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Glial and Nerve Cell Changes in Rats With Porto-caval Anastomosis

N. H. Diemer, Jill Klee, H. Schröder, and L. Klinken

Institute of Neuropathology, University of Copenhagen, 11 Frederik V's Vej, DK-2100 Copenhagen, Denmark

Summary. Nuclear size and density were determined in brain regions with different glial–neurone composition in rats up to 35 weeks after porto-caval anastomosis.

In the white matter, i.e. corpus callosum, both the total cell count and the percentage of astrocytes and oligodendrocytes were unchanged.

In the corpus striatum, where the glial/neurone ratio is about 1, the number of nuclei registered as astrocytes increased, and after 35 weeks astrocytes comprised 29% of glial cells (compared with 15% in controls). However, the number of oligodendrogial nuclei decreased simultaneously, leaving the total glial number unchanged. In the animals with longest experimental period there was a 15% loss of neurones.

In a region with higher glial/neurone ratio, i.e. the Purkinje cell layer, the neurones showed a similar reduction, whereas the number of Bergmann astrocyte nuclei increased less than striatal astrocytes.

A small group of animals with pronounced signs of encephalopathy had a higher loss of neurones and, furthermore, the glial number in corpus striatum and callosum was reduced, due to loss of oligodendrocytes.

Despite the use of perfusion fixation, the size of astrocyte nuclei increased, this was reversible, as only slight changes were seen after 35 weeks.

A possible explanation of the increase in astrocyte nuclear count and decrease in oligodendroglial count could be that nuclei normally considered to be oligodendroglial are transformed into nuclei with morphological characteristics of astrocytes.

Key words: Porto-caval anastomosis – Glial–Neurone changes – Porto-systemic encephalopathy.

Introduction

Porto-caval anastomosis may be indicated to prevent haemorrhage from oesophagal varices in patients with portal hypertension.

These patients with porto-caval anastomosis may develop post-shunt encephalopathy (McDermott and Adams, 1954; Sherlock et al., 1954). The neuropathological findings, which do not differ qualitatively from those in cases of hepatic encephalopathy without porto-caval anastomosis, consist primarily of astrocyte proliferation and Alzheimer type II changes (von Hösslin and Alzheimer, 1912; Adams and Foley, 1953; Victor et al., 1965; Erbslöh, 1958; Shiraki, 1968).

Quantitative investigations have only been performed in brains from cases of liver disease without porto-caval anastomosis (Adams and Foley, 1953). Thus the quantitative significance of the shunting per se in unknown.

In an earlier investigation on CCl_4-induced hepatic encephalopathy, an increase in the number of astrocyte nuclei was found, the total number of glial cells being unchanged. It was suggested that the increase in the number of astrocyte nuclei may be due to a direct transformation of cells normally considered to be oligodendrocytes (Diemer, 1977a).

In the present study rats were subjected to porto-caval anastomosis in order to investigate whether the number of astrocytes increases in the course of the resulting porto-systemic encephalopathy, and whether this is accompanied by a reduction in the number of oligodendrocytes. The counts were performed in a region without nerve cells (corpus callosum), in a region with approximately equal number of glial and nerve cells (corpus striatum), and in a region with a preponderance of glial cells (ganglionic layer of cerebellum).

Materials and Methods

Experimental Animals. Twenty-six 5-month-old male Wistar rats, mean weight 340 g (range 310–370 g) with free access to water and commercial rat pellets. The porto-caval anastomosis was per-

Table 1. Experimental period, weight changes, and plasma NH_4^+-concentration

Group	Number of animals	Weight at sacrifice in percent (range)	Plasma NH_4^+-concentration µmol (±S.D.)
Controls			
Age: 5 months	16	100 (93–108)	58 ± 20 (n = 50)
5–7 weeks PCA	4	92 (90–100)	–[a]
15–22 weeks PCA	8	101 (97–109)	327 ± 87 (188–392)
33–35 weeks PCA	7	118 (95–144)	168 ± 46 (126–236)
Animals with continuous weight loss, 7–15 weeks PCA	3	67 (55– 77)	491 (485–500)
Controls, sham-operated			
Age: 13 months	4	134 (124–139)	–[a]

[a] Not obtained for technical reasons

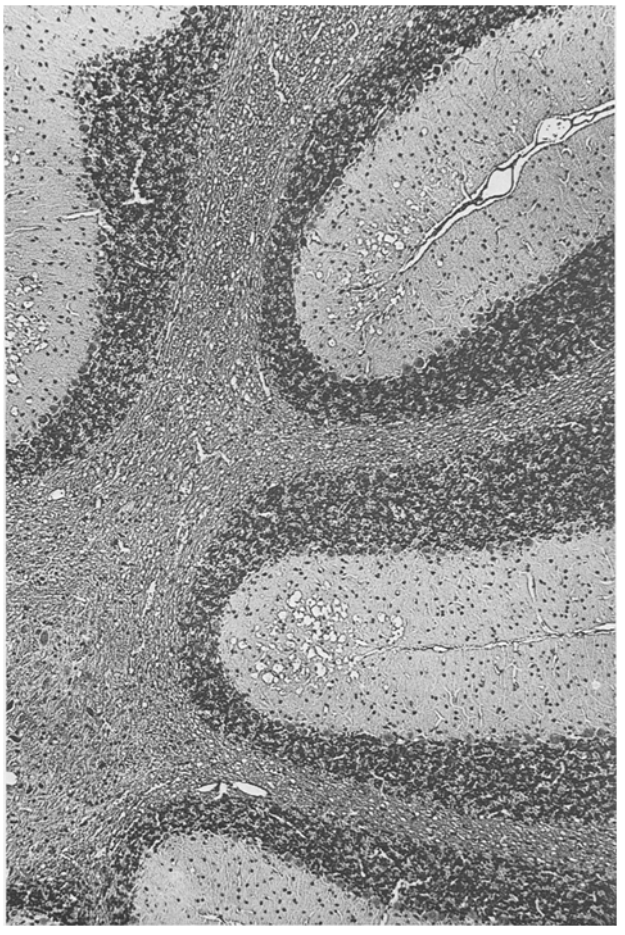

Fig. 1. Section from cerebellum of a PCA-rat with continuous weight loss. In the depths of sulci there is vacuolisation and loss of Purkinje cells. H.-E. ×40

formed as described by Lee and Fisher (1960), Bismuth et al. (1963), and Kyu and Cavanagh (1970). The time from the occlusion of the portal vein to the reestablishment of the circulation in the inferior vena cava averaged 12 min (range 8–15).

Two died during the experimental period for unknown reasons, and two were excluded to inadequate perfusion fixation. The control group comprised sixteen normal 5-month-old males, and four 13-month-old sham-operated males. All animals were weighed and examined clinically once a week.

In the four sham-operated animals the portal vein was dissected free, the pyloric vein ligated, and the vena cava occluded for 12 min.

Four animals were sacrificed 5–7 weeks after PCA, eight animals after 15–22 weeks, and seven animals after 33–35 weeks (Table 1). Three animals with continuous weight loss were sacrificed 13, 15, and 15 weeks after PCA.

Perfusion and Histological Technique. The methods employed have been described by one of us (Diemer, 1975b, 1977a, b). Thoracotomy was carried out in ether anesthesia and 5 ml blood taken from the left ventricle for duplicate determinations of the arterial plasma ammonia concentration (Bergmeyer, 1970; Gerhardt, 1973).

Counting of Glial Cells in the Corpus Callosum. This was performed on cerebral sections at the level of the decussation of the anterior commissure (section A 7190 µ, Fig. 18b, König and Klippel, 1963) using an ocular grid (×500). The subtypes of glial cells were determined according to the criteria laid down by Ling et al. (1973).

Counting of Nerve and Glial Cells in the Corpus Striatum, and Measurement of the Myelin Percentage. Klüver-Barrera stained sections were used. These were taken 700 µm anterior to the decussation of the anterior commissure (corresponding to section A 7890 µ, Fig. 16, König and Klippel, 1963). The slides were placed on the scanning board of the Classimat® and, using an ocular grid covering 0.01 mm² and 40× objective, the oligodendrocytes, astrocytes and nerve cells were counted, while all other cells (macrophages, endothelial cells) were rejected. Only cells within the grey matter were counted. The exact area of grey matter in each counting square was obtained by subtraction of the area of myelin, determined on the Classimat®.

Counting of Purkinje Cells and Bergmann Astrocytes. Using a microprojection apparatus 90× enlargements were obtained of sections stained by Bodian's method. The whole line through the nuclei of the Purkinje cells (stratum gangliosum) was then traced on drawing paper. The length of this line was measured twice using a perimeter. All Purkinje cells on this line were counted in a microscope at a magnification of 250×. On the next section, stained with haematoxylin-eosin, the number of Bergmann astrocytes corresponding to the crest and depth of the so-called folia VIII (Larsell, 1952) were counted.

Determination of the Area of Sections of Cerebrum and Cerebellum. It was checked that the degree of shinkage after histological processing was equal in the control and PCA animals by means of determining the areas of cerebral frontal sections and cerebellar sagittal sections on the Classimat.

Determination of Nuclear Size. The nuclear sizes of 25 oligodendrocytes, 50 astrocytes and 25 neurones from each animal were measured in the sections stained with cresyl violet, using the Classimat, and the true value was calculated using Sholpo's (1957) correction. The diameters found were inserted in Floderus' (1944) formula, which

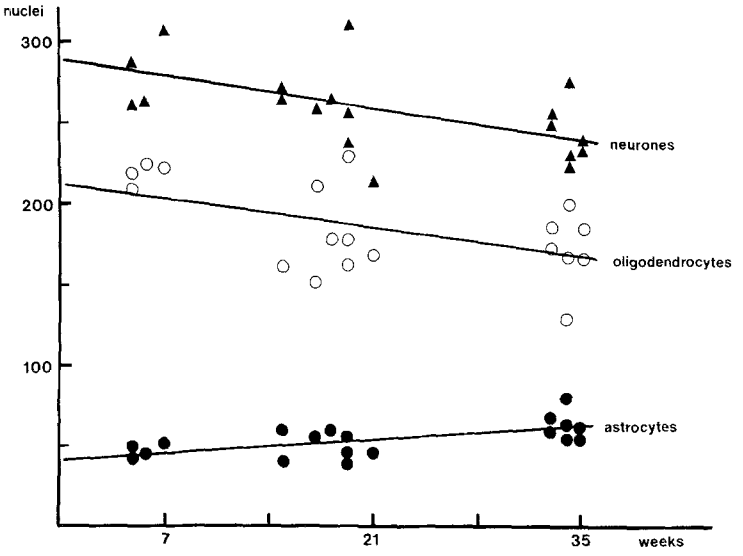

Fig. 2
Variations in the number of astrocyte, oligodendrocyte and neurone nuclei of basal ganglia grey matter plotted against time. Abscissa: weeks after PCA. Ordinate: Cells per mm^2 in a 7.45 µm thick section. Regression equation for neurones: $y = -1.331x + 289.1$ ($P < 0.02$). For oligodendrocytes: $y = -1.257x + 212.3$ ($P < 0.05$). For astrocytes: $y = 0.648x + 41.43$ ($P < 0.005$)

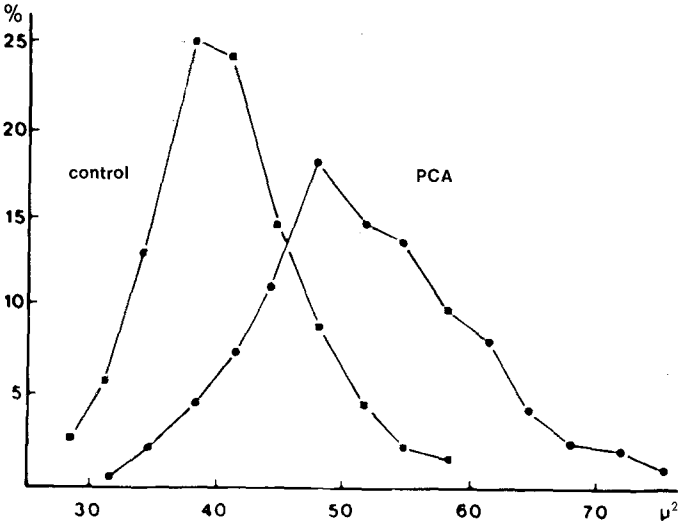

Fig. 3
Percentage distribution of astrocyte nuclear areas in corpus striatum of controls and rats 15–22 weeks after PCA. (Total nuclear area determinations: 600 + 600)

corrects the number of nuclei measured per square unit for the thickness of the section, the nuclear diameter and the height of the smallest discernible nuclear fragment.

Statistics. Due to the relatively small section thickness a number of nuclei have their greatest part outside the histological section. The measured sizes are smaller than the true ones resulting in a skewness of the distribution curves, which also showed to be of different shape (Figs. 3 and 4). Likewise it was not surprising that not all values of cell density in the experimental groups were normally distributed, a situation not uncommon in biologic materials. Due to such circumstances all values were expressed as median values (50-percentile) with 25- and 75-percentiles (1st and 3rd quartiles). Correspondingly, a non-parametric significance test, Mann Whitneys rank sum test was used.

Results

Clinical Findings. The animals were less active than usual during the first days after the operation, but apart from this their behaviour was normal. After operation there was a loss of weight which was most marked between 5 and 7 weeks. Thereafter most of the animals gained weight, and in the groups which lived for 15–22 weeks after PCA the mean value was 10% below the preoperative weight, but about 15% below that of the sham-operated animals (Table 1).

From 5–15 weeks after the operation, most of the animals had slightly increased muscle tone, manifested by an increased curvature of the spine. This seemed to decrease as the weight curve began to rise, and in animals which were observed for more than 15 weeks the muscle tone was normal.

Five of the animals continued to lose weight, and two of these died 8 and 10 weeks after PCA. In addition to increased muscle tone the animals in this group also showed ataxia, with unsteady and clumsy gait, and a tendency to fall over on one side when the cage was moved.

The plasma ammonia concentration in normal rats was 58 ± 20 µmol and the haematocrit value was

approximately 50% as determined in 50 animals. In the operated animals observed for 15–22 weeks the plasma ammonia concentration was increased to 6–7 times the normal, and the haematocrit was reduced to about 40%. In the animals with longest experimental period the plasma ammonia concentration was 3 times the normal (i.e. 180 µmol), while the animals with continuous weight loss had the highest plasma ammonia levels, the highest value found being 500 µmol. In this group the haematocrit value was reduced to about 20%.

General Pathological Findings. Adhesions in the abdomen made it impossible to dissect out the portocaval anastomosis in one-third of the animals. In the remaining animals the portal vein and the inferior vena cava were opened to check the anastomosis, which in all cases was found to be patent.

The liver in the experimental animals was frequently smaller and softer than that in the control animals. In the majority of cases the normal microscopic architecture was preserved. There was gross liver atrophy in the animals from the group with continuous weight loss. In one of these animals microscopy revealed centrilobular necrosis in almost all the lobuli. In the second animal the portal spaces were generally enlarged, due to a marked dilatation of the branches of the portal vein, and showed a peliosis-like picture indicating previous necrosis. In the third of these animals there was very marked atrophy, but no necrosis.

There were no gross changes in the kidneys. In the majority of animals microscopy revealed a slightly increased number of mesangial cells, with an increased matrix in the glomeruli. In a number of animals there were also precipitates resembling bile pigment in the glomeruli.

No gastric ulcers were seen.

Neuropathological Findings. On gross examination all brains appeared normal. Microscopically they showed features of good perfusion-fixation: an empty and dilated capillary bed and no shrunken dark neurones surrounded with clear spaces.

There was slight to moderate lobulation of the astrocyte nuclei in both cerebral cortex and corpus striatum, especially 15–22 weeks after PCA. The nuclei of the oligodendrocytes were normal. No abnormal granules were seen.

A small part of the neurones (4–5%) showed strongly stained cytoplasm with accentuated staining of the neuronal processes but most frequent with a normally sized nucleus and nucleolus. Such neurones were only rarely seen in the controls (about 1%).

In the group with continuous weight loss there was vacuolization of the molecular layer in the depths of the sulci of the cerebellum (Fig. 1). In these areas there was loss of both Purkinje cells and Bergmann's astrocytes and a few "empty" baskets was observed. Furthermore, especially in the cerebellum, neurones (Purkinje cells) with homogenization and eosinophilia of the cytoplasm were seen.

No mitoses, Alzheimer type I astrocytes or Opalski cells were seen. Bodian and Klüver-Barrera stained sections showed no changes in the axons or myelin sheats.

Quantitative Investigations of Corpus callosum. Table 2 shows the cell density in corpus callosum in controls and in animals from the different experimental groups. The total number of cell nuclei/0.01 mm^2 was unchanged except in the animals with continuous weight loss in which a 10% reduction was found, mostly due to loss of oligodendrocytes (60% of the reduction).

In the other groups, the number of oligodendrocytes and astrocytes showed no significant changes, on average there were about 10% astrocytes and 85% oligodendrocytes, the remaining cells being endothelial cells and pericytes.

There were no significant differences in oligodendroglial nuclear area between the groups (Table 2).

Quantitative Investigation of Corpus striatum. Table 4 shows the results of the corrected nuclear counts in the corpus striatum. After only 5–7 weeks there was an increase in the number of astrocytes per counting field and this increase continued, such that the animals with the longest experimental period had the greatest number, the increase being by about 90%. There was a maximal decrease of 20% in the number of oligodendrocytes after 35 weeks. There were no significant changes in the total number of glial cells in the first three groups. By contrast, in the three animals with continuous weight loss there was a reduction in the number of glial cells in particular due to a marked fall in the density of oligodendrocytes.

15–22 weeks after PCA the loss of neurones was 15%, and in the animals with continuous weight loss it was 20%. Animals 33–35 weeks after PCA had a relatively higher change in the number of neurones than in glial cells, resulting in an glial/neurone ratio of 0.98.

In the group with continuous weight loss this was reversed, resulting in a reduced glial/neurone ratio (0.83).

The corrected number of astrocytes, oligodendrocytes and neurones in the three largest PCA-groups were plotted against weeks of PCA, as shown in Figure 2.

The values were subjected to a linear regression analysis. Significance testing of the regression coeffi-

Table 2. Counted number of cell nuclei/0.01 mm² in corpus callosum, and measured area of oligodendroglial nuclei. All numbers are median values with 25- and 75-percentiles

Group	Oligodendrocyte nuclei/0.01 mm²	Astrocyte nuclei/ 0.01 mm²	Endothelial and pericyte nuclei/ 0.01 mm²	Total number of nuclei/0.01 mm²	Area of oligodendroglial nuclei (μm²)
Controls ($n = 4$)	18.90 (18.40–20.31)	1.96 (1.82–2.40)	1.06 (0.78–1.16)	21.96 (21.26–23.25)	27.84 (27.45–28.30)
15–22 weeks PCA ($n = 4$)	18.79 (16.92–19.96)	2.00 (1.40–2.28)	0.80 (0.76–0.93)	21.18 (19.68–23.04)	27.50 (26.25–28.80)
33–35 weeks PCA ($n = 4$)	18.94 (17.92–20.41)	1.86 (1.35–2.53)	0.94 (0.86–1.14)	21.72 (21.32–23.12)	28.20 (26.60–28.60)
Animals with continuous weight loss ($n = 3$)	17.54 (16.53–17.92)*	1.48 (0.85–1.81)	0.66 (0.51–0.75)	19.64 (18.27–20.11)**	26.10 (24.72–29.00)

* $P < 0.05$; ** $P < 0.02$ (Mann-Whitneys rank sum test)

Table 3. Density of astrocyte-, oligodendrocyte-, and neurone nuclei in the grey matter of corpus striatum corrected for nuclear diameter, section thickness, percentage of myelin in the counting fields. All numbers are median values with 25- and 75-percentiles

Groups	Corrected number of astrocytes/mm²	Corrected number of oligodendrocytes/mm²	Corrected number of neurones/mm²	Glial/neurone ratio
Controls, 5 month ($n = 10$)	33 (30–38)	217 (205–273)	292 (266–304)	0.95 (0.87–1.03)
5–7 weeks ($n = 4$)	47 (45–52)*	221 (211–223)	281 (261–301)	0.95 (0.90–0.99)
15–22 weeks ($n = 8$)	51 (41–59)**	171 (160–203)*	261 (204–273)*	0.91 (0.81–0.92)
33–35 weeks ($n = 7$)	62 (59–70)**	176 (169–191)**	238 (230–258)*	0.98 (0.93–0.98)
Cont. weight loss ($n = 3$)	46 (41–53)*	143 (108–187)**	232 (217–264)**	0.83 (0.65–0.91)*
Control, 13 month ($n = 4$)	29 (27–31)	226 (207–243)	282 (265–287)	0.91 (0.88–0.94)

* $P < 0.05$; ** $P < 0.01$ (Mann-Whitneys rank sum test)

Table 4
Nuclear areas of astrocytes and oligodendrocytes and nuclear diameter of neurones in corpus striatum in rats with porto-caval anastomosis and in control rats. All numbers are median values with 25- and 75-percentiles

Group	Astrocyte nuclear area, μm² (Sholpo's factor: 1.13–1.14)	Oligodendrocyte nuclear area, μm² (Sholpo's factor: 1.10)	Neurone nuclear diameter μm (Sholpo's factor: 1.16)
Control, 5 months ($n = 10$)	39.9 (36.4–43.3)	24.0 (22.8–27.5)	11.6 (11.4–12.2)
5–7 weeks ($n = 4$)	37.6 (34.9–39.2)	23.4 (21.8–24.5)	11.4 (11.3–11.8)
15–22 weeks ($n = 8$)	50.0 (47.8–54.4)**	25.7 (23.9–27.5)	11.6 (10.6–11.8)
33–35 weeks ($n = 7$)	43.4 (39.8–45.4)*	24.1 (22.5–25.3)	11.3 (10.9–11.8)
Cont. weight loss ($n = 3$)	58.6 (52.6–61.6)**	26.1 (24.0–27.9)	11.7 (11.4–11.9)
Control, 13 months sham-operated ($n = 4$)	41.4 (35.4–44.0)	25.2 (22.5–27.7)	11.9 (11.4–12.4)

* $P < 0.05$; ** $P < 0.01$ (Mann-Whitneys rank sum test)

cient for each cell type showed that the changes in cell number were related to the length of period after PCA. The regression lines are seen in Figure 2, and the regression equations and significance levels appear in the legend to the figure.

The area of the oligodendrocyte nuclei in the region remained unchanged during the experimental period (Table 4). In contrast the astrocyte nuclear was increased. This was especially true of the first group, where the increase in nuclear volume was by about 60%. In the group of animals with continuous weight loss the volume increased by about 75%.

There was no significant change in the size of the neuronal nuclei.

Table 5. Nuclear densities in the cerebellum of PCA rats. Counted and corrected number of Purkinje cell/mm. Counted number of Bergmann astrocytes per Purkinje cell and per mm of stratum gangliosum. All numbers are median values with 25- and 75-percentiles

Group	Counted number of Purkinje cells/mm	Corrected number of Purkinje cells/mm	Counted number of Bergmann astrocytes per Purkinje cell	Corrected number of Bergmann astrocytes/mm
Control ($n = 10$)	14.5 (14.1–14.9)	5.2 (5.1–5.4)	4.3 (3.6–5.3)	31.8 (26.6–39.2)
5–7 weeks ($n = 4$)	14.3 (13.7–14.9)	5.3 (5.1–5.5)	5.6 (4.9–5.6)	38.8 (35.2–40.2)
15–22 weeks ($n = 8$	12.1 (10.2–13.0)	4.4 (3.7–4.7)*	6.6 (6.3–6.9)	38.8 (37.0–40.6)*
33–35 weeks ($n = 7$)	12.1 (10.1–13.8)	4.4 (3.7–5.0)*	7.1 (6.5–8.0)	43.6 (39.9–49.1)**
Animals with continuous weight loss ($n = 3$)	11.8 (11.7–11.9)	4.1 (4.1–4.2)**	7.5 (6.1–8.7)	41.3 (33.6–47.9)*
Sham-operated controls, 13 months ($n = 4$)	14.2 (14.0–14.4)	5.1 (5.0–5.1)	4.1 (3.9–4.4)	29.7 (28.2–31.9)

* $P < 0.05$; ** $P < 0.01$ (Mann-Whitneys rank sum test)

Figure 3 shows the distribution curves of astrocyte nuclear areas from control animals and animals 15–22 weeks after PCA. The distribution curve for the control animals shows a typical skewness, with obvious divergence between the median and mean values. The curve for the PCA animals indicates a greater variation in nuclear areas, and is clearly displaced to the right. Semilogarithmic plotting did not transform the values into normally distributed ones.

Quantitative Investigation of Cerebellum. In animals with an observation period of 15 weeks or longer, there was a reduction in the number of Purkinje cells per millimeter. Thirty-five weeks after PCA there was a loss of 15% (Table 5). In the animals with continuous weight loss, with an observation period of only 7–15 weeks, the Purkinje cell density was reduced by 20%.

The number of Bergmann astrocytes per millimeter was significantly increased after 15–22 weeks, and reached a maximum of 37% increase at 33–35 weeks after PCA. In the animals with continuous weight loss there were vacuolated areas in the molecular layer, but in contrast a less marked increase in the number of Bergmann astrocytes per millimeter of Purkinje cell layer. This could be accounted for by the loss of astrocytes (and Purkinje cells) in the vacuolated areas.

Measurements of the neurone diameter in the various groups revealed no significant changes during the experimental period (Table 6).

In contrast, the size of the Bergmann astrocyte nuclei was increased, most markedly in the animals with continuous weight loss, where the volume increased by up to 80%. In the animals with an observation period of 35 weeks after PCA the increase in the size of the nuclei of the Bergmann astrocytes was not significant.

Table 6. Classimat® determinations of nuclear sizes in cerebellum: Diameter of Purkinje cell nuclei, area of Bergmann astrocyte nuclei. All numbers are median values with 25- and 75-percentiles

	Purkinje cell nuclei Sholpo's factor: 1.17 measured diam. μm	Bergmann astrocyte nuclei Sholpo's factor: 1.13 measured area μm²
Controls, 5 months ($n = 10$)	12.3 (11.6–12.6)	37.9 (33.9–40.2)
5–7 weeks ($n = 4$)	12.0 (11.3–12.2)	40.3 (39.5–45.4)*
15–22 Neeks ($n = 8$)	12.1 (11.6–12.7)	45.2 (42.6–51.5)**
33–35 weeks ($n = 7$)	12.0 (11.6–12.4)	38.8 (33.8–43.5)
Animals with continuous weight loss ($n = 3$)	12.7 (11.7–13.4)	52.0 (51.9–54.4)**
Sham-operated Controls, 13 months ($n = 4$)	12.4 (12.0–12.5)	37.9 (34.7–44.4)

* $P < 0.05$; ** $P < 0.01$ (Mann-Whitneys rank sum test)

Figure 4 shows the distribution of 400 astrocyte nuclei from the control group, and 400 from the group with 15–22 weeks' observation; the curve is uniformly displaced to the right without the appearance of a new nuclear class.

Discussion

The clinical and pathological reactions to portocaval anastomosis (PCA) differ widely between species (Bollmann, 1961), and even the shunt operation in rats gives different clinical results. In Lee et al.'s (1974) review of the changes in the rat following PCA, the weight loss after PCA ranges from 5–35%, and the time at which the animals regain their preoperative weight varies from about 2 weeks to 35 weeks.

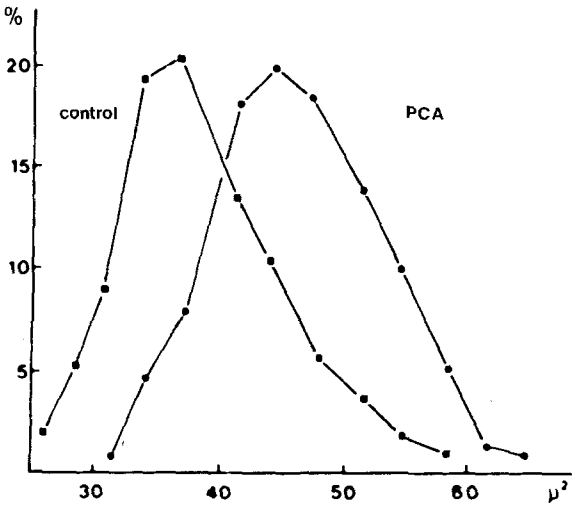

Fig. 4
Percentage distribution of Bergmann astrocyte nuclear areas in the Purkinje cell layer of controls and rats 15–22 weeks after PCA. (Total nuclear area determinations: 400 + 400)

An explanation of these differences has been put forward by Kline et al. (1966) and Bismuth et al. (1965), who demonstrated that the weight loss following PCA was greatest in the animals with the largest shunt opening and that these animals did not produce as many collaterals to the liver. If the anastomosis closed during the postoperative course an increase in weight and a fall in plasma ammonia concentration took place (Kline et al., 1971). Zuidema et al. (1962a, b) and Orloff et al. (1963) found that a total shunt led to higher plasma ammonia levels than a subtotal shunt, and Kline et al. (1966, 1971) showed that the neuropathological changes with development of Alzheimer type II astrocytes, were more pronounced after complete shunting.

This increase in the number of observed astrocyte nuclei is assumed to be the result of an actual astrocyte proliferation in numerous investigations of porto-systemic (Kline, 1966, 1971; Norenberg et al., 1974) or hepatic encephalopathy (Lahl, 1974; Mossakowski et al., 1971; Lapham, 1961). However, the differential counts in this and a previous investigation (Diemer, 1977b), which revealed no increase in the total number of glial cells, indicate that alternative explanations of the increase in the number of astrocyte should be considered. Especially this aspect of hepatic encephalopathy will be discussed here. One possibility is that some oligodendrocyte nuclei or glial percursor cell nuclei are stimulated by the changed metabolism after PCA and transformed into nuclei with the morphological characteristics of astrocyte nuclei.

Ling et al. (1973) described medium shade and light oligodendrocytes in the rat cortex and corpus callosum besides the common dark oligodendrocytes. Similar investigations of the corpus striatum have not been performed, but supposing a similar percentage of light and medium shade oligodendrocytes (5–6%), changes of such glial nuclei cannot account for the observed increase of astrocyte nuclei in corpus striatum and in the Purkinje cell layer where only a few oligodendrocytes are found.

Glial precursor cells have been described as "multipotent glial cells" (Vaughan an Peters, 1968), "glioblasts" (Privat et al., 1972; Sturrock, 1976) or "free subependymal cells" (Ling et al., 1973). Transformation of these cells has earlier been proposed as the source of the increase in the number of astrocytes (Gutierrez and Norenberg, 1975; Diemer, 1977). A transformation of these cells into astrocytes involves differentiation of the cytoplasm as they contain no glial filaments or glycogen granules. Against this assumption speaks the fact that glial nuclear changes similar to those demonstrated in this investigation were found to be fully reversible after short term hyperammonemia (Diemer and Laursen, in press). Supposing that the observed changes in the number of astrocyte nuclei are not due to transformation from other glial cell types, two other possible explanations exist: division of astrocytes or transformation of astrocyte nuclei.

The current view on hepatic encephalopathy assumes that a real astrocyte proliferation takes place on the basis of cell divisions (Adams and Foley, 1953; Victor et al., 1965; Erbslöh, 1958; Kline et al., 1966, 1971). However, no astrocyte mitoses have been reported and it has been proposed that the proliferation takes place by amitotic division (Stadler, 1936; Adams and Foley, 1953; Lapham, 1962). Recently, it has been shown that astrocytes can divide mitotically (Cavanagh, 1970; Diemer and Klinken, 1976). The duration of an astrocyte mitosis, however, is so short

that the possibility of a sufficient number of cell divisions cannot be entirely ruled out.

The remaining possibility is that not all astrocyte nuclei in the normal brains are recognized as such. A part of these may be counted as oligodendroglial nuclei due to similarities in size and chromatine pattern. This possibility involves no astrocytic divisions but a nuclear enlargement with dispersion of chromatine.

The 29% astrocyte nuclei found in the PCA rats should then represent the full number of astrocytes. This is equivalent to the 27% found in rats with CCl_4-induced liver cirrhosis (Diemer, 1977b) and the 30% found in both cortex and striatum of pigs with PCA and temporary hepatic artery clamping (Diemer and Tønnesen to be published). These astrocyte percentages correspond to those found in semithin/ultrathin sections from rat cerebral cortex by Ling and Leblond (1973) who counted about 30 astrocytes and 70 oligodendrocytes per 100 neurons. Thus there is evidence suggesting that normal small astrocyte nuclei are enlarged after PCA. This is in accordance with the fact that nuclei normally counted as astrocytic enlarge in the perfusion fixed brains as illustrated in Figures 3 and 4. This was also shown by Cavanagh and Kyu (1971) who found enlarged astrocyte nuclei in perfusion fixed rats 10–35 weeks after PCA.

The metabolic changes in rats after PCA seem to activate the astrocytes resulting in lobulation of the nucleus, swelling of the processes, an increase of the number of organelles and folding of the capillary basement lamina (Zamora et al., 1973). The dry mass of the astrocytes is increased (Watson, 1972), and the permeability of the astrocyte membrane to horseradish peroxidase is also increased (Laursen and Westergaard, 1977).

Supposing that no astrocyte divisions took place, the animals with a constant total glial number would also have a constant oligodendrocyte number. However, the animals with continuous weight loss and liver atrophy – presumably due to lack of collaterals to the liver (Latham, 1975) – showed a reduction of glial cells due to loss of oligodendrocytes. Another sign of severe cerebral affection in these animals was vacuolisation of the cerebellar molecular layer. Electron microscopy has shown that these vacuoles are the distended processes of Bergmann astrocytes (Cavanagh et al., 1972).

All animals in the material with an altered astrocyte/oligodendrocyte ratio had also a slight loss of neurones. Most investigations on hepatic encephalopathy has focused on demonstrating astrocyte changes but nerve cell changes and loss have been described by a few investigators both in human (Victor et al., 1965) and experimental hepatic encephalopathy (Lahl, 1974; Mossakowski, 1971; Kline, 1966, 1971; Diemer, 1977b). In PCA rats Cavanagh et al. (1972) described homogenization and loss of Purkinje cells as also found by us, and electron microscopy (Zamora et al., 1973) showed 10% degenerated axons, possibly as a sign of a "dying-back" process.

Concerning the pathogenesis of the glial and nerve cell changes investigations on the ammonia toxicity and changed ammonia metabolism in porto-systemic/hepatic encephalopathy are dominant. As shown, also by us, the plasma ammonia concentration is increased 5–7 fold and the glutamine content in the brain raised (Patel et al., 1972). The increased glutamine content is found in the glial compartment and may well be localized to the dilated endoplasmatic reticulum of the astrocytes (Cremer et al., 1975). Norenberg (1976) found increased glutamate dehydrogenase activity in the astrocyte processes.

Thus at least part of the glial changes and possibly the changes of the astrocyte nuclei seem to be related to the detoxification of ammonia.

The enlargement of astrocyte nuclei, normally regarded as oligodendrocytes can explain the often very high number of astrocyte nuclei found in human hepatic encephalopathy (Brun et al., 1977) and after even short periods of experimental hepatic encephalopathy (Kline et al., 1971). Only in severe human cases of hepatic encephalopathy including Wilsons disease this explanation is not sufficient. Certainly, in this situation, astrocyte division takes place in the focal areas with spongy degeneration, necrosis, neurone loss and vascular proliferation.

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