The Ultrastructure of Some Dinosaurian Egg Shells on.the Basis of Data Obtained by Scanning-Electron Microscopy

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(translator unknown)

Knowledge of the life of organisms which existed in ancient geological times is not limited only to study of fossil remains of these organisms. There is also great interest in research of the different stages and evidences of their past life activity. Among related evidences, there exist also documents of paleontological history, by which we may establish determination of traits; for example, the reproductive cycle of these and other living things.

The famous Soviet paleontologist Academician of the Ukrainian SSR, O. S. Bialov, who for many years has been working very hard on the study of the different stages of life of ancient organisms, proposed a special classification, particularly for evidence of past manifestations of their multiplication – *augerisignia*, which probably occur at the same time, and *natisignia*, by evidence of birth (Bialov, 1971). In a series of such paleontological documents, a prominent place is held by the fossil eggs of Veterovata (according to the classification of O. S. Bialov), traces of which are visible; particularly, a class of eggs of reptiles, Reptilova, which includes different orders; among which are Testudinovida (tortoise eggs), Dinosauridovida (dinosaur eggs) with different genera; for example, *Dinosauridovam*, etc.

Sirugues, 1967, 1968; Lapparent, Lavocat, 1955; Müller, 1968. Studies of some dinosaur egg shell fragments from the Jurassic deposits of Portugal.

A particularly large collections of finds of remains of eggs from various dinosaurs is provided by Asia. Here primarily, we should mention sites in the Mongolian Peoples Republic. The first dinosaur eggs in Mongolia were found in 1923 by American paleontologists in the district of Bayn Dzak (otherwise Sharabak-Usu) located in the Southern Gobi. In 1946, colleagues of the Mongolian Paleontological Expedition of the USSR found a complete nest with a clutch of eggs. Later, a very large collection of dinosaur eggs of different dimensions and shapes, with countless fragments of eggs, was found by Soviet, Mongolian and Polish paleontologists, not only at Bayn Dzak, but at a series of other localities in Southern Mongolia. A prominent Soviet paleontologist, the organizer and leader of the first paleontological expeditions of the Academy of Sciences of the USSR in Mongolia, I. A. Efremov, for some time had expressed doubt that these eggs should be considered dinosaur eggs and assumed that in due course they could be assigned to large tortoises, which actually existed in large numbers in the Late Cretaceous of Mongolia (Efremov, 1954; Rozhdestvensky, 1952). Subsequently, however, these doubts were laid to rest and definite confirmation of finds in Mongolia, even the majority, as belonging to dinosaurs was obtained, when fragments of embryo dinosaur skeletons were found in some eggs (Sochava, 1972; Mezhevska, 1976). Organized in 1967, the famous Soviet-Mongolian Geological Expedition of ANSSSR and AN MNR distinctly added to the accumulation of material on dinosaur fauna of Mongolia and, specifically, led to the opening of new massive buried deposits of dinosaur eggs, in some places, moreover, of extremely large dimensions (Sochava, 1969; Martinson, 1974).

In 1950 in the region of the town Laiyan (Shantung Province) in China, in a series of localities, many remains of skeletons and dinosaur eggs were collected. There, in subsequent years, the Special Expedition of the Academy of Sciences of the Chinese Peoples Republic took place, as a result of which there were found a few tens of eggs, distributed in nests and individually. These eggs are divided into 4 types, of which 3 are positively attributed to dinosaurs. Also, as in Mongolia and the majority of other places, the Chinese accumulations of dinosaur egg remains are confined to Upper Cretaceous deposits (Young Chung-Chien, 1954, 1959). A site of dinosaur egg remains is known also in the USSR. It is confined to the southern part of the Zaisan Coomb in Kazakhstan, where in 1959 debris of dinosaur egg shells was found by the geologist V. S. Erofeev on the left bank of the Tayzhuzgen River, and by geologist Iu. V. Tsenovsky in the region of the settlement of Karabulak. Both finds were taken from red clay of the North Zaysan layer of the Upper Cretaceous. The shell thickness of the eggs found in these localities are of equal dimensions, 2 mm, but their external sculpture varies so much that the supposition arises concerning the species affiliation of this shell to dinosaurs (Bazhanov, 1961; Bazhanov and Kozhamkulova, 1960).

Accumulation of material for fossil dinosaur egg shells led necessarily to the separation of taxonomic affiliation of these sites, compelling them to pay direct attention to a detailed study of the morphology of the shell data. Such a study led in several directions. Attention was paid to the basic configuration of more or less whole eggs in conformity with their dimensions and volumes. The mechanical shell strength and its probable physiological protective aspects were estimated. The studies of greatest detail touched upon the features of shell structure, and also on its mineral content and biochemical features (Voss-Foucart, 1968; Kolesnikov and Sochava, 1972). It turned out that calcium carbonate, modified as calcite, makes up the basis of dinosaur egg shells. In the shell membrane and the porous layer of the shell were found a series of amino acids which are known from the albumin parts of eggs of contemporary reptiles and birds (Martinson, Sochava, and Kolesnikov, 1971).

At present, the main tendency in the study of dinosaur eggshell structure is divided into microstructural and ultrastructural levels. For the first time the microstructure of this shell was subjected to study in the material from Mongolia (Straelen, 1929). I. A. Sadov studied the same material later (1970) and arrived at an important conclusion concerning it, that in bird eggshells, for example, we may divide them into genera and even species. This application to dinosaur eggshells is obvious. It is clear that in every case a detailed study of the property of the microstructure of the shell may be of greatest importance for judging the phylogenetic direction for a specific egg-laying creature.

Study of dinosaur egg microstructure from China (Laiyan, Shantung Province) showed that of the two types of shell encountered there, one was characterized by a thickness of 2 mm and a two-layered structure. The upper layer was porous or prismatic, having a fibrous structure, and is divided into three zones corresponding to their density and organic content. The lower layer is papillary and takes up 1/3 of the general shell thickness. This layer is dense; it consists of closely set papillae. Both layers are perforated by aeration channels, which grow wider in the lower section of the porous layer and contract in the papillary zone. Shell remains of another type also occur in the site at Laiyan. The generally small shell thickness corresponds to it, and the absence of the porous layer and the unvarying width of the opening of the aeration channels (Chow, 1951).

In the following years, A. V. Sochava carried out a series of studies of shell microstructure for a large number of dinosaurs (1969, 1971, 1972). It was established that the shell is divided according to it’s structure into two main types – single layer and double layer, corresponding to the analogous division of the shell of the Chinese dinosaurs. In the two-layer shell, with an internal papillary and external porous layer, the aeration channels become narrow, of equal width (angusticanicular type) and expanding. In single-layer shells there are many very dense channels (multicanicular type). The eggshells from Mongolia and Kazakhstan (evidently also from China) belong to the general type, designated the ornithoid type, which have a structure similar to bird shells. The second type, the testudoid, is characteristic of dinosaur shells from southern France. It resembles the type of structure of tortoise eggshells (Sochava, 1971).

The significant progress mentioned above in the study of morphological traits in eggshells of extinct animals, in the case of dinosaurs, with the use of the tool of optical microscopy, established the suitability of deeper study based on utilization of contemporary methods of electron-microscope research. In recent years, these methods have produced even more applications in the field of paleontology. To carry out the most accurate and precise micromorphological research in paleontological subjects, utilization of the scanningelectron microscope has special significance (Barskov, 1973). In this connection, it is impossible to avoid mentioning the important part played by the special journal "Biomineralization Forschungsberichte", in which were published reports especially concerning the ultrastructure of the skeletal formation of different creatures. These reports concerned the ultrastructural formation of egg shells of contemporary and extinct reptiles and birds (Erben, 1970).

In addition to the paper mentioned above on eggshell microstructure research in Asiatic dinosaurs, it is necessary to point out that an analogous investigation was conducted on suitable material in southern France (Dughi and Sirugue, 1958, 1964; Schwarz et. al., 1961; Schmidt, 1967; Erben, 1970).

In a series of publications elucidating the results of this investigation, the paper by A. Müller is of special interest (1963), in which the data of the electron-microscopestudy of dinosaur eggshells is published, and which was conducted by the Geological Institute of the Gorny Academy in Freiburg. In a cross-section of a dinosaur eggshell from Southern France, there were discovered the characteristic calcite prisms of cylindrical or conical shape, which were arranged vertically relative to shell surface and over the extent of the entire external layer of the shell. Apparently homogeneous, the prisms appear under the electron microscope to consist of points of miniature crystalline fibers. Obviously, these prisms are connected to the main shell section at a very late stage of its formation. Here, as matrix, it probably serves as a fibrous restraining substance, the fibrils of which have regular orientation. This determines the posterior distribution of the crystals forming between them (epitaxial process). As a whole, it was established that according to the character of the microstructural elements, the dinosaur egg shell has the same structures as the contemporary reptile (Müller, 1963).

Recently, another short report was published on the study of egg shell structure in some Cretaceous reptiles and birds (Mezheevska, 1976). In the processing of the data in the work, scanning and transmitting electron microscopes, and also a polarization microscope were employed. In the studies of the egg shell fragments from Mongolia, one turned out to be attributed evidently to a dinosaur, the others to tortoises and lizards, and also birds. The shells of some eggs were clearly bulging, which is considered a teratological phenomenon. The author of this paper assumes that, electron-microscope studies of the shell can assist in clarifying the taxonomic position of fossil eggs.

We also undertook a study of dinosaur egg shells by using a scanning electron microscope. The material that we had available was collected by Soviet paleontologistsin Mongolia, in the region mentioned above, Bayn Dzak and Zaysan Coomb by the Tayzhuzgen River. We obtained the Mongolian shell specimens from the Paleontological Institute of AN SSSR, and the Zaysan specimens from the Institute of Zoology AN Kazakh SSR. We take the opportunity to express our thanks to the designated institutions.

In order to conduct electron-microscope shell research, very small sections (2-3mm) were separated from the specimens at our disposal, which were then glued to small copper disks (stands) so that external and internal shell surfaces were equally suited for scanning (photography or direct research). The adhesive sections were covered by a thin layer of gold in a vacuum evaporation chamber of type Zeiss NVA 1. The material thus prepared was studied by means of an electron microscope of type JEOL JSM 50 A in the Department of General Zoology and Comparative Anatomy of the University of Budapest.

Shell specimens No. 1 and No. 2 (fig. 1) collected at the Tayjuzgen River in Zaysan Coomb (Kazakhstan) were very little separated from each other. In surface sculpture, such sculpture is fairly typical for dinosaur eggs, not only at the Zaysan site, but also at the borders of Mongolia. Emerging on the entire surface are round or oval tubercles (Spec. No. 2) or separate tubercles which alternate with low and rather short ridges (Spec. No. 1). They represent constructive elements which increase shell strength with comparatively little thickness. At the same time, such external irregularity of the shell, which is normal for dinosaur eggs, provided better attachment of the egg surface with the fragmentary particles of the base and prevented it from rolling out from the clutch in the nest.

In Specimen No. 1, the crest in the surface sculpture is partially orientated in a single direction, but the rest are orientated in two directions and they are rarely united with each other. The crest surfaces and the spaces between them appear smooth under slight magnification, more rounded and aeration pores are not apparent. With magnification up to 1000 and more (fig. 2 and 3) the surface appears to be made up of very fine crystalline grains more or less equal in size (only a few stand out of comparatively large dimensions). These grains are very loosely scattered everywhere and are grouped closely together. Cavities appear between them, which evidently lead to a row of points of entry for concealed aeration channels. These openings (outlets) appear as independent micropores, which are generally rather numerous. It is possible that they take the place of the very rare but coarser external pores on the eggshells of other species.

The internal surface of Specimen No. 1 (figs. 4 and 5), characteristic of the papillary layer, is made up of papillae closely packed together, which even when magnified 1000 times (fig. 4) produces an effect of a loose, but more or less homogenous texture. On the other hand, under much greater magnification, it appears that the papillae seldom run together into large groups. These papillae are round and terminate mainly at one level at the inner surface. Somewhere between them internal pores appear. Next to these sections, islets of smooth surface appear with very thin, small porosities. As always, from within the shell surface, a great number of adhesive brownish-red ferruginous small particles of grit are attached. In some places the papillae (fig. 5) are lying side by side. In these it is possible to observe the structure of the lower base of the sphero-crystals with the distinct layering of the Karastan isospherites. The remaining small crystallites arranged adjacently are of more even parts; here the openings of the inner pores are also visible. In more level sections, the sphero-crystals are tightly joined and their bases do not stand out in the form of papillae.

The papillary layer is 6 times thinner than the porous layer. In the place of contact, the isospherites are radially in contact, so that durability of texture is provided. In the fracture of Specimen No. 1 (fig. 6) laminar structures with straight-line grains, orientated to the right, approximately parallel with each other and located perpendicularly to the surface, are clearly apparent.

The external surface of Specimen No. 2 (figs. 7-8) is characterized by rather even but distinctly raised protuberances. The protuberances are rather high, not quite in contact with each other. Under low magnification the surface appears smooth ("bisque-like"). Parts of external pores are in the depressions between the tubercles. Under great magnification, an extremely dense combination of crystals is revealed, partially coalescing, partially making contact with each other only at the radial border. At the point of contact, depressions are visible (micropores). In all, the external surface of Specimen No. 2 appears more even, compared to the shell surface in Specimen No. 1 (fig. 8).

The inner surface of Specimen No. 2 is entirely formed of more or less spherical isospherites (fig 9 and 10). The adjoin each other very tightly and only at the opening places of surface discharge, the perforations of the inner pores, are the isospherites separate (fig. 9). At pronounced magnification, there were discovered distinct layers of concentrated and increasing sphero-crystals which are tightly joined together at separate points of contact. Between them (sphero-crystalline papillae) depressions are seen, separated by connecting strips proceeding from one isospherite (papillae) to the next. The internal pores are clearly observed (fig. 10). In the fracture the given shell turns out to be mainly related to that which was observed in the fracture of Specimen No. 1, but the laminae for Specimen No. 2 are thinner and incline sharply to the oblique (fig 11).

From the two shell fragments from Mongolia which we studied, only Specimen No. 3 shows some relationship with those specimens from Kazakhstan (fig. 12, No. 3), but the tuberosity characteristic in the given cases are rarer in the Mongolian fragment, although the protuberances themselves are smaller and lower than in Specimen No. 2 from Kazakhstan. Somewhere on the surface of Specimen No.3 the tubercles coalesce into small short crests. The shell surface between them is extremely porous and very rough.

At great magnification the external shell surface of No. 3 brings to our notice the smoothness of a substantial group of crystallites,which appear as if melted. They are united at the radial borders but do not coalesce. In a row with such crystallite unions on the risings [nodes], distinct depressions appear randomly in different places (figs 13 & 14).

The internal surface of Specimen No. 3 is characterized by a large array of pores. There are no distinct papillae, but in reality they occur in a state of tight fusion with each other. Subsequently, no typical isospherites are seen. The main part of the inner surface differs in the extent of the fixed tendencies of the rather distinct flagellum-shaped coarse grained strands, between which are fossae and pores of different depths. Between these calcite bands, close grained heaps are evident, often separated from larger ones but in a series of instances forming heaps and further accretions (fig. 15 & 16).

In the fracture of Specimen No. 4, it is clear that the porous layer is very dense. Narrow aeration channels are very straight, exactly perpendicular to the shell surface and of identical diameter in their entire extent (the angustocanicular type). The density of the porous layer is determined by the compact distribution of the crystalline structures and strongly orientated to their even facets. They have the appearance not so much of laminate formations as of monoliths, of independent rectangular blocks (fig. 17).

The internal appearance of Specimen No. 4, which came from Mongolia, as did Specimen No. 3, differs in that straight ribs [ridges] in high relief traverse the external surface, which are orientated parallel to each other, in unbroken manner to a large extent (fig. 12, No. 4). In the depressions between these ribbing elements of surface relief, and also on their lateral sections, pores are rather densely distributed. The shell surface is very rough in the depressions.

When we magnify the external surface of Specimen No. 4, one of the rising sections is clearly visible with a smooth, level keel (figs. 18 and 19). Its laminae are rather distinct. In the depressions lie rows of loose uneven crystalline lumps of different sizes (here also are attached small grains of sand or rock, sandy dust, which is also observed in other preparations).

The entire surface of Specimen No. 4 is smooth, "sugary", and with pores visible in some places. Here and there we discover sections where isospherites are very evident (fig. 20). On these sections, they develop as formations which are convex and smooth at the base and round-spherical and drawn out with radial edges at the places of contact (fig. 21). The radial projections do not make such contacts with all isospherites, because at their very base they stand out to some extent. Furthermore, with the transition to exospherite, the position of these outgrowths and the edges of the prisms of the porous layer are set; this layer is tightly compressed and deep.

In the fracture of Specimen No. 4, a pattern appears, roughly related to that which may be seen in the fracture of Specimen No.3. In this instance there are also very perceptible large crystalline blocks, which form rows from which compact masses of crystal-prisms of the porous layer are made up.

Dinosaur egg shell specimens of ornithoid type from Kazakhstan and Mongolia are constructed according to a general plan. The two-layer structure and the presence of narrow aeration channels are innate for all of them. These channels have an opening of similar width for their entire extent (angusticanalicularshell, according to the classification of A. V. Sochava, 1969). Research into the general morphological character of such a shell was supplemented by research into the slides of the crystalline structure of the calcareous material of the component shell using the polarizing microscope (Sochava, 1971). The lower (internal) layer of the shell, called the papillary, consists of cones of sphero-crystals. Their bases are isolated from each other (more or less) and located below the level of the shell membrane. They are called isospherites and are not preserved everywhere. Above, their presence was noted on some parts of the specimens investigated. The upper parts of the papillae are made up of exospherites, which are tapered at the edges of the papillary layer and columnar in their formation of the porous layer, or transformed into prisms, as the exospherites here appear as prisms, firmly joined by surface ribbing [ridges]. The latter have radial configurations on the tangential sections in the region of the porous layer.

A.V. Sochava (1969) made the suggestion that the shell of ornithoid structure might be peculiar to the eggs of ornithischian dinosaurs (Ornithischia) and those of testudoid structure, to the saurischian dinosaurs (Saurischia).

Consequently, researches into the ribbed shells from the Zaysan Coomb and the Gobi which belong to the angusticanicular type, as a variant of the double-layered ornithoid shell, may be classified as hadrosaurs or, in any case, to Hadrosauridae, which were very widely distributed among the Ornithischian dinosaurs in the Late Cretaceous. The American paleontologists have another opinion on this score. Their opinion is that an egg with a shell of such a type could be assigned by them to other related ornithischian dinosaurs and referred to by a determined genus and species *Protoceratops andrewsi* (Brown and Schlaikjer, 1940). The criterion for this is that the remains of these dinosaurs were found together with the remains of the eggs under consideration. On the other hand, in the past the distribution of *Protoceratops* was comparatively narrowly localized [geographically], but such a shell is very widely represented at diverse Asiatic sites. At the same time, corresponding to one of the taphonomic locations, the remains of eggs and animals that deposited them must not have been buried together at that time (Efremov, 1950; 1954). Thus, the supposition about hadrosaur as the origin of the shell described here is highly probable.

At present, the reasons shown above for the taxonomic classification of the shell studied here may not be specifically determined as to the level of species or even genus of this material. On the other hand, a comparative analysis of the results of this microstructural research leads to the conclusion of obvious differences between the Zaysan and Gobi specimens. Their descriptions above and the pertinent scanning review of the attached scanophotography is convincing. It is possible to believe that these specimens belong to different genera of the Hadrosauridae, that they agree with the approximate geographic separation of the given sites (Zaysan-Gobi), and also with the different geologic ages. The Zaysan material is stratigraphically limited to the very highest limit of the Maastrichtian (or even of the Danian stratum, if we might add the following to the Cretaceous), but the Gobi specimens are limited to the beginning of the Maastrichtian and may also be at the boundary with the Campanian.

Whereas the determination of the differences between shell specimens from Kazakhstan and Mongolia allow us to consider their species or even genera, some differences in structure of a unique morphological characteristic observed in different Kazakhstan and Mongolian specimens (Specimens No. 1 and No. 2 in one instance and No. and No. 4 in the other) probably should be considered manifestations of individual variants. The data provided at present for study of thin microstructural formations of different paleontological objects attest to very great occasional individual variations of these specimens. Quite justly because of the remarks of N. V. Tolstikova (1974), it is not necessary in every case to explain the character and tendency of such variation in order not to assume that individual microstructural features were taxonomic.

The discussion of the material included and presented here leads us to state that the use of electron-microscopic methods in paleontological research clearly raises the level of morphological analysis.

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