A quantitative histological study of the indusium griseum and neostriatum in elderly mice

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INTRODUCTION

The effects of ageing on the number of neurons and glia in the indusium griseum and on the number of glia in the neostriatum during the median lifespan (22 months) of the ASH/TO strain mouse have already been described (Sturrock, 1979, 1980). By setting up a large colony of mice it is possible to obtain a small number of mice which survive well beyond the median lifespan. This paper gives the results of a quantitative histological study of the indusium griseum and neostriatum of ASH/TO mice aged 25, 28 and 31 months, including changes in mitotic activity and cell death.

Changes in the number of mitotic and pyknotic cells in the indusium griseum up to 22 months of age have already been reported (Sturrock, 1979) but so far no qualitative study of cell division and cell death has been undertaken in the neostriatum. The glia to neuron ratios and the percentages of each type of glial cell are different in the indusium griseum and neostriatum (Sturrock, 1980) and it therefore seemed worth investigating whether mitotic activity and cell death also differed between the two regions. To enable a meaningful comparison of these parameters between the two regions the neostriatal study was extended to include mice aged 6, 9, 12, 15, 18 an d 22 months.

MATERIALS AND METHODS

Male ASH/TO mice aged 25, 28 and 31 months were used in the present study. Three animals at each age were anaesthetised by an intraperitoneal injection of Sagatal and killed by vascular perfusion with Bouin's solution after flushing with physiological saline. A further three animals at each age, anaesthetised with Sagatal, were killed by vascular perfusion with a mixture of 2 % paraformaldehyde and 3 % glutaraldehyde in an 0·165 M phosphate buffer after the vascular system had been flushed out with physiological saline. After death, the animals were left in polythene bags overnight at 4 °C. The following morning the brains were removed from the skulls. Those brains which had been fixed in Bouin's solution were bisected in the mid-sagittal plane, dehydrated and embedded in paraffin wax. The left halves were serially sectioned at 6 μm in the coronal plane and stained with haematoxylin and eosin while the right halves were serially sectioned at 6 μm in the parasagittal plane and stained with Lapham's stain (Lapham, Johnstone & Brundjar, 1964).

The brains fixed in mixed aldehydes were bisected in the mid-sagittal plane and 1–2 mm thick coronal slices were cut rostral to the anterior commissure. These slices were rinsed in 0·165 M phosphate buffer, postfixed in OsO_4 for two hours, dehydrated and embedded in Spurr's resin.

Semithin (1 μm) coronal sections, obtained using a Ralph knife, were mounted on slides and stained with 1 % toluidine blue.

6 μm sections

The total number of neurons and glia and the number of mitotic and pyknotic cells in the indusium griseum were estimated from the sets of coronal sections as described previously (Sturrock, 1979). In order to estimate the percentage of mitotic and pyknotic cells in the neostriatum three sections were examined in each set. The sections were selected using easily recognised anatomical landmarks so that each set of three was as nearly as possible identical. The three sections used were those used in an earlier quantitative study of the subependymal layer (Sturrock, 1985) and consisted of the section in which the junction of the anterior limb of the anterior commissure joined the cortico-hypothalamic tract, the section in which the anterior and posterior limbs of the anterior commissure united, and the section which lay exactly halfway between the other two.

The three chosen sections were scanned systematically from medial to lateral in strips using an eyepiece graticule at a magnification of $\times 630$. Each glial nucleus, mitotic cell and pyknotic cell was recorded. On average 2100 glial nuclei were counted in each set of three sections. The only exception was the 31 months group where no mitotic cells were found in the nine sections initially scanned. A further two sections were scanned in each of the three sets of sections. The sections examined were those equidistant from the first and second section and from the second and third section. Care was taken in each case to exclude mitotic and pyknotic cells of the adjacent subependymal layer.

The neostriatal mitotic counts were repeated in three sets of serial sections from ASH/TO mice aged 6, 9, 12, 15, 18 and 22 months using sets of slides prepared earlier (Sturrock, 1979).

1 μm sections

These sections were used for differential counts and for estimates of the glia to neuron ratio in the neostriatum as described previously (Sturrock, 1980). The glia to neuron ratio in the indusium griseum was calculated using the estimates of the total neuron and glial number of paraffin sections since neurons are not evenly distributed along the indusium griseum and in any case only a small number of neurons and glia are present in each section.

The number of each type of glial cell per 100 neurons in the neostriatum was estimated for each animal and the mean and standard error for each age group was calculated. Since the glia to neuron ratio in the indusium griseum was estimated from the 6 μm sections while the percentage of each glial type was found from the 1 μm sections the number of each type of glial cell per 100 neurons was calculated by multiplying the mean glia:neuron ratio by the mean percentage at each age. It was not therefore possible to estimate a standard error.

RESULTS

The results of the earlier studies (Sturrock, 1979, 1980) are included in each Table since in isolation the results obtained from 25 to 31 months would be meaningless. The source of the numbers is indicated in each case.

As noted previously (Sturrock, 1980) the number of glial cells per 100 neurons increased in both the indusium griseum and neostriatum between 6 and 9 months and thereafter remained constant up to 18 months (Table 1). Between 18 and 22

Table 1. *Number of glia per 100 neurons (± SEM) in the indusium griseum and neo-striatum: the level of significance of analysis of variance is shown by P*

Age in months	Indusium griseum	Neostriatum
G6*	60±4	48±2
G9*	70±2	58±3
G12*	68±7	60±1
G15*	69±3	60±1
G18*	71±3	61±2
G22*	97±6	65±3
G25	95±6	66±5
G28	98±3	47±4
G31	97±5	47±4
P	< 0·001	± 0·01

* From Sturrock, 1980.

Table 2. *Percentage of mitotic and pyknotic cells (± SEM) in the indusium griseum and neostriatum: the level of significance of analyses of variance is shown by P*

Age in months	Indusium griseum		Neostriatum	
	Mitotic	Pyknotic	Mitotic	Pyknotic
6*	0·06±0·04	0·14±0·05	0·02±0·02	0·06±0·06
9*	0·12±0·02	0·31±0·05	0·02±0·02	0·08±0·05
12*	—	0·09±0·04	0·06±0·04	0·12±0·01
15*	0·05±0·05	0·08±0·03	0·03±0·03	0·04±0·04
18*	0·08±0·02	0·30±0·04	0·02±0·02	0·10±0·03
22*	0·21±0·02	0·48±0·02	0·04±0·03	0·07×0·02
25	0·04±0·02	0·03±0·01	0·03±0·03	0·07±0·02
28	0·05±0·02	0·03±0·01	0·04±0·03	0·02±0·02
31	0·08±0·02	0·02±0·01	0·007±0·007	0·11±0·03
P	< 0·01	< 0·001	NS	NS

* Results for indusium griseum from Sturrock, 1979.

months there was a significant increase in the glia to neuron ratio in the indusium griseum but not in the neostriatum. The number of glia per 100 neurons in the indusium griseum remained constant from 22 to 31 months but in the neostriatum the number of glia per 100 neurons returned to the 6 months level between 25 and 28 months. Analyses of variance showed that both sets of changes were statistically significant.

The percentage of mitotic and pyknotic cells in both regions was very low (Table 2). In the indusium griseum there were significant variations in the percentage of both mitotic and pyknotic cells with increasing age. No such variations were found in the neostriatum but it should be noted that at 31 months of age 15 sections of neo-striatum (5 from 3 animals) had to be examined before one mitotic cell was found.

Estimates of total neuronal and glial number in the indusium griseum (Table 3) showed no significant variation in neuron number but an increase in the number of glia, mainly between 18 and 22 months of age.

In 1 μm sections it was possible to identify the type of glial cell which was dividing using established morphological criteria (Sturrock, 1984). At the medial border of the neostriatum most mitotic cells had a cytoplasmic density similar to (Fig. 1) or

Table 3. *Estimated total number of neurons and glia (± SEM) in the indusium griseum with age: the level of significance of analysis of variance is shown by P*

| Age in months | Indusium griseum | |
	Neurons	Glia
6*	2788 ± 117	1648 ± 56
9*	2902 ± 276	2010 ± 118
12*	2761 ± 93	1870 ± 183
15*	2867 ± 125	1977 ± 174
18*	3010 ± 111	2135 ± 57
22*	2511 ± 89	2438 ± 169
25	2601 ± 152	2475 ± 178
28	2628 ± 188	2549 ± 112
31	2919 ± 170	2860 ± 299
P	NS	< 0·001

* From Sturrock, 1979.

Table 4. *Percentage of each glial type (± SEM) in the indusium griseum and neostriatum: the level of significance of an analysis of variance is shown by P*

| Age in months | Indusium griseum | | | Neostriatum | | |
	Oligo-dendrocytes	Astrocytes	Microglia	Oligo-dendrocytes	Astrocytes	Microglia
G6	20·4 ± 0·3	61·1 ± 1·3	18·5 ± 1·1	25·7 ± 0·7	53·1 ± 1·2	21·2 ± 0·5
G9	21·5 ± 0·2	60·8 ± 0·4	17·8 ± 0·6	28·9 ± 1·9	47·8 ± 4·4	23·4 ± 2·4
G12	20·8 ± 1·1	60·6 ± 2·9	18·6 ± 1·8	30·3 ± 1·0	51·0 ± 1·3	18·8 ± 2·2
G15	18·9 ± 1·0	62·5 ± 0·9	18·5 ± 1·3	26·8 ± 0·5	52·9 ± 3·0	20·4 ± 2·5
G18	17·1 ± 0·6	63·9 ± 1·0	19·0 ± 0·7	27·0 ± 0·8	56·6 ± 1·0	16·7 ± 0·3
G22	14·0 ± 0·8	62·7 ± 1·9	23·4 ± 1·1	26·1 ± 2·0	50·3 ± 1·9	23·6 ± 0·3
G25	24·3 ± 1·4	57·9 ± 3·1	17·9 ± 3·0	38·4 ± 2·7	37·9 ± 1·0	23·8 ± 1·7
G28	25·4 ± 1·0	60·0 ± 2·6	14·7 ± 2·7	37·8 ± 4·5	44·3 ± 6·0	18·0 ± 1·6
G31	25·3 ± 2·7	62·9 ± 4·8	11·7 ± 2·2	35·8 ± 5·5	41·7 ± 3·2	22·5 ± 2·1
P	< 0·001	NS	< 0·01	< 0·05	< 0·01	NS

slightly darker than (Fig. 2) the adjacent neuropil. These were identified as mitotic subependymal cells and in 6 μm sections were excluded from the counts of neostriatal mitosis. The cytoplasm of mitotic astrocytes was paler than that surrounding neuropil (Fig. 3). Figure 4 shows a mitotic cell with an irregularly vacuolated cytoplasm which is characteristic of microglial cells in the aged brain. The mitotic cells shown in Figures 5 and 6 both had a densely stained cytoplasm and were in contact with myelin sheaths. This is more obvious in Figure 6 which shows a mitotic cell from the induseum griseum where myelin sheaths are sparse. The pale area in the cytoplasm had a shiny gunmetal blue appearance under the microscope which is a characteristic feature of lipofuscin. An attempt to re-embed this section for electron microscopy was unfortunately only partially successful. The lack of contrast in staining made it impossible to produce a micrograph suitable for publication and the nucleus did not appear in the section. Electron microscopy did confirm that the cytoplasm was in contact with the two myelin sheaths and the pale area was identified as consisting of lipofuscin granules.

In the indusium griseum the percentages of oligodendrocytes and microglia varied
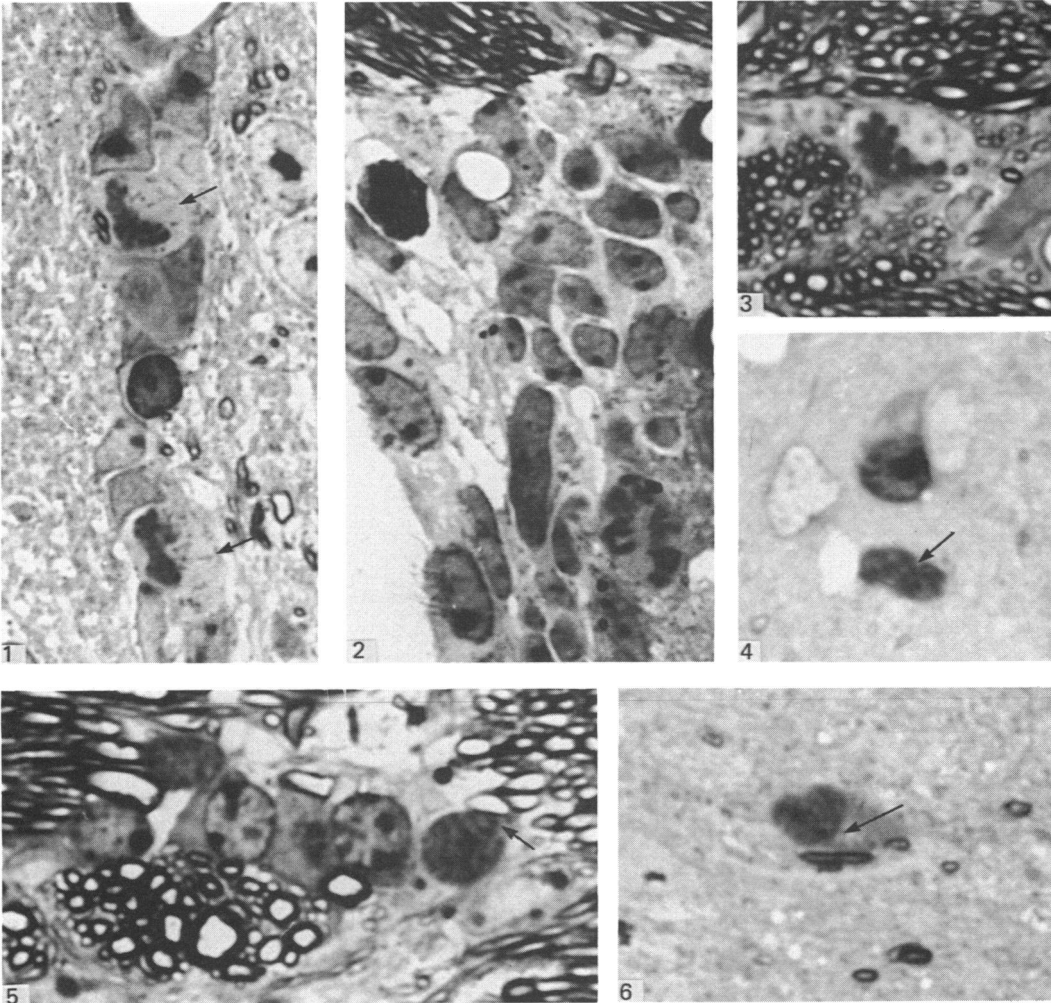
All Figures are from 1 μm sections stained with toluidine blue at ×1500 magnification. All sections are from 28 months old mice.

Fig. 1. The arrows indicate two mitotic cells in late telophase. Their cytoplasm is similar to that of the surrounding neuropil. They are lying in the subependymal layer along a coarctation of the lateral ventricle and are subependymal cells.

Fig. 2. This shows a darker mitotic subependymal cell.

Fig. 3. The pale cytoplasm of this mitotic cell enables it to be identified as an astrocyte. It is in the neostriatum adjacent to the corpus callosum.

Fig. 4. The mitotic cell indicated by the arrow is in the neostriatum. The pale area to the left is a large cytoplasmic vacuole, typical of those found in microglia in the aged brain. Figs 4–7 are photographically slightly under-developed in an attempt to show the chromatin pattern in cells with dark cytoplasm.

Fig. 5. The cytoplasm of this mitotic cell (arrow) is in contact with a group of myelin sheaths and is probably an oligodendrocyte.

Fig. 6. This telophase from the indusium griseum has a dark cytoplasm in contrast with two myelin sheaths. The pale area (arrow) is caused by shiny lipofuscin granules.

Table 5. *Estimated number of each type of glial cell per* 100 *neurons in the indusium griseum and neostriatum: the level of significance of an analysis of variance is shown by P*

Age in months	Indusium griseum			Neostriatum		
	Oligo-dendrocytes	Astrocytes	Microglia	Oligo-dendrocytes	Astrocytes	Microglia
G6*	12	37	11	15 ± 0	22 ± 1	13 ± 1
G9*	15	42	12	17 ± 1	27 ± 1	14 ± 2
G12*	14	41	13	20 ± 1	26 ± 1	14 ± 1
G15*	13	43	13	18 ± 1	27 ± 2	15 ± 2
G18*	12	45	14	19 ± 1	29 ± 1	12 ± 1
G22*	14	61	23	19 ± 2	27 ± 1	18 ± 1
G25	23	55	17	26 ± 3	25 ± 1	16 ± 1
G28	25	59	14	18 ± 3	21 ± 3	9 ± 1
G31	25	61	11	17 ± 4	19 ± 1	11 ± 1
P	—	—	—	NS	< 0·01	< 0·01

* From Sturrock, 1980.

significantly with age (Table 4). In the case of the oligodendrocytes and microglial population this was due to a decrease between 12 and 22 months followed by an increase between 22 and 25 months. Microglia increased significantly between 18 and 22 months then decreased between 22 and 31 months. The percentage of astrocytes did not vary significantly. In the neostriatum there was a significant increase in the percentage of oligodendrocytes and a significant decrease in the percentage of astrocytes between 22 and 25 months. The percentage of microglia did not vary with age.

When the number of each glial cell type per 100 neurons was estimated a different pattern emerged (Table 5). In the indusium griseum the number of oligodendrocytes was substantially higher from 25 to 31 months than in younger animals. The number of astrocytes was greatest from 22 to 31 months whilst the number of microglia increased between 18 and 22 months then decreased continuously up to 31 months. In the neostriatum the number of oligodendrocytes did not vary significantly with age. The number of astrocytes increased between 6 and 9 months and thereafter remained constant until 22 months of age, after which astrocyte number gradually declined. The number of microglia increased between 18 and 22 months then decreased up to 31 months.

DISCUSSION

Previously the increase in the number of glial cells in the indusium griseum between 18 and 22 months was interpreted as a response to neuronal loss (Sturrock, 1980) but an analysis of variance indicates that the reduction in neuron number at 22 months is not statistically significant and this is borne out by the fact that the number of neurons in the indusium griseum at 31 months is not significantly different from that at 6 months. It was not possible to estimate the number of neurons in the neostriatum but since the only change in the glia to neuron ratio in the neostriatum after 9 months of age was a decrease in the number of glia per 100 neurons between 25 and 28 months it seems unlikely that there was any significant decrease in the number of neostriatal neurons. Curcio & Coleman (1982) have shown that there is no loss of neurons from the cortical barrels of C57BL/6 mice up to 33 months of age.

The increase in glial number in the induseum griseum between 18 and 22 months corresponded with an increase in the percentage of mitotic cells at 22 months. The mitotic index in the indusium griseum from 25 to 31 months was similar to the 6–18 months level and the number of glia per 100 neurons remained constant from 22 to 31 months. This indicates that the increase in glial number at 22 months is a sudden, isolated event mainly brought about by local proliferation.

There is a substantial increase in the number of oligodendrocytes in the indusium griseum of the 25–31 months old mice in contrast to younger animals. It may be significant that the only mitotic cell found in 1 μm sections of the aged indusium griseum was an oligodendrocyte (Fig. 6) despite oligodendrocytes making up only 25 % of the glial population. Why oligodendrocytes should increase in number in the indusium griseum is not clear. Oligodendrocyte proliferation has been shown to occur in mature animals following experimental demyelination (Arenella & Herndon, 1984) and trauma and implantation (Ludwin, 1985) and there was also a substantial increase in the number of oligodendrocytes in both limbs of the anterior commissure of the same mice between 22 and 25 months of age (Sturrock, 1987) which was associated with an increase in the percentage of myelinated axons. Changes in myelination do not seem to present a likely reason for an increase in oligodendrocyte number in the indusium griseum although some oligodendrocytes whose cell bodies lie in the indusium griseum participate in myelination of the medial longitudinal striae (see Fig. 1 in Sturrock, 1983). Astrocytes were most numerous between 22 and 31 months. This could be a response to a decrease in neuronal volume or a reduction in the dendritic tree. Geinisman, Bondareff & Dodge (1978) found that in the dentate gyrus of aged rats there was hypertrophy of astrocyte processes but no increase in astrocyte number while Landfield *et al.* (1977) found both a hypertrophy of astrocyte processes and an increase in astrocyte number in the hippocampus of aged rats. The increase in microglia at 22 months corresponds with the increase in the number of pyknotic nuclei found at this age and, perhaps significantly, is followed by a continuous decline in the number of microglia from 25 to 31 months when very few pyknotic nuclei are present.

Since the decrease in microglial number is balanced by an increase in oligodendrocyte number the possibility that the differences are due to faulty identification of oligodendrocytes and microglia in 1 μm sections must be considered. Microglial nuclei have a distinct clumping of heterochromatin around the nuclear envelope which makes them easy to identify and, with increasing age, large dense inclusions and vacuoles are present in the cytoplasm which makes identification in old animals even more reliable than in young animals (Vaughan, 1984). It is unlikely that the changes noted in glial number are due to errors in identification.

In the neostriatum the decrease in glia per 100 neurons between 25 and 28 months is caused by a decrease in number of astrocytes and microglia. The decrease in microglial number in both the indusium griseum and neostriatum contrasts with the increase in microglial number found in the rat auditory cortex by Vaughan & Peters (1974). The rats they examined were 27 months old Sprague–Dawley rats which have a median lifespan of 27 months but about 10 % survive past 32 months (Peters, Feldman & Vaughan, 1983). The increase in microglial number in these rats may correspond with the increase found in the indusium griseum of 22 months old mice which have also reached their median lifespan and there may be a later decrease in microglial number in rat auditory cortex as well.

The decrease in glial number in the neostriatum may be due to cell death, although

there is no evidence of any alteration in mitotic activity or cell death in the neo-striatum except for a reduction in the number of mitotic cells at 31 months. The fall in number could be explained by migration of glial cells from the neostriatum or a reduction in glial precursors migrating into the neostriatum from the adjacent sub-ependymal layer. Lewis (1968) demonstrated that subependymal cells migrate through the neostriatum as well as by way of the corpus callosum. The cellularity of the subependymal layer declines from 22 months of age (Sturrock, 1985) in the same mice and this supports the suggestion that the fall in neostriatal glial cell number is due to a reduction in migration from the subependymal layer.

Curcio & Coleman (1982) found no change in glia to neuron ratio up to 33 months in C57BL/6NNia strain mice which have a similar lifespan to ASH/TO mice. Heumann & Leuba (1983), however, found that in white Swiss mice there was a gradual increase in glial number in the cerebral cortex up to 2 years of age. Interest-ingly a similar gradual increase in glial number has been reported in the buccal ganglion of the snail *Planorbis corneus* (Pentreath, Radojcic, Seal & Winstanley, 1985), a species in which there is no neuronal loss with age. Conversely Miller, Alstona, Mountjoy & Corsellis (1984) found no increase in glial cells in the ageing human hippocampus but in this study glial identification was based on nuclear size which Curcio & Coleman (1982) found to be an unreliable criterion.

Mitotic activity is greater in the indusium griseum than in the neostriatum at all ages examined. It was suggested (Sturrock, 1979) that most mitotic cells in the adult brain outside the subependymal layer were astrocytes. Although a very small number of mitotic cells have been identified as oligodendrocytes and microglia the majority are astrocytes and in support of this the mean overall percentage of mitotic figures in the indusium griseum, neostriatum and anterior commissure (0·077; 0·030; 0·014 respectively) bear a similar relationship to each other as do the percentages of astro-cytes in each region (61·4; 48·4; 9·4).

It may be appropriate at this stage to summarise the results of the quantitative histological results obtained so far, using the same sets of histological sections from ASH/TO mice aged up to 31 months. There is no loss of nerve cells from the indusium griseum and probably none from the neostriatum but there is a substantial loss of neurons from the locus caeruleus after 25 months of age (Sturrock & Rao, 1985). The number of glia per 100 neurons increases in both the indusium griseum and neo-striatum between 6 and 9 months. In the indusium griseum the number of glia per 100 neurons remains constant from 9 to 18 months, increases markedly between 18 and 22 months and thereafter remains constant up to 31 months. In the neostriatum the number of glia remains constant from 9 to 25 months before falling between 25 and 28 months. In both limbs of the anterior commissure the number of glial cells falls from 9 to 18 months then returns to the 9 months level between 22 and 25 months and then remains constant up to 31 months (Sturrock, 1987). The subependymal layer shows a loss of cellularity after 25 months (Sturrock, 1985).

There does not appear to be any overall pattern of brain ageing. The only common features seem to be an increase in oligodendrocytes in most regions between 22 and 25 months and a decrease in microglial cells between 25 and 31 months. Changes in glial number differ both in the ages at which they occur and also whether glial number increases or decreases. This seems to indicate a considerable degree of plasticity in the brain throughout life.

SUMMARY

A quantitative histological examination of brains from mice aged 25, 28 and 31 months of age showed that there was no loss of neurons from the indusium griseum and the number of glia per 100 neurons also remained constant. In contrast the number of glia per 100 neurons in the neostriatum fell between 25 and 28 months. The percentage of oligodendrocytes was significantly higher than in younger mice but the percentage of microglia fell in both regions from 25 to 31 months.

Unlike the indusium griseum, in which there was a significant variation in the mitotic and pyknotic indices with age, the neostriatum showed no variation in the mitotic and pyknotic indices between 6 and 31 months. The mitotic index was always lower in the neostriatum.

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