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THE INCREASE OF THE NUCLEAR LOBULATION OF THE PINEALOCYTES IN AGING MICE: ELECTRON-MICROSCOPIC KARYOMETRIC STUDY

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SUMMARY

The age-dependent changes of the nuclei of the mice pinealocytes were studied elecron microscopically. The morphometrical results showed that 1) the perimeter of the pinealocyte nuclei increased during 5 to 8 months of age (3 to 5 & 8 months; $P<0.05$), then gradually decreased to 24 months with advancing age (5 & 8 to 24 months; $P<0.05$), and 2) the mean number of the nuclear segments per cell increased according to age ($r=0.793$, $N=22$, $P<0.0001$). These findings may reflect the age-associated changes in the cytokinetic and metabolic activity of the pinealocyte nuclei.

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Key words: pineal organ, aging, karyometry, mouse

INTRODUCTION

The pineal organ and the suprachiasmatic nucleus seem to be involved in the organization and coordination of circadian systems in vertebrates[12], which synchronize with daily changes in light and darkness associated with rotation of the earth. Although the physiological significance of the human pineal gland still remains enigmatic, human pineals also appear to play a role in the neuroendocrine function affected by environmental stimuli. In the context of the imaginary very long-term interplanetary travel, which advances years, changes in pineal function would undoubtedly occur in animals, and also in humans, where light stimuli of course become out of physiological LD-phase on the earth.

During the course of the electron-microscopic study of the age-associated changes of the mouse pineal gland[6], we noticed the increased nuclear lobulations, or foldings, of the pinealocytes in senility. If this change is ascertained quantitatively, this would suggest that a common mechanism exists between the nuclear segmentation during maturation process of the polymorphonuclear leukocytes, which are highly differentiated and cannot reproduce by mitosis, and the increase of the nuclear lobulation of the pinealocytes with progressing age, although the time-scale of each phenomenon is quite different.

Peculiar nuclear structures of the pineal parenchymal cells called 'Kernkugeln[1]' have

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been variously interpreted. These findings were thought in the past to indicate a secretory activity with some physiological significance[3]. By electron microscopy, the nuclear membrane of the pinealocytes was shown to have many foldings and to date the nuclear pellets of the pinealocyte are recognized as an artefact in light-microscopic sections due to obliquely sectioned cytoplasmic invaginations into the nuclei[9)12)]. However, the functional significance of these findings and the correlation with aging still remains to be investigated.

In the context of environmental biology and aging, the investigation was designed to estimate the age-related morphological changes in nuclear shape of mice pinealocytes using three rather simple factors consisting of perimeter, area and number of segments.

MATERIALS AND METHODS

A total of 22 male C57BL/6Crj mice was used in this study, which were kept in a windowless room under strictly controlled conditions (12 h light/12 h dark, at 55±5% humidity and 22±2℃, food and water given *ad libitum*). They were anesthetized with ether and decapitated at 11 : 00 on October 29. The pineal glands were fixed by immersion in a 2% paraformaldehyde-0.5% glutaraldehyde. Both anesthesia and fixation were always performed in the same way. After embedding in a single Poly/Bed 812 block, thin sections from the peripheral region ("cortex") of the pineals were stained with uranyl acetate and lead citrate and examined using Nihondenshi JEOL-100B and Hitachi H-600 electron microscope. After selecting the sampling area with the greatest ratio of parenchyma/perivascular space, the whole field of the one grid square of No. 200 mesh was scanned and photographed at low magnification (2, 100 to 3,300×), followed by a calibration by grating. The montages of the grid-image made from about 25 sheets of printing paper was then traced on the tracing paper (Dupont Cronaflex*) to make a line drawing. The morphometry was done by KONTRON MOP-DIGIPLAN (sensitivity ; 0.1 mm). In each gland, 30-50 pinealocytes containing the nuclear components in the cytoplasm, including light and dark pinealocytes, were measured regarding the perimeter, the area and the number of lobulation of each nucleus. Analysis about the darker and smaller interstitial cells (glial cells) is excluded in this report. Fig. 1 shows the schematic drawing of the nucleus of the aged mouse pinealocyte with the definition of the nuclear lobulation used in this study. The mean of these variables represented the indices of each mouse. Data were recorded in the 32 bit minicomputer (VAX11/750) using interactive database DATATRIEVE[2)]. SPSS-X release 2.2 (VAX/VMS V4.4) was used for statistical analysis[11)] ; one way analysis of variance followed by a least-significant difference

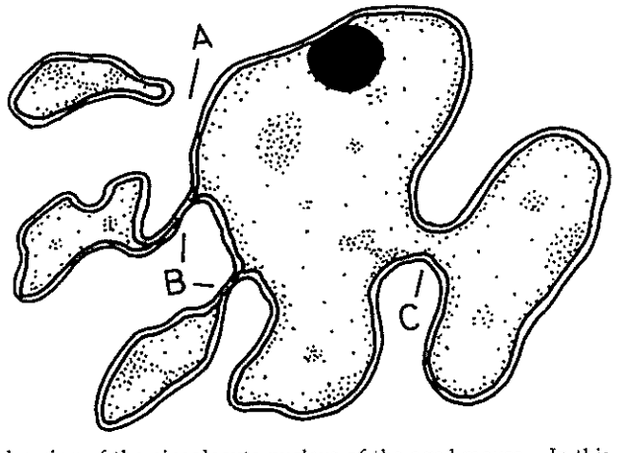

Fig. 1 Schematic drawing of the pinealocyte nucleus of the aged mouse. In this study A and B were conveniently defined as a lobulation and C was not calculated as so. In this case the number of the lobes is 4.

method (LSD) and a Student-Newman-Kreuls method (SNK), Pearson's correlation test and linear regression test.

RESULTS

The pineal gland of the mice is a parenchymatous organ chiefly consisting of the pinealocytes in all the age groups. Although some authors regard light and dark pinealocytes as separate cell types, the present authors have now the impression that they represent immersion-fixation artefact and/or different stages of one cell type and did not analyse them separately. The shape of the pinealocyte nuclei becomes more complex due to increase of the foldings of the nuclear membrane electron microscopically with advancing age (Fig. 2), however, even at 24 months of age neither karyorrhexis nor nuclear pyknosis is seen and euchromatin still predominates in the pinealocyte nuclei. In thick sections (1 μm) stained with toluidine blue, by light microscopy, the nuclei of pinealocytes are more convoluted and invaginated with increasing age, compared with more vesicular appearance in 5 to 8 months.

The chronological change of the mean perimeter of the pinealocyte nucleus by electron-microscopy is shown in Fig. 3. The statistical difference is found between each age group by ANOVA (degree of freedom=5, $F=3.94$, $P<0.02$) and the variable at age 5 & 8 months is significantly larger than at age 3, 18 and 24 months by LSD method ($P<0.05$) and the variable at age 5 months compared to age 24 months by SNK method ($P<0.05$). The mean area of the pinealocyte nucleus at age 5 months is also significantly larger than that at age 24 months

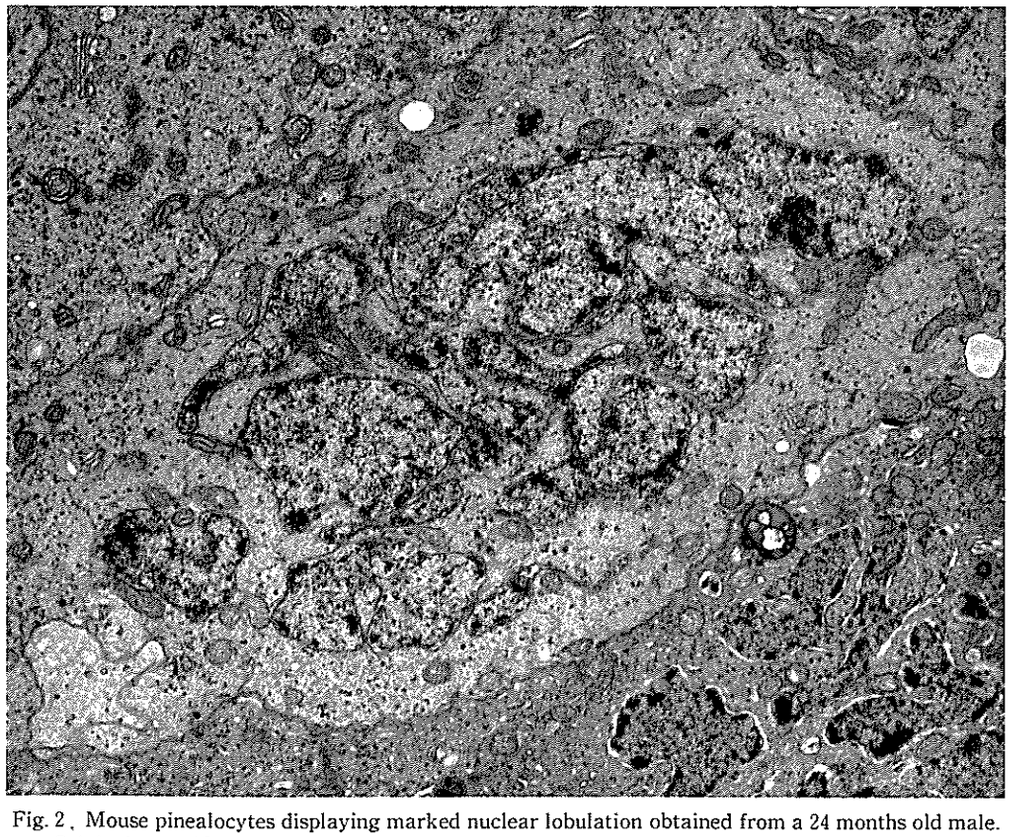

Fig. 2 . Mouse pinealocytes displaying marked nuclear lobulation obtained from a 24 months old male. Original magnification ×12,800

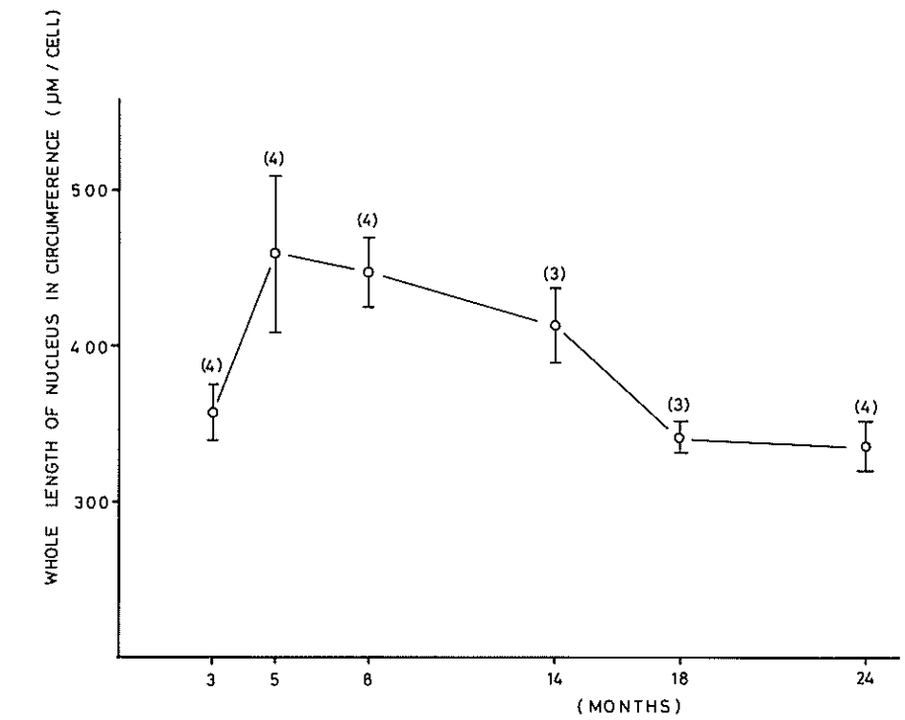

Fig. 3 Age-related change in the mean perimeter of the mice pinealocyte nuclei. Mean±SE ( ); number of animals

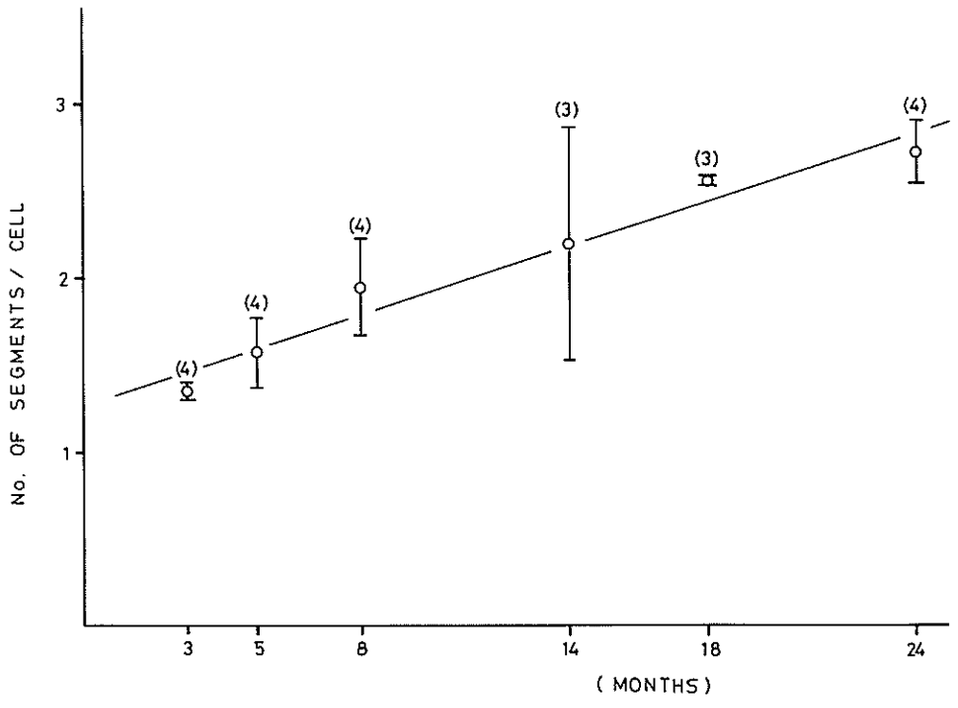

Fig. 4 Linear increase in number of the nuclear segments of the mice pinealocytes according to age. Mean±SE ( ); number of animals

($P<0.05$). Fig. 4 shows the linear increase in number of the nuclear lobulations, as difined in Fig. 1, according to age ($r=0.793$, $N=22$, $P<0.0001$). The regression line is $Y=0.065X+1.27$. The difference is also significant by ANOVA ($df=5$, $F=6.16$, $P<0.005$) and the number of segments at age 18 & 24 months is significantly larger than that at age 3 & 5 months by LSD and SNK test ($P<0.05$).

DISCUSSION

The present study confirmed age-related morphological changes of the mice pinealocyte nuclei hitherto not shown by electron-microscopic quantitative data. The perimeter and area of the nuclei increased during young adult and adult life (5 to 14 months), which probably reflected the hypertrophy of the pineal parenchymal cells ('functionelle Kernschwellung', see Gedigk and Totović 1984 ; Izawa 1925). The number of lobes of the nuclei increased linearly from birth to old age, although it is difficult to interpret the finding and this issue cannot be resolved now.

The postnatal cytogenetic activity of the pineal parenchymal cells is generally believed to continue to decrease. Quay and Levine[10] showed that postnatal mitotic activity in the rat pineals drops from a neonatal maximum to a low or trace levels by two weeks. Wallace et al[13] indicated by autoradiographic study that the postnatal development of the pineal is due to cellular hyperplasia in the young animal and hypertrophy in the adult. These and the present results seem to provide the evidence consistently supporting the hypertrophy of the pinealocyte nuclei in the adult superimposed on the progressive baseline decrease in cytogenetic activity.

Even in advanced senility neither nuclear pyknosis, karyorrhexis, nor increase of heterochromatin was observed electron microscopically. Meanwhile the number of nuclear segmentation of pinealocytes increased linearly. Johnson[8] also described increase of pinealocytes with nuclear invaginations and with nuclear inclusions in rat with advancing age. It must be too hasty to hypothesize that there exists some common basis of cellular development and functional maturation between polymorphonuclear leukocytes and pinealocytes, however this finding strongly indicates the highly differentiated and sophisticated status of the pineal parenchymal cells. It is also worthy of note that some domesticated inbred strains of mice (C57BL/6J) are reported to have neither detectable melatonin, nor NAT activity in their pineal glands at any time of day or night due to a genetic defect[4]. Although in this study no mitotic figure was found in total of 1,037 pinealocytes, the possibility still remains to be elucidated that the mammalian pinealocytes are in the facultative proliferative state. Our electron-microscopic analysis on the age-associated changes of the pinealocyte nuclei showed one of the unquestionably unique aspects of the pineal parenchymal cells and more detailed study with DNA analysis is expected to be in progress to investigate the cytogenetic mechanism of the mouse and other mammalian pinealocytes.

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