

# Geological Problems in the Question of Living Bacteria in Paleozoic Salt Deposits<sup>1</sup>

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## ABSTRACT

*The microscopical investigation of micro-cuts of salts reveals regularly shaped inclusions, which, by their form and size can be distinctly identified as bacteria and bacterial spores. The question as to whether these biogenous structures were still vital motivated us to carry out further appropriate experiments.*

*The geological, petrological and mineralogical character of the salt to be investigated is of decisive importance in determining the age of these bacteria. Rock salt specimens were chosen with utmost care, and only those of evident primary genesis were used.*

*If the salt proved to be of primary genesis on the basis of mineralogical tests, we could then reasonably hold the age of the biogenous elements enclosed therein to be identical to that of the rock salt.*

*Our salt specimens were taken from German Zechstein, Nova Scotia Carboniferous, Saskatchewan Middle Devonian, New York Silurian, as well as Siberian Lower Cambrian. The special mineralogical data will be exposed in detail.*

*Further convincing arguments will be brought forth to prove that the imprisoned bacteria are of Paleozoic age.*

*A new chapter in the investigation of life is opened with the bringing to light of living micro-organisms preserved in Paleozoic rock salt.*

Microscopic investigations of salt crystal micro-cuts resulted in our observing regular shaped inclusions, whose size and shape clearly identified them as bacteria or bacterial spores. The question as to whether these biogenous structures were still capable of living was the motive of our further research.

Three kinds of problems arise hereby: methodological, biological, and geological in nature. Each of the three in turn, presents us with a multitude of individual problems. The purpose of this paper is to clear up the geological questions, whereby the methodological and biological matters should be touched on briefly, having already been thoroughly dealt with at international congresses.

We first developed a special bacteriological method by means of which the sterile isolation of the bacteria within the salt can be assured, and which excludes the slightest trace of secondary contamination. Then these bacteria were allowed to multiply in an appropriate culture, thereby providing their vitality.

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Of decisive importance in determining the age of these bacteria is the question of the geological, petrological, and mineralogical data concerning the rock salt to be investigated. In this respect, only pieces of salt that had been thoroughly tested for primary genesis were employed.

The first successful investigations were carried out on Zechstein salts of the "middle rock salt" (Na  $\beta$ ), from the Werra series of German Zechstein. The samples came from a depth of some 50 feet above a Thuringian stratum. Drilling core samples of 6 cm inch diameter were processed. Petrologically, these are light grey, irregularly medium-grained rock salt whose average grain diameter was from 2 to 5 mm and whose layering is hardly visible.

In addition to rock salt, the microscope revealed kieserite skeletons (up to 5 mm in diameter), polyhalite aggregates (up to 3 mm in diameter), lengthy polyhalite crystals (approx. 2.5 mm long), and also partly corroded anhydrite (up to 5 mm long). Traces of sylvine were to be found in the samples, in minute inclusions (from 0.05 to 0.1 mm in diameter), as well as traces of clay substance, lying principally in kieserite and polyhalite. Local, cloudy dispersions could be seen in tiny liquid inclusions.

The following is the chemical analysis of the mineral content in weight-per cent: rock salt 96.10, polyhalite 1.22, kieserite (prevalent) anhydrite (little), scattered clay traces 2.68. The bromine content per 100 NaCl was 0.0223 weight per cent. This can be said to be a normal salt of this horizon, on the basis of the bromine content. The works of Kühn (16), and D'Ans and Kühn (5), confirmed by Dittrich in 1962 (6) indicate that the middle Werra rock salt in undisturbed formations contains from 0.020 to 0.023% bromine. Middle rock salt, according to its position in the profile must be considered to be a very great rock salt bed, intercalated between two potash horizons. The rather uniform bromine concentration throughout a great thickness (50 to 60 m) is the sign of a long period of low concentrated infiltrations during the precipitation of the salt. The infiltrations containing little salt of descended origin, counteracted the increase in the bromine content to be expected from evaporation, and reduced the concentration from a carnalite region to a polyhalite region. The occasional low-concentrate infiltrations have their petrographical expression in the heavy corrosion of the kieserite, giving the impression of a skeleton, or of holes, and also in the corrosion of the anhydrite, whose few extended crystals are jagged or fringed, or even completely wasted away. The polyhalite must also be primary, having effected a partial pseudomorphosis of the anhydrite in early diagenetic-synsedimentation. Thus, also, the salt stone from the German Zechstein is to be considered primary. The grains of the rock salt could have become coarse, to a certain extent, due to diagenesis and recrystallization, though these processes were certainly not very intensive, since the distribution of the few tiny liquid inclusions has remained cloudy and disorderly.

The stone gives the impression of density and solidity, with no appreciable loosening of the salt stone structure due to boring for samples, and this in spite of the relatively small core diameter.

If the salt must be considered primary genetic on the basis of the mineralogical research data, then one has a certain right to assert the identity of the ages of the biogene structures within the rock salt and this salt itself. Our task was facilitated in the case of the Zechstein salt when Permian gymnosperm spores were found therein. (13, 14, 17, 18).

After having succeeded in the isolation of living bacteria from these primary-genetic Zechstein salts, we proceeded in investigating salts from other Paleozoic salt deposits by the same methods.

The second group of rocks studied were Carbonic salts from Pugwash, Nova Scotia. A piece of rock salt weighing 10,480 gr. was cut out with a diamond saw and bacteriologically prepared. Stratigraphically, we may consider this piece Windsor, thus belonging to the upper Mississippian.

Macroscopically, the rock salt is white to light grey in color, irregularly fine- to medium-grained with granules of about 2 mm in diameter. It shines, furthermore, like clouded glass and is non-sparry. It is sprinkled with some grey, dense (i.e., extremely fine-grained) isolated groups of a few millimeters in diameter. There is not the vaguest trace of layering to be seen.

Darker grains with uneven edges can be observed swimming in the light rock salt mass, and whose crystals reveal a chaotic grain structure. Under microscopic analysis the former prove

to be principally fine, elongated anhydrite crystals (0.2 x 0.04) on the average), in addition to aggregates containing a little clay, and in which the anhydrite is either feathery or rosette-form. The entire salt rock is slightly powdered with clay substances in which rhombic carbonate particles can be recognized (about 0.014 mm in diameter, probably dolomite). Numerous tiny liquid inclusions swarm through the salt and appear often like a string of beads, less frequently like canal systems. The strings seem to run through many grains at the same time and to prefer certain planes.

The partial chemical analysis indicates: NaCl 94.15%, KCl 0.03%, Br 0.003%. The remaining 5% is made up of anhydrite, a little clay (dolomite, etc.) as well as rock moisture (liquid inclusions). A part of the KCl is probably adsorbed on the clay, and another part dissolved in minute liquid inclusions. The bromine content is 0.0032% per 100 NaCl.

It is our experience that a medium- to fine-grained structure is typical for primary salt stone, while sparry salt masses must be considered secondary. Liquid inclusions in oceanic salt stone are quite common and in themselves are nothing special. However, typical marks of recrystallization may be seen in the inclusions that string themselves through many grains. These strings of inclusions indicate the former films of brine or correspond to the former dehydration channels, in the case of the preferential arrangement. The channels are now crossed by the grain limits of subsequent recrystallization. According to its bromine content, the salt does not yet correspond to the very first rock salt that was precipitated out of the sea water when the saturation point of NaCl was reached. Boeke reports (2) that a concentration of from 0.004 to 0.005% would have been necessary, while Braitsch and Herrmann (3, 4) advance 0.007%. However, the infiltration of crystal water of gypsum liberated in an early diagenetic transition of metastable primary gypsum into stable anhydrite, thus causing an early diagenetic, moist recrystallization of the rock salt, might explain the relative decrease of the original bromine value in the contact of this kind of rock salt with anhydrite. The bromine content of the rock salt is somewhat reduced in this recrystallization when a certain portion of the bromine is lost together with the expelled brine. This must have been the case here, for, first of all, the many liquid inclusions ordered along specific planes and spanning a number of grains, indicate recrystallization and special dehydration channels; furthermore, the Nova Scotia rock salt, both below and above, is overlain with massive deposits of anhydrite (19), which must have been at least in part primary-metastable gypsum. These deposits of anhydrite, which correspond to the next lower level of concentration of sea water evaporation, show that after having deposited the rock salt the concentration diminished. The absence of layers in the rock salt is either evidence for the fact of a fluctuation prevailing in the evaporating basins, which already in the primary deposit hindered the constitution of layering; or, for the fact that diagenetic recrystallizations, which were rather intensive during the anhydration of the neighboring gypsum, obliterated the layering. It is probable that both took place, due to the precipitation process explained above. Greater turbulence during the depositing of relatively thin rock salt layers embedded between anhydrite packets is possible, since a relatively strong reverse current is to be assumed over the bar on the floor of the basin ("bar" in the sense of Ochsensus' bar theory) causing the loss of the highly concentrated brine which would normally have produced potash salts.

Summarizing, we may say that the macroscopic grain development of Pugwash salt stone indicates primary precipitation material, and the relatively low bromine content, and the absence of layering are to be explained by the special genetic conditions of this salt deposit.

The next rock investigated bacteriologically was middle Devonian salt from Saskatchewan, Canada. The pieces to be studied were lower lying rock salt from approximately 15 meters below the potash deposits at a depth of 1,000 meters. It is a coarsely sparry, light grey rock salt, having a diameter of from 2 to 3 cm on the average. A few detritic agglomerates of from 0.5 to 1.0 mm in diameter are to be found dispersed irregularly within the rock. No layering is to be seen. The chemical analysis shows an NaCl content of 98.28%, KCl 0.15% (predominantly in inclusions) and 1.5% of detritic substances and inclusions. The bromine content per 100 NaCl is 0.0102.

The rock salt crystals are hypidiomorphic. The little detritic substance is found mostly in clumps within the crystals, though there are also short, thin, loose stripes of it near the limits of the grains. The individual grains within the aggregate have a diameter of 0.01 to 0.05 mm and

are often in the form of elongated prisms (anhydrite, quartz). The true (illitic) clay substance lying between them is found in the form of extremely fine flakes. According to its geological position and related bromine content, this rock salt belongs to the lower or middle part of the anhydrite section. Our previous investigations of the deposits force us to conclude that the sea water of the Canadian salinary must have suffered serious metamorphosis, i.e., primarily transformed into a state poor in sulphate, and in the potash regions it seems that intensive posthumous restructuring processes must have been effected by the action of infiltrating water. The low bromine content is also a witness to this fact. An infiltration of this kind, far below the potash horizon, is, indeed, hardly imaginable. On the other hand, the very coarse sparry structure, which, as far as we know, is commonly found in this deposit, is by no means an ordinary primary formation. The probability is, that under the massive covering (carrying pressures up to 250 atmospheres), it was strongly recrystallized, or perhaps also transcrystallized under the action of mobilized and totally expelled solution remains. It is not a wonder that under these pressure brine inclusions in the salt are uncommon; it is to be assumed that they had been mostly transformed into clay during the recrystallization. Occasionally, a liquid inclusion was to be seen with libella as a negative cube having a length along its edge of 0.06 mm. The crystals are not uniformly oriented to one another, are grown together unevenly, and constitute, therefore, a structure solid enough to survive even the great relaxation caused by the removal of samples.

The Silurian rock salt from the Cayuga rock mine in Myers, N. Y., may be suspected to have suffered a slight posthumous influence. The rock salt, which was also studied bacteriologically, is irregularly grainy to sparry and whitish grey in color. The diameter of the grains is from 3 to 10 mm. It is quite pure and only occasionally is an anhydrite inclusion to be located, and layering not at all. The chemical content was: NaCl 98.67%, anhydrite, traces of clay and inclusions 1.33%. The bromine content per 100 NaCl was 0.0049. The rock is practically free of potash.

The salt crystals are joined together in uneven dovetails, and contain numerous liquid inclusions of the usual types (clouds, stripes, mud, and tiny negative crystals). A few anhydrite crystals are found in irregular dispersions, from 0.05 to 0.25 mm in diameter. The largest crystal, an exception, has a diameter of 0.75. The anhydrites are partly idiomorphic and partly corroded. The bromine content lies somewhat under the value normally to be expected for primary rock salt of oceanic origin. The salt might have been formed by a process similar to the one described for Pugwash rock salt, i.e., early diagenetic infiltration with liberated crystal water from the transformation of gypsum into anhydrite. The conditions in the deposit (ref. Lotze 19) also confirms the probability of this explanation. Thus we must assume an early diagenetic transcrystallization. The structure of this sample is also to be characterized as dense, the crystals being so solidly grown together and dovetailed.

Finally, we studied lower Cambrian rock salt of the Angara series (cmjan) from Irkutsk, Siberia, taken from a depth of 230 meters. It is a brownish-yellow, coarse sparry salt stone, with grains from 2 to 5 mm in diameter, for the most part constituted by rock salt. The latter contains tiny grey detritic agglomerates, sprinkled to cloudy, at millimeter distances, and which the microscope reveals to be anhydrite, quartz, and clay. The rock salt content is 98.57% weight, the detritic substance in inclusions 1.43%. The bromine content per 100 NaCl is 0.0181% weight. The salt is practically free of potash. The yellowish color might be imputed to the presence of iron oxide, but this does not appear in the micro-cuts. The diameter of the detritic aggregates is from 0.5 to 1.0 mm, on the average. These are often extended and lie on the grain boundaries, though they are also to be found lying directly in the coarse sparry salt. The individual grains (quartz, anhydrite, clay) within the aggregate are from 0.01 to 0.03 mm in diameter on the average, but finer ones are also present in clumps of flakes. The rock salt contains minute liquid inclusions in the usual manner, i.e., in the form of swarms or stripes, although sometimes also as coarse negative cubes and as canal-like systems.

The bromine content indicates that the brine, corresponding to the lying portion of the polyhalite region, must have been concentrated. The coarse sparry salt crystals are unevenly and densely dovetailed, thus producing a solid resistant structure in spite of the relatively small diameter of its grains (6 mm). The stability of this structure may also be imputed to the rather unimportant relaxation of the rock after extraction from such a modest depth, where the pressure is only 70 atmospheres.

Concerning the mineralogical findings of the salts investigated bacteriologically, we may say, in summary, that the isolated living bacteria are of primary genesis. In any case it is extremely improbable that at a later stage foreign waters from the rock strata neighboring the salt deposits could have brought in the bacteria.

The compactness of the pieces is also a powerful argument against a secondary contamination posterior to their extraction from the rock group. The irregularly medium- to fine-grained structures seem significant as regards the bacteriologically irreproachable extraction of the samples. Even if the rock salt should have relaxed during its extraction from the rock group as a result of the sudden absence of mountain pressure, or even if it burst into fine particles along the grain boundaries into which traces of air might then penetrate, the grain bonds would still act as a perfect filter. The angularity and the numerous ramifications of these bonds protect the interior of the salt specimens bacteriologically, in the same way as would the filter effect a modern bacteria pump.

The bacteria inhabited the seas or parts of the sea, which, once dried out, left the salt deposits behind. During the precipitation of the salt, the microorganisms must have been imprisoned in the developing salt crystals, within which they can be seen today, in micro-cuts. The important finding is that the bacteria are not located at the edges of the crystals, in cracks, but in the very center of the salt crystals.

In order to imitate the natural process of dehydration, we experimented with drying and salting methods. If a culture bouillon of non-spore producing bacteria is slowly dried, then these bacteria cannot be brought back to life, even by a subsequent addition of fresh bouillon, for they were killed by the desiccation. A different result is produced if a culture solution is enriched by a weekly addition of 1 gram of sterile salt, until a supersaturated solution is reached. If this substratum is slowly dried until the salt is thoroughly dry and crystalline, then the same bacteria can be shown to possess the identical vital and reproductive capacities after 5 years of total desiccation when the salt crystals are placed in a fresh nutritional solution.

Counter-controls were also carried out. Pieces of salt were heated for 4 hours to a temperature of 200 degrees C. before being studied bacteriologically. This salt was sterile.

In addition, pieces of salt were studied that had been obtained by boring to a depth of over 4,300 meters, i.e., out of the Holstein Zechstein. These salts lay 1,000 meters deeper during the Mesozoic era than they do today, so that they must have had a temperature of about 160 degrees C. due to the geothermal level. These pieces also had no life in them.

In conclusion, there is the question of carrying pressures to which our bacteria in the salt stone are subject. At a depth of 1,000 meters and more, the overburden pressure is at least 250 atm. However, this is relatively little compared to the pressures bacteria receive in the laboratory: up to 4,000 atmospheres. Furthermore, we may be sure that due to the particular architecture of the crystal structure, the bacteria have been quite well protected from possible damage caused by mechanical pressure.

The evidence of living microorganisms preserved in the rock salt of the paleozoic, and recently found also in the Mesozoic era, opens a new chapter in the science of life.

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