ACTA ZOOL. CRACOV. | 30 | 5 | 53—70 | KRAKÓW, 30. IV. 1987

Konstantin E. Mikhailov

The principal structure of the avian egg-shell: data of SEM studies

[with Plates VIII—XVI and 2 text-figs]

Zasadnicza struktura skorupy jaja ptasiego: wyniki badań na skaningowym mikroskopie elektronowym

Abstract. The egg-shell structure of 5 species of *Ratitae* and 13 species of *Neognathae* was studied using SEM. The results confirm the value of considering the regularities of egg shell formation from the point of biomineralization. The growth of crystals in the egg-shells is induced and controlled by the organic matrices, which act as templates. The vertical macro--units of egg-shell growth (cone + column) are of polycrystalline structure and represent the system of hierarchically co-ordinated blocks of various size levels. Crystallites of all levels possess a "sheath", composed of organic sheets, which if chemically destroyed, can result in the consecutive decomposition of prisms and wedges into structural elements. The complex arrangement of egg shell growth units is caused by biologically predetermined polysynthetic twinning of submicroscopic plate crystallites. The results confirm Erben's (1970) data on the distinctions in the structure of *Ratitae* and *Neognathae* egg-shells. Some peculiar characteristics in the egg--shells of *Passeriformes* were found.

CONTENTS

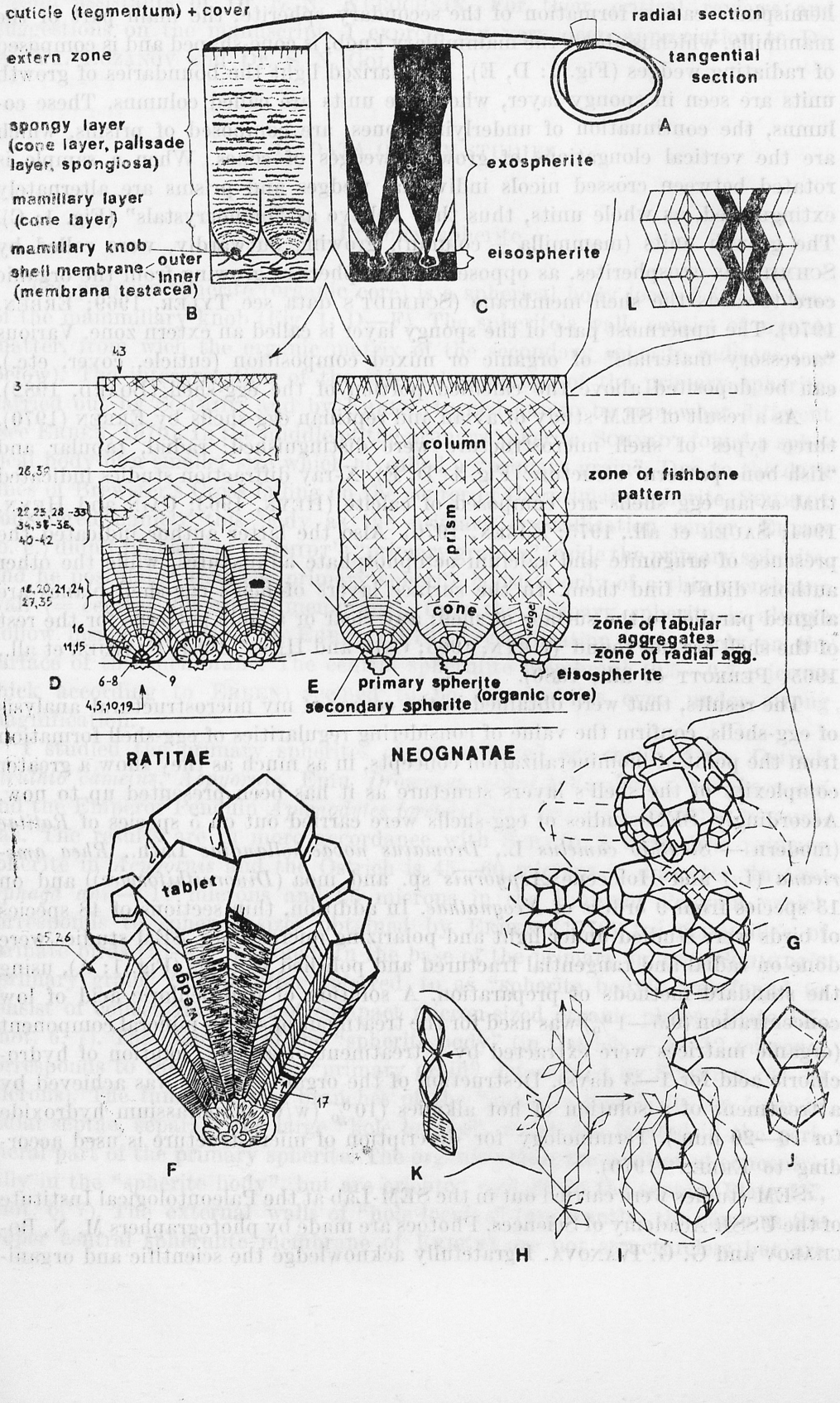
I. INTRODUCTION

Biomineralization study has revealed common laws for very different groups of animals and plants. The organic matrix concept includes the principles of nucleation and crystal growth control by organic matrices, which results in

the formation of various types of biocrystalline structures, specific for different groups of animals and plants (WILBUR, 1980; BEVELANDER, NAKAHARA, 1980; BARSKOV, 1982; KRAMPITZ, 1982; GOLUBEV, 1981, 1983). In all cases which have been studied, calcium carbonate in the form of calcite and/or aragonite is accumulated in organic tissues of animals and of some algae groups as polycrystalline aggregates (WATABE, 1974, 1981; WATABE and DUNKELBERGER, 1979; CRENSHAW, 1982). It is natural, therefore, to expect that the same situation is true for egg-shells as well (ERBEN, 1970). However, up to now (POOLEY, 1979) there are still some reflections of traditional views which limit the identification of egg-shell formation and crystal growth to the inorganic system (the organic matrix is considered as a passively included component). Ideas of this kind, historically based on studies using polarized light, have up to now limited, to some extent, the systematic and ecological interpretation of egg-shell microstructure.

An avian egg-shell is composed of large crystalline macro-units growing vertically outwards from the surface of the outer shell membrane. In radial cross-sections being examined under a light non-polarizing microscope, the growth units show their individuality only in their lower part (mammillae), but above the mammillary layer they are seen as single layer — spongy layer (Fig. 1, B; terminology synonymy from TYLER, 1969). The base of the growth unit is a round capsule, called a mammillary knob, which can be distinctly seen from the inner surface of the egg-shell after removing the shell membrane (Plate IX, Phot. 4, 5). In the center of the mammillary knob there is an organic core (primary spherite). Radial crystal growth shape of an outwardly expanding

Fig. 1: A — scheme of egg with two sections of shell. B—C — Radial section of avian egg-shell and terminology (B — under non-polarized light microscope; C — in polarized light). D—E — Scheme of *Ratitae* and *Neognathae* egg-shell microstructure on radial section according to SEM-data (after ERBEN, 1970, with changes); figures correspond to the numbers of photographs. F — Three-dimensional picture of mammilla structure (mammillary knob + cone); primary spherite, surrounded by radial crystallites, is inside mammillary knob (below); within the wedge, cords (double arrow) of subrhombic plates are built up to form polygonal tablets, viewed tangentially; the figures correspond to the photos number. G—K — Scheme of "real" structure of prism G — Structural elements of different sizes (in form of polyhedral and subglobular blocks) hierarchically co-ordinated in volume of prism; H—I — Principal scheme of a complex three-dimensional twinning of the subrhombic plates (H), which result in formation of blocks of the next sizes levels (I); screw (spiral)-like patterns can be seen in structure of elements of all levels (G—I); axis of "screw symmetry" is parallel to shell surface (vertical arrow); J — Way of realization of "pentagonals" on the bases of subrhombic plates (factual correlations are three-dimensional); K — Subrhombic plates are composite crystals and consist of subunits (below); "ridges" of plates are elongate crystallites (dark lines, above), which grow along the organic fibres contouring the lanceolate sheets (= templates of subrhombic plates; see the text); arrow indicates kink-point of contacting fibers; the marked angle possibly corresponds to angle of irrational twinning of subrhombic plates. L — More simple way of subrhombic plates twinning in prisms of extern zone of egg-shell (*Aepyornis*); twinning elements are painted; factual correlations are three dimensional, i. e. planes, which cover large faces of plates, are orientated in three different directions analogical to cross-lammellae structure


cuticle (tegmentum) + cover
extern zone
spongy layer (cone layer, palisade layer, spongiosa)
mamillary layer (cone layer)
mamillary knob
shell membrane outer inner (membrana testacea)
radial section
tangential section
A
exospherite
eisospherite
B
C
L
43
3
26,39
22,25,28-33 34,35-38, 40-42
18,20,21,24 27,35
16
11,15
6-8
9
4,5,10,19
D
column
prism
cone
vesicle
zone of fishbone pattern
zone of tabular aggregates
zone of radial agg.
eisospherite
primary spherite (organic core)
secondary spherite
E
RATITAE
NEOGNATAE
tablet
vesicle
17
F
G
K
H
I
J

hemisphere causes formation of the secondary spherite; the main part of the mammilla, which is above the mammillary knob, is cone-shaped and is composed of radiating wedges (Fig. 1: D, E). In polarized light the boundaries of growth units are seen in spongy layer, where the units are called columns. These columns, the continuation of underlying cones, are composed of prisms, which are the vertical elongations of growing wedges of cones. When a sample is rotated between crossed nicols individual wedges and prisms are alternately extinguished, as whole units, thus they behave as "monocrystals" (Fig. 1; C). The growth units (mammilla + column), growing outwardly, were called by SCHMIDT as exospherites, as opposed to eisospherites growing from the organic core towards the shell membrane (SCHMIDT'S data see TYLER, 1969; ERBEN, 1970). The uppermost part of the spongy layer is called an extern zone. Various "accessory materials" of organic or mixed composition (cuticle, cover, etc.), can be deposited above the calcified portion of the egg-shell (BOARD, 1982).

As a result of SEM-study of avian and reptilian egg-shells by ERBEN (1970), three types of shell microstructure were distinguished: radial, tabular and "fish-bone pattern" structure (Fig. 1: D, E). X-ray diffraction studies indicated that avian egg shells are composed of calcite (HEYN, 1963; CAIN and HEYN, 1964; SAUER et all., 1975; ERBEN, 1970). Also the latter author indicated the presence of aragonite and calcium octophosphate admixtures, while the other authors didn't find them. In the surface layers of hen's egg-shell planes are aligned parallel to the surface of shell; any clear or simple patterns for the rest of the shell are not found (HEYN, 1963; CAIN and HEYN, 1964; FAVEJEE et all., 1965; PERROTT et all., 1980).

The results, that were obtained in the course of my microstructural analysis of egg-shells, confirm the value of considering regularities of egg-shell formation from the point of biomineralization concepts, in as much as they show a greater complexity of the shell's layers structure as it has been presented up to now. Accordingly, SEM-studies of egg-shells were carried out on 5 species of *Ratitae* (modern — *Struthio camelus* L., *Dromaius novae-hollandiae* Lath., *Rhea americana* (L.) and Holocene *Aepyornis* sp. and moa (*Dinornithiformes*)) and on 13 species from 9 orders of *Neognathae*. In addition, thin sections of 48 species of birds were studied under light and polarizing microscopes. SEM-studies were done on radial and tangential fractured and polished sections (Fig. 1: A), using the standard methods of preparation. A solution of hydrochloric acid of low concentration (0.5—1%) was used for the treatment of shell's mineral component (organic matrices were extracted by a treatment of a 0.1% solution of hydrochloric acid for 1—3 days). Destruction of the organic matter was achieved by a treatment of a solution of hot alkalies (10% (w/v) of potassium hydroxide for 10—20 min.). Terminology for description of microstructure is used according to ERBEN (1970).

SEM-studies were carried out in the SEM-Lab at the Paleontological Institute of the USSR Academy of Sciences. Photoes are made by photographers M. N. BOCHAROV and G. G. IVANOVA. I gratefully acknowledge the scientific and organi-

zation assistants of Dr. E. N. KUROCHKIN. For their critical reviews and suggestions on the manuscript, I express my very deep appreciation to Dr. A. Yu. ROZANOV and Dr. S. N. GOLUBEV.

II. DATA OF SEM-STUDIES

A. Primary spherite

The primary spherite (organic core) is a spherical body located in the base of the mammillary knob (Fig. 1: D—F). The spherite's walls consist of organic matter, from wich the organic matrix of the secondary spherite radiates (see below). The interpretation of the internal structure of the primary spherite, carried out by SCHMIDT and by ERBEN, turned out to be somewhat different (see ERBEN, 1970). In the middle of the primary spherite, SCHMIDT found a spherical body of a smaller size which he called a "primary grain". Due to the dark lines of Brewster's Cross falling on the center of the primary spherite, SCHMIDT considered namely this body as the primary crystallization center. ERBEN (o. c.) didn't find any of SCHMIDT'S "primary graines" inside the primary spherite, and he postulated that the primary spherite consists only of a thin membrane wall (= central spherulite membrane), thus the primary spherite is always hollow inside. According to ERBEN (1970), crystallization begins only on the surface of this membrane. The central spherulite membrane (0.5—0.7 microns thick according to ERBEN) seemed to be structureless even under strong magnification.

I studied the primary spherites of unincubated egg-shells of the Ostrich *Struthio camelus*, *Aepyornis*, Emu *Dromaius novae-hollandiae*, *Crotophaga ani* and the Emperor Penguin *Aptenodytes forsteri* CRAY based on prepared mammillae. The results are in more accordance with SCHMIDT'S data. The primary spherite in *Aepyornis* and the Ostrich is 45—60 microns in diameter. In *Crotophaga ani* — 11 microns and 25 microns in the Emperor Penguin, which corresponds to spherite sizes obtained by ERBEN (1970) for mammillae of carinate birds (13—27 microns). In the base of the primary spherite SCHMIDT'S "primary grain" (herein after referred to as "spherite body") was found to consist of concentric layers of compact micron-sized organic plates (Plate IX, Phot. 6, 7). The diameter of the "spherite body" (in Ostrich — 8—12 microns) corresponds to the size of the "primary grain" determined by SCHMIDT (8—10 microns). The funnel-shaped branches of the "spherite body", in the form of radial septae, separate the large "hole locules", which are located in the peripheral part of the primary spherite. The organic plates are positioned concentrically in the "spherite body", but are oriented radially in the septae (Plate IX, Phot. 6, 7). The external walls of "hole locules" (apparently, the same as the proper central spherulite membrane of ERBEN) are not structureless but are

composed of narrow organic plates, arranged concentrically (Plate IX, Photo 6). Contrary to ERBEN (1970), the radiating crystallites grow not only on the external walls of the primary spherite, but also inside the radial septae, which separate the "hole loculus" (never inside "hole locules"; Plate IX, Phot. 7, 8). This explains the presence of Brewster's Cross in the center of the primary spherite as observed by SCHMIDT while studing thin sections of egg-shells in polarized light. The "hole locules" in the peripheral part of the primary spherite were observed by me in non-incubated egg-shells. Everything points to the fact that the "locules" are originally hollow and that their origin is connected with the process of spherite formation, and not the result of calcium carbonate resorption by the embryo during incubation, as was considered by ERBEN (1970).

The divergence of my and ERBEN'S (1970) data can probably be explained by the fact that in contrast to my study using non-incubated eggs, ERBEN used the shells of incubated eggs. But in the incubated eggs the "spherite body", which is tightly connected with the shell membrane, may peel away, together with the shell membrane from the egg-shell, or may decompose in the natural way during the incubation period. The primary spherites of the ratite birds that I studied had no accessory concentric rows of small "hole locules" (described by ERBEN for egg-shells of *Anser anser* and *Pavo cristatus*), adjoining to the outside of the central spherulite membrane (external spherite wall). It is possible that these small "locules" really occur as a result of calcium resorption by the embryo from the mineralized parts of a mammillary knobs, which are closest to the primary spherite. It has been indicated by previous authors that where the shell membrane contacts the primary spherite, fibers of the former penetrate the matter of the latter (TEREPKA, 1963; SIMONS, WIERTZ, 1963; BELLAIRS, BOYDE, 1969). Filaments, which compose the fiber core, are connected directly which the filaments of the organic matter of the spherite (WYBURN et all., 1973). The submicroscopic rhombic crystals grow along the contact line of the shell membrane fibers and the organic matter of the spherite (WYBURN et all., 1973). On some primary spherites preparations, I observed a concave body (as seen from the inner surface of egg-shell looking outwardly) underlying the "spherite body" and composed of numerous layers of organic plates (Plate IX, Phot. 9).

B. Eisospherite

The traditional view that the eisospherite is equivalent to the secondary spherite (basal part of exospherite) as an outwardly and inwardly growing from organic core semispheres, in my opinion, is only partly correct. Contrast to the secondary spherite composed of the radially orientated crystallites, the eisospherite contains aggregates of plates with their flat faces orientated mostly transversely to the radial axes from the organic core. These aggregates are formed

between the fibers of the outer shell membrane and are based directly on organic plates of the "spherite body" and/or its underlying concave body. These eisospherite plates often form irregularly-shaped structures (Fig. 2: A, B).

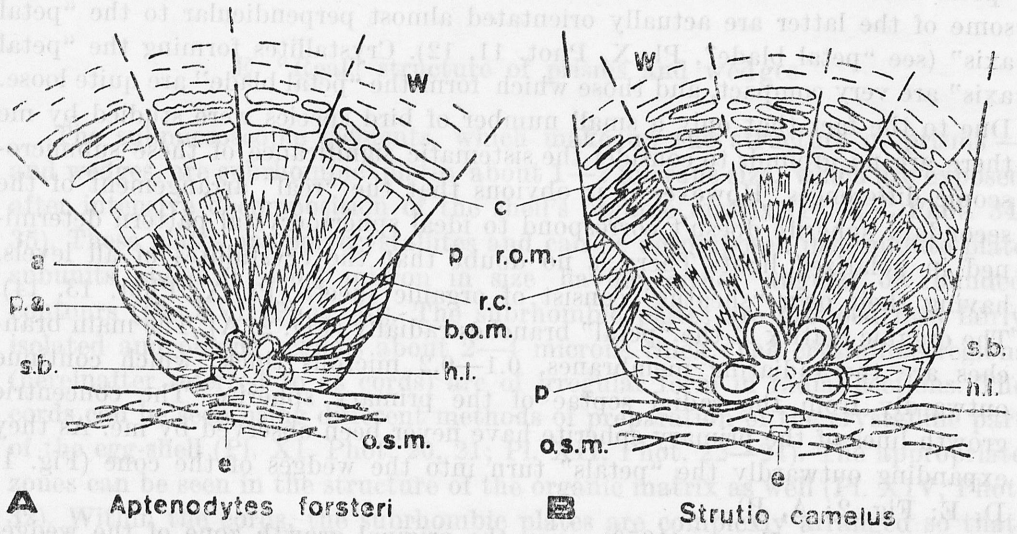


Fig. 2. Mammillary knob (primary and secondary spherites) in the *Neognathae* (A) and in the *Ratitae* (B). Indexes: o.s.m. — outer shell membrane (membrana testacea), s.b. — "spherite body", h. l. — "hole locules", e — plates of eisospherite, p. a. — petal-like aggregates ("petals"), b. o. m. — branching organic matrix of the "petal", r. c. — radiating spicular and prismatic crystallites, a — "petal axis", p — large plates consiting of "fused" radial crystallites, r.o.m. — large radiating organic membranes ("sheath" of "petals"), w — wedges (initial stage of growth). c — crystalline cords in the wedges (tabular structure)

C. Secondary spherite. Zone of radial aggregates, or radial structure

The results of my studies on the secondary spherite surrounding the primary spherite (organic core) confirm the data obtained by ERBEN (1970). The secondary spherite consists of large, petal-like crystallites (hereinafter referred to as "petals") radiating from the primary spherite (Pl. IX, Phot. 4; Pl. X, Phot. 10, 11). These "petals" became pointed proximally and enlarged distally. They correspond to bundles of spicular-prismatic and spicular-tabular lamellae covered by a single organic "sheath", which was described by ERBEN (lamellae are 1.5—3 microns wide; Pl. X, Phot. 11, 14, 15). I consider these "petals" to be discrete units in order to stress their morphological isolation under small magnification. Viewed from the underside of the mammillary knob, the "petals" are seen to make up a typical corolla (Pl. IX, Phot. 4, 5; Pl. X, Phot. 10). Spicular-prismatic and spicular-tabular lamellae consist of smaller spicular crystallites, 0.3—0.5 microns thick, which are aligned with the long axis of the lamellae (Pl. IX, Phot. 8; Pl. X, Phot. 14, 15).

Lamellae in Ostrich and Emu shell are radially oriented and only slightly deviate from the long "petal" axis in its distal part (Pl. X, Phot. 14). In contrast, in Emperor Penguin shell only some of the lamellae are orientated along the "petal axis" and the some — obliquely to it. In the distal part of the "petal", some of the latter are actually orientated almost perpendicular to the "petal axis" (see "petal blade", Pl. X, Phot. 11, 12). Crystallites forming the "petal axis" are very compact, and those which form the "petal blade" are quite loose. Due to the fact that only a small number of bird species were studied by me there are no grounds to consider the sistematic significance of these submicroscopic differences. However it is obvious that the "real" arrangement of the secondary spherite doesn't correspond to ideal spherrocrystal pattern determined in polarized light. There is no doubt that the crystallites of all levels, having their own "sheaths" consist of organic sheets (Pl. X, Phot. 13, 14). The organic matrix of the "petal" branches radially (Fig. 2: A). The main branches are the radiating membranes, 0.1—0.2 microns thick, which continue outwardly from the radial septae of the primary spherite. The concentric growth lines of the primary spherite have never been observed by me. As they expanding outwardly the "petals" turn into the wedges of the cone (Fig. 1: D, E; Fig. 2: A, B).

According to ERBEN (1970), even the original growth zone of the wedges is made up of modified elements of the radial structure, i. e. of radially orientated plate aggregates which contain "fused" spicular-prismatic and spicular-tabular crystallites (Pl. XI, Phot. 16). This zone, being well developed in the egg-shells of many the *Neognathae* (Fig. 2: A; ERBEN, 1970, Fig. 2) wasn't determined by me in egg-shells of the *Ratitae* (Fig. 2: B), in which the "fusing" of radial elements into the larger plate aggregates was observed only in the distal part of laterally radiating "petals".

D. The tabular structure in cones and the "fish-bone pattern" structure in the spongy layer

Transverse striations can be distinctly observed in the radial sections and fractures of the cones and indicate the tabular structure (Pl. XI, Phot. 18,20, 21). The vertical boundaries of prisms are well marked in the spongy layer of the *Neognathae* and very poorly expressed in the *Ratitae*. The oblique cleavage lines either cross several prisms or are orientated in the opposite directions in the adjacent prisms that identify the "fish-bone pattern" structure (Fig. 1: D, E; Pl. VIII, Phot. 1; Pl. XIV, Phot. 29, 29a). Moreover, the structure of prisms is observed as a fine-foliated pattern, composed of numerous parallely orientated lamellary units and subunits, which are the boundaries of the "fish-bone pattern" lines. It's noteworthy that the patterns of the oblique lines observed on the broken radial surfaces of egg-shells, and which indicate the "fish bone pattern" structure, occur as a result of cleavage of the "real" biocrystalline structure

of the prisms along the cleavage planes of calcite composite crystalls. The "fish-bone patterns" don't exhaust all peculiarities of the biogenetic structure (see below).

E. "Real" structure of prisms and wedges

The submicroscopic elements, which make up the structure of prisms — and wedges, are subrhombic plates about 1—2 microns size, which are exposed after intensive decomposition of the shell's organic matrix (Pl. XV, Phot. 34, 35). These plates are polycrystallites and can be decomposed into minute plate subunits about 0.1—0.3 micron in size having either angular or rounded contours (Pl. XVI, Phot. 40). The subrhombic plates are grouped into fairly isolated and oblong regions about 2—4 microns thick. The crystalline regions (hereinafter referred to as cords) are of irregular form in radial sections. The cords can be seen using different methods of preparation of the crystalline part of the egg-shell (Pl. XI, Phot. 20, 21; Pl. XII, Phot. 22—24). The appropriate zones can be seen in the structure of the organic matrix as well (Pl. XIV, Phot. 32). Within the cords, the subrhombic plates are complexly arranged so that, during decomposition of the organic matrix of the cords, the aggregates of plates can be observed in the form of either geometrically proportional polyhedral blocks or subglobular blocks, of equal size (3—5 microns) (Pl. XV, Phot. 36—38; Pl. XVI, Phot. 41, 42). The independence of these blocks is thus relative. The cords are located inside of the wedges in the plane always parallel to the plane of wedge accretion (Pl. XI, Phot. 21). In tangential view the cords are build up to form polygonal tablets (Pl. XI, Phot. 17) which correspond to the accretion lines of wedges when seen in radial view (Pl. XI, Phot. 20). The wedges have their own organic "sheath" (Pl. XIII, Phot. 27), which is composed of "intercrystalline matrix" of the accreting tablets (Pl. XIII, Phot. 25). Judging from the photoes of the forming egg-shell (Creger et all., 1976; Fujii, 1974) the tablets are pentagonals, in which one of the angles falls on the center of a cone and the sides run in the radial directions indicating the boundaries of the wedges when seen in radial view (Fig. 1: F).

The prisms possess both horizontal and oblique (in inter-opposite directions) cords, sometimes forming a cris-cross patterns; accretion lines are interrupted in prisms and are not clear (Pl. XII, Phot. 22). The subrhombic plates of different cords (on the next larger structural level — polyhedral and subglobular blocks about 3—5 microns in size) are built up to form the larger polyhedral and subglobular blocks, which are of discrete sizes: 8—10 microns, 15 microns and 30 microns; the largest ones correspond to the width of prism (Pl. VIII, Phot. 36, 38, 39; Pl. XVI, Phot. 41; Fig. 1: G). Note that larger blocks (8—10 microns and over) were distinguished by me only in the egg-shells of the *Ratitae*.

The analysis of numerous photoes makes it certain that crystallographic axes of the subrhombic plates are orientated complexly within the volume of the

prism. Some elements of screw (spiral) symmetry can be seen in the arrangement of crystallites, which are of about the same size (Pl. XV, Phot. 34—36, 37). The arrangement of the subrhombic plates shows their twinning (Pl. XII, Phot. 23; Pl. XVI, Phot. 40) as proposed by HEYN (1963). Some faces of the polyhedral blocks are regular pentagonals (Pl. XV, Phot. 37, Pl. XVI, Phot. 41). This fact is of interest, because in crystallography there is a strict theorem, according to which, a monocrystal can not be pentagonally symmetrical. But the pentagonal symmetry of the biogenetic crystals is caused namely by twinning, i. e. pentagonal symmetry is realized in the polycrystalline aggregates (GOLUBEV, 1981, 1983). It is necessary to note that a biologically pre-determined polysynthetic twinning is characteristic of all skeletons, and is the unique mechanism in the formation of various structural types (MUTVEI, 1978, 1979; GOLUBEV, 1981, 1983). The specific way of twinning is different in the wedges, and in the prisms of the spongy layer and those of the extern zone, thus causing the different structural types (Fig. 1: H—L). Crystals twinning in egg-shells is also biologically pre-determinated, as illustrated by the comparison of photos 31, 32 (Pl. XIV) with photo 40 (Pl. XVI). The subrhombic plates consist of elementary crystals (Phot. 40), which, probably, grow on the network of interlaced organic matrix filaments, which compose fibers and sheets (see SIMONS and WIERTZ, 1963). The "ridges" of the plates are elongated crystallites which grow up along the organic fibers, which are about 0.1 microns thick (Pl. XIV, Phot. 31). These fibers contour the lanceolate-shaped sheets (templates of the subrhombic plates, Phot. 32), which compose the "intercrystalline matrix" of the cord. Running outwardly (crossing horizontal cords) fibers interlace to form spiral-like figures; spiral's step correspond to one lanceolate membrane (Pl. XIV, Phot. 31, 32). Interlacement of fibers results in appearing elements of screw symmetry in crystalline structure. The point of contact of interlacing fibers (= point of contact of two lanceolate membranes) marks the appearing so-called irrational twinning (see KLASSEN-NEKLUDOVA, 1960) of subrhombic plates, which contour the pentagonal faces (Fig. 1: K—J).

As an ideal case, we can consider the wedges and prisms as composite "monocrystals" (as can be seen in polarized light) the spaced lattice of which includes the system of the screw (spiral) symmetry axes. The simple diffraction methodics cannot show such a complicated crystalline arrangement. However it should be emphasized that the "real" structure of prisms and wedges when considered as composite "monocrystals", contains plenty of accessory features. Some holes must exist between the subglobular and polyhedral blocks. These holes are probably filled in by the accessory elements (see arched-shaped plates on the periphery of subglobular blocks — Pl. XV, Phot. 36). Moreover, the crystalline lattice of prisms and wedges includes the streamlined network of organic membranes of different thickness and orientation, which separate different blocks. However, it is necessary to note that these membranes are not separate lamellae but only boundary zones of the "intracrystalline matrix" of appropriate blocks where mineralization doesn't occur (Pl. XIII, Phot.

26, 28). Also, the proper defects of the crystalline lattice seem to be extremely essential.

Geometrically regular angular faces (rhomb, pentagon) and three-dimensional polyhedrons, both of which characterize the shell's crystalline structure, always build up to form rounded contours and subglobular blocks respectively. Such a phenomenon is characteristic of biogenetic crystals and is realized due to the conjugation of accessory elements with polycrystalline rhombic plates by twinning in different crystallografic planes (GOLUBEV, 1983).

F. Extern zone

The structure of the shell's extern tone is very diverse both in the *Neognathae* (ERBEN, 1970; BOARD, 1982) and the *Ratitae* (my unpublished data) and will be discussed in a separate paper.

III. DISCUSSION

Egg-shell mineralization is a typical matrix process, i.e. the process in which the inorganic crystalline structure of the biomineralized skeleton is regulated by biological mechanisms. This regulation is the basis on which the systematic interpretation of the shell microstructural features are possible (see below). The vertical egg-shell units of growth are undoubtedly of polycrystalline structure and include the system of hierarchically co-ordinated blocks of various sizes. Blocks and crystallites of all sizes have organic "sheaths" the decomposition of which results in the consecutive destruction of prisms and wedges into the structural elements. The arrangement of shell growth units is caused by the biologically pre-determined polysynthetic twinning of cristalline elements. The cleavage pattern is determined by definite parametres of the biocrystalline structure (i. e. by the position of the cleavage faces in a definite polysynthetic twin). These parameters differ both in the wedges and prisms of the same egg-shell, and probably, in the prisms of the shells of different groups of birds (the oblique lines of the "fish-bone pattern" structure in the *Neognathae* are more vertical than in the *Ratitae*). The complex arrangement of submicroscopic elements turns out to be beyond the means of a simple diffraction analysis and beyond the limits of resolution of polarizing microscopy, as has been discussed repeatedly before (GLIMCHER et all, 1965; TRAVIS, 1970; TOWE, 1972).

The organic matrix of avian egg-shells includes protein-mucopolysacharide complexes (KRAMPITZ, 1982), which are characteristic of very different groups of animals and plants (GOLUBEV, 1981). Elementary skeletal crystals grow on fibrils of the "structural" proteins, in vertebrates — of collagen. Protein--mucopolysacharide complexes of egg-shell are supposed to be similar in compo-

sition to the cartilage's ones (LEACH, 1982), whereas the protein of the latter belongs to the collagen group.

Not only protein fibrils, but also discrete polysacharide plates, found in all examined mineralized biogenic structures from single-celled eukaryotes to human beings, participate in biomineralization. Moreover, the orientation of protein fibers and polysacharide plates adjusts the orientation of the twinning crystals; the presence of the "braking mechanism" of crystal growth is also apparently connected with these plates (GOLUBEV, 1983). Polysacharide plates must exist in the egg-shell's organic matrix as well; spherical inclusions (vesicles), long known in avian egg-shells, are probably the indirect manifestation of these plates. The vesicles are better developed in the spongy layer than in cones; they are absent in the secondary spherite. The sizes of vesicles (0.1—0.5 microns) differ in the egg-shells of different birds (BEKING, 1975) and are poorly developed in the egg-shells of the *Ratitae*.

It can be well seen on the photos made by POOLEY (1979) (Pl. XIV, Phot. 33) that the vesicles are spheroidal films. They form horizontal and bolique lines as well as systematic bunches, and they are connected with fibres and sheets. After etching with EDTA-gluteraldehyde, some subrhombic spaces were exposed around vesicles, and were interpreted by POOLEY (1979) to be a result of etching the calcite "monocrystal" along c-axes. But these spaces correspond completely to the subrhombic plates, whereas the pyramidal projections, also alined with c-axes, and left after etching, seem to be lanceolate sheets of the cord matrix (compare with phot. 32). After the additional treatment of the same sample with sodium hypochlorite (organic matter destroyed) the "projections" are no longer seen (POOLEY, 1979, Fig. 11). Similar vesicles are observed in the lumen of many gland cells of the "uterus" (WYBURN et all., 1973). Though such structures were considered earlier as a fixation artifact, they could just as well be the shed tips of microvilli. "Bulbous tips are not a constant feature of microvilli and could indicate a functional specialization". Polysacharide particles (B-glycogen particles) were found inside the bulbous tips (WYBURN et all., 1973). It is worth noticing that similar vesicles are apparently connected with the nucleation of mineralization in cartilage (SCHRENK et all., 1982).

IV. SYSTEMATIC AND PHYLOGENETICAL ASPECTS

At present, microstructural features of polycrystalline skeletons are used successfully in systematic and phylogentical studies of invertebrates, e. g. molluscs (KOBAYASHI, 1980). As for avian egg-shell, the first similar attempts go back to numerous works of von NATHUSIUS, the data of which are summarized by TYLER (1964). The various features of egg-shell structure were used for systematic purposes by SADOV (1940), SCHWARTZ, SCHMIDT, TYLER, and others (see TYLER, 1969; ERBEN, 1970). Three types of egg-shell structure were distin-

guished by ERBEN (1970), who then outlined the phylogeny of sauropsid egg-shells.

Avian egg-shell includes all three types: radial, tabular, and "fish-bone pattern" structures. The extern zone can consist of a modified "fish-bone pattern" structure, and/or of quite peculiar and rather diverse layers (BOARD, 1982; my unpublished data). The same three structural types are known from dinosaur egg-shells (ERBEN, 1970), whereas only a tabular structure was found by ERBEN (o. c.) in the growth units of crocodilian egg-shells. Later, in *Alligator* there was found some crystalline bodies analogous to avian mammillary knobs, which vanished by the end of incubation (FERGUSON, 1981/1982); the existence of radial crystallites inside these bodies, however, is still unknown. The vertical growth units of turtle egg-shells are entirely made up of radial crystallites, which are, moreover, composed of aragonite. ERBEN (1970) postulated the homology of the zone of radial aggregates in all sauropsids (it must have been lost in the *Crocodilia*) and considered it as the original zone in egg-shell evolutionary development. Without going into details of this matter, it should be noted that there are some principal distinctions in the zone of radial aggregates in both turtles and birds. Unlike the turtles, the avian radial structure is composed not of aragonite but calcite (HEYN, 1963; CAIN and HEYN, 1964; SAUER et all, 1975; POOLEY, 1979), which indicates the existence of various types of protein-mucopolysacharide complexes. The analysis of the aminoacid composition of shell membrane and shell matrix in turtles and birds confirms it (KRAMPITZ et all., 1972). According to my data, the secondary spherite arrangement in avian egg-shells is more complex. Three hierarchical levels of co-ordinated units exist: 1) spicular crystallites (the smallest), 2) spicular-tabular and spicular-prismatic lamellae, and 3) petal-like aggregates (the largest). The structural elements often deviate, especially on the "petals" boundaries, from the strictly radial axes (see secondary spherite). Taking into account that for the present time the formation of the secondary spherite even in birds is unknown, the homology of the radial structure in birds and turtles must be considered as uncertain. Hard shell in turtle eggs might appeared independent from the archosaurian linage.

My results confirm ERBEN'S (1970) data on the distinctions between the *Ratitae* and the *Neognathae* (all groups of the *Ratitae* were studied except *Apteryx*). Firstly, the parameters of the biocrystalline structure of the spongy layer (see above) in the *Ratitae* and the *Neognathae* are different. This corresponds to the distinctions in the aminoacid composition of the shell matrix (and of the shell membrane) of these groups (KRAMPITZ et all., 1974); these authors found a considerable similarity between *Casuarius* and *Dromaius*, and less — between *Struthio* and *Rhea* (*Ratitae*); *Apteryx* is separate from them. The mammillary knob structure is a second important distinction. In the *Ratitae* the knob is difficult to distinguish morphologically from the base of the cone and is made up of two types of structures: 1) the radial structure inside the "sheath" and 2) the tabular structure (Fig. 2: B; Pl. XI, Phot. 19). In the *Neognathae*

the mammillary knob is made up of only the radial structure (Fig. 2: A). In contrast to the *Ratitae*, the "petals" of the *Neognathae* form the typical corolla (Pl. IX, Phot. 4, 5). Finally, the tabular structure, in comparison with the radial one, is expanded much more vertically in the *Ratitae* then in the *Neognathae*, which can be seen by the form of the mammilla (Fig. 1: D, E). These distinctions, and other more special ones, can be seen in the Table I. The structu-

Table I

Differences in structure of egg-shells of the *Ratitae* and the *Neognathae*

Layers, units of macro--structure	Features	
	Ratitae	*Neognathae*
Spongy layer	Lines of "fish-bone pattern" run subhorizontally and are expressed less then in the *Neognathae*	Lines of "fish-bone pattern" run subvertically and are expressed more then in the *Ratitae*
Mammillary knob	Made up by two types of structures (radial and tabular structures); morphologically is poorly separated from the base of cone	Made up by one type of structures (radial structure); morphologically is well separated from the base of cone; viewed from underside, the typical corolla, which consist of "petals", is well distinguished
Cones	Zone of tabular aggregates is expanded vertically and sharply prevails over zone of radial aggregates (Fig. 1 : D); the shape of mammilla is much elongated	Zone of tabular aggregates developed relatively less than in the *Ratitae*; vertical and lateral growth of cone are roughly equal (Fig. 1 : E)
Eisospherite	Poorly expressed	Well expressed
Primary spherite	Equal in size with secondary spherite or larger	Smaller than secondary spherite in size

ral features of the pore canals of the *Ratitae* egg-shell will be discussed separately. Due to the insufficient study of *Neognatae* egg-shells it is difficult now to use egg-shell microstructural features to determine systematic relationships within this group. However, egg-shells of the *Passeriformes* deserve special attention. Among all the studied species (*Pica pica* (L.), *Motacilla alba* L., *Oriolus oriolus* (L.), *Turdus pilaris* L.) I didn't find with certainty the tabular structure in the zone corresponding to cones. Only "fish-bone patterns" with "plate cleavage units" orientated almost subvertically can be seen on the fractured radial sections (Pl. VIII, Phot. 2). The mammillary knobs are extremely small and located far away from each other (see also Phot. 5). Wedges, as morphologically separate bodies, seem to be developed only laterally, forming a base for a "loose" spongy

layer. The presence of the tabular structure in the wedges is doubtful; at any rate it is very much reduced. Only further studies can show if these specific characteristic hold for the whole order *Passeriformes*.

Paleontological Institute,
USSR Academy of Sciences
117321 Moscow, Profsoyuznaya 113, USSR

REFERENCES

Barskov I. S., 1982. Барсков И. С. 1982. Биоминерализация и эволюция. Палеонт. ж., **1982**, (4): 5—13.

Becking J. H., 1975. The ultrastructure of the avain egg-shell. Ibis, **117** (2): 143—151.

Bellairs R., Boyde A., 1969. Scanning electron microscopy of the shell membranes of the hen's egg. Zeitschr. Zellforsch. Mikrosk. Augt., **96**: 237—249.

Bevelander G., Nakahara H., 1980. Compartment and Envelope Formation in the Process of Biological Mineralization. In: The Mechanisms of Biomineralization in Animals and Plants, Tokyo, Tokai University Press, p. 19—27.

Board R. G., 1982. Properties of avian egg shells and their adaptive value. Biolog. Rev. Cambridge Philos. Soc., **57** (1): 1—28.

Cain C. J., Heyn A. N. J., 1964. X-ray diffraction studies of the crystalline structure of the Avian egg shell. Byophys. Journ., **4**: 23—39.

Creger C. R., Phillips H., Scott J. T., 1976. Formation of egg-shell. Poultry Science, **55**: 1717—1723.

Crenshaw M. A., 1982. Mechanisms of Normal Biological Mineralization of Calcium Carbonates. In: Biological Mineralization and Demineralization, Berlin: Springer-Verlag, Dahlem Konferen., p.: 243—257.

Erben H. K., 1970. Ultrastructuren und Mineralisation rezenter und fossiler Eischalen bei Vögeln und Reptilien. Biomineralization, **1**: 1—65.

Favejee J. Ch. L., van der Plas L., Schoore R., Floor P., 1965. X-ray diffraction of the crystalline structure of the avian egg shell: some critical remarks. Biophys. Journ., **5**: 359—361.

Ferguson M. W. J., 1981/1982. The structure and composition of the egg-shell and embryonic membranes of *Alligator mississippiensis*. Trans. Zoolog. Soc. London, **36** (2): 99—152.

Fujii S., 1974. Further morphological studies on the formation and structure of Hen's egg-shell by scanning electron microscopy. Journ of Faculty of Fisheries and Animal Husbandry. Hiroshima University, **13**: 29—56.

Glimcher M. J., Daniel E. J., Travis D. F., Kamhl S., 1965. Electron optical and x-ray diffraction studies of the inorganic crystals in embryonic bovin enamel. Journ. Ultrastruct. Res. (Suppl.), 1—77.

Golubev S. N., 1981. Голубев С. Н. 1981. Реалные кристаллы в скелетах кокколитафорид. Москва, Наука.

Golubev S. N., 1983. Голубев С. Н. 1983. Двойнкование кристаллов в скелетах организмов. Палеонт. ж., **1983**. (2): 3—11.

Heyn A. N. J., 1963. The crystalline structure of calcium carbonate in avian egg shell: a TEM study. Journ. Ultrastruct. Res., **8**: 176—188.

Klassen-Nekludova M.V., 1960. Классен-Неклюдова М. В. 1960. Механическое двойникование кристаллов. Москва, АН СССР .

Kobayashi J., 1980. Various Patterns of Biomineralization and Its Phylogenetic Significances

in Bivalve Molluscs. In: The Mechanisms of Biomineralization in Animals and Plants. Tokyo, Tokai University Press., p.: 145—155.

KRAMPITZ G. P., 1982. Structure of the organic matrix in mollusc shells and avian egg-shells. In: Biological Mineralization and Demineralization, Berlin: Springer-Verlag, Dahlem Konferenzen, p.: 219—232.

KRAMPITZ G. P., ERBEN H. K., KRIESTEN K., 1972. Über Aminosäurenzusammensetzung und Structur von Eischalen. Biomineralisation, **4**: 87—99.

KRAMPITZ G. P., KRIESTEN K., FAUST R., 1974. Über die Aminosäuren — Zusammensetzung morphologischer Eischalen — Fraktionen von *Ratitae*. Biomineralisation, **7**: 1—13.

LEACH R. M. 1982. Biochemistry of the organic matrix of the egg-shell. Poultry Science, **61**: 2040—2047.

MUTVEI H., 1978. Ultrastructural characteristics of the nacre in some gastropods. Zool. Scripta, **7**: 287—296.

MUTVEI H., 1979. On the internal structure of the nacreous tablets in molluscan shell. Scanning Electron Microscopy, II, AMF O'Hare, Illinois, p.: 457—462.

PERROTT H. R., SCOTT V. D., BOARD R. G., 1980. Crystal Orientation in the Shell of the Domestic Fowl: An Electron Diffraction Study. Calcified Tissue International, Springer--Verlag, **33**: 119—124.

POOLEY A. S., 1979. Ultrastructural relationships of mineral and organic matter in avian egg--shells. Scanning Electron Microscopy, II, AMF O'Hare, Illinois, 443—482.

SADOV I. A., 1940. Садов И. А. 1940. Строение скорлупы яиц птиц и рептилий. Сборник научных работ комсомольцев-биологов, Ин-т эвол. Морфол. им. академика А. Н. Северцова, 139—148.

SAUER F. G., SAUER E. M., GEBHARDT M., 1975. Normal and abnormal Patterns of Struthious Egg-shells from South West Africa. Biomineralisation, **8**: 32—54.

SCHRENK R. K., HUNZIKER E., HERRMANN W., 1982. Structural Properties of Cells Related to Tissue Mineralization. In: Biological Mineralization and Demineralization, Dahlem Konferenz., Berlin Springer-Verlag, p.: 143—160.

SIMONS P. C. M., WIERTZ G., 1963. Notes on the structure of membranes and shell in the hen's egg. Zeitschr. Zellforsch., Mikrosk. Augt., **59**: 555—567.

TEREPKA A. R., 1963. Organic-inorganic interrelationships in Avian egg-shell. Exptl. Cellul. Res., **30**: 183—192.

TOWE K. M., 1972. Invertebrate shell structure and organic matrix concept. Biomineralisation, **4**.

TRAVIS D. F., 1970. The comparative ultrastructure and organization of five calcified tissues. In: Biological calicification, cellular and molecular aspects, New York, p.: 203—312.

TYLER C., 1964. "Wilhelm von Nathusius 1821—1899 on Avian Egg-shells". The Berkshire Printing Co. Ltd., The University Reading.

TYLER C., 1969. Avian egg-shells: Their structure and characteristics. International Review of General and Experimental Zoology, New York and London, Academic Press, **4**: 81—130.

WATABE N., 1974. Crystal growth of calcium carbonate in biological systems. Journ. of Crystal Growth, **24/25**: 116—122.

WATABE N., 1981. Crystal growth of calcium carbonate in invertebrates. In: Progress in Crystal Growth and Characterization, Oxford, Pergamon Press, **4** (1/2): 99—147.

WATABE N., DUNKELBERGER G., 1979. Ultrastructural studies on calcification in various Organisms. Scanning Electron Microscopy, II, AMF O'Hare, Illinois.

WILBUR K. M., 1980. Cells, Crystals and Skeletons. In: The Mechanisms of Biomineralization in Animals and Plants. Tokyo, Tokai University Press, p.: 3—11.

WYBURN C. M., JOHNSTON H. S., DRAPER M. H., DAVIDSON M. F., 1973. The ultrastructure of the shell-forming region of the oviduct and the development of the sehll of *Gallus domesticus*. Quarterly Journal of Exp. Physiology, **58**: 143—151.

STRESZCZENIE

Przy użyciu skaningowego mikroskopu elektronowego badano struktury skorupy jaj 5 gatunków *Ratitae* i 13 *Neognathae*. Wyniki potwierdziły przewidywane prawidłowości formowania się skorupy z punktu widzenia biomineralizacji. Wzrost kryształów w skorupie jest wywołany i kontrolowany przez organiczne matryce, które działają na zasadzie szablonów. Pionowe jednostki wzrostu skorupy (stożek i kolumna) mają strukturę polikrystaliczną i przedstawiają system hierarchicznie ułożonych bloków o różnej wielkości. Krystality wszystkich wielkości mają "pochewki" składające się z organicznych płytek, których chemiczne zniszczenie może powodować ciągły rozkład słupów i klinów na elementy strukturowe. Złożony układ jednostek wzrostu skorupy jest powodowany przez biologicznie zdeterminowane wielosyntetyczne odbicia submikroskopowych płytkowych krystalitów. Wyniki potwierdzają dane ERBENA (1970) na temat różnic w budowie skorupy jaj *Ratitae* i *Neognathae* (fig. 1 i 2, tabela I). Stwierdzone zostały też szczególne cechy skorupy jaj *Passeriformes*, które jednak wymagają dalszych badań.

Remark

During the investigations, described in this paper, the standard etcher — 10% solution of KOH — was used for decomposing the organic matrix. But the most recent experiments (undertaken after sending the paper to the Editor) consisting in etching the egg-shell and the abiogenic calcite with hot solutions of KOH and H_2O_2 have convinced the author that the alkaline solutions in some cases decompose the organic matrix whereas in the other ones — the crystal. Therefore it is necessary to admit, that blocks with pentagonal faces and subglobular blocks (Pl. XV Phot. 34—38; Pl. XVI Phot. 41—42) do not determine specific structure of the egg-shell.

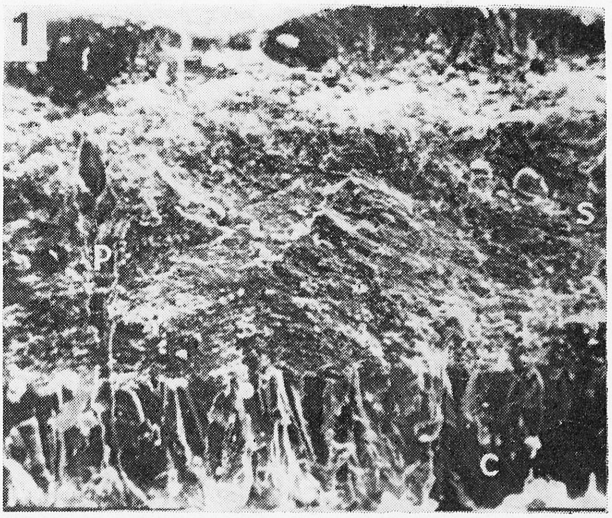
Redaktor pracy: prof. dr Z. Bocheński

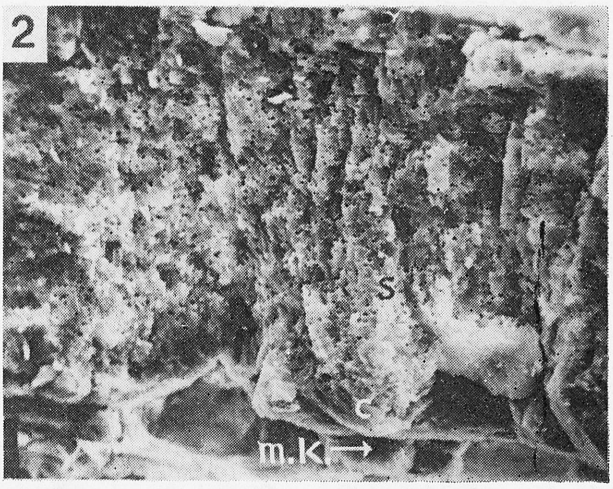
Plate VIII

Phot. 1. Fractured radial surface of egg-shell in *Dromaius novae-hollandiae*. Oblique striations indicate "fish-bone" structure in spongy layer (s). Note strongly vertically expanded wedges of cones (c). Pore canal (p) seen on the left (×136)

Phot. 2. Fractured radial surface of egg-shell in *Pica pica*. Note subvertical orientation of "plate units" of "fish-bone" structure in spongy layer (s); note well expressed vesicles. Cones (c) poorly developed and produced mainly laterally. Mammillary knob (m. k.) small and sparse (×675)

Phot. 3. Fractured radial surface of extern zone of egg-shell in *Aepyornis* sp. (Pleistocene, Madagascar). Subvertical plate elements cross one another (analogically as cross-lamellae structure in mollusc shell). Treatment: 1% acetic acid for 6 sec. (×180)


1
P
S
C


2
S
C
m.K

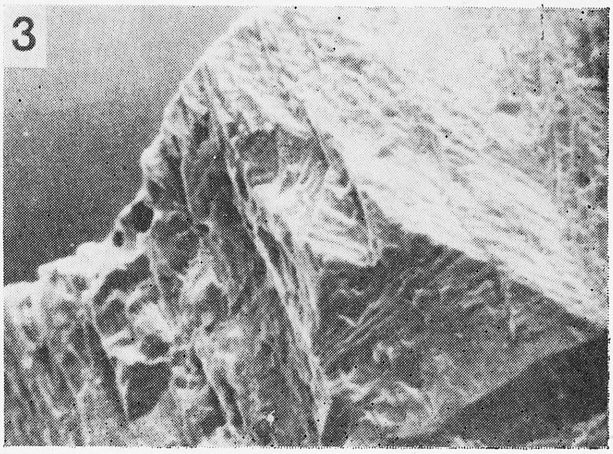

3

Plate IX

Phot. 4, 5. Mammillary knob and eisospherite. Inner view of egg-shell surface after removing shell membranes. Radiating petal-like crystallites form typical corolla. Extern part of "petal" based on wedge (w) with tabular structure

Phot. 4. *Crotophaga ani*, shell of incubated egg. The space, left after decomposition of primary spherite by the natural way, seen in the center of the mammilary knob ($\times$ 900)

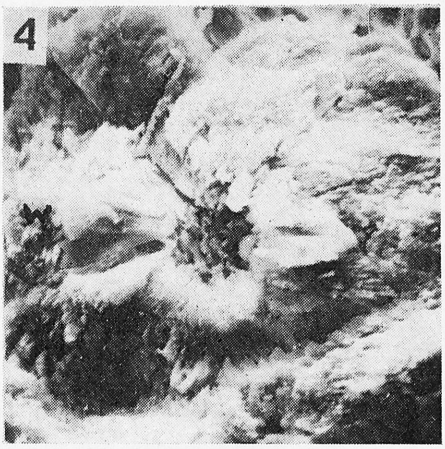
Phot. 5. *Motacilla alba*, shell of non-incubated egg. Mammillary knob (k) very small and sparse; wedges (w) developed laterally. Treatment: 10% KOH for 10 min ($\times$ 450)

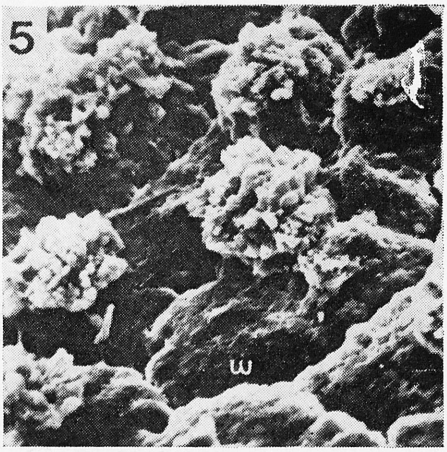
Phot. 6—8. Structure of primary spherite: "spherite body" (s. b.), radial septae (r), multilayered wall of primary spherite (m), "hole locules" (h. l.)

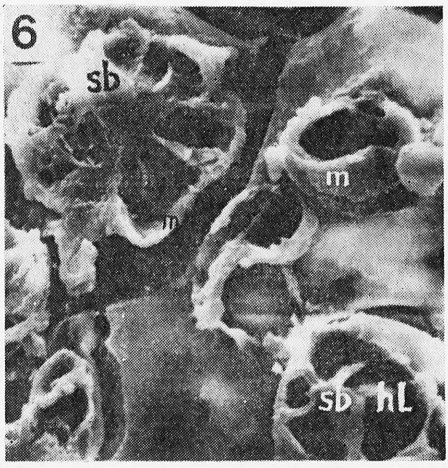
Phot. 6. Outer view on shell membrane in *Aepyornis* sp. after complete decalcification of egg-shell. Treatment: 0.1% HCl for 3.5 days; ($\times$414)

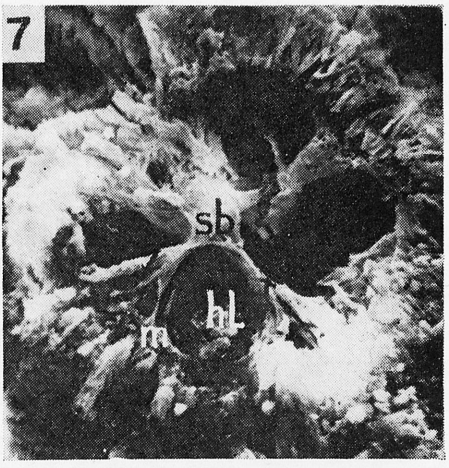
Phot. 7—8. Inner view on egg-shell inner surface in *Struthio camelus* after removing shell mebranes. Radial aggregates (spicular crystallites "fused" into spicular-prismatic lamellae) formed (arrow) into radial septae of primary spherite. Phot. 7 — ($\times$765), Phot. 8 — ($\times$1800)

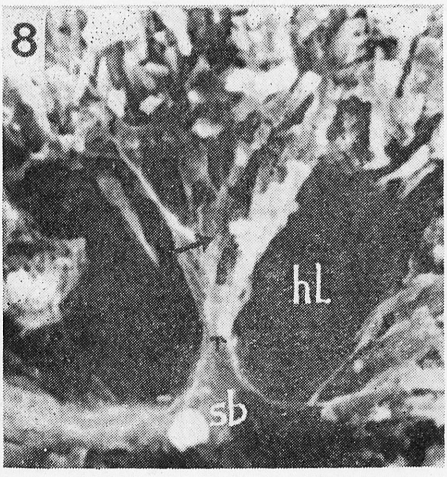
Phot 9. Inner view on egg-shell inner surface in *Struthio camelus*. Note concave body, consisting of organic plates, below "spherite body" of primary spherite ($\times$1350)


4


5
w


6
sb
m
m
sb hL


7
sb
hL
m


8
hL
sb

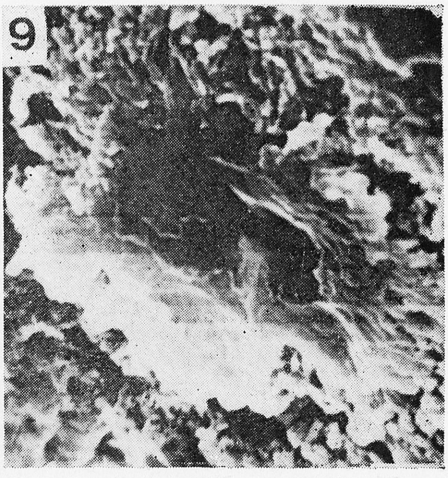

9

Plate X

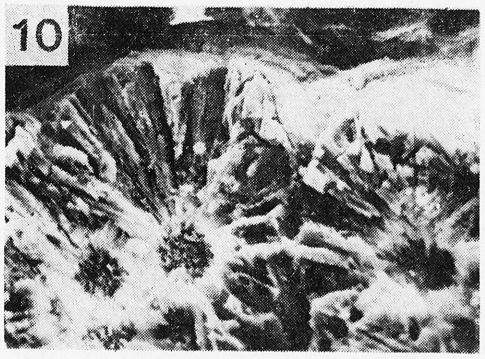
Phot. 10. Mammillary knobs in *Aptenodytes forsteri*. Inner view on egg-shell surface after removing shell membranes and primary spherite. Note corolla consisting of large petal-like crystallites ("petals"). Treatment: 10% KOH for 10 min. (×360)

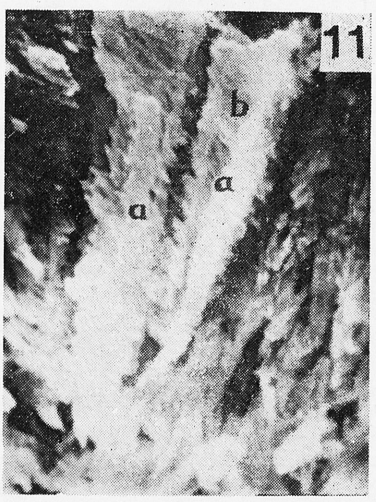
Phot. 11, 12. Broken surface of mammilary knob in *Aptenodytes forsteri*. Details of secondary spherite structure (organic component partly removed). Note "petal axis" (a) consisting of compact plate elements, and "petal blade" (b) consisting of spicular-prismatic crystallites oriented obliquely to the "petal" axis. Treatment: 10% KOH for 10 min. Phot. 11 — (×3150), Phot. 12 — (×4050)

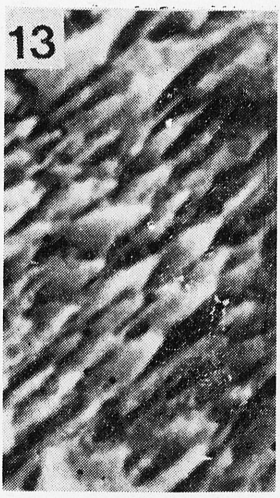
Phot. 13. Organic matrix of secondary spherite in *Aepyornis* sp. Radial view of broken mammilary knob. Note organic "sheaths" of spicular-prismatic lamellae. Treatment: 0.1 HCl for 6 sec. (×3600)

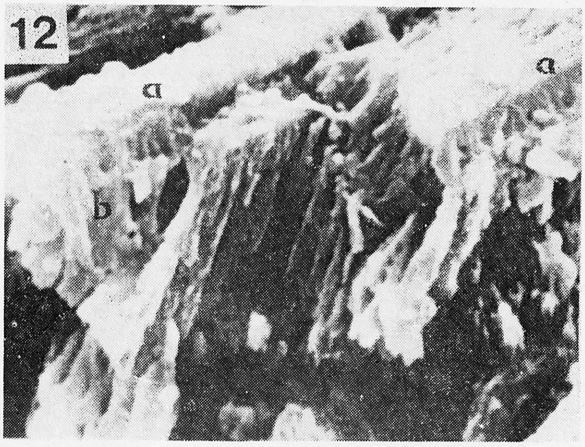
Phot. 14. Broken mammillary knob in *Struthio camelus*. Note petal-like aggregates provided with organic "sheaths" (arrow). "Petal" forms bundle of spicular-prismatic crystallites consisting of more smaller spicular crystallites (upper right). Below right, outer wall of primary spherite seen (×1600)

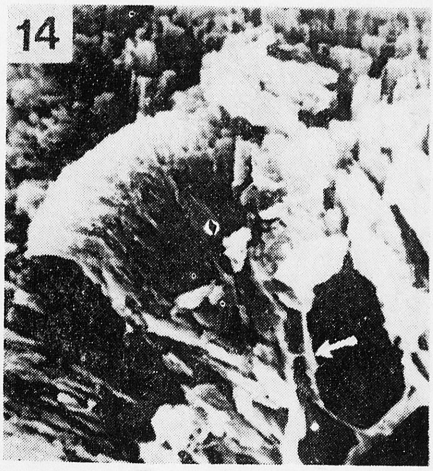
Phot. 15. Details from phot. 14. Bundle of spicular-prismatic lamellae. Each lamella consisting of spicular crystallites of smaller size (×3150)


10


11
b
a
a


13


12
a
a
b


14

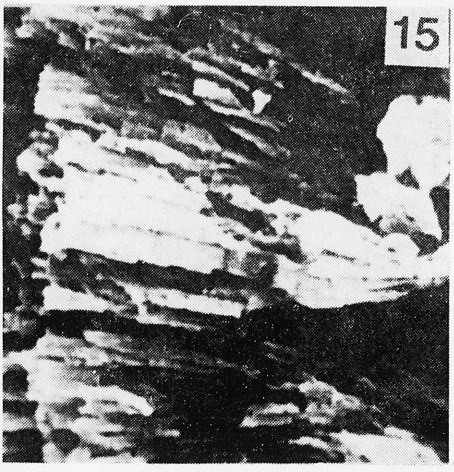

15

Plate XI

Phot. 16. Upper part of broken mammillary knob near the base of cone in *Aptenodytes forsteri*. Note rows of large plates consisting of "fused" tabular and spicular crystallites (modificated radial structure on the boundary with tabular structure). Treatment: 10% KOH for 10 min. (×3600)

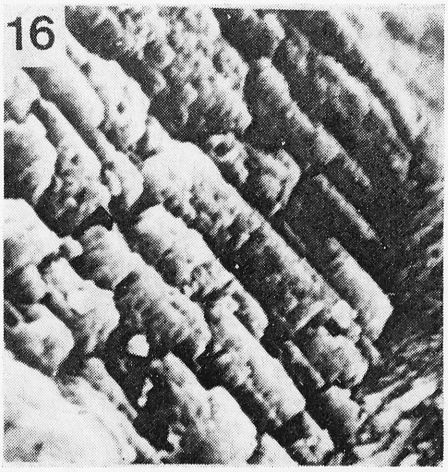
Phot. 17. Fractured tangential surface of cone in *Struthio camelus*; dorsal view. Note polyhedral tablets composing wedge. Treatment: 10% KOH for 10 min. (×225)

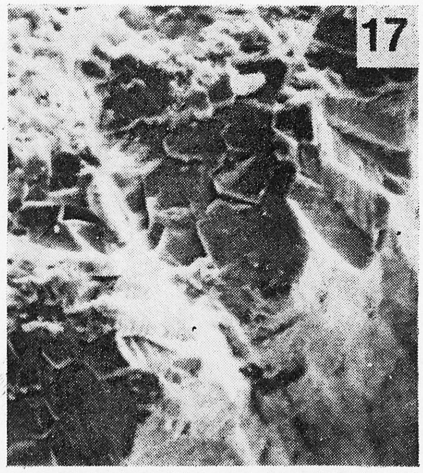
Phot. 18. Fractured radial surface of cones in *Struthio camelus*. Boundaries between wedges well seen; transverse striations indicate well developed vertically tabular structure. Mammilary knob poorly isolated from the base of cone (compare with phot. 2, 4, 5); (×108)

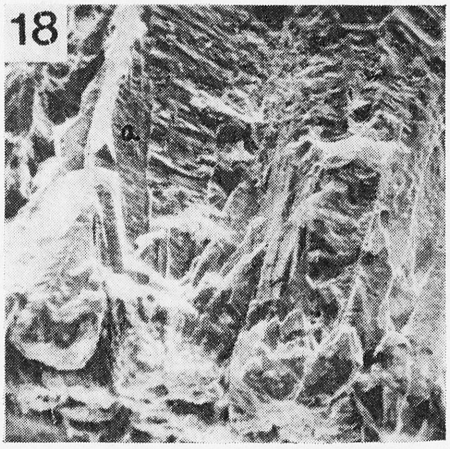
Phot. 19. Details from phot. 18. Radiating aggregates (wedges) with tabular structure. Note cords in the right wedge (w). Secondary spherite with radial structure situated inside the mammillary knob (compare with photos 4, 5, 10). Organic plates of primary spherite seen in knob base (arrow); (×315)

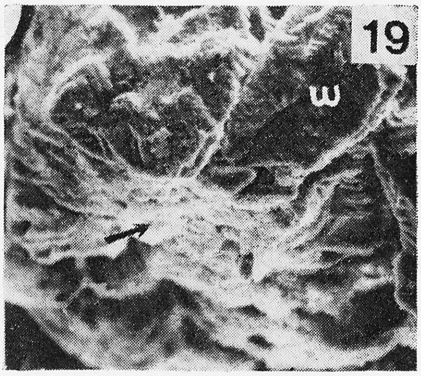
Phot. 20. Polished radial surface of cones in *Struthio camelus*. Note well expressed horizontal accretion lines. Treatment: 1% HCl for 6 sec. (×250)

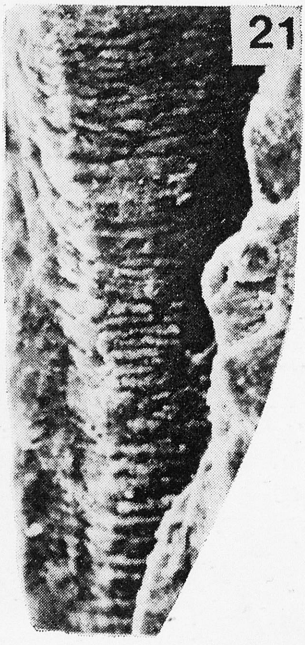
Phot. 21. Zone of cones. Radial section of egg-shell in *Aepyornis* sp. Outer shell surface parallel to the upper edge of the photo. Elongated aggregates of irregular form (cords) seen within wedges. Cords formed into "brick-wall"-like pattern. Treatment: 1% HCl for 7 sec. (×400)


16


17


18
α


19
ω


21

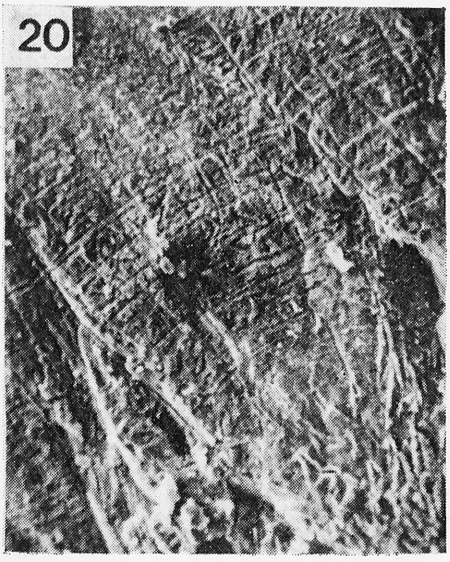
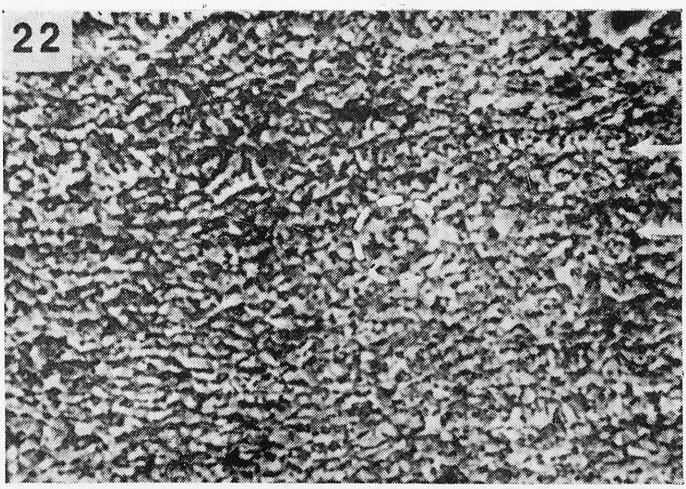

20

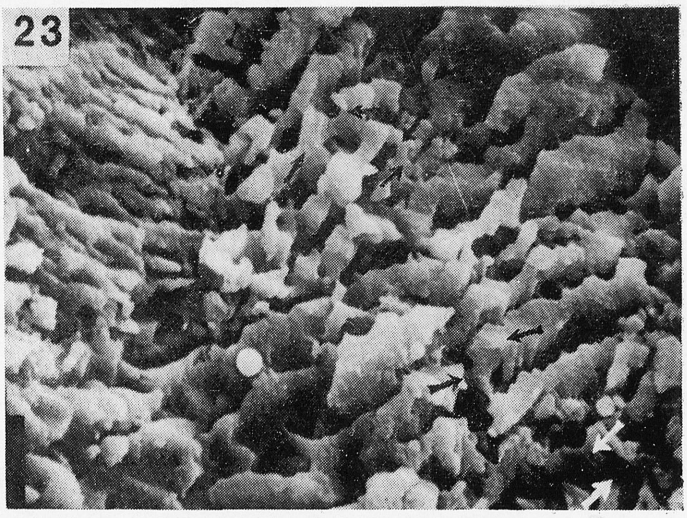
Plate XII

Phot. 22. Polished radial surface of spongy layer in *Struthio camelus* (outer surface of egg-shell parallel to the upper edge of the photo). Obliquely and horizontally oriented cords sometimes formed into criss-cross figures; horizontal accretion lines poorly expressed (arrows; compare with phot. 20). Note "sections" of subglobular blocks (dotted line). Treatment: 1% HCl for 5 sec. (×675)

Phot. 23. Fractured radial surface of spongy layer in *Struthio camelus*. "Intercrystalline matrix" decomposed. Note the pattern resembling that of phot. 22. Cords begin to be decomposed into subrhombic plates and smaler plate elements (arrows). Treatment: 10% KOH for 10 min. (×1630)

Phot. 24. Zone of cones on radial section of egg-shell in *Struthio camelus*. The pattern similar to that of phot. 21. Cords begin to be decomposed into minute plate elements. Treatment: 10% KOH for 10 min. (×1360)


22


23

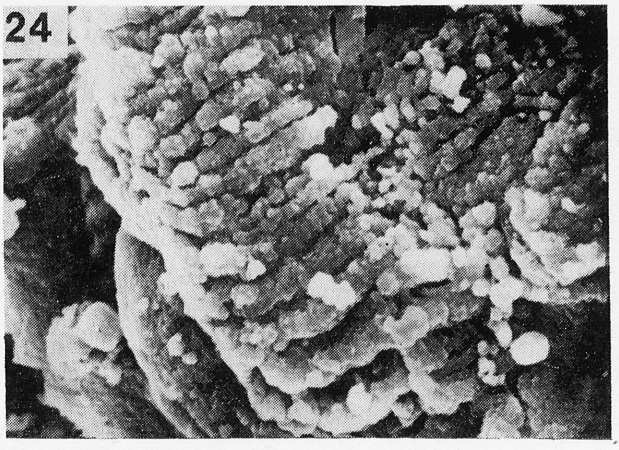

24

Plate XIII

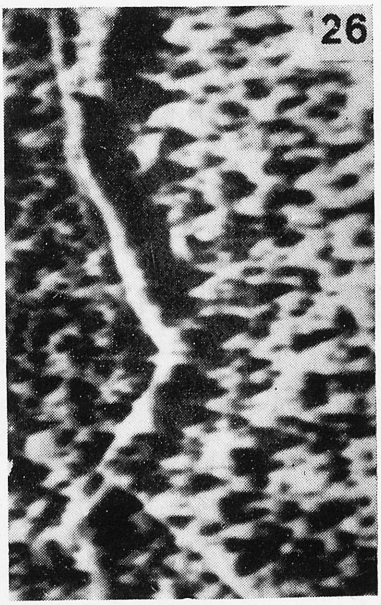
Phot. 25. Organic matrix of cones on decalcificated tangential section of egg-shell in *Aepyornis* sp. Note cross section of pore canals and membranes ("intercrystalline matrix") separating (arrows) "intracrystalline matrix" of polyhedral tablets. Treatment: 0.1% HCl for 1.5 days (×450)

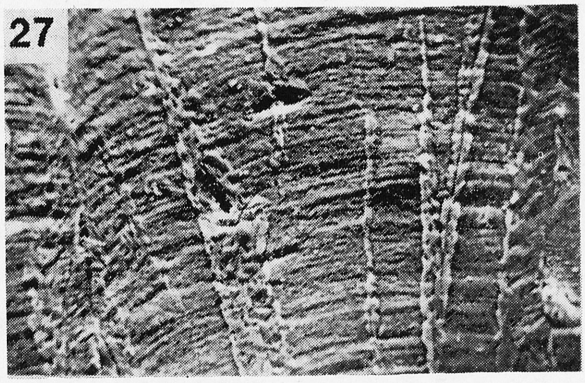
Phot. 26. Details of phot. 25. Membranes separating tablets consist of units similar to those of "intracrystalline matrix" (×3600)

Phot. 27. Decalcificated radial section of cones in *Aepyornis* sp. (outer surface of egg-shell parallel to the upper edge of the photo). Radial membranes separating wedges well seen. Treatment: 0.1% HCl for 1 day (×220)

Phot. 28. Organic matrix of spongy layer. Decalcificated tangential section of egg-shell in *Struthio camelus*. Orientation of units of organic matrix different in particular prisms. Membranes (arrow) separating prisms consist of units similar to those of "intracrystalline matrix". Treatment: 0.1% HCl for 15 hours (×1800)


25


26


27

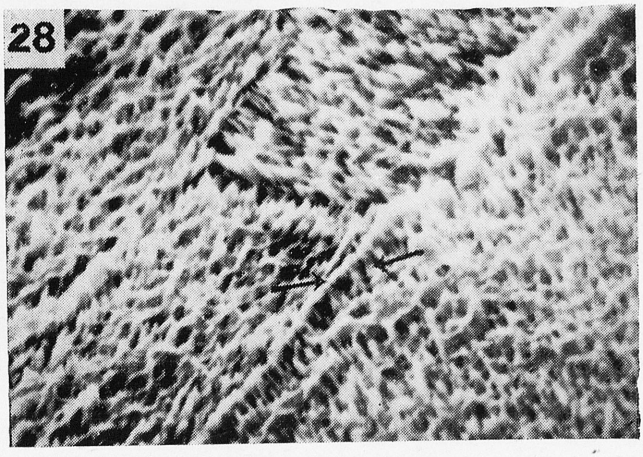

28

Plate XIV

Phot. 29 and 29a. Fractured radial surface of spongy layer in *Struthio camelus* (outer surface of egg-shell parallel to the upper edge of the photos). Oblique striations of "plate units and subunits" indicate the "fish-bone" structure. Phot. 29 — (×450), Phot. 29a — (×610)

Phot. 30—32. Organic matrix of spongy layer. Decalcificated radial section of egg-shell (outer surface of egg-shell parallel to the upper edge of photos)

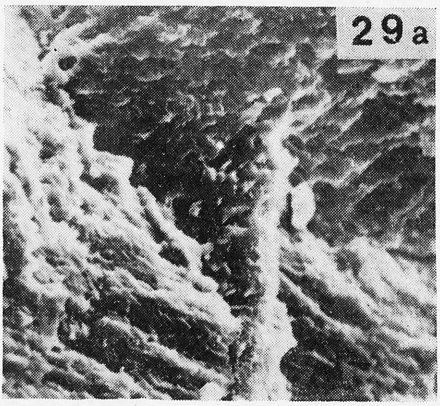
Phot. 30. *Dromaius novae-hollandiae* (partly decalcificated sample). Note screw-like arrangement of organic matrix (arrow). Thickest horizontal membranes (double arrow) are "intercrystalline matrix" of cords. Treatment: 0.1% HCl for 1 day (×1360)

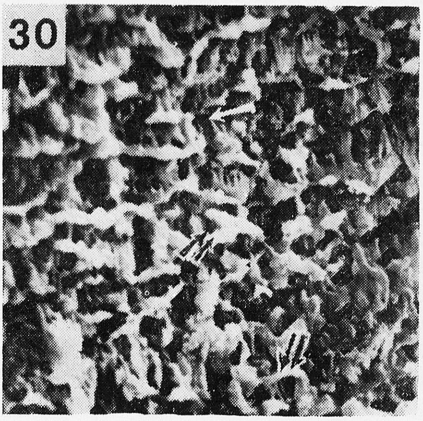
Phot. 31. *Struthio camelus*. Note screw-like arrangement of lanceolate holes (= imprints of crystalline subrhombic plates), and interlacement of organic fibers well seen in points of fiber contact (arrows), Treatment: 0.1% HCl for 1 day (×4500)

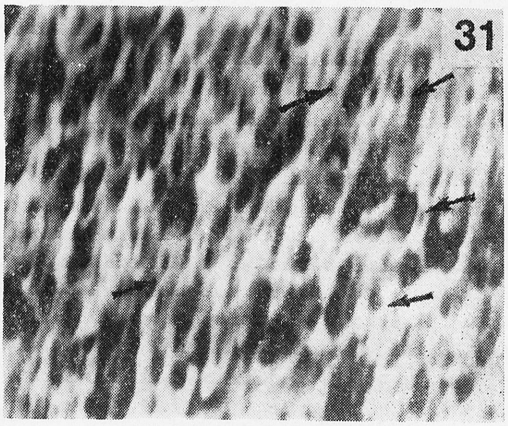
Phot. 32. *Struthio camelus*. Subvertical lanceolate membranes (templates of crystalline subrhombic plates) compose "intracrystalline matrix" of cords (see horizontal rows). Interlacement of fibers, surrounding lanceolate membranes, well seen in points of fiber contact (arrows). Treatment: 0.1% HCl for 1.5 days (×5000)

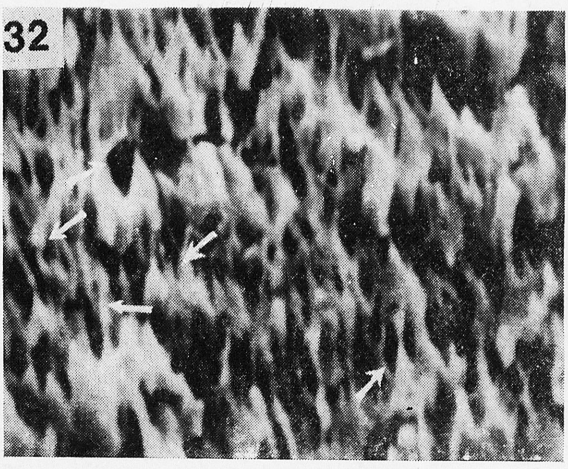
Phot. 33. Decalcificated radial section of spongy layer in *Pelecanus erythrorhynchos*. Symbols: VL — vesicle linings, P — pyramidal projections. Treatment: a solution of 0.01 EDTA, 2% glutaraldehyde and 0.1% sodium phosphate buffer pH 7.4 for 1/2 hour. Bar = 1 micron, (from Poolley, 1979)


29


29a


30


31


32

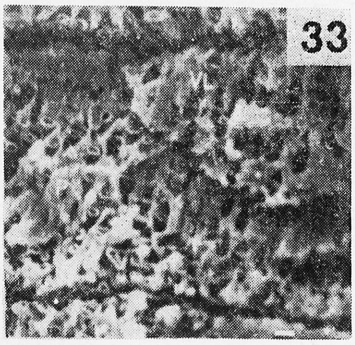

33

Plate XV

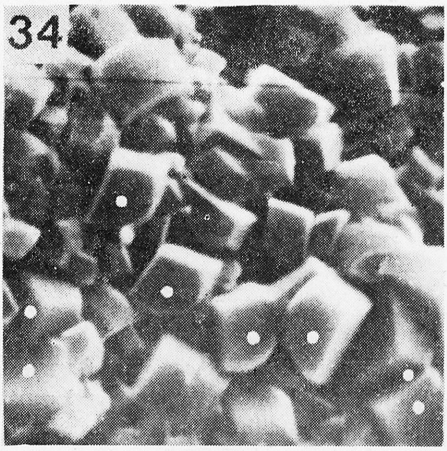
Phot. 34—38. Fractured radial section of egg-shell in *Struthio camelus* after decomposition of organic component to a different extent (outer surface of egg-shell parallel to the upper edge of photos). Note hierarchically co-ordinated blocks of different size levels

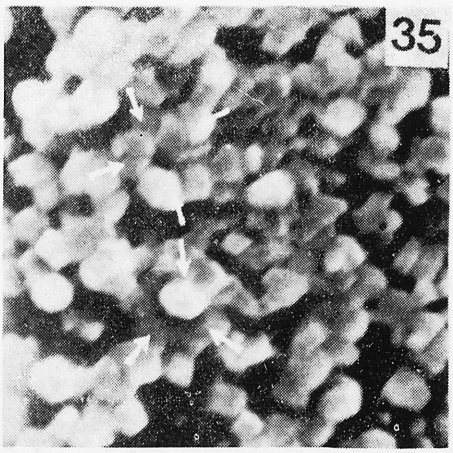
Phot. 34. Spongy layer. Note subrhombic plates and polyhedrons, both with partly rounded contours, and elements of "screw symmetry" (dotted line). Treatment: 10% KOH for 10 min. (×3200)

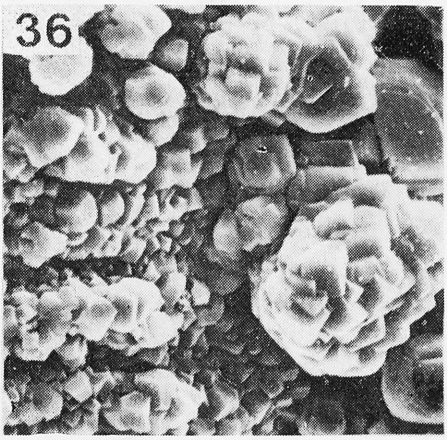
Phot. 35. Zone of cones. Note smallest subrhombic plates and some circle figures (arrows). Treatment: 10% KOH for 15 min. (×4540)

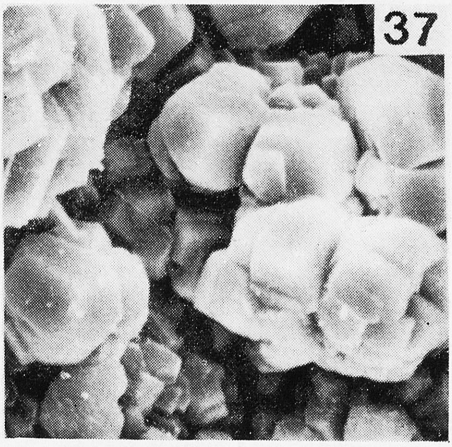
Phot. 36—38. Spongy layer. Note polyhedral blocks of 3—5 microns in size, and subglobular blocks of various size. Note screw-like arrangement of structural elements inside subglobular blocks. Treatment: 10% KOH for 15 min. Phot. 36 — (×900). Phot. 37 — (×1700). Phot. 38 — englared part of phot. 36; regular geometrical "faces" often exposed in circular arc forms (arrow) (×1800)

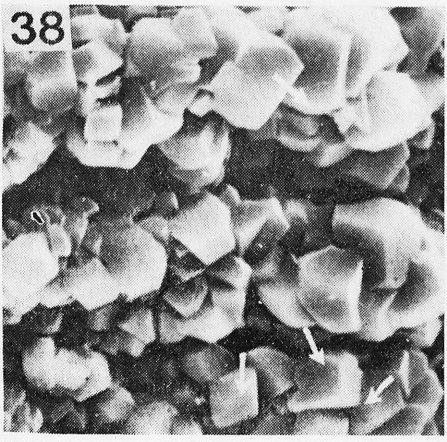
Phot. 39. Tangential section of spongy layer in *Struthio camelus*. Oblique view on fractured surface (organic component partly decomposed). Subglobular block of about 10 microns in size including structural elements of several cords (cords beginning decomposition). Note fine arrangement of microscopic elements (arrow). Treatment: 10% KOH for 10 min. (×2700)


34


35


36


37


38

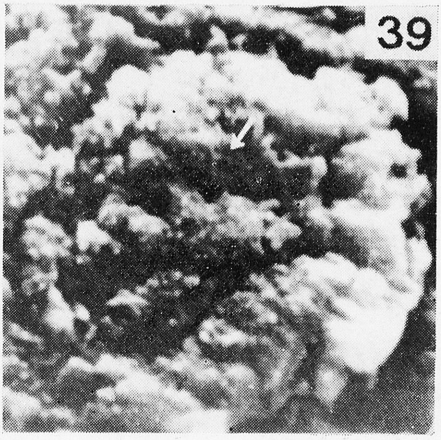
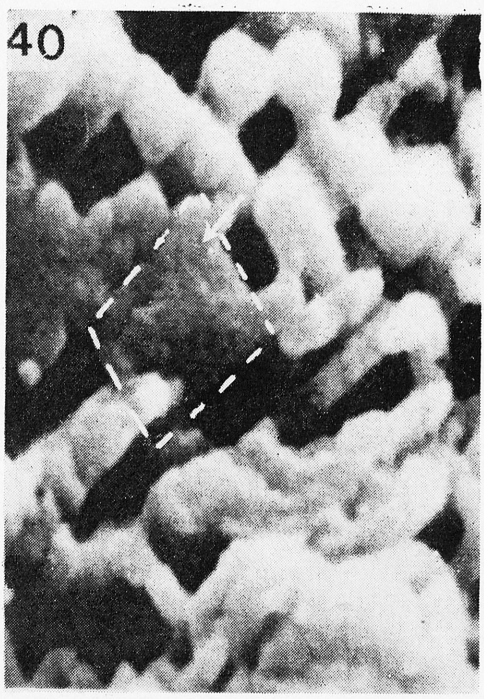
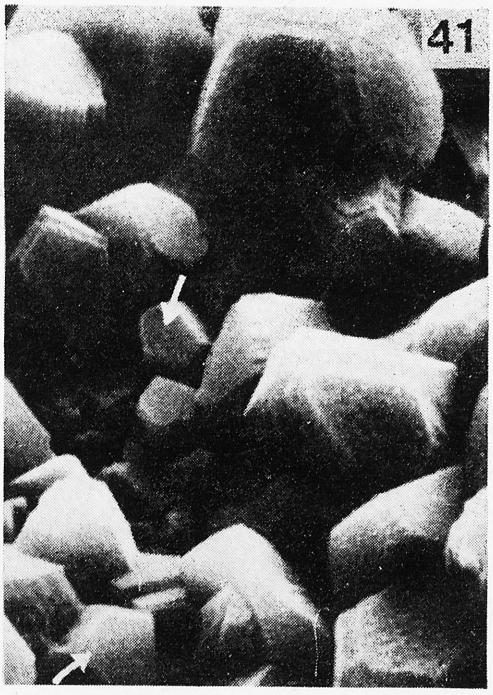
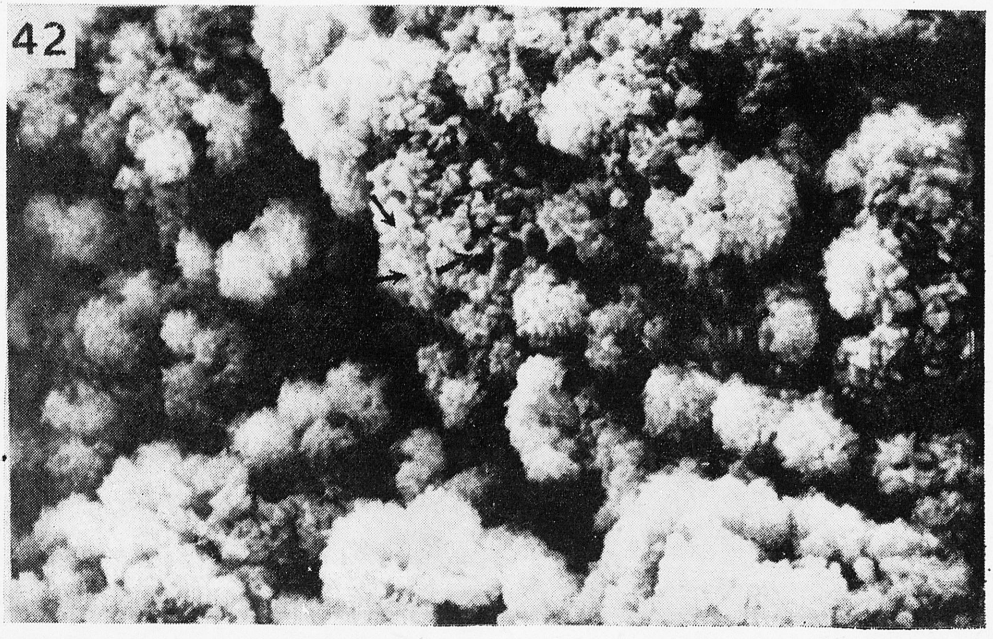

39

Plate XVI

Phot. 40—42. Fractured radial surface of spongy layer in *Struthio camelus*. Organic component decomposed to different extent. Phot. 40. Subrhombic plate (dotted line) consisting of smaller plate subunits; "ridges" of plate are elongated crystallites forming kink in the top of "rhomb" (arrow) compare with phot. 31, 32. Treatment: 10% KOH for 20 min. (×7500) Phot. 41. Polyhedral blocks of different size levels are revealed in "real" structure of spongy layer. Note pentagonal faces (arrows) of blocks. Treatment: 10% KOH for 10 min. (×3000) Phot. 42. Subglobular blocks located within cords (subhorizontal lines), consist of minute plate elements (= subunits of subrhombic plates). Treatment: 10% KOH for 20 min. (×2250)


40


41


42