Journal of Neuropathology and Experimental Neurology

Vol. 44, No. 6
November, 1985
pp. 559–577

Neuropathological Classification of Huntington's Disease

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Abstract. In postmortem brain specimens from 163 clinically diagnosed cases of Huntington's disease (HD) the striatum exhibited marked variation in the severity of neuropathological involvement. A system for grading this severity was established by macroscopic and microscopic criteria, resulting in five grades (0–4) designated in ascending order of severity. The grade correlates closely with the extent of clinical disability as assessed by a rating scale. In five cases of clinically diagnosed HD there were no discernible neuropathological abnormalities (grade 0), suggesting that the anatomical changes lag behind the development of clinical abnormalities. In eight cases, neuropathological changes could only be recognized microscopically (grade 1). The earliest changes were seen in the medial paraventricular portions of the caudate nucleus (CN), in the tail of the CN, and in the dorsal part of the putamen. Counts of neurons in the CN reveal that 50% are lost in grade 1 and that 95% are lost in grade 4; astrocytes are greatly increased in grades 2–4. These studies indicate that analyses of the CN in grade 4 would reflect mainly its astrocytic composition with a component of remote neurons projecting to the striatum. Because of the relative preservation of the lateral half of the head of the CN in grades 1–2, these regions would reflect early cellular and biochemical changes in HD.

Key Words: Caudate-nucleus; Corpus striatum; Huntington's chorea; Morphometry; Neuronal atrophy; Putamen.

INTRODUCTION

Huntington's disease (HD) is inherited through an autosomal dominant gene located on chromosome 4 (1). The symptoms are progressive and characterized by both behavioral and motor disturbances which usually appear in adult life. The duration of the disease is about 17 years; its prevalence is estimated to be between 5 and 7.5 per 100,000 persons (2, 3).

Anton (4) and Lannois (5) were among the first to recognize clearly that the corpus striatum (caudate nucleus, putamen and globus pallidus) was abnormal in HD. Their findings gained acceptance between 1904 and 1911 (6, 7). In addition, neuropathological alterations have been noted in other regions of the brain (8–10). These areas

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Supported in part by NINCDS Grant 16367 (Huntington's Disease Center Without Walls) (RHM, EDB, EPR), NIMH/NINCDS 31862 and Hereditary Disease Foundation (Brain Tissue Resource Center) (TJS), The Fonds National Suisse de la Recherche Scientifique, Switzerland (JPV), the Alexander von Humboldt-Stiftung, Bonn, Federal Republic of Germany (EPR, during sabbatical leave at the Neuropathological Institute, Free University of Berlin, Director: Prof. J. Cervós-Navarro), and the Massachusetts Huntington Disease Foundation of America (RHM).

include the subthalamic regions, pons and medulla oblongata (7), spinal cord (7, 11–13), amygdala (14, 15), cerebellum (11, 13, 14, 16–18), superior olive (12), and claustrum (10, 12). Although most observers have suggested the opposite, Pfeiffer (19) and Dunlap (20) found the putamen to be more involved than the caudate nucleus (CN) in HD. Dunlap (20) also noted atrophy of the white matter. In general, these reports have been based on analyses of a small number of brains (up to four). The studies of Dunlap (20) (17 brains), Stone and Falstein (8) (6 brains), and Forno and Jose (12) (14 brains) are the exceptions.

The neuropathological features that are now commonly accepted as characteristic of HD may be summarized as follows: the neostriatum (CN and putamen) undergoes diffuse atrophy (loss of neurons with astrogliosis) (10); the CN is more severely involved than the putamen (12). The globus pallidus also is affected, but to a lesser extent (11, 21, 22). In the cerebral cortex subtle changes occur (10, 12, 23).

Several investigators state that there are variations in the degree of severity of neuropathological changes in the striatum in HD (12–14, 20–22, 24–27). We have conducted a systematic neuropathological study of clinically diagnosed HD patients and have also been impressed with the variation we found in the extent and severity of the neuropathological changes. We report here a grading system using macroscopic and light microscopic examination that allows comparisons to be made among cases of HD and between HD and control brains. The designation of a grade may assist in the interpretation of neurochemical analyses in HD brains at different degenerative stages, in the interpretation of quantitative cytologic observations of different regions of the HD brain, and in the understanding of clinicopathological relationships.

MATERIALS AND METHODS

The grading system was developed through the examination of 238 half-brain specimens donated to the Brain Tissue Resource Center, McLean Hospital. The fresh brain was divided in the mid-sagittal plane at the time of the autopsy. One half of the brain was deep-frozen for biochemical studies and the other half was immersion-fixed for three weeks in 10% buffered formalin. The weight of each fixed half brain was recorded.

After separation of the brainstem and cerebellum by a transverse cut through the midbrain at the rostral border of the third cranial nerve, the fixed cerebral hemisphere was sectioned coronally and the brainstem and cerebellum were sectioned transversely at 0.5-cm intervals. For microscopic examination, 14 tissue blocks were taken systematically. The procedure was as follows:

Brain Regions Examined Microscopically: The four samples of cerebral isocortex and underlying white matter were: 1. the anterior frontal region, Brodmann area (BA) 9; 2. the precentral gyrus (BA 4); 3. the parietal region (BA 7); 4. calcarine area (BA 17 and 18). 5. The hippocampal region included: dentate, parahippocampal and occipitotemporal gyri with the tail of the CN and lateral geniculate body. Samples 6 to 9 were selected from the subcortical nuclei: 6. *level CAP* (C: caudate nucleus; A: nucleus accumbens septi; P: putamen), taken at the caudoputaminal junction; 7. *level GP* (GP: globus pallidus) with claustrum, CN, globus pallidus, paraventricular and supraoptic nuclei with nucleus basalis; 8. amygdala; 9. thalamus (centro-median nucleus), with the body of CN and rostral substantia nigra. Four levels of the brainstem were examined: 10. mesencephalon with substantia nigra and red nucleus (rostral border of the third cranial nerve); 11. upper pons; 12. lower pons; 13. medulla oblongata. A sample from the cerebellum, 14. included the cerebellar cortex with dentate nucleus.

Histopathological Methods: Paraffin sections (5–6 μm) were stained respectively with Luxol fast-blue hematoxylin and eosin (LH&E) for general tissue survey and assessment of myelin, cresyl violet (CV) (Nissl method) for cytoarchitectural evaluation, and the Bodian silver method for axons and neurofibrils. In the grading, the sections stained with these standard methods were used. In twelve representative cases, blocks CAP (#6) and GP (#7) were pro-

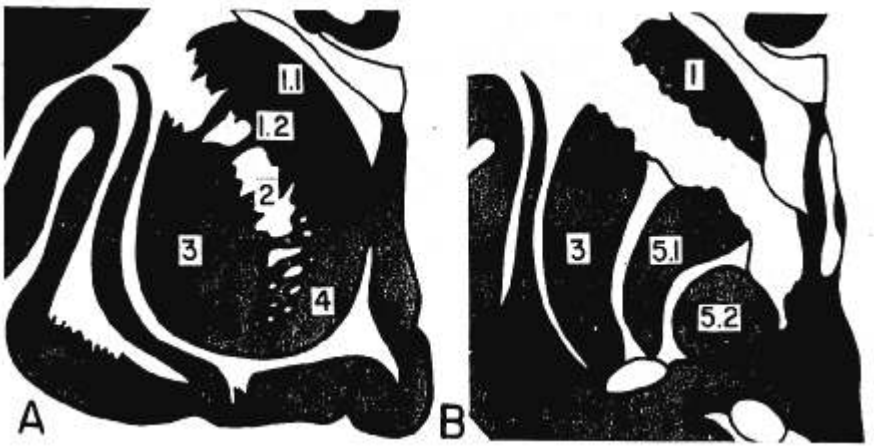

Fig. 1. Areas assessed in grading neuropathological changes in Huntington's disease. A. Block #6, level Caudate-Accumbens-Putamen (CAP). Head of caudate nucleus (1), medial (1.1), lateral portion (1.2). Gray matter bridges traversing internal capsule between head of caudate nucleus and putamen (2). Putamen (3). Nucleus accumbens septi (4). B. Block #7, level Globus Pallidus (GP). Head of caudate nucleus (1), putamen (3), globus pallidus (5), lateral (5.1), medial segment (5.2).

cessed with the peroxidase–antiperoxidase (PAP) method for glial fibrillary acidic protein (GFAP). GFAP antibody was kindly supplied by Amico Bignami, M.D., West Roxbury Veterans Administration Medical Center, Boston, MA 02132. For negative controls, normal rabbit serum was used in place of the primary antibody. An old cavitated cerebral infarct with an extensively gliotic border was used as a positive control.

Neuropathological Evaluation and Grading: The variation that was observed from case to case in our studies was far more striking in the structures demonstrated in levels of CAP and GP than in any other regions of the brain. These are shown diagrammatically in Figure 1. The planes of section for these levels were obtained as follows: with the anterior commissure as the external landmark on the medial surface for the first coronal section, the initial section was cut coronally, perpendicular to the long axis of the cerebral hemisphere and passing along the posterior border of the anterior commissure. The cross-section so obtained corresponds to level GP. Level CAP was obtained at the caudo-putaminal junction where the anterior limb of the internal capsule (IC) approaches the basal portion of the external capsule.

The neuropathological evaluation of each case included an assessment of the degree of severity of the disease process based upon both macroscopic and microscopic features. The macroscopic feature which proved to be the most useful indication of disease severity was the extent of atrophy as determined by the shape and size of the striatum at levels CAP and GP.

The histopathological features of relative neuronal loss and relative gliosis were evaluated by visual impression, and by cell counting in 31 cases (see below) comparing the case with normal control material. Both the neuronal density and astrocytic gliosis were rated as follows: within normal limits (0); slight (+); moderate (++); severe (+++); and very severe (++++). The usual number of cells in neostriatum per field at ×312 magnification for the rating (0) is: 18 neurons and 18 astrocytes without visible cytoplasm or processes as seen with LHE or CV stains; (+): 12 neurons and 25 astrocytes many of which are reactive as indicated by visible cytoplasm or processes; (++): nine neurons and 32 astrocytes, the majority of which are of reactive type; (+++): six neurons and 40 reactive astrocytes, and (++++): two neurons and 65 reactive astrocytes.

The macroscopic and microscopic evaluations were done independently by two of us (JPV, EPR), after which the results were compared and discussed. In instances of disagreement, or

when the assessment indicated a degree of severity that fell between two grades, we chose the more severe one in assigning the final grade. The neuropathological examinations in all instances were carried out without reference to the clinical data.

Quantitative Assessment of the Grade: A. Macroscopic Measurement of the Caudate Nucleus and Internal Capsule—The maximal width (perpendicular to ependymal surface) of the CN was measured macroscopically to the nearest 0.2 mm at the level of the head (level CAP), the body (level 9) and the tail (level 5) of CN. The width of the IC (level CAP) was also measured macroscopically. Those structures which were indistinguishable macroscopically such as the tail of the CN in the most severe degrees of atrophy, were coded as 0.2 mm.

B. Cell Counting—A quantitative assessment of the cellular population of the head of the CN was undertaken. Cases were randomly selected for each grade of severity. Seven age-matched control cases without neurological disease, six cases of grade 3 and five cases each for grades 4, 2 and 1 were included in cell counting studies. Only two grade 0 cases could be included because blocks of tissue at the proper level were not available for the other three grade 0 cases.

For cell-counting, the CV-stained sections for level CAP were used. The counts were made in the CN along one drive from the lateral border of the subependymal glial layer to the medial border of the IC. The location of the drive was obtained as follows: A line was drawn along the lateral border of the IC, with a dorsal limit at the junction of the IC and external capsule, and a ventral limit at the junction of the IC and the basal white matter. From a point on this line one-third of the distance from its superolateral end, a perpendicular line was drawn medially, which traversed the CN. The counts were made along this line, the lateral limit of which was made by a line drawn along the medial border of the IC.

We used a Zeiss microscope giving a magnification of ×312 and a Zeiss camera lucida to produce a series of drawings of microscope fields, each covering a surface of 0.961 mm^2 of the slide. Each field was defined by means of an eyepiece grid, and the first 0.12 mm from the ependymal surface were skipped. Neurons, astrocytes, and oligodendrocytes were recorded on the drawings. Determination of the number and location of each of these cell categories was made by a digitizing grid. All "dead" areas (e.g. vessels) larger than 0.0025 mm^2 on the slide were deleted.

Clinical Features: The medical records for the 163 cases with the clinical diagnosis of HD were reviewed. The following features were recorded when available: sex, age at death and a rating of physical disability (28). The reliability measures for the disability rating are reported elsewhere (28).

Statistical Methods: The correlations of the neuropathological grade with other variables were performed by non-parametric Spearman correlation coefficient. Other correlations were by Pearson coefficient. Multiple group comparisons of numbers of neurons were computed by one way analysis of variance. Adjustment for multiple comparison of means between groups was done by the Bonferroni method (29).

RESULTS

Neuropathological Diagnosis

Table 1 presents the neuropathological diagnosis of the 238 brains of which 163 had been clinically diagnosed as HD. As already indicated, a marked variation in severity was noted among the 158 pathologically confirmed cases of HD (the five grade 0 cases, by definition, are not included in this number). Four cases could not be given a classification of severity due to artifacts at both level CAP and GP; the pathologic changes in the body and tail of the CN and in the putamen were consistent with severe HD, however.

Grading System

We distinguished 5 grades (0 to 4) of neuropathological severity in HD to which 159 brains were assigned. The neuropathological characteristics of the individual

TABLE 1
Total Cases Examined Neuropathologically

Huntington's disease (HD)	153
HD with Alzheimer disease	5
Total pathologically confirmed HD	158
Clinically diagnosed HD not pathologically confirmed (grade 0)	5
At risk for HD	8
Controls without neurological disease	30
Total without neuropathological abnormality	43
Other neurological disorders	37
Total	238

grades are summarized in Table 2. Judgment as to the type and extent of the astrocytic reaction was based upon the examination of the LH&E-stained sections. The representative sections stained by GFAP confirmed the impressions gained from study of the LH&E-sections.

The grades are defined as follows:

Grade 0: The five cases so classified were those in which there was substantial clinical evidence for the diagnosis of HD, yet no gross or microscopic abnormalities that could be related to HD were discerned on examination of the brain (Figs. 2A, 3A, B).

Grade 1: Eight cases were classified as grade 1. In these, there were no macroscopically distinguishable alterations at levels CAP and GP (Fig. 2A). Microscopically, however, there was a moderate fibrillary astrocytosis which was much easier to assess than the neuronal loss. At level CAP, the medial half of the head of the CN was especially affected (Fig. 3C) and to a lesser extent the dorsal half of the putamen. When the lateral half of the head of the CN (Fig. 3D) was affected, the changes were always less severe than in the medial half.

At level GP, the CN showed a more diffuse involvement than in level CAP; nevertheless, the changes were less severe in the paracapsular portion of the nucleus. The putamen showed a slight astrocytosis throughout, slightly more evident dorsally. However, its neuronal density was apparently normal, as were the remaining anatomical structures in levels CAP and GP.

Although the tail of the CN (level #5) was not used in assessing the grade, it was our experience that neuronal loss and gliosis were more distinct in this part of the CN than in the head.

Grade 2: Thirty-eight cases were classified as grade 2. Macroscopically, atrophy of the head of the CN at level CAP was evident, but the convex outline of its ventricular surface (Fig. 2B) was retained, as was the medially convex configuration of the IC. The lateral ventricle was slightly enlarged.

At level GP the atrophy of the CN was more conspicuous than at level CAP. The putamen was slightly atrophic at both levels CAP and GP. The globus pallidus was unremarkable macroscopically.

Microscopically, level CAP showed evidence of neuronal loss with concomitant fibrillary astrocytosis in both the medial half of the head of the CN (Fig. 3E) and dorsal portion of the putamen. These changes were also present, but less pronounced, in the lateral half of the head of the CN (Fig. 3F). The putamen was less involved ventrally, showing only scattered fibrillary astrocytes.

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TABLE 2
Characteristics of the Neuropathological Grades of Huntington's Disease

	Caudate nucleus (level CAP)								
	Medial			Lateral			Putamen (level CAP and level GP)		
Grade	Atrophy	ND	FA	Atrophy	ND	FA	Atrophy	ND	FA
0	0	0	0	0	0	0	0	0	0
1	0	+–++	+–++	0	0–+	0–+	0	0	0–+
2	+	++	++–+++	+	+	+–++	+	+–++ (sup)	+–++
3	++–+++	+++	+++	++–+++	++	++	++	+++ (sup) ++ (inf)	+++ (sup) ++ (inf)
4	++++	++++	++++	++++	++++	++++	+++	++++	++++

ND = neuronal depletion, FA = fibrillary astrocytosis.
Atrophy is judged on macroscopic examination, ND and FA are judged on microscopic examination.
Sup = superior, inf = inferior.
0 = within normal limits, + = mild, ++ = moderate, +++ = severe, ++++ = very severe.
Levels CAP and GP are defined in Figure 1.

TABLE 2
Continued

Grade	Globus pallidus (level GP)						Nucleus accumbens (level CAP)		
	Lateral			Medial					
	Atrophy	ND	FA	Atrophy	ND	FA	Atrophy	ND	FA
0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0
2	0	0	+–++	0	0	0	0	0	0
3	+	+	+–++	+	0–+	0–+	0–+	0–+	0–+
4	++	+–++	+–++	++	+–++	+–++	+	0–+	+–++

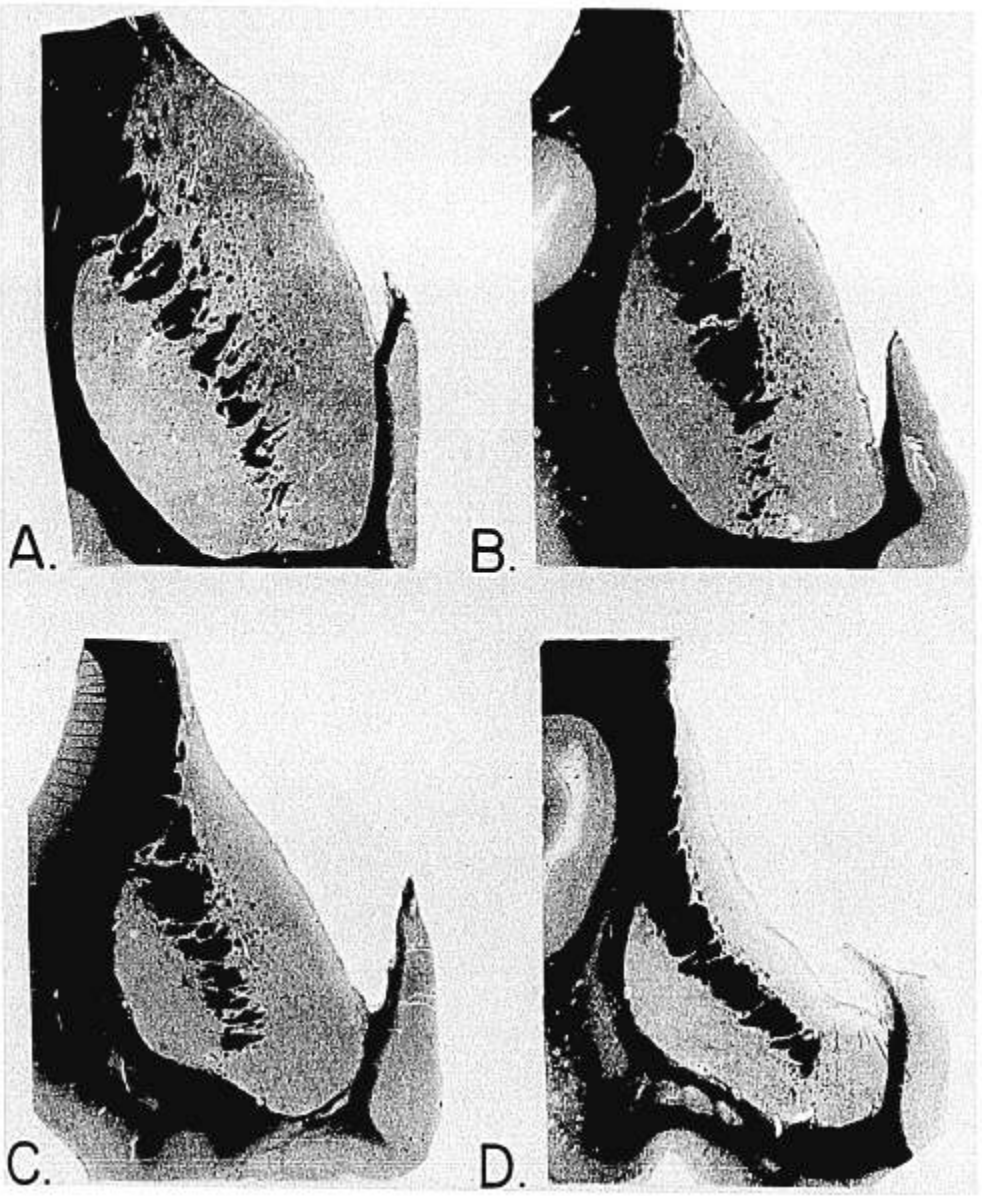


Fig. 2. Coronal sections at level CAP (see text and Fig. 2A) showing the respective grades. Luxol fast-blue hematoxylin and eosin stain (LH&E). ×2. A. Control, and grades 0 and 1. No abnormality on gross examination. B. Grade 2. The caudate nucleus is atrophic, but maintains its convex medial outline. C. Grade 3. The striatal atrophy is moderate to severe and the medial outline of the caudate nucleus is now flat, forming a nearly straight line. The cross-section outline of the anterior limb of the internal capsule has likewise lost its medial convexity. The putamen is atrophic. D. Grade 4. Very severe atrophy of the caudate nucleus and putamen, with markedly concave medial outline of both caudate nucleus and internal capsule.

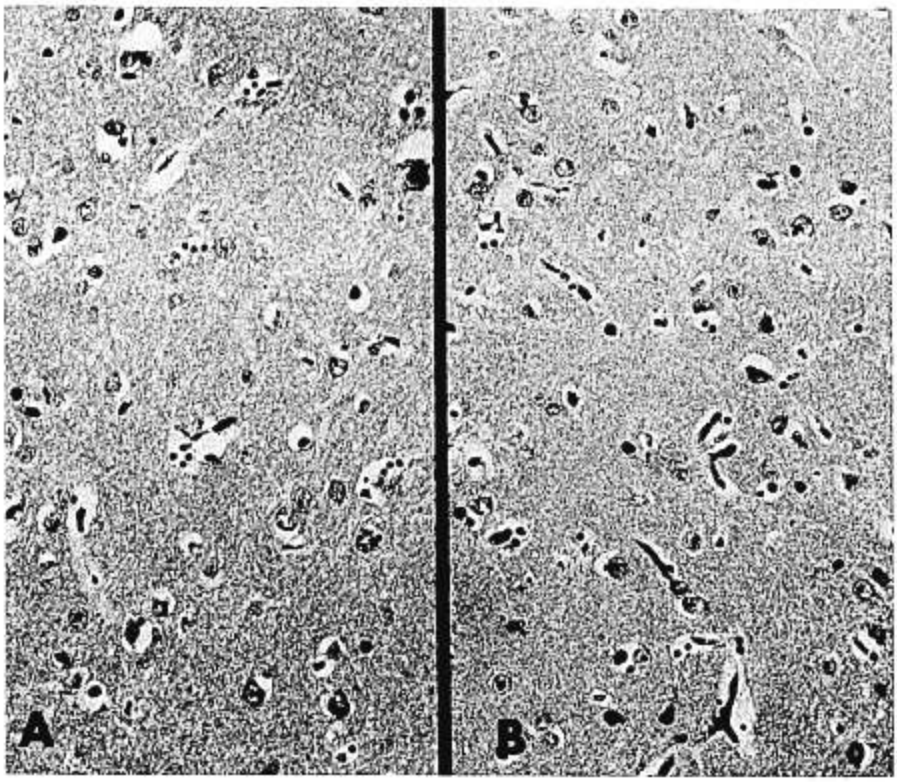


Fig. 3. Photomicrographs of representative regions of the head of the caudate nucleus (A–J) at level CAP (see text and Fig. 2A). A, C, E, G, I: medial; B, D, F, H, J: lateral. LH&E. ×320.

A and B: Age-matched control.

C and D: Huntington's disease, grade 1.

E and F: Huntington's disease, grade 2.

G and H: Huntington's disease, grade 3.

I and J: Huntington's disease, grade 4.

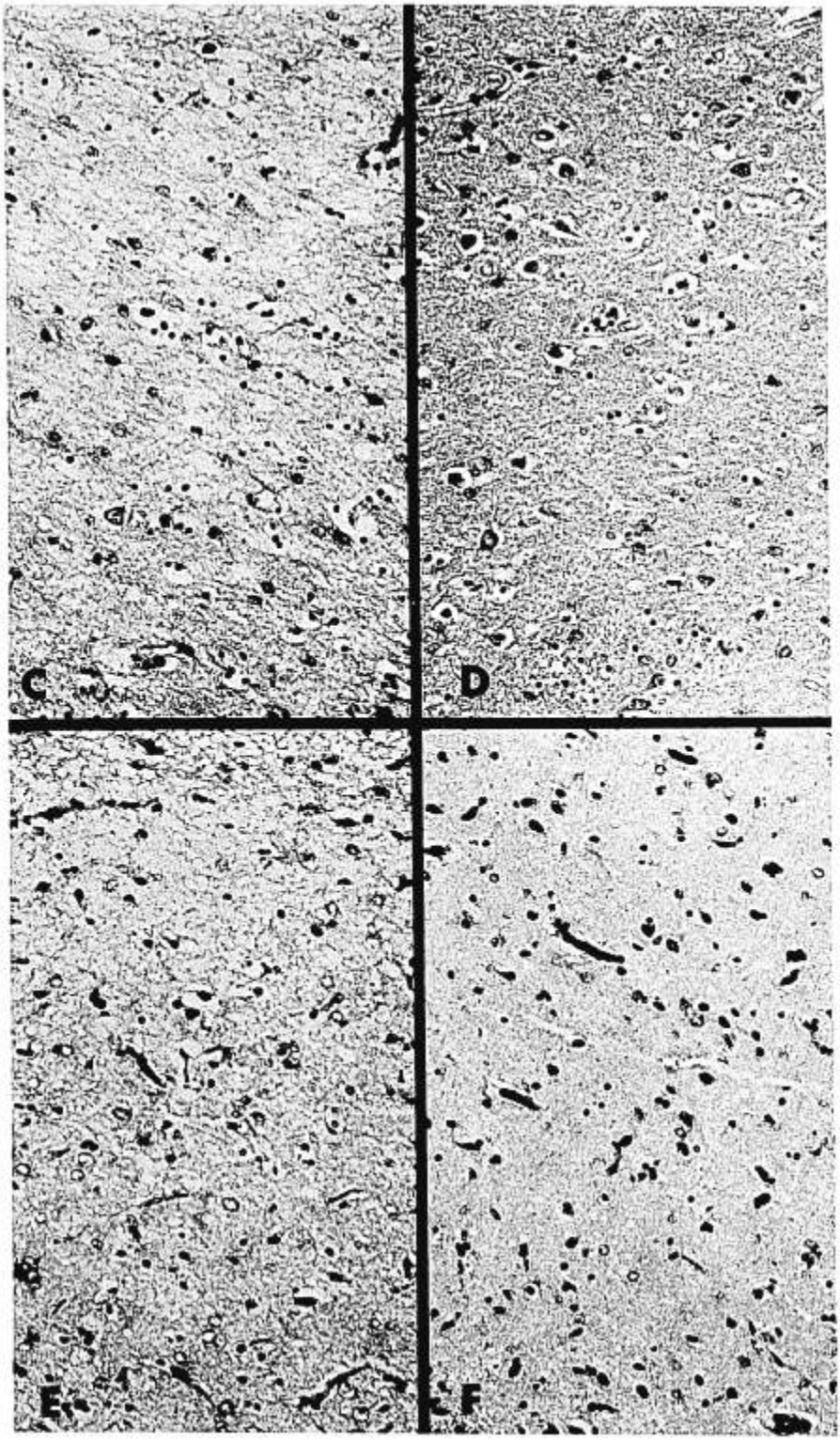
There is a progressive neuronal depletion and fibrillary astrocytosis through grades 1, 2, 3 and 4 in the medial portion of the head of the caudate nucleus (C, E, G, I) and through grades 2, 3 and 4 in its lateral portion (F, H, J). In grade 1, the lateral half of the head of the caudate nucleus (D) shows minimal changes if any at all.

The medial portion of the head of the caudate nucleus (C, E, G, I) is more severely involved than is the lateral portion (D, F, H, J). In grade 4, these differences in severity within the caudate subdivisions are less evident because of the severity of the striatal involvement (I, J).

The gray matter bridges between the head of the CN and the putamen (level CAP) were normal or showed at most a slight fibrillary astrocytosis which was greater dorsally. The nucleus accumbens was unremarkable.

At level GP, the CN displayed a marked neuronal loss and astrocytosis, with the paracapsular region being least involved. The putamen showed a moderate fibrillary astrocytosis and a slight neuronal loss throughout, but especially in its dorsal half. The globus pallidus showed minimal or no changes.

Grade 3: Ninety cases were classified as grade 3. Macroscopically, at level CAP the CN was shrunken and showed some yellow-brown discoloration. The anterior



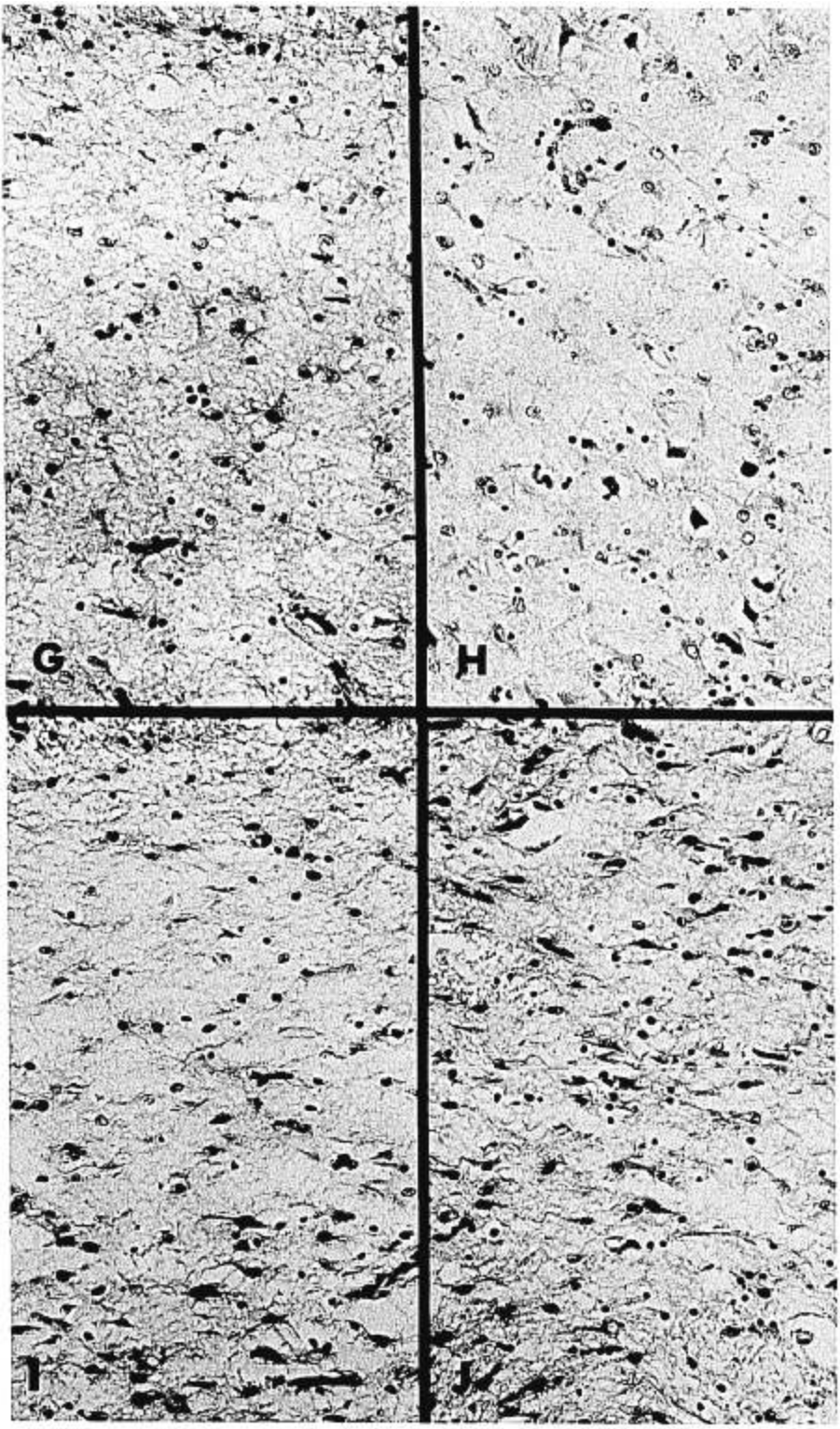


TABLE 3
Macroscopic Measurement (mm) of Caudate Nucleus and Internal Capsule Mean ± Standard Deviation

	Head of CN (level CAP)	Body of CN (level 9)	Tail of CN (level 5)	IC (level CAP)
Control	9.5 ± 1.5 (10)	3.5 ± 0.7 (10)	1.75 ± 0.3 (10)	6.0 ± 1.0 (10)
0	9.8 (2)	3.8 (2)	2.0 (2)	5.3 (2)
1	7.4 ± 1.5 (5)	3.3 ± 0.8 (5)	1.1 ± 0.4 (4)	4.4 ± 2.1 (5)
2	5.3 ± 1.9 (15)	1.9 ± 0.7 (13)	0.7 ± 0.4 (11)	3.9 ± 1.1 (13)
3	4.2 ± 1.1 (46)	1.7 ± 0.7 (46)	0.6 ± 0.3 (46)	3.0 ± 0.7 (46)
4	2.6 ± 1.6 (13)	1.0 ± 0.7 (12)	0.3 ± 0.1 (13)	2.0 ± 0.3 (7)

Number of cases in parentheses.
CN = caudate nucleus, IC = internal capsule.
CAP = caudate-accumbens-putamen (see text).

horn of the lateral ventricle was correspondingly enlarged, and the medial outline of the head of the CN formed a straight line (Fig. 2C). The IC, which was approximately half of its normal thickness also presented a straight-line configuration as did the adjacent medial border of the putamen. A slight degree of medial convexity or concavity of these structures was considered to be compatible with grade 3. The putamen was moderately decreased in size; the nucleus accumbens was normal.

At level GP the CN was reduced to a thin strip. Both the putamen and globus pallidus were moderately decreased in size.

Microscopic examination at level CAP of the CN showed severe neuronal loss and fibrillary astrocytosis in the medial half (Fig. 3G) and these were moderately severe in the lateral half of the CN (Fig. 3H). The difference in the extent of involvement of the medial and lateral subdivisions of the CN was much less striking than in grades 1 and 2.

Moderate neuronal loss with fibrillary astrocytosis involved the gray matter bridges between the CN and the putamen, especially dorsally. The nucleus accumbens usually showed no abnormalities; in a few instances, however, there was a slight neuronal depletion with corresponding astrocytosis.

At level GP both the neuronal loss and fibrillary astrocytosis were severe and diffuse in the CN, again with some paracapsular preservation.

Grade 3 also was characterized by neuronal loss and gliosis in the putamen. The superior half of the putamen showed severe neuronal depletion and a severe concomitant fibrillary astrocytosis; these changes were less striking at the base.

The lateral segment of the globus pallidus showed a slight to moderate fibrillary astrocytosis especially in the portion adjacent to the putamen. The medial segment of the globus pallidus was unremarkable; nevertheless, fibrillary astrocytes were occasionally present. In one case there was striking fibrillary gliosis in the temporal (30) part of the claustrum; the insular (30) part was unremarkable. In all the other cases in our series we found no abnormalities.

Grade 4: Eighteen cases were classified as grade 4. At level CAP, the CN was extremely shrunken, and yellow-brown in discoloration; it presented a concave appearance (Fig. 2D). The anterior horn of the lateral ventricle was correspondingly widened. The IC was medially concave, and its width was decreased to about one-third of that normally expected. The putamen was markedly atrophic with a concave

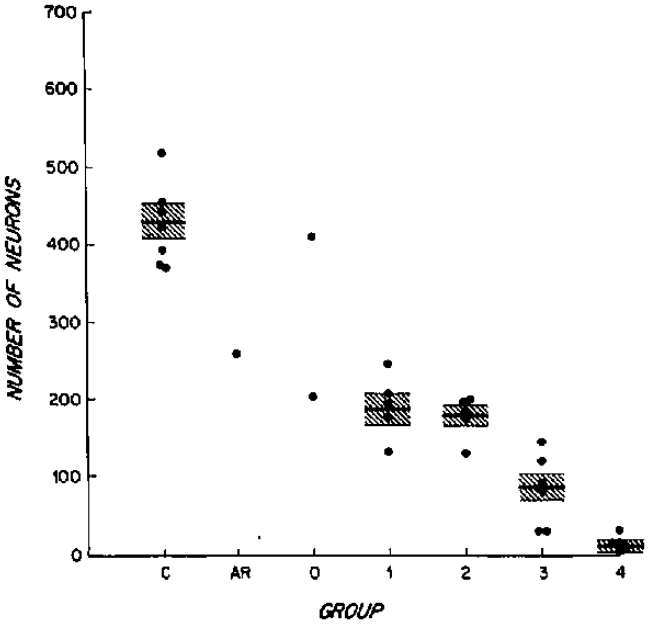


Fig. 4. Cell counts of the number of neurons were made in the head of the caudate nucleus along one drive from the lateral border to the internal capsule. Cases were randomly selected from each grade and from matched control material. The mean of each group is indicated by the heavy line and standard deviation by shaded area. The number of neurons is reduced to half the control complement in grade 1 and grades 2, 3 and 4 show progressive loss of neurons. Only two grade 0 and one at-risk cases were counted and this number is too small for meaningful interpretations.

medial outline. The nucleus accumbens was smaller than normally expected but appeared relatively prominent in comparison with the adjacent CN and putamen.

At level GP, the CN was reduced to a thin medially concave strip. The putamen was markedly decreased in size and showed widened perivascular spaces in its ventral portion. The external medullary lamina of the globus pallidus was indistinct. The globus pallidus was markedly atrophic (approximately half the size of that of age matched controls).

Microscopically, nerve cell depletion and fibrillary astrocytosis were extremely severe and diffuse throughout the CN (Fig. 3I, J) and putamen at both levels CAP and GP. The bridges between the head of the CN and the putamen displayed both neuronal loss and astrocytosis, but less severely than in the main part of the CN or putamen. The nucleus accumbens showed slight to moderate fibrillary astrocytosis especially dorsally. The globus pallidus showed fibrillary astrocytes especially in the lateral segment and its neurons were more closely packed together than in age-matched controls.

Quantitative Assessment of the Grade

The weight of the fixed half-brain correlated negatively with the grade ($r = -0.31$, $p < 0.002$) (Table 6). The mean half-brain weight was 560.4 g (539.6 g for females and 580.4 g for males). The grade of neuropathological severity of HD correlated with the macroscopic measurements of the head ($r = 0.61$, $p < 0.001$), body ($r =$

TABLE 4
Differences in Number of Cells Between Medial and Lateral Caudate*

	N	Neurons	Astrocytes
Controls	7	31	34
HD grade			
0	2	31	23
1	5	8	78
2	5	−13**	39
3	6	0.3**	53
4	5	−4**	15

* Mean cell counts of medial minus lateral caudate.
** $p < 0.01$ compared to controls.
HD = Huntington's disease.
N = Number of cases examined.

0.64, $p < 0.001$) and tail ($r = 0.63$, $p < 0.001$) of the CN and of the IC ($r = 0.67$, $p < 0.001$). The mean macroscopic measurements are shown in Table 3.

Cell Counting

Grade 1 has lost more than half of its neurons, and grade 4 more than 95% (Fig. 4). The two cases designated grade 0 displayed different neuronal cell counts; one appeared normal, and one appeared to have fewer than the normal number of neurons. The counts for grade 0 cannot therefore be interpreted meaningfully. For the present report, neuronal cell counts were done for only one individual at 50% risk for HD. According to the family report, this 73-year-old man may have been showing subtle signs of HD at the time of his death by suicide, however he had not been evaluated medically and was not diagnosed as HD. The gross examination of the brain was unremarkable but the number of neurons more closely resembled the numbers found in grade 1 than the normal control neuronal complement. Cell counts must be done on a larger number of patients at-risk before a definitive statement can be made as to the possible neuronal loss in this group.

The severity of neuronal cell loss displayed a decreasing mediolateral gradient. The control brains contained, on average, 31 more neurons in the medial half of the CN than in the lateral half (Table 4). In grade 2, a significant loss of neurons had occurred in the medial half of the CN, when compared with the lateral half, so that there were actually more neurons remaining in the lateral CN than in the medial CN. In grades 3 and 4 neuronal loss was diffuse throughout the CN.

The number of astrocytes in grades 1 and 2 is increased by 16% and in grades 3 and 4 by 28% (Table 5). The density of oligodendrocytes is increased in grade 0, 1 and 2 but then decreases in grades 3 and 4.

The grade was strongly inversely correlated with the count of the number of neurons ($r = -0.79$, $p < 0.001$) and oligodendrocytes ($r = -0.60$, $p < 0.001$). The grade was weakly correlated with the number of astrocytes ($r = -0.33$, $p < 0.06$) as a result of the large variability among the cases within each grade.

Clinical Features

Table 6 summarizes the clinico-pathological features. A rating of physical disability was made for 118 persons. The rating of physical disability at the time of death was

TABLE 5
Mean Cell Counts ± Standard Deviation

	Astrocytes	Oligodendrocytes
Control	425.6 ± 39.0	637.1 ± 129.9
Grade 0	426.0 ± 111.9	1,128.7** ± 166.8
Grade 1	496.0 ± 104.0	994.8** ± 183.3
Grade 2	496.2 ± 57.8	1,005.4** ± 263.7
Grade 3	544.8 ± 150.7	871.7 ± 313.6
Grade 4	546.6* ± 126.3	568.2 ± 155.4

* $p < 0.05$, ** $p < 0.01$ compared with control.

negatively correlated with the grade ($r = -0.49$, $p < 0.0001$) indicating a strong relationship between low level of function and high atrophy grade. The mean disability ratings for each grade are presented in Table 6. The age of death was known for 160 of the 163 cases with a mean of 55.7 years (SD = 12.4). The mean age of death for each grade is presented in Table 6. The grade 4 cases had the earliest age at death.

DISCUSSION

One hundred and sixty-three HD brains were collected, processed and evaluated by means of a standardized protocol. A system was designed for assigning a five point grading scale for the severity of striatal neuropathological involvement of HD, with 0 indicating no abnormality and 4 signifying very severe involvement.

The criteria for the grading system indicate the progressive degeneration of the CN and putamen, where the brunt of the degenerative change takes place in HD (6–8, 11, 13, 20–22, 25, 26, 31). The paraventricular portion of the CN and the dorsal putamen display early fibrillary astrocytosis and neuronal loss. As the disease progresses, the degenerative changes move laterally and basally. The neuropathological changes in the neostriatum are diffuse and more obvious in the caudal regions, especially in the cases of lesser severity, as previously noted (25). Indeed the tail of the CN was a very informative area for the diagnosis of HD in grade 1. In summary, the neuropathological changes in the neostriatum increased gradually along the antero-posterior, latero-medial, and ventro-dorsal axes in grades 1 through 3, with very severe degeneration throughout the striatum in grade 4.

Regional variation in the neostriatum in HD, similar to our observations, has been previously noted. The anterior part of the putamen has been reported to be relatively preserved when compared by neuron count with the posterior part (21); nerve cell loss was most evident in the middle and posterior parts of the putamen (20), and the dorsal portion of the putamen was much more involved than the ventral portion (13, 24). McCaughey (25) found that nerve cell loss and astrocytosis were especially severe in the dorsal levels of the anterior neostriatum and that only minor changes were present in the ventral caudo-putaminal junction. He also observed that "the medial half of the head of the caudate nucleus sometimes showed a little more glial reaction than the lateral portions." Forno and Jose (12) also concluded that the CN was almost always more severely affected in its paraventricular portion and that the anterior ventral portion of the neostriatum was relatively spared. Our findings have been consistent with these observations. However, in disagreement with other authors (13, 14), none of our graded cases of HD showed more neuronal

TABLE 6
Clinicopathological Features*

Grade	Half-brain weight (in grams)	Age at death	Disability rating
0 (5)	620 (3)	56 (5)	63 (4)
1 (8)	625 (5)	58 (8)	48 (6)
2 (38)	571 (21)	59 (37)	38 (29)
3 (90)	560 (66)	56 (88)	28 (66)
4 (18)	496 (9)	47 (18)	21 (12)
Total (159)	560	56	31

* Means for each group, number of cases in parentheses.

loss and gliosis in the anterior third of the neostriatum than in its more caudal parts. Similarly, we did not find any cases where the lateral putamen was more involved than the medial portion (13).

The weight of the fixed half-brain in this series is closely related to the grade. However, the mean weight is somewhat higher than that previously reported (20, 25) which may be due to the number of cases from grades 0 to 2 included here.

The grade 4 cases died at an earlier age than did the other grades (Table 6) and this agrees with the severe atrophy previously noted (17, 31) in cases of early onset. The rating of the physical disability (28) was highly correlated with the neuropathological grade. In the neuropathological grade 0 cases, the mean disability rating indicates that the patient has experienced dysarthria, choreiform movements and frequent falls, and this is somewhat surprising in view of the absence of neuropathological findings. In grade 1, the physical disability rating indicates that the patient requires assistance with ambulation; in grade 2, ambulation is minimal even with assistance. For grade 3 the patient is confined to a wheelchair, and in grade 4 the patient is bed-ridden.

The macroscopic measurement of the head, body and tail of the CN are highly correlated with the grade. Our measurements have likewise indicated a close correlation between the grade and the thickness of the anterior limb of the IC. This may indicate that extensive loss of frontal lobe connections represented in the anterior limb of IC (30) is a characteristic feature of HD.

In grade 1 the impression on microscopic examination was of only slight neuronal loss, the extent of which could only be appreciated by cell counting procedures. The loss of neurons is dramatic in grades 2 and 3 and by grade 4 only 5% of the original complement remain. We did not find total absence of neurons in the affected regions in any of our cases, although this has been reported (33). Reactive astrocytosis, as judged in LH&E- and CV-stained sections and confirmed by GFAP staining in 12 representative cases (4 grade 0, and 2 each of grades 1–4), parallels neuronal loss. The topographic sequence of the involvement of the neostriatum is reflected both in the loss of neurons and reactive astrocytosis. We found also that there are decreased numbers of oligodendrocytes in the grade 4 cases. The loss of these cells may be an effect of previous neuronal cell loss and hence of myelinated axons.

The globus pallidus was atrophic in grades 3 and 4. Microscopically, the globus pallidus appeared much less affected than would be expected from the degree of macroscopic atrophy. The neurons in the globus pallidus were found to be more densely packed in grade 4, suggesting that although tissue bulk decreases, neurons

are relatively preserved. This contrasts with the dramatic loss of neurons found in the CN and putamen and suggests that in the globus pallidus the atrophy is due to loss of neuropil, and hence of fiber connections, rather than of nerve cell bodies. These changes in the globus pallidus have been previously reported (11, 12, 17, 20–22, 24, 32, 33). The absolute number of neurons in the globus pallidus has been found to be decreased by approximately 40% (34).

The nucleus accumbens was remarkably preserved in this series; such degenerative changes as were seen in this structure were slight and were seen almost exclusively in grade 4. Unlike the observations of others (10, 12), we found the insular claustrum to be normal in all samples; in one case of grade 3 we found marked fibrillary astrocytosis in the temporal claustrum. As others have previously reported, the cerebral cortex in our HD cases did not exhibit any noteworthy histopathological changes (20, 25).

In our series, there were five cases with clinically recognizable HD but without neuropathological evidence of the disease. Several other cases of slight neostriatal involvement have been reported (14, 20, 25–27, 35). The development of anatomic changes in HD appears to lag behind the clinical manifestations. Neuron counts for the grade 0 cases failed to reveal a consistent abnormality (Fig. 4). The lack of neuropathological evidence of the disease in grade 0 underscores the necessity of a thorough review of clinical features in evaluating these cases.

This study suggests that a broad range of neuropathological severity in the involvement of HD brains may be graded by a simple systematic evaluation of the striatum. These variations should be considered in the immunohistochemical, biochemical, cytometric and receptor studies of HD brains. Analyses of neostriatal samples in grade 4 would mainly reflect the astrocytic composition, perhaps with a component resulting from remote neurons projecting into the area. Studies of grade 1 or grade 2 samples from the lateral half of the head of the CN may more nearly approximate the early changes in HD. The quantitative analysis of the grading system yielded a significant difference in the content of neurons and glial cells in each grade. The morphometric studies of HD of recent years do not indicate precisely the severity of the grade, although we may assume that for the most part they have been based on cases of grades 3 and 4 which represent 67% in our series (32, 34, 36–41). We believe that it would be useful in the future for investigators to report the grade of involvement to facilitate comparison between observations.

ACKNOWLEDGMENTS

We thank Drs. Tessa Hedley-Whyte, Raymond D. Adams and Roger Williams for the advice given during preparation of this study and Evelyn Basso, Timothy Wheelock and Daniel De Stefano for technical assistance. We also thank Michelle Germain, Norma Terrin and Adrienne Cupples for data entry and analysis and Peter Paskevich without whom cell counting would have been much more difficult. We also express our appreciation to the numerous pathologists who referred case material to the Brain Tissue Resource Center; this work would not have been possible without their assistance.

ADDENDUM

Since submission of our manuscript, a report of a morphometric study of the putamen in Huntington's disease has appeared (Roos RAC, Pruyt JFM, de Vries J, Bots GThAM. Neuronal distribution in the putamen in Huntington's disease. J Neurol Neurosurg Psychiat 1985;48:422–5). Our impression that the ventral anterior part of the putamen is relatively spared in HD receives quantitative corroboration in this article.

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(Received 18 March 1985/Accepted 20 June 1985)
MS85-12