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The cells of Cajal–Retzius in the developing human brain

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In the molecular layer of the cerebral cortex of the developing mammalian brain a special type of cell occurs which has received little study in recent years. This type of cell was studied extensively by Cajal (1890, 1891, 1929) in small animals and by Retzius (1893, 1894) in the brains of human foetuses 6–8 months old. More recently Conel (1939, 1944, 1947, 1951), using cresyl violet stains and preparations by Cajal and Golgi-Cox methods, has followed the fate of these cells in the postnatal human infant brain. These observers and Veratti (1897) have adduced evidence for the neuronal nature of this cell. Thus it possesses a myelinated axon running a long horizontal course in the molecular layer and which sends short sprouts down among the cortical neurons of lamina II. Furthermore, it commonly shows a series of sprouts passing towards the meningeal layer with which each makes contact in prominent expansions. These latter fibres may sometimes actually penetrate the investing pial coat (Retzius). In size, these cells may sometimes be as big as Betz cells although usually they are smaller. Their shape has been variously described as fusiform, triangular, pyriform, or stellate.

They do not appear to survive into late infantile life. Conel (1951) noted their appearance in the sixth postnatal month, when they appeared to be degenerating. Cajal, although he actually refers to an 'adult' type as well as a foetal type, found them in human brains only up to the second postnatal month, when he noted loss of their ascending branches which make contact with the pia mater. Cajal even believed that their horizontal fibres might persist indefinitely, a view contested by Conel who, in keeping with the neuron theory, could not believe that fibres might persist in the absence of a cell body. Fox & Inman (1966) have reported the presence of the Cajal–Retzius cells in adult dog brain.

With the use of histochemical methods for demonstrating various enzymes, these cells are readily identified and their evolution can be followed. Cells with similar morphological and histochemical characteristics are situated on the external surface of the hypothalamic region. They appear to form a special class of neuron whose development and maturation proceeds in advance of those of the rest of the brain and which, as the latter matures, are superseded and lost.

MATERIALS AND METHODS

The cortex of thirty embryos and foetuses varying in age from 6 to 40 weeks and of eight infants ranging in age from full-term birth to 6 months was examined.

Methods, described in the text-books of Pearse (1960), Burstone (1962) and Barka & Anderson (1963), were used to demonstrate the sites of activities of the following

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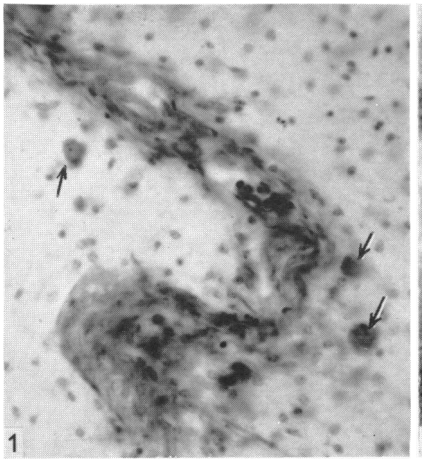
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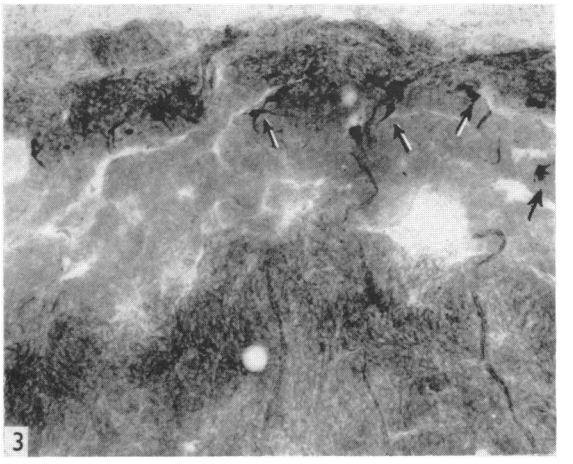
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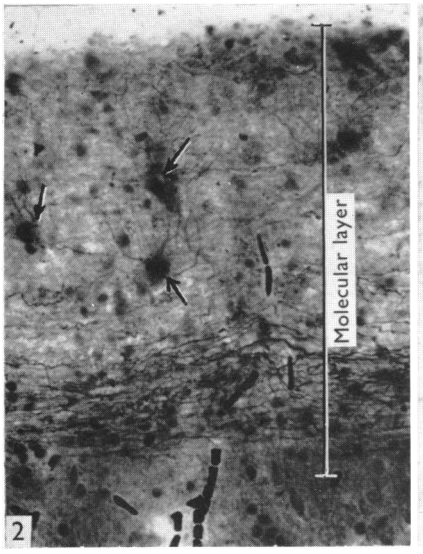
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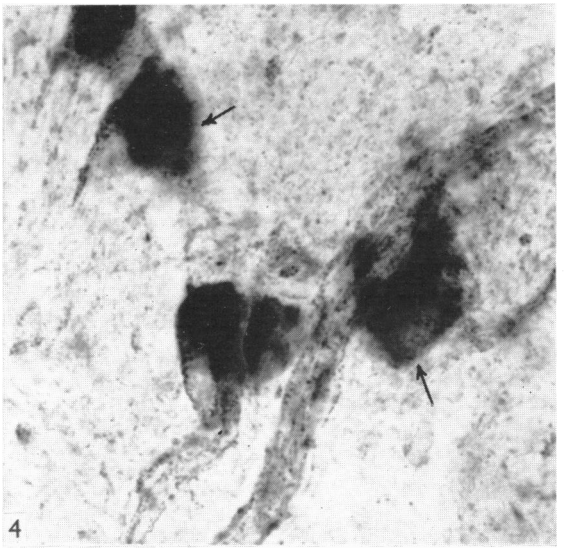
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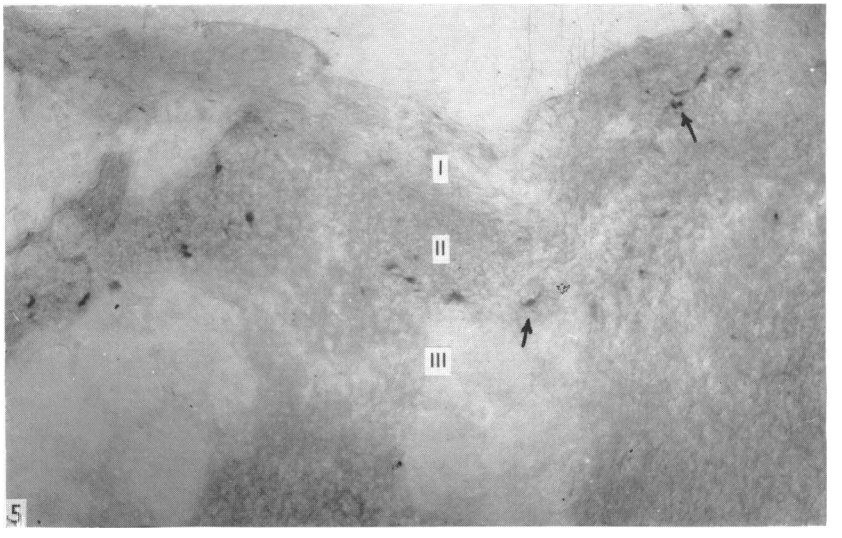
I
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enzymes: NADPH and NADH diaphorases; various dehydrogenases such as succinate, 3-hydroxybutyrate, glutamate, alphaglycerolphosphate and alcohol (with MTT, NBT, TNBT as hydrogen acceptors); various phosphatases such as acid phosphatase (Barka & Anderson pararosanilin method), alkaline (Pearse's modified azo dye), glucose-6 (Wachstein & Meisel), 5-nucleotidase (Wachstein & Meisel), ATPase (Wachstein & Meisel, Padykula & Herman); non-specific esterases (Holt & Whithers, Barka & Anderson); cholinesterase (Gerebtzoff modification of the Koelle method): monoamine oxidase (Glenner, Burtner & Brown). Standard methods of staining were haematoxylin-eosin, Nissl, PAS and Bielschowsky.

RESULTS

There is little to add to the morphological description of these cells by Cajal and Retzius except to mention the presence of a nucleus and nucleolus. These cells are identified by their presence in the molecular layer, their large size, dendritic attachment to the pia arachnoid and their horizontal fibres which are the first identifiable axones in the cerebral cortex (Fig. 1, 2).

The cytoplasm, dendrites and axones of the cells of Cajal–Retzius contain NADH and NADPH- diaphorases and succinate, glutamate and 3-hydroxybutyrate dehydrogenases, eserine-resistant and eserine-sensitive esterases and acid phosphatase (Figs. 3–5), but there is no detectable activity of alkaline phosphatase, ATPase, 5-nucleotidase and monoamine oxidase.

There are large isolated cells in the molecular layer and in the limiting meningeal membrane of His in the telencephalic vesicle of the 7-week-old embryo (25 mm C.R. length) which contain esterase which could presumably be precursors of the cells of Cajal–Retzius of their location, enzymic content and size.

The cells of Cajal–Retzius are identified morphologically and enzymically between the fourth month of foetal life until shortly after birth. Their size and appearance are always the same, and they are distributed in the molecular layer of the cerebral cortex in all lobes.

Cells similar in appearance, size and enzymic content to the cells of Cajal–Retzius also appear during the fourth month of foetal life on the external surface of the hypothalamus.

In the earlier weeks of their appearance the cells of Cajal–Retzius appear to be concentrated in the external part of the molecular layer, attached by dendrites to the pia-arachnoid. During the last 4 months of foetal life many cells lose this attachment

Fig. 1. The cells of Cajal–Retzius (see arrows) in the molecular layer of the cerebral cortex of an 18-week-old human foetus. Nissl stain. × 250.

Fig. 2. The cells of Cajal–Retzius (see arrows) in the molecular layer of the cortex of a 24-week-old human foetus, with their horizontal fibres in the deeper part of this layer. Bielschowsky method. × 250.

Fig. 3. NADH-diaphorase activity in the cells of Cajal–Retzius (see arrows). 24-week-old human foetus. × 100.

Fig. 4. Cells of Cajal–Retzius attached to blood vessels on the molecular layer of a 30-week-old human foetus. NADH-diaphorase. × 370.

Fig. 5. Cells of Cajal–Retzius (see arrows) present in lamina I, II and III of the cerebral cortex region of the insula, presumably migrating. NADPH-diaphorase. × 50.

and progress more deeply into the molecular layer adhering to blood vessels with their body or processes (Fig. 4). In the frontal, parietal and occipital lobes the cells of Cajal–Retzius are not identified in the cortex deeper than the region between the molecular and external granular layers. However, in the insula they are seen to penetrate deeply into the cortex, particularly during the latter months of foetal life (Fig. 5). In the gyrus temporalis inferior they cross the molecular layer to form or join the nodules of cells which constitute the so-called digitations of Retzius. In the hippocampus the cells of Cajal–Retzius eventually form or join the pyramidal layer.

DISCUSSION

The function of these cells is unknown, nor is there any plausible explanation for their presence during intra-uterine life and their atrophy and eventual disappearance shortly after birth. There is some evidence that they migrate deeper in the cortex in the insular region and in the parahippocampal gyrus and hippocampus, where they contribute cells to the digitations of Retzius and to the pyramidal layer in the gyrus dentatus. The cells ('diencephalic cells') seen near the surface of the subthalamic region could be the same as the cells of Cajal–Retzius because of their similar morphological and enzymical structure.

The presence of eserine-sensitive esterase in the cells of Cajal–Retzius and diencephalic cells suggests cholinesterase activity. This is also indicated by the use of the Gerebtzoff method which demonstrates the presence of activity of acetylcholinesterase in the cytoplasm of the cells of Cajal–Retzius. The presence of activity of the diaphorases and of succinate, glutamate and 3-hydroxybutyrate dehydrogenase indicates use of Krebs' cycle and links with protein and lipid metabolism as seen in adult neurones. This suggests the use of aerobic pathways for the energy requirements of these cells and of cholinesterase for neuronal function.

The enzymic composition of the cells of Cajal–Retzius and of the 'diencephalic cells' is as demonstrated with histochemical techniques similar to the chemical activity in adult neurones. The presence of these enzymes suggests that these cells are presumably some of the earliest functional neurones in the developing human brain.

The migration of these cells into the cavity appears to occur along blood vessels. Because of their size, it is tempting to think of the cells of Cajal–Retzius as possible precursors of the large pyramidal cells, such as the Betz cells, which are identified during the 18th week (150 mm C.R. length) (Boulton & Moyes, 1912), 1 month after the cells of Cajal–Retzius.

SUMMARY

The cells of Cajal–Retzius are neurones which appear in the molecular layer of the human cerebral cortex, during the fourth month of gestation and which begin to disappear at birth. These cells oxidize carbohydrates, proteins and lipids for their energy requirements and are the site of cholinesterase activity which indicates potential excitability. They thus appear to be the first recognizable neurones in the brain.

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