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Intraventricular macrophages in the lateral ventricles with special reference to epiplexus cells: a quantitative analysis and their uptake of fluorescent tracer injected intraperitoneally in rats of different ages

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ABSTRACT

The labelling of epiplexus cells associated with the choroid plexus in the lateral ventricles was examined in rats of different ages with the fluorescent dye, rhodamine isothiocynate (RhIc). A quantitative study was also attempted; this showed that the number of epiplexus cells and their related cells, namely supraependymal and free-floating cells, increased with age. The mean absolute number of epiplexus cells ranged from ~ 700 in the newborn to ~ 2200 in rats of 17 d of age; thereafter it remained unchanged. The number of free-floating cells also increased substantially but showed considerable individual variation. Following i.p. injection, the tracer was rapidly taken up by the epiplexus cells. This provided strong support for their phagocytic nature. In the newborn (1 d) and developing (13 d, 17 d) rats, RhIc-labelled epiplexus cells were first observed 3 h after the injection. In adult rats, labelled cells were not observed until 12 h after injection. In either case, the fluorescence in the epiplexus cells gradually increased with time. It is suggested from this study that the blood-CSF barrier in the choroid plexus in postnatal rats is incomplete, thereby allowing a rapid transvascular diffusion of the injected RhIc into the blood circulation. The fluorescent dye which enters the ventricle by way of the choroid epithelium is subsequently taken up by the epiplexus cells. Such an unimpeded passage, however, is reduced in the adult rats, probably due to the maturation of the blood capillaries as well as the choroid epithelium.

INTRODUCTION

Epiplexus cells were first described by Kolmer (1921) who stated that they were macrophage-like cells residing on the epithelium of the choroid plexus of lower vertebrates. This view was later shared by Ariëns-Kappers (1953). The ultrastructure and monocytic origin of the epiplexus cells in various animals were subsequently studied by many authors (Carpenter et al. 1970; Hoyosa & Fujita, 1973; Chamberlain, 1974; Peters, 1974; Allen, 1975; Sturrock, 1978, 1979, 1983; Ling, 1979, 1981, 1983, 1985; Peters & Swan, 1979; Ling et al. 1985, 1988; Maxwell et al. 1988, 1992; Kaur et al. 1990). The phagocytic nature of the cells was confirmed experimentally by Carpenter et al. (1970), who injected carbon particles and thorotrast into the lateral ventricles in cats, and by Ling (1979), who reported that these cells were labelled following an i.v. injection of carbon suspension in postnatal rats. From his observations, Ling (1979) speculated that these labelled cells represented newly recruited epiplexus cells that were derived from monocytes which had endocytosed carbon particles in circulation.

Recently, Leong & Ling (1992) reported the presence of labelled amoeboid microglial cells in the supraventricular corpus callosum in postnatal rats following an i.p. injection of the fluorescent dye, rhodamine isothiocynate (RhIc). Since both epiplexus cells and amoeboid microglia are part of the mononuclear phagocyte system in the nervous tissue (Oehmichen, 1978; Sturrock, 1978; Ling et al. 1982) it seems likely that they could also be labelled with RhIc. There were 3 main objectives in the present study: (1) to determine and compare the total number of epiplexus cells in postnatal and adult rats since

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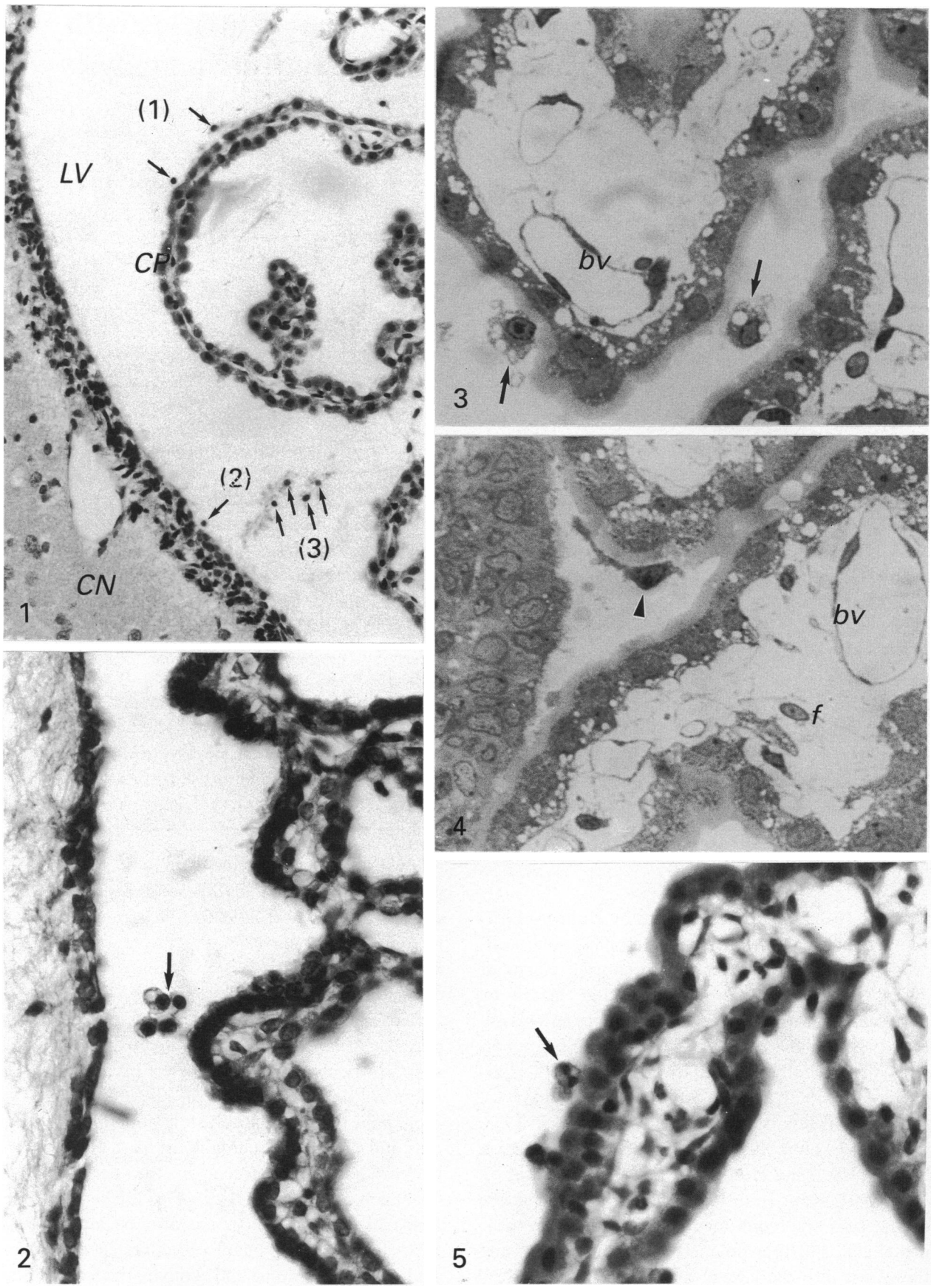

Fig. 1. Portion of lateral ventricle (LV) containing choroid plexus (CP) in a 49-d adult rat. Epiplexus cells (1) were unevenly distributed on the surface of the choroid epithelium. A supraependymal cell (2) is closely adherent to the ependyma, bordering the caudate nucleus (CN). Free-floating cells (3) are seen in the lumen between the ependyma and the choroid plexus. H & E stain. ×270.

quantitative information about epiplexus cells is lacking, the only available data being the work of Sturrock (1978); (2) to demonstrate the uptake of RhIc by epiplexus cells; and (3) to ascertain whether RhIc labelling of epiplexus cells is age related. Other related cells including supraependymal and free-floating cells were also studied.

MATERIALS AND METHODS

A total of 68 Wistar rats of either sex aged 1, 13, 17 and 49 d were used in this study. Of these, 1 was used for general morphological study, 9 for quantitative analysis and the remaining 58 for the fluorescent labelling study. In the quantitative study, the rats were divided into 3 age groups: newborn (1 d), developing (17 d) and adult (49 d). For the fluorescent labelling study, the rats were divided into 4 age groups: 1, 13, 17 and 49 d.

General morphological features

One rat, aged 1 d, was perfused with 2% paraformaldehyde and 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). After perfusion, the brain was removed and kept in the same fixative at 4 °C overnight. The brain was then rinsed with 0.1 M phosphate buffer (pH 7.4), trimmed into small pieces and postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 1 h. The tissues were then dehydrated, cleared and embedded in Araldite. Semithin sections of 1.0 μm were cut using a Reichert Ultracut E microtome and stained with 1% methylene blue. They were viewed and photographed in a Leitz Aristoplan photomicroscope.

Quantitative study

Each of the age groups consisted of 3 rats. Under ether or chloral hydrate (3.5 or 7%) anaesthesia, the rats were perfused with Ringer's solution for a few minutes until the liver and lungs were clear of blood. They were then perfused with 10% neutral formalin for 15 min. After perfusion, the brain was removed and kept overnight at 4 °C, in the same fixative. The brain was then dehydrated in an ascending series of alcohol, cleared with toluene and embedded in paraffin wax. 7 μm thick coronal serial sections which included parts of the lateral ventricles and their choroid plexuses were cut and stained in haematoxylin and eosin (H & E). All the epiplexus, supraependymal and free-floating cells in both lateral ventricles were counted. The values, which represented the total count of each cell type, were corrected using Abercrombie's (1946) formula. The nuclear diameters of 60 randomly selected cells from the 3 cell types were measured in each age group. The mean diameters of the cell types of the respective age groups were then calculated.

Fluorescent labelling study

In the 4 age groups studied, the first group (1-d-old) consisted of 22 rats and the remaining 3 groups, 12 animals each. Under ether anaesthesia, each rat was given an i.p. injection of 5 μl of 1% RhIc in normal saline per gram body weight. For the 1-d-old rats, the animals were killed 15 min, 1, 3, 6 and 12 h and 1, 2, 6, 15, 30 and 60 d after the RhIc injection. In the older age groups, the animals were killed 1, 3, 6 and 12 h, 1 and 2 d after RhIc injection.

Under ether or chloral hydrate (3.5 or 7%) anaesthesia, the animals were perfused with 0.1 M phosphate buffer for a few minutes until the liver and lungs were clear of blood. This was then followed by 6% paraformaldehyde fixative in a similar buffer at pH 7.4 for 15 min. After the perfusion, the brain was removed and postfixed in a similar fixative containing 10% sucrose overnight at 4 °C. Frozen serial sections of 40 μm thickness were cut coronally at and caudal to the level of the optic chiasma. Sections which included the 2 lateral ventricles and containing choroid plexuses were mounted on gelatinised slides, air dried and coverslipped with a nonfluorescent medium, Entellan (Merck, D-61 Darmstadt, Germany). They were viewed and photographed in a photomicroscope equipped with a mercury lamp for fluorescence microscopy. A wide-band ultraviolet excitation filter was used.

Sections from longer surviving rats (i.e. 30 and 60 d) receiving RhIc injection at 1 d of age, were counterstained with cresyl fast violet after being viewed and photographed by fluorescence microscopy. Some sections from animals of different age groups

Fig. 2. A cluster of 4 epiplexus cells (arrow) with darkly stained nuclei on the choroid epithelium in a 1-d-old rat. H & E stain. × 540.

Figs 3, 4. Semithin sections of the choroid plexus of a 1-d-old rat. The connective tissue stroma of the choroid plexus contains blood vessels (bv) and fibroblasts (f). The epiplexus cells with vacuolated cytoplasm were either round (arrows, Fig. 3) or elongated (arrowhead, Fig. 4). X780 (Fig. 3) and X780 (Fig. 4).

Fig. 5. An epiplexus cell (arrow) which appears to be undergoing mitosis. 1-d-old rat. H & E stain. × 340.

Table. *Cell counts of epiplexus (EPC), supraependymal (SEC) and free-floating cells (FFC) in the lateral ventricles in rats of different ages after correction with Abercrombie's (1946) formula*

Age/cell type	Rat 1		Rat 2		Rat 3		
	Left	Right	Left	Right	Left	Right	Mean ± S.D.
1 day							
EPC	720	741	669	630	758	813	722 ± 65.1
SEC	359	344	413	459	352	322	375 ± 51.1
FFC	71	82	173	145	148	176	133 ± 45.3
17 days							
EPC	2084	2178	2256	2078	2505	2556	2276 ± 208
SEC	817	678	495	586	693	624	649 ± 109
FFC	397	477	567	574	346	455	469 ± 90.8
49 days							
EPC	2140	2044	2561	2473	1670	1803	2115 ± 354
SEC	1158	1079	772	817	782	1141	958 ± 186
FFC	1139	1092	736	694	2624*	1856*	1357 ± 748

All counts were in H & E sections. *See remarks in text.

killed 1 d after receiving RhIc injections were also treated in the same manner. This was to confirm the identification of the epiplexus cells.

OBSERVATIONS

General morphological features

In all the rats examined, the epiplexus cells were distributed widely over the free surface of the choroidal epithelium in H & E stained sections (Fig. 1). The majority of the cells occurred singly (Fig. 1), but occasionally in clusters (Fig. 2). In semithin sections, the epiplexus cells were characterised by their round or ovoid nuclei with coarse chromatin clumps (Fig. 3). The cell outline was either round (Fig. 3) or elongated (Fig. 4). In the former, the cytoplasm was often vacuolated (Fig. 3). Supraependymal cells were less frequently observed (Fig. 1). On the other hand, free-floating intraventricular cells were common in adult rats (Fig. 1); they usually occurred in clusters of a few cells, although single free-floating cells were also observed. A few occasional epiplexus cells in the newborn rats appeared to be undergoing mitosis (Fig. 5).

Quantitative study

The number of epiplexus, supraependymal and free-floating cells were comparable in both lateral ventricles of the same rat in the different age groups (see Table 1). The mean numbers of all 3 cell types increased considerably after birth (Fig. 6). By 17 d of age, the total number of epiplexus cells in each of the lateral ventricles had reached the adult level, i.e. ~ 2200 cells, which was about 3 times that of the newborn rats. The value remained relatively unchanged in the adults (49-d-old). Remarkably, in 1 of the adult rats (rat 3) the number of free-floating cells far exceeded that of the other 2 rats of the same age group (asterisks in Table).

The relative proportion of epiplexus, supraependymal and free-floating cells varied in the 3 different age groups (Fig. 7). Epiplexus cells made up more than 50% of the total population of the intraventricular macrophages in all ages. A remarkable change was the upsurge of the frequency of free-floating cells from 10% at birth to 30% in the adult.

Fluorescent labelling study

All rats turned pinkish within a few minutes after the injection of RhIc but remained physically active.

1-d-old rats. No labelling was observed in the epiplexus cells in this group of rats which were killed 15 min after RhIc injection. The choroid plexus in the lateral ventricles, however, emitted a weak fluorescence. At 1 h after the injection, the fluorescent intensity in the choroid plexus increased considerably (Fig. 8). At a higher magnification, the cytoplasm of the epithelial cells of the choroid plexus showed intense fluorescence. The labelling of the choroid epithelium, however, was not even, some areas being extremely bright and other areas only weakly fluorescent.

RhIc-labelled epiplexus cells emitting a weak fluorescence were first detected consistently over the surface of the choroid epithelium 3 h after the RhIc injection (Fig. 9). In animals killed 6 and 12 h after the

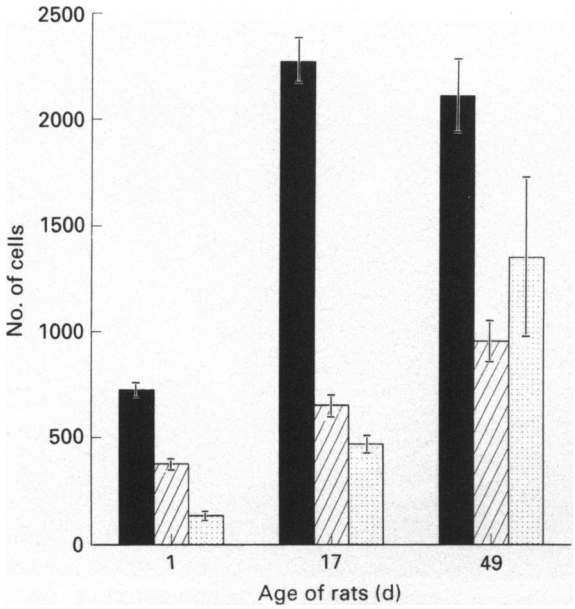

Fig. 6. Histogram showing the mean numbers of epiplexus, supraependymal and free-floating cells in the lateral ventricles in 1-, 17- and 49-d-old rats. Note the steady increase in the mean number of all 3 cell types after birth. ■, epiplexus cells; ▨, supraependymal cells; ⊡, free-floating cells.

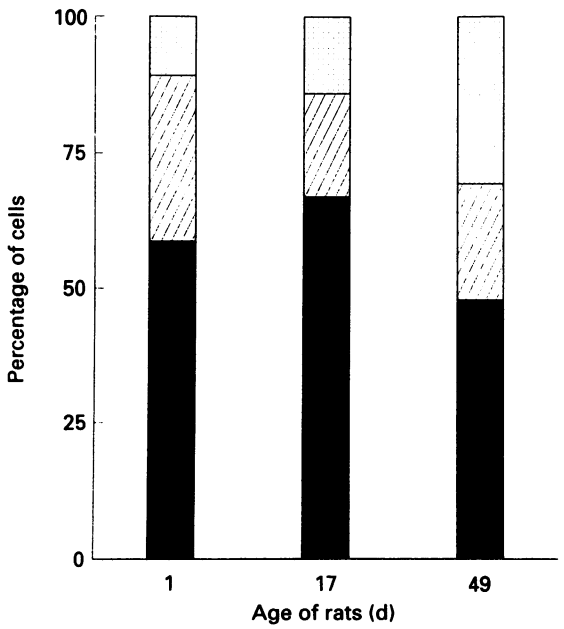

Fig. 7. Histogram showing the relative proportions of epiplexus, supraependymal and free-floating cells. These varied in the 3 different age groups. Epiplexus cells made up more than 50% of the total population of the intraventricular cells at all ages. ⊡, free-floating cells; ▨, supraependymal cells; ■, epiplexus cells.

RhIc injection, the number and fluorescence intensity of the labelled epiplexus cells appeared to increase progressively. In rats killed 1 d after the injection, the labelling of the epiplexus cells was extremely intense (Fig. 10). The labelled cells occurred either singly or in clusters of a few cells. In rats which were killed 15 d after the injection, a large number of labelled epiplexus cells with an intense fluorescence could still be observed (Fig. 11). However, 1 month after the injection, the fluorescence in the choroid plexus was confined to some small isolated particles scattered in the epithelial cells. There were no RhIc-labelled epiplexus cells. This was confirmed when the same sections were counterstained with cresyl fast violet (Figs 12, 13). In rats killed 2 months after the injection, the RhIc in the choroid plexus had diminished, although on higher magnification, some very fine fluorescent grains could be visualised in the epithelial cells (Fig. 14). RhIc-labelled epiplexus cells were absent.

13 and 17-d-old rats. In rats killed 1 h after the i.p. injection of RhIc, the choroid epithelial cells were clearly labelled with RhIc. On the other hand, RhIc-labelled epiplexus cells were not observed (Fig. 15). RhIc-labelled epiplexus cells were first seen 3 h after the administration of the fluorescent dye (Figs 16, 17). The RhIc-labelled epiplexus cells in the 17-d-old rats, however, exhibited a weaker fluorescence when compared with that of 13-d-old rats. The fluorescent intensity of the labelled cells increased with advancing time. Thus in rats killed 1 d after the RhIc injection, a large number of epiplexus cells were intensely labelled (Fig. 18).

49-d-old (adult) rats. RhIc-labelled epiplexus cells were not observed 6 h after the injection (Fig. 19). The earliest time interval at which the epiplexus cells were labelled was 12 h after the injection of RhIc, but the number of cells labelled was rather low (Fig. 20).

In the sections taken from rats of different age groups killed 1 d after RhIc injection and counterstained with cresyl fast violet, virtually all the epiplexus cells present were confirmed to be labelled.

Epiplexus cells associated with the choroid plexus displayed diverse morphological forms which were age related. In 1-d-old rats, most of the epiplexus cells were round (Fig. 10), with occasional ones that were oval or elongated. In 13- and 17-d-old rats, more ramified cells bearing a variable number of processes were observed (Figs 16, 18). In adult rats, the ramified epiplexus cells prevailed (Fig. 20).

DISCUSSION

The present study has provided a detailed quantitative analysis of the intraventricular macrophages in the lateral ventricles of newborn (1 d), developing (17 d) and adult (49 d) rats. It is evident that the actual number of all 3 cell types increased with the advance in age. The number of epiplexus cells showed a considerable increase after birth (3-fold within 17 d).

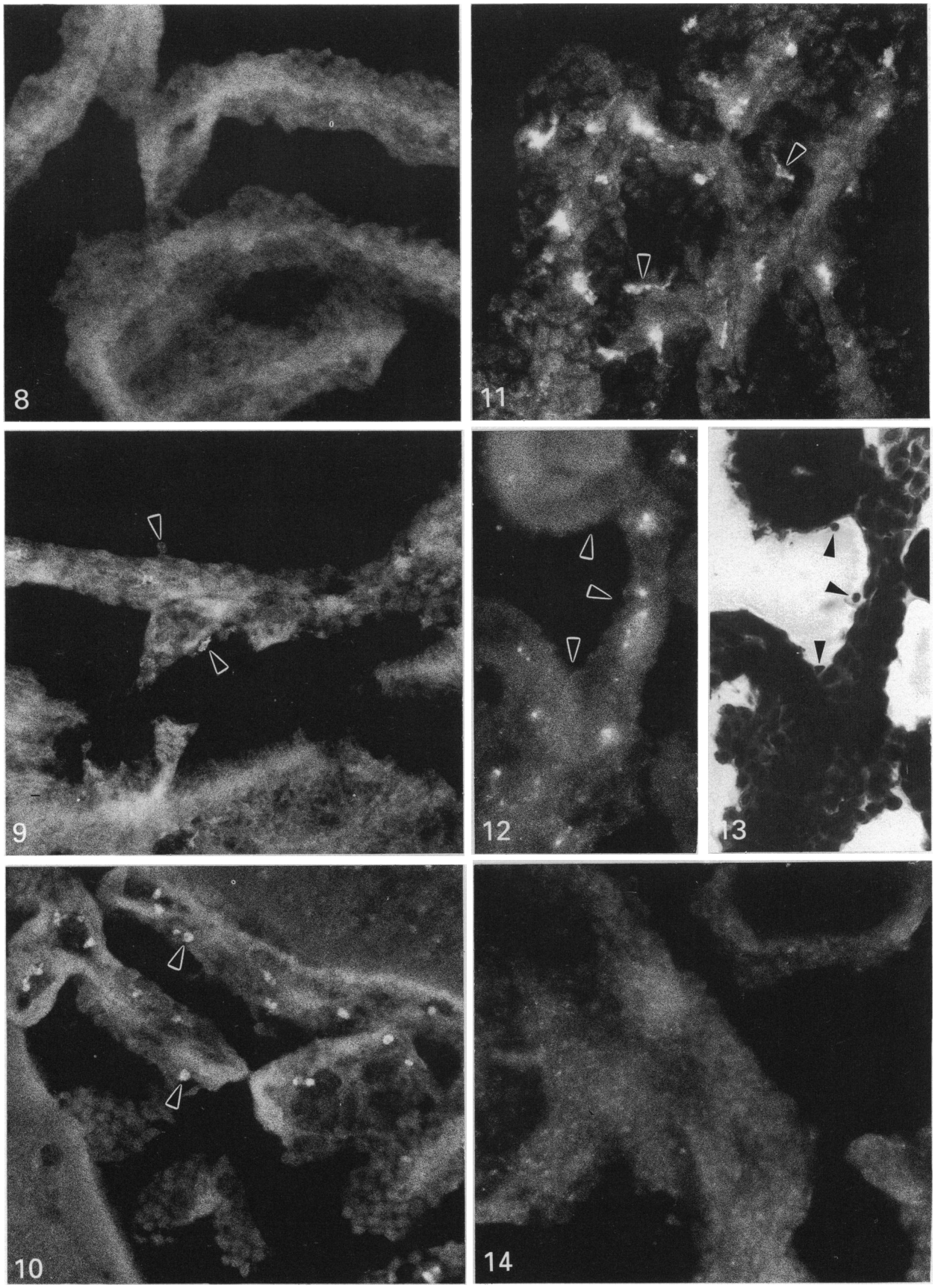

Fig. 8. Choroid plexus in the lateral ventricle of a 1-d-old rat 1 h after an i.p. injection of RhIc. The choroid plexus is clearly labelled with the fluorescent dye. Labelled epiplexus cells are absent. ×220.

A possible explanation for such a prominent increase is a proliferation of this cell type since some epiplexus cells appeared to be undergoing mitosis in H & E stained sections in the newborn rats. The number of epiplexus cells, however, remained relatively unchanged after 17 d of age through to adulthood. This finding differs from that of Sturrock (1978), who reported an initial increase in the mean number of epiplexus and supraependymal cells which reached a peak at 8 d postnatum; after that the numbers declined up to 25 d postnatum. The present study also showed that the mean number of free-floating cells increased from ~ 100 cells in early postnatal rats to more than 1000 cells in adult rats. Sturrock (1978), on the other hand, showed that the number of free-floating cells rapidly declined after birth. The discrepancy may be due to individual variation and to the different counting methods used.

The present study has also shown that there was little variation in the number of cells in newborn rats but that this was more variable in adult rats. Two possible explanations may be offered for this observation: (1) the development of the ventricular system may vary considerably from rat to rat; (2) CSF infection during the development of the choroid plexus could have induced the increase in the number of intraventricular cells which may serve as scavenger cells.

Previous studies had shown that epiplexus cells could pick up India ink (Ariëns-Kappers, 1953) and carbon particles (Ling, 1979) introduced intraventricularly or intravenously, indicating that they are active macrophages. The present study has confirmed the phagocytic nature of the epiplexus cells. This was demonstrated by the endocytosis of RhIc which had been administered i.p. It is noteworthy that the uptake of RhIc was independent of age, suggesting that they may serve as ventricular scavenger cells throughout life. The active phagocytic role of these cells may have clinical significance in the response to the presence of intraventricular microorganisms during intracranial infections (Bleier & Albrecht, 1980).

It is postulated from this study that the RhIc which had been injected i.p. entered the blood circulation to reach the endothelial lining of the blood vessels and subsequently into the connective tissue spaces in the choroid plexus. The extravasated RhIc would then be transported across the epithelial cells to be endocytosed by the phagocytic epiplexus cells residing on the ventricular surface of the epithelium. Supporting this view is the fact that the walls of the blood vessels in the choroid plexus are known to be fenestrated (Maxwell & Pearse, 1956; Van Deurs, 1980); this would facilitate a rapid transport of the RhIc in circulation. Furthermore, the fluorescent dye was concentrated in the cytoplasm of choroid epithelial cells soon after the injection of RhIc. The endocytosis of RhIc, most probably derived from the epithelial cells, by the epiplexus cells, thus indicates a close functional relationship between the 2 cell types.

The present study has shown that the largest number of epiplexus cells emitting a strong fluorescence occurred 1 d after the RhIc injection. In newborn (1 d) and developing (13 and 17 d) animals, epiplexus cells were first labelled 3 h after the injection. In adult (49-d) rats, the cells were first labelled 12 h after the injection. The cause of the delay in the labelling of epiplexus cells in the adult rats is uncertain. It is speculated that the fully developed blood vessels or choroid epithelium in the adult may impede a rapid passage of the injected tracer into the ventricular system before being taken up by the epiplexus cells. But since virtually all the epiplexus cells were consequently labelled independent of age difference, it may be suggested that the blood–CSF barrier is not efficient in the choroid plexus. This is compatible with the view of Hofer (1958) who stated that the blood–brain barrier is incomplete in the circumventricular organs.

The present study has confirmed the diverse

Fig. 9. Choroid plexus in the lateral ventricle of a 1-d-old rat 3 h after an i.p. injection of RhIc. Epiplexus cells (arrowheads) emitting a weak fluorescence are first detected over the surface of the choroid epithelium. × 220.

Fig. 10. Intensely labelled round epiplexus cells (arrowheads) associated with the choroid plexus in the lateral ventricle of a rat 1 d after an i.p. injection of RhIc at 1 d of age. Note that the fluorescence of the labelled epiplexus cells is greatly enhanced when compared with that at 3 h postinjection (cf. Fig. 9). × 220.

Fig. 11. Choroid plexus in the lateral ventricle of a rat 15 d after an i.p. injection of RhIc at 1 d. A large number of labelled epiplexus cells (arrowheads) emitting an intense fluorescence are present. × 270.

Fig. 12. Choroid plexus in the lateral ventricle of a rat 1 month after an i.p. injection of RhIc at 1 d. The choroid plexus emits an uneven weak fluorescence with small isolated fluorescent particles scattered in the epithelial cells. × 340.

Fig. 13. Same section as in Fig. 12 counterstained with cresyl fast violet. Epiplexus cells (arrowheads) are present but they are not labelled with RhIc when viewed under the fluorescence microscope (see Fig. 12 arrowheads). × 340.

Fig. 14. Choroid plexus in the lateral ventricle of a rat 2 months after an i.p. injection of RhIc at 1 d. The fluorescence in the choroid plexus is greatly reduced (compare with Fig. 12). Extremely fine fluorescent grains are discernible in the choroid epithelial cells. × 270.

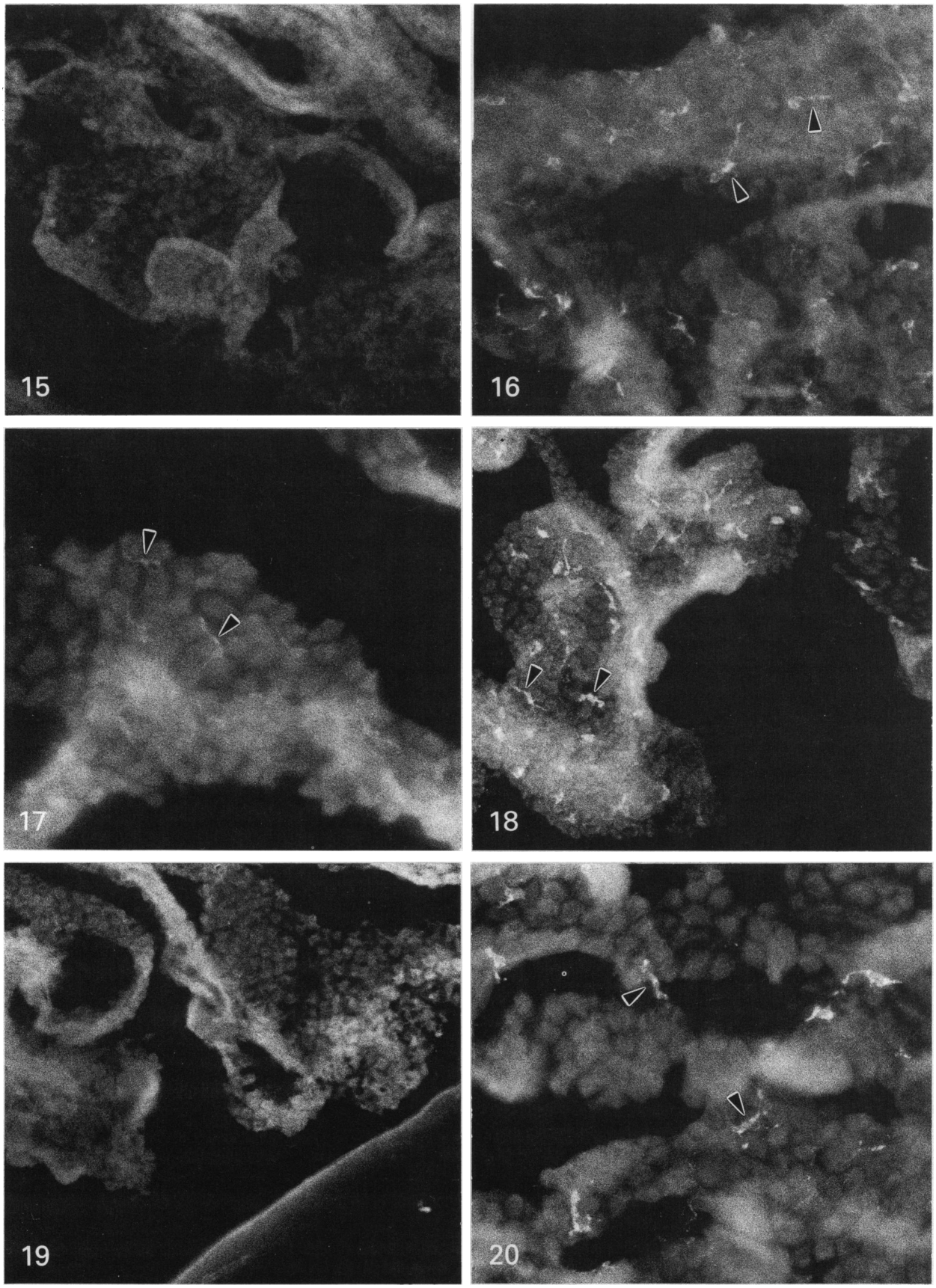

Fig. 15. Choroid plexus in the lateral ventricle of a 13-d-old rat 1 h after an i.p. injection of RhIc. The choroid plexus is unevenly labelled. Labelled epiplexus cells are absent. × 220.

morphological forms of epiplexus cells in the choroid plexus in rats. In 1-d-old rats, most of the epiplexus cells were round while in 13- and 17-d-old rats the cells became more ramified, bearing a variable number of processes. In adult rats, most of the cells were branched or ramified. It has been speculated that this diversity of form probably reflects different degrees of activation or states of maturation or stages in the development of these cells (Sturrock, 1979; Bleier & Albrecht, 1980). From the present study, it is surmised that the round cells represent an early developmental stage while the branched or ramified cells represent the mature form. In the latter, the cells may be more adaptable to their ambient environment since they may have to be exposed to a relatively greater flow rate of CSF. Under such circumstances long interdigitating processes would be advantageous in providing more extensive anchorage, resisting dislodgement of the cells (Mitchell, 1979). It is also possible that the long processes of epiplexus cells may be used as probes for detecting foreign substances which might have to be endocytosed (Hosoya & Fujita, 1973). In this respect the ramified cells would have a greater surface area for uptake of foreign materials (Mitchell, 1979). Supporting this view is the observation that leaked erythrocytes in the ventricles were sometimes seen trapped by the branched epiplexus cells during phagocytosis in 6-aminonicotinamide-treated rats (Ling, 1985).

The reason for the absence of fluorescent dye in the epiplexus cells 1 or 2 months after RhIc injection remains speculative. It is possible that the epiplexus cells which were labelled earlier had migrated to other sites or that the fluorescent dye taken up by the cells had been digested by the lysosomes known to be present in these cells (Ling, 1979).

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Fig. 16. A large number of labelled epiplexus cells bearing long processes (arrowheads) in a 13-d-old rat 3 h after an i.p. injection of RhIc. × 340.

Fig. 17. Choroid plexus in the lateral ventricle of a 17-d-old rat 3 h after an i.p. injection of RhIc. The labelled epiplexus cells exhibit a weaker fluorescence (arrowheads) when compared with that of a 13-d-old rat (cf. Fig. 16). × 340.

Fig. 18. Choroid plexus in the lateral ventricle of a 17-d-old rat 1 d after an i.p. injection of RhIc. Numerous epiplexus cells are intensely labelled (arrowheads). × 340.

Fig. 19. Choroid plexus in the lateral ventricle of an adult (49-d-old) rat 6 h after an i.p. injection of RhIc. Labelled epiplexus cells are absent. × 170.

Fig. 20. Choroid plexus in the lateral ventricle of an adult (49-d-old) rat 12 h after an i.p. injection of RhIc. The labelled cells appear elongated and ramified (arrowheads). × 340.

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