were distorted (fig. 1) and there was moderate enlargement of the third ventricle. No other abnormality was evident macroscopically in the cerebral hemispheres and brain stem. The cerebellum showed atrophy of the vernal folia anteriorly.

*Microscopic Findings:* The leptomeninges showed slight fibrous thickening with occasional collections of round cells. Small leptomeningeval blood vessels were congested. The cerebral cortex showed signs of disease in all areas, with some foci more severely affected. In general, the changes consisted of neuronal loss, pigmentary atrophy of a small proportion of the surviving cells and others that had dark, pyknotic nuclei. Occasionally, neurons were swollen, with only peripherally preserved Nissl substance. None contained neuro-fibrillary tangles. The cell loss was irregular, and in some areas was pseudo-laminar in distribution. The deeper layers of the cortex were more affected than others, and spongy vacuolation of the neuropil was a feature of the more heavily involved regions. This change was most pronounced in the depths of the gyri which showed relative sparing of their crests. Axonal swellings were rare in the cerebrum, in marked contrast to the findings in the cerebellum. Moderate fibrous astrogliosis was associated with the neuronal loss, while there were fewer numbers of protoplasmic, Alzheimer II astrocytes. The astrocytic proliferation was more pronounced in the more severely affected areas, where, in addition, pleomorphic microgliocytes occurred in moderate numbers. Astrocytic and oligodendrocytic satellites were increased in number around the larger neurons. Much less common were microglial clusters and neuronophagic nodules. Small parenchymal capillaries and veins were conspicuous within the damaged areas and the occasional presence of a typical plaque of kuru.
Diag. 1. Shaded areas indicate distribution of most severe cerebral lesions for both cases.

completed the picture. These plaques varied between 20 and 70 micra in diameter, were markedly argyrophilic and consisted of a dense center surrounded by many delicate radiating fibrils. They stained well with P.A.S. but were more difficult to identify with hematoxylin-eosin, cresyl violet and phloxine-tartrazine stained sections.

Although the lesions described above were present in all areas of the cerebral cortex, they were more pronounced in the frontal and temporal lobes than posteriorly. The greatest involvement was found in the paramedian cortical structures, particularly in the cingulate gyri and to a slightly lesser degree in the medial aspects of the superior frontal gyri. The orbital gyri were moderately involved, and the insular cortex showed lesions of intermediate severity. The temporal lobes, particularly the hippocampal and parahippocampal gyri, were the next most involved areas, while the occipital lobes and the convex surfaces of the cerebral hemispheres were relatively spared. The distribution of the severe lesions is depicted in Diagram 1. The symmetry of the disease process is noteworthy. In general, subcortical and longer association and projection white matter tracts showed no changes except for a partial loss of myelinated fibers of the corpus callosum, anterior commissure and non-specific pulvinar radiations. The core of the anterior commissure retained its normal dark appearance in the Loyez stain for myelin, while the superficial fibers of this structure were paler than normal* (fig. 2). The non-specific thalamic radiations from the pulvinar to areas 18 and 19 were paler than in normal adult standards.

* It is interesting to note that these systems of fibers do not acquire stainable myelin at the same time during the development of the human nervous system. In a four-month-old infant, the peripheral fibers of the anterior commissure are already quite dark, while it will take many more weeks before any stainable myelin can be detected by the Loyez method in the core of this structure (Yakovlev and Lecours 26)).
available at the Warren Anatomical Museum. In myelin preparations the superficial or limbic fibers of the corpus callosum, which are normally darker than the deeper commissural fibers of this structure, were relatively pale so that the appearance is the reverse of normal.

Severe lesions were found in the basal ganglia. In the caudate nuclei there was a loss of both small and large neurons, satellitosis and neuronophagia, and well formed fibrous astrocytes and pleomorphic microglia were numerous. Pigmentary atrophy and occasional plaques were other findings. Similar changes were found in the lentiform nuclei, with the putamen having been more affected than the globus pallidus on both sides. The medullated fibers of the corpus striatum were paler than in the Warren Museum's standards, but this change, if significant, was not well developed. The thalami also showed severe changes similar to those found in the caudate nuclei and the medial and anterior nuclei were more affected than others. The sub-thalamic and red nuclei were much less involved. In contrast, the hypothalamus showed severe lesions, particularly near the midline, where there was well-marked fibrous gliosis.

The brain stem showed relatively few changes. The substantia nigra was normal. The middle cerebellar peduncles were paler than normal due to loss of medullated fibers (fig. 3). In the medulla, the inferior olivary nuclei showed a moderate loss of neurons and gliosis, as well as the presence of a few plaques. The cervical spinal cord showed abnormal pallor of the olivo-spinal tracts, but no other change. Cortico-spinal tracts were not affected at any level.

Striking lesions were present in the cerebellum, which were more advanced in the anterior portion of the vermis. The leptomeninges overlying the cerebellum were thickened and blood vessels were congested. Parenchymal lesions exhibited a thinning and excessive cellularity in the molecular layer, increase in the number of small blood vessels, clusters of microglia and of numerous astrocytes with well formed vertically oriented fibers (fig. 4). Occasional plaques, dendritic expansions of Purkinje cells and microglial brush
Fig. 3. Case 1. Pallor of the middle cerebellar peduncle, section no. 3980, Loyez stain; × 2.

Fig. 4. Case 1. Dense fibrous astrogliosis due to hypertrophy of the Bergmann fibers in the molecular layer of the cerebellum, section no. 3937, phosphotungstic acid hematoxylin stain; × 500.
formations were also noted in the molecular layer. There was a moderate loss of Purkinje cells, and chromatolysis was present in a small proportion of the remainder. Many of the Purkinje cells showed most pronounced axonal torpedoes in the vermis (fig. 5). These structures were often multiple, and were best seen in the Gros-Bieschowsky silver stained preparations. Bergman's astrocytes were greatly increased in number and size, and were markedly fibrous. Typical plaques were numerous as they were in the underlying granule
layer. There was a marked loss of granule neurons, especially in the vermis, with a lesser reduction in their number in other areas. The flocculonodular lobe showed lesions of moderate severity. The folial and central white matter of the cerebellum revealed slight pallor and fibrous astrocytic hyperplasia. The dentate nuclei showed pigmentary degeneration of many neurons and a fibrous gliosis. The most affected area of the dentate nucleus was its supero-medial portion.

Case 2. RPSL-EX1-41-69. Gross Findings: The brain weighed 915 grams, and showed slight thickening of the basal leptomeninges. The cerebral hemispheres, brain stem and cerebellum showed no unusual features, externally. The gyral pattern of the cerebrum was normal, the sulci were not widened, and there was no alteration in the consistency of the brain. The blood vessels were congested and their walls were free of atheroma. On section, the lateral walls of the lateral ventricles were deformed by the convevity produced by the shrunken heads of both caudate nuclei. The cortical ribbon, white matter of the hemispheres, lentiform nuclei, thalami, brain stem, and cerebellum were normal in appearance.

Microscopic Findings: The histopathological features were similar in all essentials to those in Case 1. The leptomeninges were thickened by fibrous tissue which contained numbers of amieloid fibroblasts, a few macrophages and some round cells. Cortical lesions were present. In these, there was a loss of neurons, pigmentary degeneration of remaining nerve cells, satellitosis, astrocytic gliosis (fibrous and protoplasmic), pleomorphic microglial proliferation, vacuolation of the ground substance and a prominence of small vessels. “Kuru plaques” were common (figs. 6, 7). The deeper layers of the cortex were more severely affected, and the process was more intense in the valleys of the convolutions. The disease process was more advanced anteriorly than posteriorly in the cerebrum, and the medial aspects of the frontal lobes were most intensely involved (fig. 8), particularly at the limbus of the hemispheres. The temporal lobe was affected to the same degree as in Case 1, but the parietal and occipital lobes were somewhat more affected. The orbital surfaces of the frontal lobes and the hippocampus were less involved than in Case 1. Circumscribed areas of neuronal loss were noted in the parahippocampal gyri. There was a loss of both large and small cells in the caudate nuclei with an associated astrogliosis (fig. 9), and a pleomorphic microgliosis. The process became less severe as the caudate nuclei were traced posteriorly. A singular and noteworthy finding was the presence of a large plaque within the centrum semi-ovale, well demonstrated by the Gros-Biel schowsky silver method (fig. 10). The lentiform nucleus, particularly the putamen, showed lesions of a similar type, and contained plaques.

Pallor of the intrinsic fibers of the putamen was more conspicuous than in Case 1. The thalamic involvement was similar to that in Case 1, but the third ventricle was not widened to the same degree. The hypothalamus was less affected. In the brain stem, changes were few, except for the substantia nigra where small numbers of pigmented neurons had disintegrated and melanin seen within macrophages. Rod-shaped microgliaocytes and fibrous astrocytes were noted in the tectum. The cerebral peduncles were dark in contrast to the medullary pyramids which were slightly paler than is normal.

The cerebellar vermis was severely involved by the disease process and showed astrocytic and microglial proliferation with brush formations in the molecular layer. There were Purkinje cell losses, dendritic enlargements, and proliferation of the Bergman’s astrocytic layer. Axonal swellings and torpedoes and many characteristic plaques were seen in the granule cell layer. The supero-medial portions of the dentate nuclei revealed more severe neuronal losses and glial reaction than was seen elsewhere. The cerebellar hemispheres were much less severely affected, with minimal Purkinje cell loss in the depth of the folia. The flocculonodular lobes were moderately involved. There was no significant perivascular cuffing and neurofibrillary tangles were not observed.

Gapless serial section, the unique feature in the handling of these kuru specimens, permitted accurate study of the distribution and severity of lesions.
Fig. 7. Case 2. Typical plaque in superior frontal gyrus; note fibrillary outer ring, section no. 1804, periodic acid-Schiff stain; $\times$ 320.

Fig. 8. Case 2. Gliosis, loss of neurons, prominence of small vessels and vacuolation of the ground substance of the cingulate gyrus, section no. 1576, periodic acid-Schiff stain; $\times$ 50.
Fig. 9. Case 2. Astrocyte gliosis in the head of the caudate nucleus, section no. 1004, periodic acid-Schiff stain; × 200.

Fig. 10. Case 2. Plaque within the centrum semiovale, section no. 1586, Gros-Bielschowsky stain; × 220.
(diag. 1) and revealed the predominance of lesions in the limbic system and paleocerebellum. Since the 2 patients were not studied neurologically in great detail, only a brief correlation of the postmortem findings with the clinical features is possible. Both cases showed the characteristic nervous system lesions of kuru. There was selective neuronal loss. Axonal swellings and dendritic enlargements in the molecular, Purkinje and granule cells layers of the cerebellum were well demonstrated by the Gros-Bielsehowsky method, and were considered to signify neuronal degeneration. Since most sections were thick (35 micra) and mounted on thick glass, doubtful histopathological findings such as relative hypercellularity were discounted. An adequate number were thinner (20 micra) and/or provided with thin cover slips. Changes unassociated with a cellular response, such as neuronal darkening and shrinkage, were not emphasized because it is difficult to distinguish such changes from artefacts (5, 19). More definite neuronal changes, e.g. those accompanied by neuronophagia, received greater emphasis. The phosphotungstic acid hematoxylin stain demonstrated diffuse fibrous gliosis which was particularly evident in the cingulate and superior frontal cortex, in the hippocampal formation and para-hippocampal gyrus and in the caudate nuclei, putamen and cerebellar vermis (fig. 1). Fibrous gliosis and pleomorphic microgliosis were out of proportion to the signs of neuronal damage. The characteristic plaques of kuru were abundant, their morphology and staining were as described by others (17, 18) and they were best stained by the silver methods. In myelin stained (Loyez) sections, the abnormal areas were not totally devoid of myelin and there was little glial reaction to the myelin loss. In contrast to previous reports, perivascular cuffing was not present in either case and there was no significant vacuolation of neurons (6).

In neuropathological terms, kuru resembles the “degenerative”, “metabolic” or “toxic” groups of diseases, and such disorders often involve functionally related systems of neurons. System involvement in kuru is manifest in the limbic and paralimbic lobes of the cerebral hemispheres as well as in the paleocerebellum. Recent clinical descriptions have emphasized the signs of cerebellar disease (16). Within the group of human degenerative diseases, the cerebral lesions of kuru bear a close resemblance to those of Creutzfeldt-Jakob disease (18), and the disease has also been compared by Seitelbelger (24) to a rare familial disorder encountered in Austria. There is no feature in the histopathological lesions which can help decide between the various etiological possibilities such as infective (2, 4, 8–11, 13, 20), immunologic (8, 15), genetic (3, 8), toxic or deficiency factors (8) which have been considered. The lesions bear a close resemblance to those found in degenerative and metabolic diseases of the nervous system including several of known etiology (23, 27) as well as to those found in the virus disease scrapie (2, 8). The transmission of a kuru-like syndrome appearing 2 years after intracerebral and intravenous inoculation of 10 per cent brain homogenates from cases of kuru into chimpanzees, as recently reported by Gajdusek, Gibbs and Alpers (11), emphasizes the possibility that a replicating microbial agent may be
involved in this extraordinary pathological reaction, which is of a type not usually thought to be of infectious origin.

SUMMARY

Two brains of patients with typical kuru have been examined in gapless serial section. Loss of neurons, fibrous gliosis and microglial proliferation in the cerebral cortex, basal ganglia and vermis of the cerebellum, which are associated with characteristic plaques, were the principal findings. The most severe lesions were found in the cingulate gyri, the heads of the caudate nuclei, medial portion of the thalamus and in the hypothalamus. Loss of myelinated fibers was evident in the corpus callosum, in the anterior commissure and in the cerebellum, where swelling of axones of Purkinje cells, and numerous plaques, were conspicuous features of the pathological changes. The vulnerability of the limbic and para-limbic lobes of the cerebral hemispheres is reported for the first time, and the disorder is presented as a system degeneration of these structures and of the paleocerebellum.

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REFERENCES