Original

**Fibrous Calcareous Concretions in the Capsules
of Human Pineal Glands**

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Abstract : We examined calcareous concretions in the capsule of 15 human pineal glands containing a large amount of the concretions in the matrix. The capsule concretions, brown to blackish-brown in color and/or translucent, were smaller than the matrix concretions, which were yellowish-white. The matrix concretions showed the typical mulberry-shaped overall appearance with scallop-shaped concentric laminations of non-fibrous structures, but the capsule concretions, which also contained Ca and P, showed a globular or oval shape with regular, rough, or no concentric laminations. The capsule concretions also showed globules of mineralized collagen fibers that resembled randomly wound threads and that were occasionally associated with minute spherical deposits showing hypermineralization in the core region as initial calcification. Na was detected as a minor characteristic element of the capsule concretions, and at a significantly higher level than that of the matrix concretions. The findings strongly suggested that a human pineal gland containing numerous matrix concretions is likely to produce mineralized deposits in the capsule.

Key words : human pineal capsule, fibrous concretions, minute spherical deposits, backscattered electron imaging, energy-dispersive X-ray microanalysis

Introduction

Non-fibrous calcareous concretions in the pineal matrix of some mammals¹⁻³⁾ and humans²⁻¹¹⁾ are formed by a fine lamination of alternating organic-rich and mineral-rich materials^{1, 11)}. These matrix concretions, known also as psammoma bodies or brain sands, show a mulberry-shaped overall appearance^{4, 6, 10)} with the characteristic scallop-shaped laminations^{1, 3, 8, 10, 11)}. Many of the matrix concretions may initially start at the calcified pinealocytes or other cells^{1, 3, 8, 11)}.

The characteristic structure of calcareous concretions was also observed in the connective tissue capsule of the pineal gland in aging rats by transmission electron microscopy¹²⁾. However, such structures or their components remain to be identified in humans, although the presence of concretions in the pineal capsule was reportedly observed by optical microscopy^{5, 8)}.

Previously¹³⁾, we reported pineal calcareous concretions and prostatic calculi in 40 deceased

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men ranging in age from 31 to 95 years. The coincident presence of these masses did not correlate with aging, but there tended to be a low negative correlation between their mineralized volumes assessed by microradiography. Human pineal glands containing a large number of matrix concretions also occasionally contain encapsulated masses⁸⁾, although these are smaller than the matrix concretions^{5, 8)}.

This study examined the capsule of adult human pineal glands¹³⁾ containing numerous matrix concretions by backscattered electron (BSE) imaging and energy-dispersive X-ray (EDX) microanalysis using a scanning electron microscope (SEM), and then by decalcified sections stained with van-Gieson, after observing the whole pineal glands by microradiography¹³⁾.

Materials and Methods

Human pineal glands were extracted from 40 deceased adult men (aged 51 to 85 years), who had agreed for their bodies to be employed for medical research, during the routine dissection course conducted from 1996 to 1998 at Showa University School of Dentistry¹³⁾. The tissues were, fixed in 10% neutral formalin and then stored in 70% ethanol. Radiographs showing the lateral view of the pineal glands were taken with a soft X-ray device (Sofron, Tokyo, Japan)¹³⁾, and then 15 pineal glands containing a large amount of calcareous concretions in the matrix body were used.

Seven pineal glands were embedded in polyester resin after dehydration in ethanol. The samples were cut sagittally into several slices with a diamond wheel and then ground with grinding stones. Contact microradiographs of approximately 200- μ m-thick slices were obtained, and the ground surfaces were polished with 5- and 0.3- μ m alumina on polishing cloths, and then ultrasonically cleaned. The sections were observed by transmitted-light microscopy (LM).

The ground surfaces were also observed by composite BSE imaging with an SEM (S-2500CX; Hitachi, Tokyo, Japan) operated at 20 keV after carbon coating. Masses showing a high BSE signal in the pineal capsule and matrix regions were analyzed qualitatively and quantitatively by SEM equipped with an EDX detection system (delta-4; Kevex Instruments, Valencia, CA, USA), at an accelerating voltage of 15 keV, a specimen irradiation current of 1×10^{-7} mA, and a beam spot of 15 nm. The central and peripheral regions in each mass showing high BSE signals were counted at 35 points (pineal regions) or 10 points (matrix regions) and their means ($\pm$ S.D.), were obtained.

Four samples out of the remaining resin-free pineal glands were sagittally cut into several slices with a diamond knife, dehydrated with ethanol, and then dried at the critical point of CO₂. Conventional secondary scanning electron (SE) micrographs and composite BSE images of the cut surfaces of the ground pineal glands or the cut surfaces of pineal concretions themselves were photographed with an SEM operated at 15 ~ 20 keV after carbon coating. The areas possessing higher molecular-weight elements in biological materials show higher BSE signals in the uneven planes of natural surfaces¹⁴⁾ and fractured surfaces¹⁵⁾, as well as the ground smooth planes^{8, 11)}.

The remaining four samples were embedded in paraffin and 8- μ m-thick sections were

Fig. 1. Ground sections of human pineal glands embedded in resin. (a) Contact microradiograph, (b) ordinary light, and (c-g) BSE images. Arrows in (a) and (b) show the same matrix concretion. V, blood vessel. PC, pineal capsule. PM, pineal matrix. C, central or core region of a capsule or matrix concretion. S, minute spherical deposits (MSD) showing high BSE signal. (a, b) Bars, 1 mm, (c, d) Bars, 100 μ m.

prepared. The sections were stained with van-Gieson for the identification of collagen fibers, and were observed by LM.

Results

The ground sections of human pineal gland embedded in resin (Fig. 1a~d) showed aggregations of radiopaque deposits in the pineal periphery as well as in the matrix body by contact microradiography (Fig. 1a). Figure 1b shows the LM photograph of part of Figure 1a. The calcareous concretions were apparent in the pineal capsule containing blood vessels as well as in the matrix. The matrix concretions were a yellowish-white color and usually showed a mulberry-like overall appearance by LM, whereas the center or whole area of the capsule concretions was occasionally brown to blackish-brown, and translucent in parts; these masses were basically circular in appearance and contained few or no concentric rings (Fig. 1b). They were also relatively smaller than the matrix concretions with a maximum diameter of up to 120 μ m.

Fig. 1 (c, d) shows the composite BSE images of the capsule and matrix concretions in the ground surfaces of the resin-embedded pineal glands. The matrix concretions had scallop-

Fig. 2. BSE images of capsule and matrix concretions in the cut surfaces of resin-free pineal glands. PC, pineal capsule. PM, pineal matrix. The arrow indicates fibrous structures with relatively high BSE signals. S, minute spherical deposits (MSD) showing high BSE signals. (a) Bar, 1 mm, (b, c) Bars, 100 μ m, (d) Bar 10 μ m.

shaped concentric laminations (Fig. 1c), whereas the capsule concretions showed several different structural patterns even between neighboring concretions (Fig. 1d). Of the three capsule concretions visible in Fig. 1d, one large high-BSE-signal concretion showed almost regular concentric laminations around the core region, whereas the other lower-BSE-signal concretion showed roughly concentric laminations containing linear structures around the loose core region. The remaining smaller concretion had an oval-shaped outline and contained minute spherical deposits (MSD) showing higher BSE signals.

Fig. 2 (a ~ c) shows the BSE images of the cut surfaces of the resin-free pineal glands. The capsule concretions were clearly distinguishable from the matrix concretions in position and size (Fig. 2a). The matrix concretions usually showed a mulberry-shaped overall appearance in the three-dimensional image (Fig. 2b), whereas many of the capsule concretions were covered with fibrous structures showing high and low BSE signals. The overall appearance was a more or less globular structure that looked like randomly wound threads (Fig. 2c, d). Some of the initial mineralizing capsule concretions contained MSD that emitted high-BSE signals (Fig. 2c).

Fig. 3 (a ~ d) shows several capsule concretions that were initially mineralized at higher magnifications on the cut surfaces of a pineal capsule. Comparison of the overall appearance of a globular capsule concretion by SEM imaging (Fig. 3a) and composite BSE imaging of

Fig. 3. SE micrographs (a, c) and BSE images (b, d) of initially mineralizing capsule concretions in the cut surfaces of resin-free pineal glands. (a, b) and (c, d) show the same regions, respectively. The arrow indicates fibrous structures showing relatively high BSE signals. S, minute spherical deposits (MSD) showing high BSE signals. (a-d) Bars, 10 μm .

Fig. 4. Capsule concretions in the cut surfaces of resin-free pineal glands by BSE imaging. Arrows indicate fibrous structures showing high BSE signals. C, core region of a capsule concretion. (a, b) Bars, 10 μm .

the same field (Fig. 3b) showed the fibrous structures as randomly wound threads on the BSE image, whereas the same structures were undistinguishable in the SE micrograph. When the cut surfaces of globular capsule concretions were similarly compared, the fibrous structures and MSD showing high BSE signals could be also distinguished in an SE micrograph of the same field (Fig. 3c, d).

Fig. 4 (a, b) represents the cut surfaces of three larger capsule concretions showing high

Fig. 5. LM photographs of the decalcified sections of pineal capsules stained with van-Gieson. S, minute spherical deposits (MSD) in the capsule concretions. (a, b) Bars, 50 μm .

BSE signals. The two oval-shaped concretions showed concentric laminations containing various linear structures around the core region showing low and higher BSE signals, respectively. The remaining globular capsule concretion comprised somewhat amorphous deposits containing linear structures in the core region. The peripheries of these three capsule concretions were surrounded with fibrous structures showing a high BSE signal.

Fig. 5 (a, b) shows the decalcified sections stained with van-Gieson for identification of collagen fibers. Globular concretions in the pineal capsules were concentrically surrounded with collagen fibers, in an arrangement that resembled randomly wound threads, with MSD detected in the central region of some capsule concretions.

Under SEM-EDX microanalysis, Ca and P as major elements and Na as a minor element were detected in the capsule calcareous concretions and mineralized blood vessels showing high or lower BSE signals on the resin-embedded ground samples. As trace elements, Mg, S, Cl, K, Al, and Si were detected. The concentrations of Ca, P, and Na, and the Ca/P molar ratio in the mineralized capsule deposits and matrix calcareous concretions were also then quantified (Table 1). These concretions ranged from 60 to 120 μm in diameter based on BSE imaging. Under Student's *t*-test, the capsule concretions contained significantly lower P content than the matrix concretions in both the central ($P < 0.01$) and peripheral regions ($P < 0.05$), although the capsule concretions contained higher Na content ($P < 0.001$).

Discussion

In this study, the capsule concretions appeared mineralized in a contact microradiograph by LM⁸⁾ and the size was usually smaller than that of the matrix concretions^{3, 8)}, but the color has not been denoted. The color difference between these pineal concretions may be based on their rough or dense compositions of organic and mineral components. That is, the concretions showing a brown to blackish-brown color might be rich in organic components and the

Table 1. Concentrations of Ca, P, and Na (weight %), and the Ca / P (molar ratio) in the capsule concretions ($n=35$) and the matrix concretions with high BSE signals ($n=10$) in human pineal gland. The concretions ranged between 60 and 120 μm in diameter. SEM-EDX microanalysis was conducted in the center and periphery of the concretions.

Capsule concretion	Center	Periphery
Ca	27.29 ± 3.50	26.32 ± 2.88
P	12.75 ± 1.63^a	12.64 ± 1.40^b
Na	1.98 ± 0.69^c	1.96 ± 0.07^c
Ca / P	1.64 ± 0.09	1.62 ± 0.04
Matrix concretion	Center	Periphery
Ca	29.68 ± 1.52	28.57 ± 1.87
P	14.22 ± 0.33^c	14.27 ± 0.43^d
Na	0.11 ± 0.02^f	0.11 ± 0.03^f
Ca / P	1.61 ± 0.09^g	1.55 ± 0.04^h

Mean $\pm$ S.D.

Ca, P, and Na : % by weight ; Ca / P : molar ratio

a vs. c, $P < 0.01$; b vs. d, $P < 0.05$;

e vs. f, $P < 0.001$; g vs. h, $P < 0.05$

translucent-rich concretions might be more mineralized.

The pineal capsule concretions showing high to lower BSE signals were calcified with Ca and P as were the pineal matrix concretions in adult humans^{4-6, 9, 10)} and aging rats³⁾, and the capsule concretions in aging rats¹²⁾. From the Ca / P molar ratios measured in this study (Table 1), the deposits showing high BSE signals may contain hydroxyapatite, as reported previously for matrix concretions^{1, 3, 7)}.

Our comparison of concretions revealed a significantly higher P content in the matrix concretions compared to the capsule concretions in both the central and peripheral regions, although there was no significant difference in Ca content. Thus, the capsule concretions tended to show a lower calcification than matrix concretions of a similar size.

The Na content in human matrix concretions has been reported as 1.1 $\sim$ 3.6%⁶⁾, 0.41%⁷⁾, and approximately 0.5%⁹⁾ by weight. In this SEM-EDX microanalysis, the Na content in the matrix concretions was approximately 0.11% by weight, which is significantly lower than the 1.75 $\sim$ 1.98% Na content in the capsule concretions (Table 1). However, data reported for matrix concretions by Galliani *et al*⁶⁾ showed a higher Na content compared with previous studies^{6, 9)} and our present data. According to Driessens¹⁶⁾, Na ions are usually present in the intercellular and cerebrospinal fluids and in plasma (140 $\mu\text{mol/l}$). Therefore, some Na ions in the capsule concretions might be contained in Na- and CO₃-containing apatite¹⁷⁾. However, further investigations will be necessary to define the presence and nature of Na in the calcified deposits of human pineal capsule.

The minute spherical deposits (MSD) showing hypermineralization in this study were occasionally observed in the core region of the capsule concretions. These deposits were similar to the hypermineralized minute globular deposits (MGD) scattered within previously reported mesenteric stone-like masses¹⁷⁾, which were mainly composed of variously calcified collagen fibers and amorphous calcium phosphate deposits containing apatite and Mg-containing whitlockite¹⁷⁾, and were proposed to be formed from amorphous calcium phosphate containing Mg¹⁷⁾. The hypermineralized MSD observed here might therefore be formed from calcium phosphate containing Na at a lower crystallization state.

The capsule concretions in aging rats¹²⁾ and several psammoma bodies in humans¹⁸⁻²⁰⁾ consisted of calcified collagen fibrils^{12, 18-21)} that would be expected to be followed by cell fragments or matrix vesicles showing an initial calcification¹⁷⁻¹⁹⁾. The capsule concretions showed various amounts of fibrous globular structures and may indeed reflect the calcification of cell remnants or matrix vesicles^{17, 19-21)}. Further, some such cell remnants might become the hypermineralized MSD.

Humbert *et al*¹²⁾ examined capsule concretions in aging rats by transmission electron microscopy and divided the concretions into two types: a multilayered concretion surrounded by numerous collagen fibrils and an extracellular concretion with incorporated collagen fibrils. Their observations¹²⁾ basically agreed with our present findings, although the rat capsule concretions were very small in size and irregular in shape. Based on previous studies^{12, 18-21)} and our van-Gieson staining, the fibers of the capsule concretions showing a randomly wound thread-like structure were identified as collagen fibers.

Previously¹⁵⁾, we reported a tendency for a low negative correlation between the masses of studied pineal glands containing a large amount of calcareous concretions from adult human males with low numbers of prostatic calculi. Therefore, in a human pineal gland that is likely to be mineralized, the mineralization would be expected to occur in the connective tissue capsule^{12, 18-21)} as well as in the matrix^{17, 19, 21)}. A larger amount of mineralized deposits in a pineal gland might be caused by longer hours of natural daylight and also by electric light, judging from the animal experimentations reported previously²²⁻²⁴⁾.

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Conflict of interest

The authors have declared no conflict of interest.

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