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Astrocytes of the Cerebral Cortex in Hepatosplenic Schistosomiasis Mansoni and in Liver Cirrhosis

A Morphological, Quantitative and Karyometric Study

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Summary. A morphological, quantitative and karyometric study of astrocytes of the cerebral cortex in patients with liver cirrhosis, hepatosplenic schistosomiasis mansoni and controls is reported. Cell proliferation was commonly seen but there was no significant increase in number of astrocytes in either cirrhosis and schistosomiasis groups. A highly significant increase in astrocyte nuclear volume in cirrhosis and schistosomiasis in relation to controls was observed. The astrocyte average nuclear volume in the cirrhotics was also significantly increased in relation to the schistosomiasis group. From the present data and those reported by other investigators it may be concluded that under normal conditions the astrocyte population is continually reforming and it proliferates in liver cirrhosis and hepatosplenic schistosomiasis. It seems that the morphological, quantitative and karyometric astrocyte changes in schistosomiasis may be the result of the same factors as those previously described for liver cirrhosis and experimental portacaval shunt.

Key words: Glia cells – Cell number – Karyometry – Cerebral cortex – Schistosomiasis – Liver cirrhosis

Introduction

Astrocyte changes in patients with liver cirrhosis and other acute and chronic liver disorders have been studied since 1912 (Hösslin and Alzheimer). Numerous studies have been published with focus on the morphological findings (Woerkom 1914; Demole and Redalié 1922; Barnes and Hurst 1925; Scherer 1933; Stadler 1936; Nicolajev 1937; Waggoner and Malamud 1942; McDougal and Adams 1950; Adams and Foley 1953; Brown 1957; Neubuerger 1957; Noetzel and Oster 1957; Kalm 1958; Boudin et al. 1959; Worms et al. 1959, 1960; Korenke 1965; Victor et al. 1965; Lahl 1967; Hagen and Lahl 1978). A few papers

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deal with the cell number (Adams and Foley 1953; Lapham 1962; Taylor et al. 1979) and nuclear dimensions (Adams and Foley 1953; Victor et al. 1965; Cavanagh and Kyu 1971a; Taylor et al. 1979).

To the best of our knowledge astrocyte behavior in hepatosplenic schistosomiasis mansoni has not been investigated as yet. This study describes the morphological, quantitative and karyometric changes seen in the astrocytes of the cerebral cortex of patients with hepatosplenic schistosomiasis mansoni due to the fibrosis of Symmers and compare them with those observed in patients with liver cirrhosis. Other points of interest in the present investigation are: 1) the comparison of two liver disorders, both of them leading to portal hypertension, but differing far as the functional state of the liver is concerned. The signs of hepatocellular failure are usually striking in cirrhosis whereas they are mild in the schistosomiasis mansoni. Thus the role of portal hypertension, collateral circulation, portal systemic shunt and liver failure in the development of the astrocytes changes can be evaluated; 2) schistosomiasis mansoni is widely distributed in Latin America, Africa and in Arabian Penninsula (Coelho 1959; Faust and Russell 1964) and spread of the disease has been demonstrated to have occurred (Faust and Russell 1964; Neves and Cunha 1978); 3) hepatosplenic involvement in schistosomiasis is important because of its frequency and its consequences. Although patients with hepatosplenic schistosomiasis are not commonly identified in clinical-epidemiological inquiries – 3 to 10% in highly endemic areas – they are frequently seen in general hospitals in endemic zones, where schistosomiasis is by far the most common cause of chronic liver disease and portal hypertension (Coutinho and Domingues 1978).

Materials and Methods

Liver and brain specimens were obtained from 56 autopsied patients in the Federal University of Minas Gerais Medical School. Eleven of these patients (control group) had died from diseases with no evidence of liver or brain involvement. Of the remaing 45 cases, most died from gastrointestinal bleeding, hepatic failure, congestive heart failure and infections, 26 patients had liver cirrhosis (cirrhosis group) and 19 patients had hepatosplenic schistosomiasis mansoni (schistosomiasis group) with liver fibrosis of Symmers (Bogliolo 1972, 1976). The brain was fixed in 10% formaldehyde solution and representative sections included frontal, temporal, parietal, occipital and insular cerebral cortex, caudate nucleus, putamen, globus pallidus, thalamus, hypothalamus, midbrain, pons, medulla, cerebellar cortex and dentate nucleus. The paraffin-embedded sections were stained with haematoxylin-eosin. Those of the cerebral cortex and basal ganglia were also stained by the Holzer and Giemsa methods.

Quantitative investigation – The astrocytes were counted in haematoxylin-eosin sections of the frontal motor, parietal, temporal and insular cortex, using a X620 magnification and a microscopic field measuring 200 micrometers. In each section an area was chosen at random and the astrocytes were counted in 50 consecutive microscopic fields. Because the molecular layer is more susceptible to technical artefacts it was not considered in the quantitative study. The layer immediately below to the molecular layer or the deep cortical layer was first examined. Then the slide was moved in a perpendicular axis to the cortical surface toward the deeper layers (in the first instance) or at right angles in relation to the subcortical white matter toward the cortical surface (in the second instance). After reaching these borders the slide was moved in a horizontal axis for 200 micrometers. The number of astrocytes per microscopic field and the total number in 50 consecutive fields were counted. Attention was also given to the presence of paired nuclei, nuclei in juxtaposition

or in groups of three or more cells. On each section the number of paired nuclei was counted in 100 consecutive astrocytes.

Karyometric investigation – An area in the cerebral cortex was chosen at random and the nuclei of 100 consecutive astrocytes were measured. The molecular layer was not included in this karyometric study. The layer immediately below the molecular layer or a deeper cortical layer was first examined. The slide was then moved in a perpendicular axis to the cortical surface, toward the deep cortical layers – in the first instance – or at right angles in relation to the subcortical white matter, toward the cortical surface – in the second instance. As these boundaries were reached the slide was then moved horizontally toward a consecutive microscopic field, and so on. In each microscopic field all the astrocyte nuclei were measured with a Zeiss micrometric ocular (X16) and a X100 oil immersion objective. In order to calculate the nuclear volume the greatest and smallest diameters of each nucleus were measured and the ellipse formula applied, $\pi/6\ d^2 \times D$ where d is the smallest and D the greatest diameter. As in each case the volume of 100 astrocyte nuclei were calculated a total of 5600 nuclei were assessed through a total of 11200 measures.

The data received statistical treatment (Student t test). In order to assess the relationship between the total number of astrocytes in 50 consecutive microscopic fields and the percentage of paired nuclei in 100 consecutive astrocytes in the cerebral cortex, the Pearson correlation coefficient (r) was calculated and its significance verified (Documenta Geigy 1965).

Results

1. Morphological Analysis

In the control group the astrocytes of the cerebral and cerebellar cortex, caudate nucleus, putamen and thalamus contained rounded, oval or ellipse-shaped large nuclei – larger than those of other glial cells – with fine and homogeneous chromatin granules; nucleoli of difficult visualization; and small brown-yellowish (haematoxylin-eosin) or greenish (Giemsa) granules around the nucleus. On the other hand the astrocytes in the globus pallidus, dentate nucleus, inferior olivary nucleus and in the brainstem showed oval or elongated irregular or lobulated nuclei, with fine and homogeneous chromatin granules; distinct nucleoli and pigment granules, as above described, around the nucleus. These granules were particularly prominent in the globus pallidus. A few paired astrocytes were observed, most of them in the cerebral cortex. Mitotic figures were not found.

In the cirrhosis and schistosomiasis groups (Figs. 1 to 4) the astrocytes were usually increased in size. Only the nucleus was clearly visible. Along the outer surface of the nuclear membrane lipofuscin-like pigment granules were frequently observed. Though these granules could be seen in all regions, they were more conspicuous in the globus pallidus and more prominent in these groups than in the control group. The shape of the nuclei was usually preserved in the slightly or moderately enlarged astrocytes whereas the strikingly enlarged ones had lobulated nuclei with folded nuclear membrane. This lobulation was more noticeable in the globus pallidus, dentate nucleus of the cerebellum and inferior olivary nucleus but was observed less frequently and in lesser degree in other sites. The chromatin pattern varied according to changes in nucleus size and shape, being scanty in the more voluminous nuclei where it was only visible apposed to the nuclear membrane. In these instances there was progressive

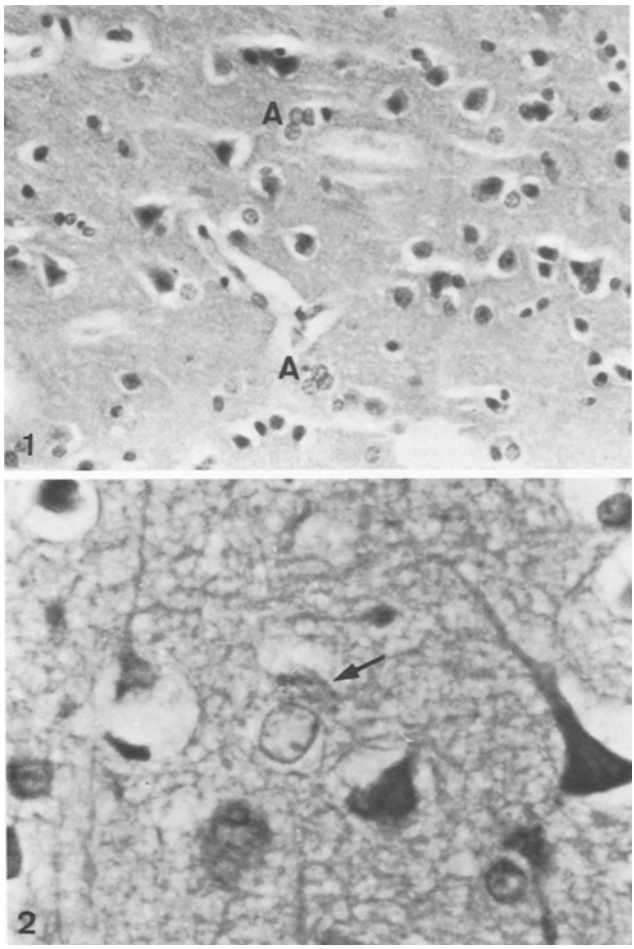

Fig. 1. Schistosomiasis group. Cerebral cortex. Astrocytes (*A*), paired nuclei (H.E., X328)

Fig. 2. Schistosomiasis group. Cerebral cortex. Astrocyte, lipofuscin-like pigment granules along the outer surface of the nuclear membrane (*arrow*). Giemsa, X819

loss of nuclear basophilia resulting in naked nuclei with distinct and thick nuclear membrane. The nucleoli, two or more in each nucleus, were more evident than those seen in the control group. Pairs of astrocyte nuclei close together were frequently seen, but groups of three and even four nuclei were not uncommon. No mitotic figures were found. Although these findings were observed in both groups, they were more conspicuous and more frequently seen in the cirrhosis group.

Examination of the astrocytes by the Holzer method for glial fibers disclosed no significant differences in the three groups. In all of them the glial fibers were abundant in subpial and subependymal regions; in great amount around blood vessels and in subcortical white matter; in variable amount (usually moder-

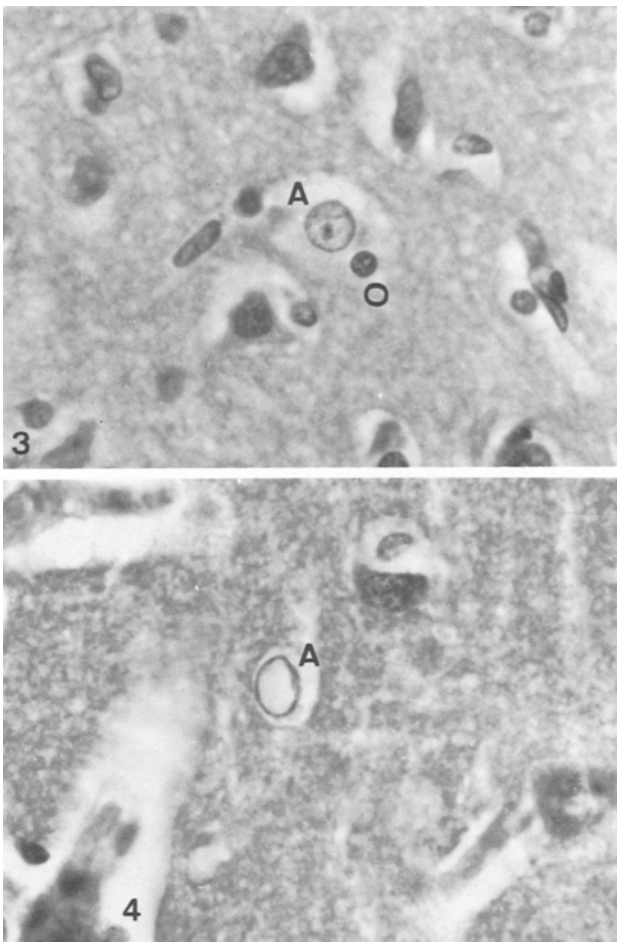

Fig. 3. Schistosomiasis group. Cerebral cortex. Moderately enlarged astrocyte but without the characteristic changes seen in Alzheimer type II cells. Oligodendrocyte (*O*). H.E. X819

Fig. 4. Schistosomiasis group. Cerebral cortex. Alzheimer type II cell (H.E., X819)

ate or great quantity) in globus pallidus; and rare or absent in the cerebral cortex, caudate nucleus and putamen.

2. *Quantitative Analysis*

In the control group the quantitative study was carried out only in five cases. The total number of astrocytes in 50 consecutive microscopic fields of the cerebral cortex varied from 173 to 251, the average being 221.07 ± 26.85. The percentage of paired nuclei in 100 consecutive astrocytes ranged from 2 to 14%, the average being $9.13\% \pm 2.10$.

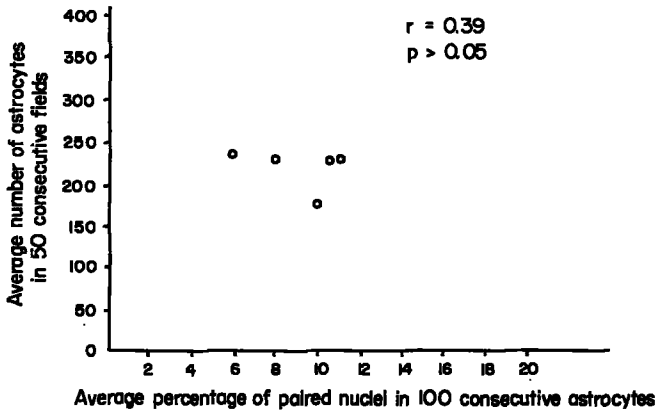

Fig. 5. Relation between the astrocytes number and the percentage of paired nuclei. Cerebral cortex, control group

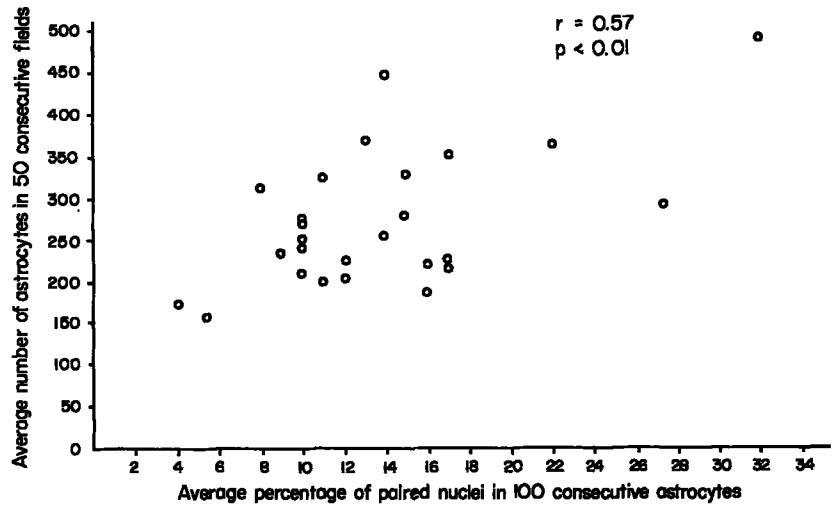

Fig. 6. Relation between the astrocytes number and the percentage of paired nuclei. Cerebral cortex, cirrhosis group

In the cirrhosis group the total number of astrocytes ranged from 136 to 491, the average being 272.81 ± 81.97. The percentage of paired nuclei ranged from 4 to 32%, the average being 13.76 ± 6.14.

In the schistosomiasis group the total number of astrocytes varied from 120 to 530, the average being 266.10 ± 77.62. The percentage of paired nuclei ranged from zero to 36%, the average being 12.26 ± 5.99.

The differences between the averages in these groups were not significant ($P > 0.05$).

The correlation between the average number of astrocytes in 50 consecutive microscopic fields and the average percentage of paired nuclei in 100 consecutive cerebral cortex astrocytes for the control, cirrhosis and schistosomiasis groups

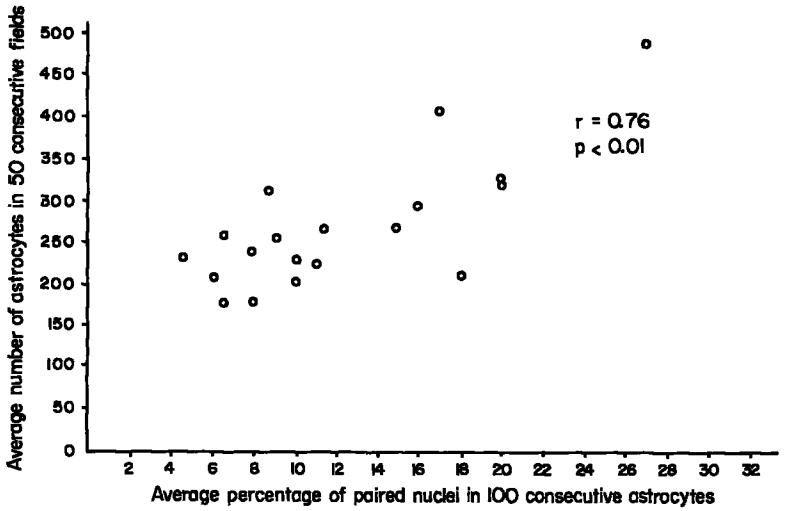

Fig. 7. Relation between the astrocytes number and the percentage of paired nuclei. Cerebral cortex, schistosomiasis group

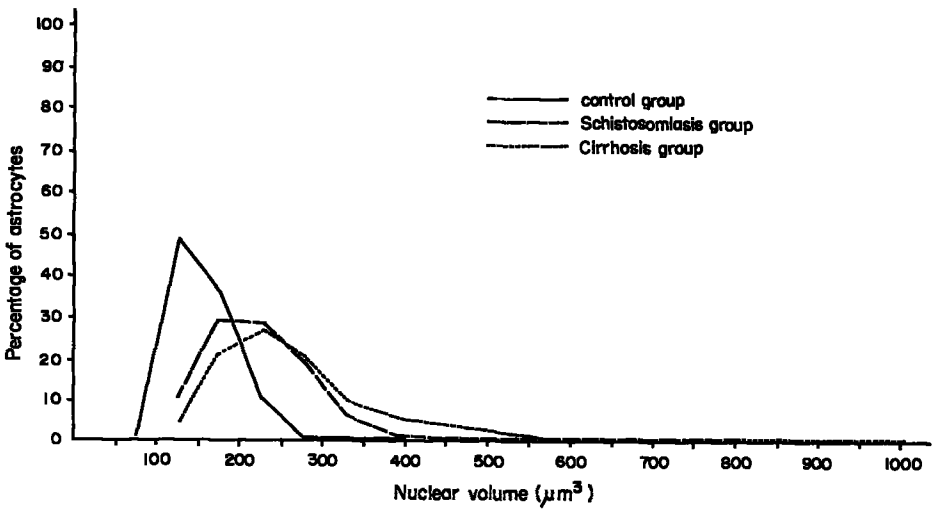

Fig. 8. Cerebral cortex astrocytes according to their nuclear volume

are shown in Figs. 5, 6 and 7 respectively. There was a highly significant positive correlation in the cirrhosis and schistosomiasis groups ($P<0.01$) but no correlation in the control group.

3. Karyometric Analysis

In the control group the values ranged from 113.78 μm^3 to 194.88 μm^3, the average being 154.38 ± 28.13 μm^3. In the cirrhosis group the volumes ranged

from 161.63 μm^3 to 575.59 μm^3, the mean and standard deviation being 274.64 μm^3 and 88.43 μm^3 respectively. In the schistosomiasis group the nuclear volumes ranged from 157.71 μm^3 to 318.92 μm^3, the average being 225.49 ± 45.86 μm^3. The differences between the averages were significant in the cirrhosis and schistosomiasis groups ($P < 0.05$) and highly significant in both cirrhosis and control groups, and schistosomiasis and control groups ($P < 0.01$).

The distribution of the astrocytes according to their nuclear volume in the control, cirrhosis and schistosomiasis groups is shown in Fig. 8. It can be noted that astrocytes in the cirrhosis group display the most voluminous nuclei and those in both the cirrhosis and schistosomiasis groups have more voluminous nuclei than those in the control group.

Discussion

Morphological Changes. In normal conditions astrocytes can be identified using different methods such as routine histological sections (haematoxylin-eosin, cresyl-violet, thionine etc.), metallic impregnations, tissue culture and electron microscopy. Because the routine microscopic preparations only stain the cell nucleus the distinction between astrocytes and other glial cells is based on the nuclear shape and size as well as its chromatin pattern (Penfield 1932; Hommes and Leblond 1967; Taylor et al. 1979). By these criteria the glial cells may be classified and counted with results similar to those using metallic impregnations (Hommes and Leblond 1967). Other workers, however, have stressed the difficulty in characterizing the different glial cells based only on their nuclear morphology (Penfield 1932; Adams and Foley 1953; Lewis 1976). In liver disorders, however, no investigator has had any difficulty in identifying astrocytes. In the cirrhosis and schistosomiasis groups there was a wide variation of the astrocyte nuclear morphology, ranging from normal appearances to the characteristic changes seen in Alzheimer type II cells. This variability has been reported in the literature (Barnes and Hurst 1925; Scherer 1933; Stadler 1936; Nicolajev 1937; Richter 1948; Noetzel and Oster 1957; Erbslöh 1958; Worms et al. 1959, 1960; Lahl 1967; Hagen and Lahl 1978).

Two other observed morphological changes, which have commonly been described by other authors, deserve mention. The first finding is the presence of pigment granules close to the nucleus (Demole and Redalié 1922; Inose 1952; Adams and Foley 1953; Greenfield 1953; Guillain et al. 1954; Alajouanine et al. 1955; Kalm 1958; Worms et al. 1959, 1960; Lapham 1962; Victor et al. 1965). Electron microscopic studies have revealed that these granules are lipofuscin (Martinez 1968; Foncin and Nicolaidis 1970; Zamora et al. 1973). The second interesting finding is the almost complete absence of newly-formed glial fibers (Hösslin and Alzheimer 1912; Greenfield et al. 1924; Barnes and Hurst 1925; Jervis et al. 1942; Richter 1948; Adams and Foley 1953; Greenfield 1953; Alajouanine et al. 1955; Lapham 1962; Gibson 1963; Korenke 1965; Tarnowska-Dziduszko and Wald 1971). As pointed by Norenberg et al. (1972), and Norenberg and Lapham (1974) this is quite different from astrocytic fibrosis with numerous newly-formed glial filaments in the perikarion and astrocyte

processes (Rio-Hortega and Penfield 1927; Luse 1958; Greenfield and Meyer 1963; Lapham and Johnstone 1964).

Quantitative Changes. Adams and Foley (1953), and Lapham (1962) found a doubling in number of astrocytes in the globus pallidus and dorsomedial nucleus of the thalamus, in cases of hepatic coma and encephalopathy. Reference to cerebral cortex astrocytes only is made by Taylor et al. (1979) who describe a patient with clinical picture of hepatic encephalopathy who died 12 years after a portacaval shunt. Autopsy disclosed mild periportal fibrosis in the liver and diffuse hyperplasia and hypertrophy of the protoplasmic astrocytes. There was a 45% increase in number of astrocytes in the insular cortex, compared to a control case. In the present investigation no significant increase in astrocyte population was observed in either cirrhosis or schistosomiasis groups but cell proliferation was evident in many cases. In half of the cirrhosis patients and in 10 from the 19 schistosomiasis patients the average figure for the number of astrocytes was above the highest figure obtained from the control group. This difference was as high 25 to 95% in 8 cirrhosis and 5 schistosomiasis patients. It is also worth bearing in mind that most authors (Adams and Foley 1953; Lapham 1962; Taylor et al. 1979) have studied patients with hepatic encephalopathy, including hepatic coma, whereas in this work some cirrhotic and the majority of schistosomiasis patients had no symptoms or signs of hepatic encephalopathy. This patient selection has to be considered when one compares the present data with those reported in the literature. The presence of paired nuclei and groups of astrocyte nuclei close together also favors the concept of astrocyte proliferation in liver disorders (Demole and Redalié 1922; Scherer 1933; Nicolajev 1937; Adams and Foley 1953; Greenfield 1953; Noetzel and Oster 1957; Worms et al. 1959, 1960; Lapham 1962; Victor et al. 1965; Lahl 1967; Taylor et al. 1979). The percentage of paired nuclei in the cerebral cortex in the cirrhosis and schistosomiasis groups were higher than that observed in the control group but the difference was not significant. There was, however, a significant correlation between astrocyte number and the percentage of paired nuclei in both cirrhosis and schistosomiasis groups.

It has been shown that astrocytes proliferate under normal as well as pathological conditions. Smart and Leblond (1961) believe that glial cells do not constitute a stable population, but rather are continuously formed from spongioblasts during even adult life. Hommes and Leblond (1967) observed continuous low-degree mitotic activity in rat glial cells (oligodendrocytes and astrocytes). As the paired nucleus is considered histological evidence of recent cell division their presence in the control group supports the findings of Smart and Leblond. Other evidence favoring this idea is also available; Hommes and Leblond (1967) noted labelled paired nuclei following labelled mitotic figures after tritiated thymidine injections. By electron-microscopy (Norenberg et al. 1972) the paired nuclei were separated by distinct and juxtaposed cytoplasmic membranes. Mitosis-related organels were also increased in number. Diploid DNA had already been reported in these paired nuclei (Lapham 1962).

Karyometric Changes. Little is known about the astrocyte nuclei size in liver disorders. Adams and Foley (1953) measured the diameter of astrocyte nuclei

in the lateral aspect of the globus pallidus in 50 cases of liver disease, 36 of them with history of hepatic coma, and in 30 controls. They found a significant increase in diameter in the hepatic coma group as compared to cases of liver disorder without coma and to controls. Victor et al. (1965) observed in cases of liver cirrhosis an increase in the astrocyte nuclear surface as compared to 2 control cases. Cavanagh and Kyu (1971a) found in 2 cases of hepatic coma a 70% increase in the average astrocyte nuclear volume, whereas Taylor et al. (1979) found diffuse astrocyte hyperplasia and increased volume of the insular cortex astrocyte nuclei in a patient who developed hepatic encephalopathy after a portacaval shunt.

This present investigation disclosed a highly significant increase in the average astrocyte nuclear volume in both cirrhosis and schistosomiasis groups. Twenty-three out of 26 cases in the cirrhosis group and 13 out of 19 cases in the schistosomiasis group had figures higher than that of the most voluminous nucleus observed in the control group, this increase being from 1 to 195% and 1.5 to 63.6% in relation to the controls, respectively. The average nuclear volume of cerebral cortex astrocytes was bigger in cirrhosis than in schistosomiasis group. Six out of 26 cases in the cirrhosis group had figures higher than that of the most voluminous nucleus in the schistosomiasis group, the increase in volume being from 0.6 to 80% in relation to the schistosomiasis group.

Although the exact causes and mechanisms involved in the development of the morphological, quantitative and karyometric changes in astrocytes in liver disorders are still to be established some evidence points to ammonia as having an important role. High blood ammonia has been found in patients with liver disease who displayed an increase in the astrocyte cell number and nuclear dimensions (McDermott and Adams 1954; Case Records of Massachusetts General Hospital – Case 44041, 1958; Boudin et al. 1959; Victor et al. 1965; Taylor et al. 1979). Bruton et al. (1970) and Lewis (1976) have observed an increased number of Alzheimer type II astrocytes in cases of metabolic block in the urea cycle due to enzyme deficiences with intermittent hyperammonemia. Hyperammonemia and an increase in astrocyte number and size have also been noted in experimental animals fed with ammonia-rich diet or after portacaval shunt (Kline et al. 1966; Doyle 1967; Cavanagh and Kyu 1969, 1971a, 1971b; Kline et al. 1971; Nance and Kline 1971; Cole et al. 1972; Zamora et al. 1973; Norenberg and Lapham 1974; Taylor et al. 1979). It is also known that an increase in blood and nervous tissue ammonia and astrocyte changes similar to those seen in hepatic encephalopathy also occur after infusion of substances, such as sulphoxymin methionin, which inhibit ammonia inactivation-enzymes (Gutierrez and Norenberg 1975, 1977).

Many workers consider the astrocyte to be the site where ammonia is accumulated and inactivated in the CNS, Alzheimer glia being an expression of astrocyte hyperactivity (Cavanagh and Kyu 1971a, 1971b; Cavanagh 1972; Zamora et al. 1973; Cavanagh 1974; Norenberg and Lapham 1974; Gutierrez and Norenberg 1977; Martinez-Hernandez et al. 1977). Lahl (1967) has suggested that the increased nuclear volume is secondary to a relative hyperhydration of the nucleoplasm due to either osmotic pressure or a specific cytotoxic effect. On the other hand Cavanagh and Kyu (1971a) and Cavanagh (1974) believe that the

nuclear enlargement is a postmortem artefact which is not seen after perfusion fixation. According to Cavanagh and Kyu (1971a) low molecular weight substances such ammonia ions cause an increase in nucleoplasm osmolarity which, on its turn, due to penetration of water in the nucleus after death is responsible for the nuclear enlargement.

As liver cirrhosis and hepatosplenic schistosomiasis share some pathophysiological changes in addition to morphological, quantitative and karyometric astrocyte changes it is possible that these glial cell changes in schistosomiasis may be due to the same factors as those described for liver cirrhosis and experimental portacaval shunt.

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