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# The PA & SAPA Bion Experiments and Proto-Prokaryotic Biopoiesis

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### Abstract

The entire range of Reich's PA and SAPA bion experiments - obtained by exposing tissue or varied crystalline or amorphous materials to extreme heats (of sterilization, autoclavation and incandescence) - is critically examined and largely replicated. We find that Reich's claim to the generation of 'PA bions' *anew* from decaying tissue (heterogenesis) or the swelling of inert materials (abiogenesis) lacks experimental proof, and that his notion of PA bions is too variable, even as a symbiotic collectivity, to allow precise taxonomical identification or a precise use. Yet, there is little doubt that in very different experiments Reich obtained diverse prokaryote-like cells which were extremely resistant to high heat. Whether these prokaryote-like cells were issued from thermally hyper-resistant spores or extremely thermophilic cells remains an open question in each specific instance examined. We also find equally wanting Reich's claim that 'SAPA bions' obtained from incandescent sand were generated *anew*. On this subject, we experimentally and formally demonstrate that it is possible to identify SAPA bions with the cyanobacterium *Myxosarcina*, and that it and a variety of closely-related oceanic sarcina also found in the SAPA experiment cultures present an unsurmised resistance to high heat and, whether in packets or as single baeocytes, exist as inclusions in carbonaceous and vitreous (quartz and quartzites) ocean sand.

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## COMMUNICATION

"The particles [into which calcinated coal disintegrates] change into vesicles indistinguishable in shape from spores. To my mind, this experiment utterly refutes the spore theory as it now exists, for no one can prove that spores can survive the temperatures generated in calcination." W. Reich, "The Bion Experiments on the Origin of Life",1938, p. 109

#### 1. Abiogenesis and compression of the biopoietic time factor

Reich's theory of the bions (*Blätschen*, or vesicles) can be summarized by the following: given all the macromolecular materials (amino acids, sugars, nucleic acids, fatty acids) of a prebiotic soup, one can greatly compress the evolutionary biopoietic time factor by forcing high energy injections (eg incandescence) onto specific substrates (sand, coal, iron, etc) that are at the same time chemically made to swell and vesiculate. The critical thought is that swelling of matter, in particular, crystalline matter, seeds the structure of a vesicle or globule which, if infused suddenly with energy and exposed to a rich protoplasmic soup, will give rise to a pre-biotic element, a bion. The blueprint of cellular life would be found in the crystalline structure of matter, and one needed both the mechanical swelling of matter and a sudden infusion of energy in order to bring it to life. The blueprint of life was a mold hidden but inherent to matter. Spores did not survive calcination; spores *were* swollen vesicles of matter ('bions') that, under the right energy conditions, would give rise to microbial life. This is the essential insight that, in Reich's mind, served as departure point for his investigation of the bions.

Proving this heretical contention was tantamount to state that cellular life is a far more common and inexorable event than modern biology would have us believe. Ultimately, if there was heterogenesis of proto-protozoa from tissue cells (as Reich claimed was the case), and if this process was mediated by formation and aggregation of PA (Packet-Amoeba) bions <sup>[1]</sup>, it was because abiogenesis of these bions or vesicles from highly energized mixtures of select molecules was possible to begin with. Given the minimum necessary mixture of organic and inorganic compounds along with enough energy, Reich reasoned that he should be able to obtain identical pre-biotic "energy vesicles", and eventually spontaneous protozoal generation analogous to that observed to occur from the "bionous disintegration" of tissue <sup>[1]</sup>. In autoclaved mixtures of lecithin with 0.1M KCl (aq. solution) he observed formation of fluid, tubular structures that could be ascribed to the intake of fluid by the lipid - a result of the KCl-induced swelling and the applied heat. If he added egg white (or aminated gelatin) and cholesterol to the autoclaved Lecithin/KCl preparation, he claimed that four different types of moving structures could now be found: bacillus-like rods, nucleus-like vesicles or cocci, celllike nucleated forms that "move about but do not display any inner motility" and amoeboid struc-

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tures with inner motility or pulsation <sup>[2]</sup>. He made the bold claim that all these abiogenically formed structures were cultivable, and - rather unfortunately from a microbiological viewpoint - applied the term 'bion' indistinctly to all of them <sup>[3]</sup>.

The cultivability of these "bionous" rods and vesicles argued against them being taken as mere pre-biotic vesicles - such as one might for example employ the term to designate Oparin's coacervates, micelles or Fox's proteinoid microspheres. In the case of coacervates or micelles (solubilized salts of fatty acids), the alkylcarboxylate ions are distributed with their negative and hydrophilic charges at the periphery for maximal repulsion, and the micellar core is occupied by the agglutinated, non-polar, hydrophobic, lipophilic portion of the molecules <sup>[4]</sup>. Whereas Reich's "energy vesicles" were, most of the time negatively, but sometimes positively, charged at their core and the lipophilic component was peripherally located. In the case of Fox's microspheres <sup>[5-6]</sup>, the peripheral component is a thermal protein layer, and - though microspheres are capable of globular replication in solution - they cannot be passaged onto semi-solid media. Moreover, none of these prebiotic micelles, globules or microspheres possess a metabolism permitting them to autonomously accumulate internal energy. Further, Reich claimed that the vibratory and rotary movements of bion vesicles, as well as their quivering displacements, were the result of internal forces, and not due to the external forces that cause Brownian motion of micron size particles in solution (see below) <sup>[7]</sup>.

If Reich were right in claiming for PA bions cultivability in agar - something which Fox's proteinoids cannot do - along with all the basic properties of cellular life (cellular and DNA replication, unity of metabolism), then a major revolution in evolutionary biology would yet have to be carried out: for, somehow, a complete teleonomic apparatus would have to be assembled in the bion vesicles in much the same way as we now know that proteinoid bodies are formed in chemically-rich prebiotic soups driven by sources of thermal and electrical radiation. Whether or not Reich's cultures were cultures of vesicles ('bions') generated *de novo* by these procedures, or cultures of unwitting bacterial contaminants is a distinct but most pertinent question - one which we will constantly strive to examine throughout the present report <sup>[8]</sup>.

In Reich's view, heat treatment of living cells or tissues would at first lead to their death by disintegration, and - in the case of prokaryotes and protozoa - stimulate their spore and cyst formation. But beyond these thresholds, treatment with greater thermal energy appeared to become an "organizer" of prebiotic soups (mixed solutions of the minimum required organic and inorganic molecules), the process that catalyzed or induced the abiogenesis of prebiotic vesicles and even nucleated protocells (proto-amoebae). Heat was both a destroyer and creator of life, as if its relation to living systems presented a kind of thermodynamic catastrophe that explained thermally-induced abiogenesis: after having disordered the molecular matter of living systems and broken down its noncovalent

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Fig. 1 - Bénard instability in superheated aqueous fluid solutions forms a regular lattice of hexagonal cells.

and covalent bonds, at higher thermal energy densities still, there would be a kind of "spontaneous instability of Bénard" (see Fig. 1) or order by fluctuation <sup>[9]</sup> that re-ordered the molecular materials back into living cells through the intermediate prebiotic stages of the bions or "energy-vesicles". According to this interpretation, life was therefore indestructible - only cells or individual systems were perishable. Given the right conditions (energy and molecular materials), vesicles would form that would absorb and withhold energy, presenting a 'spontaneous reverse potential of energy flux' and laying the ground for their further development into aggregates or packets, and the subsequent evolution of these packets into nucleated cells or primitive protista.

Thus, it seemed to Reich that time-compressed biopoiesis of prebiotic vesicles and protobiotic cells was a laboratory reality, and that his bion theory could actually explain endogenous tissue degeneration and breakdown (bion vesiculation) caused by sympatheticotonia-induced anoxia in muscle and tissue cells. To objectively prove his contention of abiogenesis, he carried out several series of infusion experiments with a variety of materials, all within the previously specified parameters (application of high heat, swelling agents, antagonistic lipids, introduction of nutrients, cultivability of inocula, etc). In autoclaved soil infusions, he observed the formation of vesicular inclusions which were capable of detaching themselves as free bluish cocci and greenish rods, and which associated themselves in diplo-, strepto- and staphylo-aggregates that, by surrounding themselves with a membrane, coalesced into "pseudo-amoebae" that he claimed were able to undergo mitosis. The same vesicular swelling and formations were observed with iron and zinc filings placed in colloidal solutions, but the rods were now absent. With dry-sterilized coal dust (autoclaved) infusions into 0.1M KCl and sterile beef bouillon, he again observed the appearance of positively-charged, large bluish cocci, bacillar rods of predominantly two sizes, and also amoeboid forms [10-11]. The same results were obtained with coke taken to incandescence <sup>[12]</sup> (benzene gas flame at 1500°C) prior to the infusion (and when peripheral blood was autoclaved <sup>[13]</sup>).

Cultures in agar of inocula from these varied infusions and incandescent preparations predominantly yielded cocci (round vesicles), whereas inoculation back to broth predominantly yielded rod and amoeboid forms. The PA cocci or vesicles stained Gram-positive, had diameters of 2 to  $6 \mu m$  <sup>[14]</sup>, and could form "packet amoeba" with a 10 µm OD. Later, in "*The Cancer Biopathy*", Reich differentiates between PA bions and ordinary cocci on the basis of size, claiming PA bions to be larg-

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er than the coccus unit of staphylococci and streptococci - that he gives as 1  $\mu$ m <sup>[15]</sup>. However, the OD of most bacterial cocci or single coccoidal units actually ranges from 0.4 to 3  $\mu$ m, fully overlapping with the most frequent size of 2  $\mu$ m given by Reich for single coccoidal PA bions. Two important notes should be taken into account, at this point. First, the questions raised by the microscopic technique employed to make the OD measurement (given a comparative high-quality of objectives employed). For example: whether the measurement of an OD for very small microbes is made to include or not the refractile ring or 'aura' of the microbes (our own measurements always exclude the refractile ring; but we suspect that Reich included this ring in his measurements), and which technique was employed in making the measurement, since the ring is always greater with brightfield than, say, with phase-contrast. Secondly, there are the questions raised by the fact that our own replication of Reich's methods to generate PA-type cocci, irrespective of source, has largely and mostly produced cell-free lysates of mitochondria <sup>[13]</sup>, or of mitochondria and plastids in the case of treated plant material, and these free mitochondrial vesicles (what Reich would call PA bions) are for the most part round vesicles with a typical OD of 0.75  $\mu$ m, and an OD range from 0.45 to 2 $\mu$ m (with fewer vesicles, in general, being greater than 1.4  $\mu$ m).

Reich also claimed that when PA cocci were added to *Bacillus subtilis* cultures, or mixed with other Gram-negative bacilli, they appeared to immobilize the latter <sup>[14]</sup>. Electrophoretic experiments indicated that the vesicles utilized in inocula that had yielded no cultures in agar were electrically neutral, and that the electrical charge of the vesicles was a precondition for their cultivability in semi-solid media <sup>[16]</sup>. Galvanization of these packet cocci and of the derived "amoeboid formations" (by repeated changes of the induced polarity) succeeded in many cases to reverse and re-reverse the electrophoretic direction of their migration <sup>[10]</sup>.

Once Reich concluded that he could observe 'spontaneous formation' of bions from preparations of molecular materials, he could now claim that it was possible to energetically compress biopoietic time. This elucidated his heterogenic theory, inasmuch as the biopoietic time factor in the laboratory setting was long when bions were generated from chemically-induced swelling and soaking of decaying tissue, and shortened dramatically when tissue was made to disintegrate by heating and/or incandescence. If vesicles capable of replication in semi-solid media could be produced from a high energy injection into the right mix of molecules, then the generation of PA bions was already the creation of a protocell, the PA bion being not a prebiotic vesicle but a prokaryote, specifically a protoprokaryote. This is to say, irrespective of how Reich conceptualized the "cell" <sup>[1]</sup>, Reich's bions must be assumed to constitute effective proto-prokaryotic cells.

So the first question which now arises in the present analysis is whether Reich did, or did not, in fact, discover a methodical series of chemical and energy-injecting steps capable of inducing or cat-

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alyzing the spontaneous organization of matter (chemical substrates) into simple, non-nucleated protocells capable of DNA replication and cellular reproduction (fission). This question, then, specifically concerns the abiogenic component of Reich's "Bion experiments".

Nothing can or should be presumed *a priori* in this query. All is to be investigated first, and one must keep an open mind: it was only yesterday that the dogma that 'life only comes from life' was buttressed by the mediatic notion that cellular spores travelled the cosmos aboard meteorites or comets - a notion that adds literally nothing to the problem of the origin of cells. Then that dogma was reinforced by the notion that DNA embodies "dictating" genes, and information only travels from DNA ro RNA to protein, with no return pathway. Yet - for there is always at least one "yet" in biology - our molecular biologists have since then encountered retroviruses and the reverse transcriptase whose code those viruses carry - and we were all forced to realize that every tissue cell, and even bacteria, has a certain concentration of this enzyme that creates DNA from RNA templates.

So then, that first question that arises in the context of Reich's "Bion experiments", can be reformulated as: did Reich in fact succeed in compressing the evolutionary time factor by the amount required to generate fully formed, energy-metabolizing, DNA-carrying, self-replicating prokaryotic cells? An answer "yes" is tantamount to claim a feat that no modern biologist, with all that is known, is willing to accept as possible. One might put bridges forth - that hydrophobic proteins can preserve fluid structure at high temperatures, that heat or other forms of radiative injection might induce high speed chemical reactions, that hydrocarbons can be regenerated upon cooling prebiotic-type solutions, that aqueous solutions acquire a lattice structure at very high temperatures, etc - but all bridges will hit a single wall: that, at high temperatures, no hybridization or stability of nucleic acid polymers is possible. Since that is not possible, all possible polymer fragments will result upon cooling, the chances of even one reconstituted series of fragments matching the minimum code required by the simplest prokaryote being infinitesimal.

The task of the open-minded scientist is, however, clearcut. First, Reich must be taken seriously with respect to his procedures and observations. These, one can, of course, confirm for oneself in the laboratory by replicating his work in part or *in toto*. Such an experimentalist attitude precludes dismissal off hand, and any light-headed notions that his techniques just were not sterile and one is dealing simply with air-infection by staphylococci, micrococci, and so on. One can certainly admit that Reich might have made mistakes, that here and there the sterility of his technique or those of his assistants was not or might not have been the best, or what, it turns out, is actually required to destroy prokaryotic spores. But these claims must be based on solid facts, new or well founded facts, that can explain Reich's own results. Which brings us to the second critical question posed by Reich's "bion experiments": could his observation of what he thought was the abiopoietic or heteropoietic assem-

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blage of PA bions in an extreme variety of preparations (that encompassed a diversity of sources - from living tissue, to putrefacting tissue, to organic and inorganic crystalline substances) have been an artifact produced by a poorly-studied, much greater resistance - than currently imagined - on the part of the spores of endospore-forming bacteria to moist heat killing?

An answer "yes" to this second question - fascinating as the case may be - would entirely abrogate the biopoietic arguments of Reich. But it would indicate that such bacteria resistant to heat killing had to be far more common, if not ubiquitous, than current microbiology has dared to contemplate. At any rate, a careful examination - analytical and experimental - of Reich's sterilization procedures and sterility techniques is a necessity, if ever either of these two questions are to be answered, one way or the other. If a thermally-resistant spore-based explanation is not eliminated by experimental studies, the conclusion of biopoiesis cannot effectively be validated. But if a thermally-resistant spore-based explanation is confirmed, then Reich did not discover cellular biogenesis. However, what then he would have encountered would be no less dramatic - that certain spore-formers can withstand conditions which are dogmatically considered to be incompatible with cellular life.

## 2. The biological characteristics of PA bions

We have seen above how Reich claimed that all living tissue (made up of eukaryotic cells) would decompose, spontaneously or in response to defined stresses, into rods (bacilli-like) and cocci (round vesicles), which together formed - in his theory - what one could call a 'bion multiplicity' connected by a notion of pleomorphism, namely, that these were different forms of the same PA bion. More specifically, Reich claimed that expression of different bion shapes was a function of culture media and the nature of the dissolved gas - anaerobic rods and small cocci predominated in putrefaction, whereas large aerobic cocci predominated in normal inflammatory responses or the induced breakdown of healthy tissue.

In Reich's view, the bions were not pre-existing infectious particles or micro-organisms, but biogenic productions - either *a-biogenic* in their formative process (ie assembled from molecular materials) or *heterogenic* (ie assembled from the molecular materials released from decomposing cells).

According to Reich, the PA bion cocci came in two varieties: the large blue ones (~1.5 to 2  $\mu$ m in diameter) observed when healthy tissue was stressed chemically, thermally or mechanically; and the smaller blackish ones (<1  $\mu$ m in diameter), observed when biopathic (diseased) tissue was equally stressed or broke down on its own in the course of an evolving process of disease. The rods varied in length between 2 and 6  $\mu$ m, and were observed only when biopathic tissue decomposed.

The critical identification provided by Reich relates to the large PA cocci. Other characteristics of these forms are extensively discussed by him: he claimed these were blue to blue-green *in vivo*,

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aerobic, observed in singlets (monococci), diplos, tetrads, irregular packets (staphylococci-like) and sarcina. The PA cocci showed a positive Gram stain reaction <sup>[17]</sup>, a fact confirmed by Du Teil <sup>[18]</sup>. In agar, they formed transparent, or blue-gray growths or, less often, yellow <sup>[19]</sup> or yellowish-white colonies - indicating that they could contain pigments. In general, first generation cocci were anionic, whereas second generation cocci (and the 'packet amoebae' in particular) were cationic <sup>[20]</sup>; upon galvanization, the 'packet amoebae' (ie sarcinoid) structures would reverse direction by becoming anionic <sup>[21]</sup>.

Reich also claimed that these PA cocci exhibited a variety of movements - vibratory and rotary motions, and oscillatory displacements - which he considered to be the result of internal forces and an autonomous deployment of internal energy (later on, he would specifically consider these movements as the result of the "orgone energy content" of the bions). We shall examine each of these characteristics in turn. However, to discuss or present the biological characteristics of the bions the way



Fig. 2- Dilution of pasteurized milk 1:3 in  $\alpha$ MEM (phase contrast, oil immersion). Hanging drop slide, 100x Ph3, 4x projective. Filmed with Sony TVR68 CCD camera, 19.6x optical zoom (320 kilopixels). Largest round vesicles are ca. 2µm in OD (print magnification: 1,900x). They are pinocytotically-extruded fat globules, and so are all smaller round vesicles with a whitish core, which appear bluish in darkfield. Also present are: (1) the aerobic, gram-positive cocci (OD 0.5-1µm) of *Streptococcus lactis, Streptococcus cremoris* and *Streptococcus thermophilus* (OD 0.7 - 0.9 µm); (2) the fermentative Gram-positive, spherical (OD < 1µm) to lenticular, staphylo-like *Leuconostoc lactis* and *mesenteroides* subsp. *cremoris*; (3) the closely related, Gram-positive, coccus-to-rod pleomorphic (predominantly rod-shaped), *Lactobacillus delbruckii* (subsp.s *lactis* and *bulgaris*) with W: 0.6 - 0.8µm and L: 2-9 µm; often also, (4) *Lactobacillus kefir* (W: 0.6 - 0.8 µm; L: 3 - 15µm); (5) the highly pleomorphic (cocci 0.5 -1µm OD; rods: W: 0.5 - 1µm; L: 0.5 - 2.6µm), Gram-negative, obligate aerobe *Alcaligenes viscolactis*, a currently unrecognized *Alcaligenes* species, likely a subspecies of *Alcaligenes denitrificans*; (6) both Gram-negative, aerobic rods - *Pseudomona fluorescens* (W: 0.7 - 0.8µm; L: 2 - 2.8µm) and *Pseudomonas pseudoalcaligenes* (W: 0.5; L 2-3µm); as well as, (7) various *Mollicutes*, in particular *Mycoplasma bovis* (W: 0.1 - 0.2µm; L: 0.2 - 0.4µm). Thus, aside from the fat globules that are secreted by mammary gland cells, milk consists of an ecological, microbiological multiplicity of fermentative and aerobic bacteria, some of which are highly pleomorphic (*Alcaligenes*, and less so, the *Lactobacilli*).

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Reich did is, for a biologist, rather insufficient and undefined. It is not a matter of taxonomy or of possible contamination that should be the main concern - but the sheer fact that the term PA-bion has too many possible senses and hardly any concrete ones. This is, in fact, generally a weakness of Reich's orgonomic functionalist method - that concepts, in having a broad enunciation, also lose or incur the risk of loosing the specificity (and thus the demonstrability) of their primary functions. It happened with the concepts of Orgone and DOR, and it happened with the concept of the PA-bions.

In our view, the direst problem with Reich's proposed biological concept of the bion is that it comports two possible applications, and neither is biologically exact. If the concept of the 'bion' is very sharpened, it runs into the inconsistencies between sharply distinct biological objets - if the bion is a fat globule, then it is not a mitochondrion or a plastid; if it is a plastid, then it is not a mitochondrion or a fat globule, and so on. And if instead the concept is kept loose and applied to various biological multiplicities, or ecological niches, then it suffers from lack of precision and inability to



Fig. 3 - Milk solution used for Fig. 2 but filtered through 0.8µm-porosity nitrocellulose membrane (hanging drop, phase contrast, oil immersion). No rod bacteria (*Pseudomonas* or *Lactobacilli*) were found. Largest milk fat globules found (eg bottom right, at 4 o'clock) had an OD of exactly 0.8µm. Only the monococcal stages of *Streptococci, Leuconostoc* and *Alcaligenes* (of <0.8µm width or OD), along with clearly visible mycoplasma, were found in the filtrate. Microscopic optics and analog video as for Fig. 2, but print magnification at 3,750x.

define the symbiotic community of any particular instance. When Reich claims that milk consists of a bion solution, he is suggesting that the relatively large, bluish, milk globules result from a normal breakdown of the mammary gland cells - much as platelets result from the breakdown of the cytoplasm of marrow megakaryocytes. In fact the fat globules of milk are pinocytotically extruded from the secretory mammary gland cells, rather than being cytoplasts, or fragments of a cell's cytoplasm,

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like platelets are. But, more importantly, these fat globules are not living systems, because they lack a unity of metabolism and cannot be cultured. Thus, the notion of milk consisting of bions cannot be reduced to the biological production that forms milk (or colostrum, for that matter) per se as a collection of fat globules, and must be extended to encompass an ecological multiplicity of prokaryotes that, from the beginning, inhabit milk (see **Fig.s 2** and **3**). But if we extend the concept of PA-bions to the microbial multiplicity found *de facto* in milk, then it appears plainly insufficient. For example, if the PA-bions are Gram-positive, then the Gram-negative *Lactobacillus* and *Pseudomonas* components of milk (as well as any mycoplasma) must be arbitrarily excluded. Moreover, if bions must be Gram-positive, the fat globules themselves must be excluded from the concept of the bion, since they are washed away (cannot be fixed) in the Gram staining procedure, and are neither Gram-negative or positive.

One runs into a parallel problem, for example, with the results of any of Reich's plant tissue preparations, whether it is a simple infusion or a boiled one (for 10-15 minutes). The lysis of plant tissue is bound to release a variety of endosymbionts according to the type of plant - not just algaelike plastids and bacterium-like mitochondria - but hydrogenosomes, a variety of other bacteria, including mycoplasma, bacterial spores and protozoal cysts, when not protozoa themselves (such as dinoflagellates). And the lysed tissue preparation will also present fertile ground for the budding of bacterial spores or protozoal cysts. Repetitions of Reich's Preparation V, were carried on in our lab for years. Whether simple or boiled infusions, they always presented (see Fig.s 4 and 5): (1) a variety of cocci, large (2-3 µm OD) and small (<1 µm OD), some single, others in diplo and packet formations, some ovoid, some spherical; (2) rod-bacilli, some of which can be recognized as spore-forming, and others are instead of the Lactobacillus or Pseudomonas type; (3) ciliated or flagellated protozoa; and (4) mycoplasma. Here, then and again, the concept of the bion loses specificity. The multiplicity can be too varied and is too undefined to be of any use to the science of biology. Had Reich, for example, followed in on the steps of Ivan Wallin, and defined a particular type of tissue decomposition as releasing, say, only plastids and mitochondria, then the concept of the bion would be referred to a specific endosymbiotic multiplicity formed by those two terms, with the proper context being the cytoplasm of a given plant cell where that symbiosis had its ecological niche. Otherwise, it is too broad a concept that simply states the obvious - that, when tissue decomposes, bacterial microbes take over, which does not advance anyone's understanding beyond Pasteur himself (and does not even require any notion of symbiosis, nor of lysis as releasing 'subcellular' elements capable of an independent life, and thus cells in their own right). The point is that the loss of specificity hinders the very understanding of how true symbiotic relationships are brought about, and - implicitly - of how does a complex aerobic eukaryotic cell arise.

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**Fig. 4A** - Grass infusion in Ringer's Solution, 2 years after sealing and storage in daylight. Monococci, coccoid packets, protozoa and rod bacteria (including a spore-former at 6 o'clock) can be seen in darkfield with a 100 watt mercury lamp. 100x apochromat, 2x projective. Filmed with Hitachi VK-C2000 CMOS camera (270 lines).



Fig. 4B - Same as Fig. 4A but kept in the dark for 4 years. Only monococci were present with an OD of  $1.5\mu$ m. Optics, technique and video are the same, except a 4x projective was used. Print magnification: 950x.

Fig. 5A - Spherical (1 to 1.5µm) and ovoid cocci, large rod bacilli and diplobacilli in a boiled fresh blade of grass infusion stored for 2 years exposed to daylight. Darkfield, oil immersion, 100 W mercury, 100x apochromat, 4x projective, Hitachi VK-C2000 CMOS camera. Print magnification: 630x.





**Fig. 5B** - Same procedure as for 5A, but stored in the dark for 2 years. Cocci and diplococci are present. Same optics and video capture as for **Fig. 5A**.. Print magnification: 840x.

Then, there is the compound problem of the use of the term bion - specifically PA-bion - to designate all the various multiplicities obtained by different methods and in different media: now the term loses significance as it applies successively to the ecological system of milk, to the lysates of plant tissue or animal tissue, to the packets obtained from the incandescence of carbon-containing and non-carbonaceous substances, etc. Exprime the term applies - and in full, given Reich's packet-et-amoeba definition that designates as such sarcinoid cocci that form a capsule - to the irregular encapsulated coccal packets obtained by taking coal and bone charcoal to incandescence, plunging it into bouillon media and growing it in liquid culture. Such encapsulated sarcinoid packets are seen

immediately after incandescence, some regular such as tetrads and others irregular and large (see **Fig. 6A**). They are highly motile and frequently quiver on the spot, stain Gram-positive and can be grown in liquid culture (see d4 packets in **Fig. 6B**). And, in still another example, Reich employs the term PA-bion to designate blood platelets, suggesting they are the 'blue bions' of blood (see discussion below and also **Fig** 7), when in fact these are normally produced cytoplasts that have no autonomous metabolism and cannot be grown as such. How can one consistently employ the term 'bion' to designate now a bluish fat globule in milk, next a bluish platelet in blood, next a coccus that forms packets that secrete capsules, has a metabolism and can reproduce, and still next a plastid or a mitochondrion that resulted from cell lysis, and so on? Irrespective of any specific findings that may be involved, and to paraphrase Prof. Sapp <sup>[22]</sup>, this is the kind of biology that suffers from optical analogism. As Sapp puts it, "seeing only what one wants to see is a problem in microscopy too". Visual or optical similarities do not imply identity of type, and even less identity of function or operation.



Fig. 6A - Sarcinoid, highly motile, encapsulated coccoid packets (see arrows) observed immediately after incandescence of bone charcoal. Darkfield oil immersion, 100 W mercury, 100x apochromat, 2x projective, Hitachi VK-C2000 CMOS camera.



Fig. 6B - Similar packet from a d4 liquid culture in 0.45µm filtered beef bouillon. Same optics and video capture as in Fig. 6A, but enlarged 2x.

In the course of our work (specifically, the accompanying papers) we shall strive to demonstrate that the only biological sense of the 'PA-type bion' - as an aerobic and protocellular element composing the eukaryotic cell <sup>[1]</sup> - that has symbiotic relevance is the sense in which it designates operationally either a mitochondrion or a plastid or chloroplast in specific and qualified instances (as to source of material, composition, method and media employed). Its best approximation would be the notion that these two endosymbionts are tributary to some form of an 'ur-symbiosis' between 'ur-

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bacteria' and 'ur-algae' (it is possible that hydrogenosomes may have to be included in this 'ur-multiplicity') which a proto-protozoon, or an archaeobacterium, imported into its cytoplasm. In this sense, despite the bluish appearance of milk fat globules, these are not bions - nor are platelets, or any other cytoplasts, bions either. Moreover, this view is, in a concrete sense, compatible with Serial Endosymbiosis Theory (SET), inasmuch as it includes indeed consideration of the possible role that mitochondrial or chloroplast precursors or 'cousins' (such as *Paracoccus denitrificans, Alcaligenes denitrificans*, various streptococcus, or certain purple nonsulfur bacteria and *Cyanobacteria*) might have in establishing cell-free symbiotic multiplicities that may actually be involved in cellular biopoiesis, specifically in the 'evolutionary' creation or modification of eukaryotic cells.

It is only tangentially, then, that this approach must concern itself with the existence of spores as biological products of spore-forming bacteria - to the extent that, when considering Reich's incandescence experiments with molecular matter (rather than the experiments that involved lysis of tissue) one also obtains a variety of bacteria, mostly of the coccoid type (monos, diplos, regular and/or irregular packets). Thus, it is important to address the problem raised by spore-forming bacteria and their thermal resistance in particular.

However, the only real link between these different experimental preparations - as we shall see through the accompanying papers - is that, whether tissue is sterilized by ordinary or extraordinary means, or matter is taken to incandescence and immersed in sterile solutions, some biological multiplicity whose composition varies from instance to instance (source and method) is systematically and consistently obtained. Whether in each case this multiplicity necessarily involves spore-forming bacteria or not, whether it involves elements that arise *de novo* by spontaneous generation or instead involves thermally super-resistant endosymbionts that are freed by cellular lysis, are questions that will constantly weave into our current investigation and its continuation, and whose answers, in large measure, we believe to have found and will present in the course of these communications.



Fig. 7 - Wright's stain of a peripheral blood smear from a normal human donor showing 4 blue-staining platelets (one on top of a stack of two erythrocytes) that measure in OD between 1.6 and 2 $\mu$ m. Platelets are cytoplasts shed by very large cells present only in bone marrow - the megakaryocytes. Oil immersion phasecontrast (100x Ph3 objective, 16x Pk projective) with monochromatic blue light. Shot with a Luminera Infinity-3 digital camera (1.5 megapixel). Print magnification: 1,600x.

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# 3. The problems posed by the heat-killing resistance of prokaryotes and endospores

Reich initially refused to assimilate his PA bions to prokaryotes, treating them as prebiotic vesicles rather than protocells. In this respect, he argued that known prokaryotes or their spores could not withstand the vigorous sterilization techniques (prolonged autoclavations) and violent heating methods (eg incandescence) that his experiments employed. He was therefore convinced that PA bions were directly produced from the 'right' solution of molecular materials, or the materials of decomposed tissue, by the high-energy treatments that he employed.

Reich noted that the "bion preparations" which exhibited the fastest electrophoretic migration were those which were moist-heated well above optimum sterilization procedures. In his epoch (the 1930's), it was thought that the highest known moist heat endured by a spore-forming bacillus (for a typical spore-forming and germinating process see the diagram of Fig. 8A; a typical spore-forming bacillus is shown in Fig. 8B) was 120°C (or 15 lbs of pressure) for 20 minutes, in the case of B. tetanus; of 140°C (36 lbs) for 30 seconds for the endospores of B. subtilis; and 140°C (36 lbs) for 1 minute for thermophillic bacteria [23-24]. Even by the early 1980's, autoclavation for 25 minutes at 121°C (15 lbs) - or dry heat sterilization at 160°C for 1 hour - were considered cut off treatments beyond which no bacterial endospore (and *a fortiori* no protozoal cyst) would survive <sup>[25]</sup>. Other researchers have claimed that the endospores of B. subtilis, B. mycoides and B. cereus may all withstand the standard autoclavation at 121°C (15lbs) for 25 minutes, and that certain clostridial spores may endure as much as 110 minutes at that temperature and pressure <sup>[26]</sup>. Today, it is known that some non-spore-forming Archaeobacteria species can grow at temperatures of up to 113°C (the hyperthermophile Pyrolobus fumarii). Only in the past 3 decades has microbiology become aware of the extraordinary powers of prokaryotes and algae. Extremely thermophilic bacteria capable of withstanding enormous pressures have been discovered and mostly are classified as Archaeobacteria. Some, like members of the anaerobic, Gram-negative Thermoproteales (eg Staphylothermus, Pyrodictum), form rods, mycoplasma-like flat discs (0.2 µm thick, 0.3 to 2.5 µm OD) or cocci-like spheres (ODs of 0.5 to 5 µm), have high salt tolerance (up to 12% NaCl) and have optimum growth temperatures of 95-110°C! They do not produce spores, but the large aggregates they form - in the case of Pyrodictum the aggregate is connected by a large network of fibers - are evocative of sporangia. It is unknown what values of temperature, pressure and time of exposure are required to destroy such prokaryotes. It has also been recently determined that autoclavation for 1 minute at 130°C is sufficient to kill B. subtilis spores [27]. The most extreme heat resistance known is that of Desulfotomaculum kuznetsovii, a thermophilic sulphate-reducing anaerobic bacillus whose spores are only reduced ten-fold in viability when moist heat-treated at 140°C (~37 lbs) for 15 minutes [27]. Yet, no known sulfate-reducing cocci are known to produce spores [28].

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**Fig. 8A** - Diagram of a typical, sporeforming process of a rod bacillus through stages I to VII (After reference 45, Fig. 8-40, p. 160).



Fig. 8B - Fast-growing culture in broth-agar of *Bacillus globisporus* (W:  $0.6 - 0.9\mu$ m; L:  $1.95 - 4.5\mu$ m in figure), whose spores (0.45 to  $0.6\mu$ m OD) could be found in a  $0.45\mu$ m porosity (nitrocellulose) filtrate. The spore shape is spherical and its position terminal. The cells swell up before producing and releasing spores. Oil immersion phase contrast. 100x Ph3 oel objective, 4x projective. Sony TRV68 CCD camera, 19.6x optical zoom. Print magnification: 2,500x.

These considerations contextualize the borderline situation of Reich's standard procedures: for autoclavation, his standard was 121°C (15lbs) for 15-30 minutes, but in some instances, up to 130°C (~25 lbs); for dry-sterilization of all the solid components, the range he used was 160°-190°C for 2-4 hours.

On one hand, the maximum temperatures of moist heat withstood by a variety of heat-resistant prokaryotic cocci (spore forming and non-spore-forming) suggest that no PA bions in Reich's autoclaved preparations can be due to the survival of such prokaryotes, at least as they have been identified. On the other hand, the sterilization and heating procedures employed by Reich were credited by him as having catalyzed the spontaneous self-assembly of large bluish cocci or vesicles having biological properties (cultivability, morphism, Gram reactions, etc) in all respects analogous to those of micrococcus or staphylococcus prokaryotes. If unwitting infection may be ruled out, then only one

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arm of the alternative may be correct to explain the results of Reich's autoclaved bion preparations it is either biopoiesis of proto-prokaryotes, or persistent infection with ubiquitous, unknown, ultrahigh-heat resistant cocci - whether it is its cells or its spores that are extremely thermophilic.

We may extend this argument to consider that the only known prokaryotes that (1) present packet formations analogous to micrococci or staphylococci, (2) are not obligate anaerobes, and (3) qualify as extreme resisters to thermal death are in fact a type of archaic bacteria - a few species of *halobacteria*. These are poorly studied, strictly aerobic *archaeobacterial cocci* that may live in 2M sodium salt concentrations and are often hyperthermophilic. We should note that they also have an ambiguous Gram-reaction, even if most stain Gram-negative. Since they are not spore formers, were one to demonstrate that PA bions are halococci, one would be either proving Reich's biopoiesis argument or identifying an unexpected thermophilic resistance on the part of vegetative cells.

If one drops the aerobic requirement from the characteristic description of PA bions, one encounters other bacterial candidates capable of withstanding high heat treatments - such as, for instance, the sulfate-reducing *Desulfosarcina*, or the obligate anaerobe *Desulfurococcus*, or the aerobic spore-forming sarcina, such as *Sporosarcina halophila* or *ureae*. However, neither *Desulfosarcina* nor *Desulfurococcus* are spore-formers, nor are they able to withstand Reich's standard autoclavations - while the spores of *Sporosarcina ureae*, found often in urine, are easily destroyed at 85°C for 10 minutes <sup>[29]</sup>. Little is known about the thermal resistance of *Sporosarcina halophila*. These facts would seem to suggest that Reich was correct, in principle, to hold that no known bacterial cells or spores could withstand his moist-heat treatments. Of course, that does not mean that such cells or spores do not exist.

#### 4. Cultivability of PA bions and microbiological pleomorphism

Reich extensively demonstrated the ability of PA-type cocci obtained from various sources to replicate in diverse microbiological media. If we are to accept his evidence that (1) his colonies in semi-solid media were not contaminant cultures and were, indeed, seeded from his primary sources, and that (2) no bacterial spores resisted his moist-heat treatments, then, as we have already emphasized, these PA bions would have to be effective proto-prokaryotic cells. As Reich presents it in his 1938 book "*The Bion Experiments on the Origin of Life*", the bion is a complex concept: it does not designate a particular lifeform, but a complex of microscopic lifeforms which he believed were connected as pleomorphisms of the same protobiont. This is a critical piece for understanding his descriptive reference - it is the multiplicity of "microbial" forms which he believes he is studying all at once. Consider the autoclaved PA bion preparations from soil, coal, moss, or animal tissue: the initial inoculations in broth exhibited both moving rods and cocci, but when Reich transferred the cultures to

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agar, mostly clusters, chains or packets of immotile cocci resulted; yet, if he transferred the cultures back to bouillon, suddenly the moving rods appeared to predominate, along with the packetamoeba formations <sup>[30]</sup>. Reich noted the existence of pleomorphism between rods and cocci in his PA bion experiments, as early as February 1937 <sup>[31]</sup>, remarking in a letter to Du Teil that "so far, I have only noticed that the rod predominates in broth while mainly cocci develop on agar".

What were these varied forms - are there known prokaryotes that can change between such forms? The answer is yes - many types of rods assume coccoidal or ovoidal shapes as a function of the conditions of the ambient and nutrient medium, and just as well vary in their states of aggregation - sometimes found only in singlets, diplos or tetrads, other times in regular or irregular packets - also largely as a function of the medium.

First, let us consider the type of structures formed by PA cocci. In figure 45 of "The Bion Experiments on the Origin of Life" (reproduced and inverted as figure 30 of "The Cancer Biopathy"), Reich shows a PA bion culture from autoclaved coal: what he calls 'packet-amoeba' are clearly highly refractile sarcina, ie regular clusters of cocci joined by division in all three planes to form packets of eight, sixteen and thirty-two cells, and surrounded by a capsule. This is what used to be called sarcinae, in Reich's time when Bergey's classification had not yet taken over microbiology and such common species of packet cocci as Micrococcus luteus, Planococus citreus or Halococcus morrhuae were called Sarcina lutea, Sarcina litoralis and Sarcina citrea, respectively. All these micro-organisms present tetradic structure, which may or may not be part of a sarcina packet or tablet structure. Today, with the use of transmission electron microscopy, we know that some sarcinoid forms, such as Lampropedia hyalina - currently classified as a tentative genus under the category of Gram-negative cocci and rods - do not have complete divisions between adjacent cocci [32], resulting in a confluent, amoeba-like structure, where all cocci are rigidly attached to form a unit that may or may not be motile. Lampropedia hyalina goes even further, and develops a complex structured envelope that encloses the synchronized cocci within the packet or tablet, typically composed of 8, 16 or 64 cells. The cocci also secrete a conjoining matrix which is enclosed by the continuous external envelope, and holds the cocci together. Lampropedia also displays "unique motion in growing cultures characterized by detachment of small groups of cells from larger groups and reattachment to other large groups" [33]. Remarkably, certain strains of L. hyalina present neither cellular confluence nor encapsulated packets, with the result that staphylo-type irregular packets are also produced [34].

Most sarcinae are not as geometrically regular as *Lampropedia*, which only divides alternately in two planes, and does so in complete synchrony. *Sarcina lutea*, now *Micrococcus luteus*, for example, engages in divisions in all three planes, but these divisions are not necessarily synchronous, and the species can produce diverse forms - such as single cocci (monococci), pairs (diplococci), tetrads, pack-

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ets of 6, 8, 10, 12, etc, cocci, or irregular clusters that are microscopically indistinguishable from staphylococci (from the genus Staphylococcus). Note that technically these diverse aggregate structures of the same microorganism are not considered to be pleomorphic transformations. But perhaps they should be, since, precisely as Reich found out with his PA bion preparations <sup>[30]</sup>, it is not just the rod to coccus transition that is affected by the nutrient medium, but the entirety of the phenotypic diversity (packets included) that largely depends upon the medium. Indeed, if we are to consider his findings and his micrographs, one easily discerns how the PA bion preparations from coal dust or soot can either form (1) regular coccoidal packets (sarcinae and tetrads), or form instead (2) staphylococci clusters or streptococci chains, together with mono and diplococcoid forms, or still further give rise to (3) rods, even motile ones that, as we know today, would have to be equipped with flagella. These three types of microbial forms constitute a single multiform multiplicity, and can all be found in the three plates that Reich presented on p. 70 of "The Bion Experiments on the Origin of Life", as figures 45-47. And the difficulty of conveying such a complex concept is underlined by the caption that Reich placed under figure 46, which describes staphylo, strepto, diplo and monococcal forms also as 'packet amoebae'. Hence, when one speaks of PA bions in Reich's language, one must refer not just to the regular sarcinoid forms that in Reich's view constituted what he called 'packet amoebae', but also to the other coccoid or bacillar forms which the same microorganisms would supposedly display as a function of the nutrient medium on which they are grown.

Pleomorphism between rod and coccus forms is today well demonstrated with the sphere-rod model of Arthrobacter, a dominant soil aerobic microorganism, and the morphogenetic induction from monococci to rods is achieved by utilizing precisely different nutrient carbon sources, some of which "will cause induction and the typical morphogenetic cycle, while other carbon sources will allow growth without morphogenesis" (see Fig. 9 [35]). Another well studied form of coccus-rod pleomorphism is that of Alcaligenes, an obligate aerobe also found in soil and milk. Remarkably, in the Arthrobacter species, we already find in the same strains all the various forms that Reich claimed for his PA bions: immotile monococci before induction; immotile zymogenous rods upon induction, followed by motile, flagellated rod forms that predominate in the log phase of culture; and, as the culture moves from the log to the lag phases, and reversion is observed, coccoid forms reappear, in diplo and tetrad forms, or in irregular staphylo-like packets, or still in strepto-like chains. For example, Arthrobacter globiformis forms, upon reversion, large, nonsarcinoid, but rather clump-like clonal aggregates or clusters of cocci, which undoubtedly would qualify - in Reich's language - as 'vesicular amoebae' (see Fig. 10 [36]), and the creation of these structures was documented by Ensign and Wolfe in 1964. However, no Arthrobacter strain has been reported to form sarcina or cubic packets. Lastly, Arthrobacter reproduces both by fission and by bud-like spherical outgrowths from segments of sep-

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**Fig. 9** - Diagram of the morphogenetic pleomorphs of *Arthrobacter*, (modified for clarity, but after Clark JB (1979) "Sphere-rod transitions in *Arthrobacter*", Fig. 5.1, p. 76, in "Developmental Biology of Prokaryotes", 1979, reference 25). Hours on the X-axis are arbitrary, since the cell cycle timing changes among the *Arthrobacter* species.



Fig. 10 - Agar-peptone (0.5%) growth of *Arthrobacter globiformis* as a function of time in hours (drawn after Ensign & Wolfe (1964), reference 26).

tate rod forms, giving - under the microscope - the distinct impression of the emergence of a vesicle or coccus from the body of a rod bacterium. Moreover - and in line with Reich's observations - we should note that whereas the *Arthrobacter* cocci are always Gram-positive, its rod forms are mostly Gram-negative.

What prevents, in many instances, a tentative identification of *Arthrobacter* strains with PA bions? In essence, that *Arthrobacter*'s size is too small (0.6 to 0.8  $\mu$ m OD, with a maximum of 1  $\mu$ m), that none of its strains produce regular packets (though sarcinoid formations can be observed in agar), nor withstand Reich's autoclavation standard (in fact they do not survive 63°C for 30'), and are not spore formers.

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# 5. PA bions and prokaryotic sarcina

Originally, Reich was very firm in trying to separate his concept of the bions and their varieties, from known microbiological "organisms" - amoebae and bacteria, whether bacillar or coccoidal. In particular, he resisted any assimilation of PA, or SAPA, bions to cocci or cocaccea. According to Reich, PA-type spherical vesicles could be produced either abiogenically from acellular preparations of chemical compounds (containing carbon, iron and silica) that were made to breakdown by swelling and heating, or heterogenically from the breakdown of various cellular preparations of plant or animal tissue. Since they could be generated either abiotically (abiogenesis) or heterobiotically (heterogenesis), they held - in his mind - the key to biogenesis. In the former, bions arose acellularly, and in the latter, they arose by discontinuous transformation of the decomposing cells into bions. Since bions have to be conceptualized as cells, as prokaryotic cells, because they are capable of autonomous replication, both abiogenic and heterogenic processes of bion generation were overt violations of the sacred biological dogma of "*omni cellula ex cellula*".

At this point of Reich's argument, bions, unlike bacterial cocci proper, were *biogenic productions*, whereas bacterial cocci were well established prokaryotic life forms that propagated mitotically or by budding, spread by infection and often germinated by sporulation. Whereas certain bions could be said to be symbiotic, in his view bacterial cocci were not. The latter only appeared when biopathic conditions favored the manifestation of nonsymbiotic, putrefacting, saprophitic, anaerobic bacteria (a coccoidal example of which Reich gave as *Staphylocccus aureus*).

Back in the 1930's and 1940's - ie before the discovery of DNA and RNA, the advent of molecular biology and the modern techniques of microbiology - one might sustain, even if with some difficulty already, this argument of a difference between bionic cocci and bacterial cocci. One might even today try to relate that difference to the distinction between simple archaic bacteria, *Archaeobacteria* and more complex bacteria, the *Eubacteria*. However, any notion held today of *Archaeobacteria* being assembled *de novo* in the laboratory from a solution of macromolecules would meet with justifiable derision in light of the complex DNA structure of these simple lifeforms, the long evolutionary scale required for the emergence of the first proto-prokaryote from the prebiotic soup, and the inability of DNA polymers to survive intact Reich's moist-heat and incandescent treatments.

Even if one assumes that Reich may be correct in suggesting the existence of heterogenic bion formations having morphologies and cultivability in all respects identical to those of bacterial cocci, one would be still hard put not to surmise *apriori* the functional identity of known cocci and packet bions, and not suspect that unwitting processes of contamination involving extremely resistant prokaryotes or their spores were at work in Reich's bion preparations. In this respect, the two main

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vulnerabilities are contamination with ordinary bacteria in air, surfaces, cloths, clothes and skin, and contamination with bacterial endospores, in particular spores that may not yet have been identified.

Contamination with ordinary bacteria is of particular relevance when Reich passaged his bouillon cultures to agar, and thus would be of greatest concern when claiming that axenic cultures of PA cocci were obtained *after passage to semisolid media*. Sterile manipulation and preparation of cells, media and vessels is not ever an easy procedure, and there are many unsterile gaps in all of Reich's bion preparations, including experiment XX<sup>[1]</sup>. Some of these gaps are systematic: Reich never employed tissue-culture hoods to effectuate the transfer of any sterile solutions or passage his cultures, nor employed self-sealing autoclave tubes (which did not exist in his time), nor longer periods of autoclavation (which he could and should have done in a systematic fashion), nor did he routinely employ 0.2 µm filtration filters in the preparation of media or solutions (Berkefelt 0.25µm filters existed back then, and he used these to retain "T-bacilli").

Contamination with spores, especially with unidentified spores, would be of crucial importance for *the bouillon cultures immediately established* after chemically-induced swelling, autoclavation or incandescence. Therefore, relating the observed PA bion forms and cultures to those of known bacteria is a necessary exercise that may well indicate the presence of either of these two forms of potential contamination of Reich's preparations, or even raise the prospect of the existence of thermally hyper-resistant spores or prokaryotic cells.

Interestingly enough, Reich himself seems to have eventually arrived - by virtue of a microfunctionalist reasoning - at the conclusion that his PA bions and the various bacterial species of cocci were identical: "The bacteriologist, for instance, sees the staphylococcus as a static formation, spherical or oval in shape, about 0.8 micron in size, reacting with a bluish coloration to Gram stain, and arranged in clusters. These characteristics are important for orgone biophysics, but are not the essentials. The name itself says nothing about the origin, function, and position of the blue coccus in nature. What the bacteriologist calls "staphylococcus" is, for orgone physics, a small energy vesicle in the process of degeneration. Orgone biophysics investigates the origin of the staphylococcus from other forms of life and follows its transformations. It examines the staphylococcus in connection with the processes of the total biological energy of the organism and produces it experimentally through degenerative processes in bions, cells, etc" [37]. This argument considerably toned down the initial refusal to reduce bionic cocci to bacteria, solely demanding a new understanding of their emergence or manifestation, or distinct functionality. But was this understanding really functional? Had Reich successfully proven that cocci arise heterogenically, and that these bionic cocci were in fact the same as typed bacterial cocci, but played different roles in different situations? Was it tenable to hold on to the view that, when normal tissue broke down, only large blue PA cocci were formed, but that when

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conditions were anoxic and putrefacient, only their variation as pus staphylococci and streptococci, along with rot bacilli, were formed - all from the same "bion multiplicity"?

Formally, an answer to these questions would simply lie in performing what is today a trivial comparison between the DNA sequence of the PA cocci obtained from each of Reich's preparations, with the DNA sequence of established bacterial cocci - along with a simple in situ demonstration that the PA vesicles formed from decomposing tissue, or from incandescent materials, were effectively DNA-carrying prokaryotic cells and not mere DNA-devoid cytoplasts or fat globules. Indeed, claims to cultivability in agar could always be falsified by unwitting contaminants, present either in the media employed or by air-infection. In fact, as we shall see ahead, in our research and reproduction of Reich's bion experiments, we found that a variety of cocci (monos, diplos, tetrads, streptos and staphylos) with different sizes (up to ~2µm) were present from the start in all the media prepared and employed by Reich - bouillon-agar, egg medium E, and potato medium IV. More astonishingly still, we've shown that Berkefelt filter filtration of bouillon through 0.45 µm porosity filters did not remove contamination with very small cocci or spores that we measured in the 0.4 µm range - and this sufficed to obtain a whole variety of monococci and coccoidal formations, whose typical coccus size varied from 0.5 to 1 µm (in the Arthrobacter range; but note that Arthrobacter is not a spore-former). Even more amazing was the fact that in sealed bouillon tubes, these cocci - or spores - resisted three autoclavations (27lbs 30'; 20 lbs 20'; 25 lbs 15') spaced apart by 24-36 hours, and were only killed by a 4th autoclavation at 27 lbs for 40 minutes! In fact, only a single autoclavation at 27 lbs 40' was sufficient to yield contaminant-free 0.45 µm-filtered bouillons. Reich failed to conduct control tests such as these, thus leaving the door open to expressing possible contaminants present in essential solutions or media.

Irrespective of these concrete questions and problems, once Reich admitted that PA bions and bacterial cocci were functionally the same, these bions could no longer continue to be conceptualized as prebiotic elements or vesicles. They were already cells, at the very least protobionts or protocells of some prokaryotic type. Here, it all depends on the accent one places upon the prefix 'proto-'. In a neo-darwinist perspective, the assumption is that practically all modern prokaryotes are 'advanced' cells that have 'evolved' far from whatever their protobionts might have been. Yet the astonishing variety of prokaryotes has suggested the enunciation of evolutionary models that place some genera as being closer to proto-prokaryotes than others. Simple facts - viz. that freezing does not kill bacteria and only inhibits their growth, that some bacterial spores have survived 2 hours of exposure to near absolute zero degrees <sup>[38]</sup>, that staphylococci and other bacteria have been found in the stratosphere since the 1950's <sup>[24]</sup>, etc - seem to further indicate that there is a high probability of encountering still extant archaic bacteria, or their spores, in extreme environments.

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These considerations prompt us to seek, irrespective of any evolutionary classification, a tentative identification of these PA vesicles and their packets with established species of globular or spherical bacteria (cocci) that have the property of forming packets. The task is somewhat pointless given (1) the diversity of sources employed by Reich to obtain these PA bions - which implies that he likely encountered many different species of cocci and rods - and (2) the diversity of different species that microscopically appear to be grossly similar in aspect and behaviour. Yet, we will be surprised by the few possibilities which will result from the exercise. The exercise is of interest, above all, because it will show that none of the possible known bacterial candidates can actually survive the thermal treatments inflicted by Reich on the preparations that he used to obtain PA bions.

Any tentative identification will have to go by a minimum list of shared characteristics, in the present case - size, color, motility, cell arrangement, aerobic metabolism, cultivability and Gram stain reaction. We may also include non-spore formers as an initial criterion. Let us say that the cells we search for, have a bacterial cell wall; their size is 1.5 to 5  $\mu$ m; the color under brightfield is blue or milky-blue; the motility not locomotive but impulsive, oscillatory, "erratic", involving quivering, flickering and rolling; the typical arrangement is not linear (excluding all *Streptococcaceae* therefore), but in irregular packets (like staphylococci) or in ordered sheets (tetrads and multiples, including cubical packets); the metabolism is aerobic or facultative; and they present a Gram-positive reaction. Leaving algae aside, these characteristics appear at first to potentially tally with several bacteria. Let us go through the list.

Sarcinoid structures - what Reich called 'packet amoebae' originally, ie in a strict sense of ordered tablets of cells, whether motile as a unit or immotile - can develop amongst the *nonspore* forming bacteria from eg *Deinococcus radiodurans, Deinococcus radiopugnans, Lampropedia hyalina, Micrococcus luteus, Micrococcus roseus, Micrococcus varians, Micrococcus morrhuae* (now *Halococcus morrhuae*), *Nitrosococcus oceanus, Planococcus halophilus, Stomatococcus mucilaginosus, Staphylococcus saprophyticus, Sarcina ventriculi, Sarcina maxima, Thiosarcina rosea* and *Thiopedia rosea*. If one upheld the necessity of the sarcinoid structure having to be enveloped by a capsule - which could be slimy, mucilagenous, or what is also called a shared "structured array envelope" - the entire list is decimated to leave only *L. hyalina, S. mucilaginosus* and *S. ventriculi*. However, this might be the correct criterion if, for example, one were to consider the possible identification of the encapsulated, sarcinoid, highly regular (tetrads, octets, etc) PA bions obtained from the autoclavation of coke <sup>[39]</sup>. However, most PA bion preparations do not result in the production of such regular or sarcinoid packets.

Let's therefore suspend the criterion of encapsulated sarcinoid packets and re-examine the above list in greater detail. Again we can right away eliminate *Sarcina ventriculi* and *Sarcina maxima* because they are strict anaerobes. Both are also nonmotile and do not form rods. We can further elim-

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inate the obligate aerobe Lampropedia hyalina, inasmuch as its Gram reaction is negative and also forms far more regular cuboidal packets than the planar sarcinoid clusters of PA bions. One can also exclude the sulfate-reducing sarcina (Gram-negative anaerobes) and dissimilatory sulphur-reducing cocci (also Gram-negative anaerobes). Based upon the colour of the colonies on agar, we can also cross off Micrococcus roseus, Halococcus morrhuae, Thiosarcina rosea and Thiopedia rosea, since their colonies are pink to violet in pigmentation; and, furthermore, their reaction to the Gram stain - with the exception of *M. roseus* - is negative. We should note that *Halococcus morrhuae* (previously classified in the family of the Halobacteriaceae) is a strict aerobe in the 2 µm OD range, capable of forming regular and irregular packets, and able to survive in very high salt concentration (up to 3M NaCl). Remarkably, the Halobacteriaceae family can be considered to be generally pleomorphic, since the rod or bacillar species of its Halobacterium genus lose the cell wall at lower salt concentrations (<1.5M) to become cocci. Moreover, they are usually Gram-negative in the rod form <sup>[40]</sup>, and so is *H. morrhuae* which, in addition, is non-motile. With the arguments of size and absence of rod pleomorphism we may also eliminate the entire Staphylococcus genus (size too small) of the Micrococcacea family, even though Staphylococcus saprophyticus can reach sizes of 1.5 µm and occasionally form tetrads and cubical packets [41]; but it is generally composed of small and heterogenous cocci, in the 0.5 to 1.5 µm range, that are nonmotile. Since the Stomatococcus mucilaginosus is a facultative anaerobe and nonmotile, it is also dropped from the list. Finally, we also drop Nitrosococcus oceanus because it is Gramnegative and only forms small regular packets (tetrads), but no tablets. (We may also eliminate the Peptococcaceae family since they are strict anaerobes, nonmotile, do not produce cubic packets or sarcina, and again are apparently incapable of rod pleomorphism.

Excepting spore-formers (such as *Sporosarcina ureae* or *Sporosarcina halophila*), these summary considerations leave us *with only a few non-sporing candidates* that could still be identified tentatively with PA bions, if the latter were a single 'micro-organism' - rather than a term designating a variety of microbiological multiplicities: some members of the Gram-positive *Micrococcus* genus; *Planococcus halophilus*; and the *Deinococcaceae*.

The most likely candidate of these three - on the presupposition of unwitting but systemic infectious contamination of the bion preparations - are members of the Gram-positive cocci *Micrococcus* genus (family of *Micrococcacea*). They are aerobes (and facultative anaerobes), with sizes ranging from 0.5 up to 2.5 µm OD <sup>[42]</sup>, occurring in singlets, pairs, tetrads, cubical packets, sarcina and irregular packets. Some are motile by one or two flagella (eg strains of *M. roseus*), and others are nonmotile. They have been isolated from soil, dust, water, salt-containing environments and food, marine muds, the skin of animals or the surfaces of plants, milk and dairy products, animal carcasses, etc, and are, therefore, as ubiquitous as staphylococci or streptococci, but without their potential

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pathogenicity. If one tries to ascertain to which species of micrococcus may PA bions correspond, the best candidates turn out to be *Micrococcus luteus* and *Micrococcus varians*, in the 1.5 to 2  $\mu$ m OD range, whose cultures always pigment yellow (arguably a partial match with PA bion cultures in agar). But no pleomorphism of coccoid to rod (induction) back to coccoid (reversion) forms is known in either case, and they do not produce a capsule. Their size range, their positive Gram-reaction and their aerobic metabolism makes them equally good candidates for a possible amalgamation with Reich's PA bions, but these two *Micrococcus* species are strictly nonmotile.

Of the other two candidates, the *Deinococcaceae* - which have no packet capsule - are nonmotile, and also lack sphere-rod pleomorphism. Conversely, the *Planococcus halophilus* (or *citreus*, which withstands a tremendous pressure, up to 40MPa) is motile by one or two flagella and, though it lacks a capsule, it has a very refractile double-layered wall. However, it does not form packets proper, only diplos, triplets and tetrads.

Of the nonsporing prokaryotes, it seems therefore that some of the *Micrococci* would be the best candidates to embody a potential identification of the PA bions. Yet, the main and obvious objection to these taxonomic correlations of PA bions with any of these nonspore-forming species of prokaryotes is that none of them are known to contain strains that are capable of withstanding the heat treatments involved in Reich's PA bion preparations. Suppose for example that PA bions were de facto identical to, say, M. luteus: then, if Reich's method in effect produced the appearance of M. luteus, this could a priori only come from either (1) improperly sterilized media components, (2) unwitting air or contact infection, or (3) effectively be created either heterogenically and abiogenically. Assuming that all media components were properly sterilized - as per Reich's claim to proper procedure with the methods already discussed - and assuming that sterile procedures were obeyed and air-infection can also be ruled out (even though Reich did not employ a tissue-culture hood for his inoculations and transfers, the air infection would have to be so massive and ubiquitous that it would be unlikely as an explanation for observations made immediately after the experimental treatment), then that hypothetical identification would reduce to point #3, and would mean that M. luteus could emerge de novo either from decaying tissue or from acellular preparations, or both. In other words, one would have to conclude that direct, observable "spontaneous generation" of complex prokaryotes was an inevitable reality, if proper sterilization and lack of infection could be firmly established in Reich's PA bion preparations.

The only *caveat* to this threefold scenario is that proper sterilization must effectively include elimination of the endospores of all spore-forming bacteria - and that is by no means a given. In this light it is most doubtful that the PA bions could generally be identified or assimilated to any known *nonspore-forming* type of cocci.

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Hence, if in our list of identifying criteria we introduce a requirement for spore-formation, one other candidate genus can be considered. This is the most relevant portion of the present exercise in tentative identification, since it permits one to venture that it might be spores of a hardy bacterium which systematically survived the violent thermal treatments inflicted by Reich on his diverse PA bion preparations. Such a possibility would then preclude any conclusion regarding the presence of a direct "spontaneous generation" of prokaryotes. The sole candidate is the *Sporosarcina* genus of the family of the *Bacillaceae*. The cells are cocci that form regular or irregular packets; they are motile and nonmotile, strict aerobes and Gram-positive. Most provocatively, both *S. ureae* and *S. halophila* present some degree of pleomorphism, with spherical cocci (OD 1-2.5  $\mu$ m) and oval rods (W: 1-2  $\mu$ m; L: 2-3  $\mu$ m, sometimes up to 4  $\mu$ m). *Sporosarcina ureae* also presents colony growths that vary from the transparent-grey to the opaque yellowish colour. Yet, few *Sporosarcina* survive 10 minutes of moist-heat at 99.5°C, and their spores, as already mentioned, are destroyed by autoclavation at 121°C (15lbs) for less than 15 minutes. The smallest size of their spores (0.8  $\mu$ m) would also preclude them from transfer across a 0.45 $\mu$ m porosity filter. It is also unclear whether they have a slime capsule or not.

Thus, if we assume that PA bions are spore-forming sarcina whose spores resist Reich's moistheat treatments, we are forced to conclude that he must have unwittingly discovered unknown strains of *Sporosarcina*. The only other alternative to both Reich's abiogenesis model and this suggestion of an unknown strain of spore-forming sarcina is an even more remote possibility than the latter - ie that vegetative prokaryotic cells or prokaryote-like endosymbionts (present free and inside plant and animal tissue, etc) exist that can withstand such extreme heat and pressure treatments.

The reader may now grasp the full extent of the quandary. One could summarize it as follows: it is indeed ascientific to simply throw at Reich's bion experiments the contention that he was looking at "staphylococci infections", as a blanket statement for infection of his cultures with contaminant microbes. In terms of the real science of microbiology, whatever it is that Reich obtained in the form he termed PA bions did not qualify as staphylococci or streptococci - not in the correct scientific acception of the terms. Rather, in their coccoidal forms, the PA bions only appeared to qualify as sarcinae, halococci or micrococci. But since no coccoidal cell can withstand the autoclavations employed by Reich, the only assumption left to avoid any admission of "spontaneous generation", is to relate those PA bions to spore-forming sarcina. Yet, no known strain spores can withstand the same moist-heat treatments.

The question is so poignant that, assuming the existence of a single taxonomical identification and after having gone through an entire list of possible microbiological candidates for Reich's PA bions, not one known species or strain tallies with the results that he reported. We are left therefore

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with the poignant question: were Reich's results with the bion preparations evidence for an "accelerated biopoiesis" (phenomenologically heterogenic or abiogenic), or were they evidence for the ubiquitous existence of very small spores of unidentified species of spore-forming sarcina?

This question can only be solved experimentally - and though it should have been incumbent upon Reich and his medical disciples to have tried to answer it, it is no less a discredit to microbiology that no microbiologist has seriously considered solving it, if for no other reason than to prove Reich's contention of biogenesis wrong.

Lastly, we would like to say a word on the matter of morphology and taxonomical rules. Originally, the term sarcina (singular; pl. sarcinae) applied to any species of cocci that that divided in three successive planes at right angles to one another to form regular cubical packets of eight cells, or multiples thereof <sup>[43]</sup>. This was therefore a strict morphological term. But its use in taxonomic classification to identify the morphological particularity of a given species resulted in tremendous taxonomic confusion. This aggravated further when the Bergey's nomenclature (in the 8th edition of the determinative manual) decided to adopt a new classification semi-based on metabolism, and limited the term 'Sarcina' to designate only *anaerobic* cocci capable of sarcinoid packet formation (placed either in the family of the *Peptococcacea*, genus *Sarcina*, or, if methanogenic, in the family of the *Methanobacteriacceae*, genus *Methanosarcina*), while placing all *aerobic* sarcina either in the genus *Micrococcus* or the genus *Halococcus*, with the exception of spore-forming sarcina, which became a genus of its own, the *Sporosarcina* <sup>[44]</sup>.

# 6. Optical artifacts and the evolutionary time-scales of prokaryotes and protozoa

Reich never abandoned the dual claim to accelerated biopoiesis in the form of the "spontaneous generation", *de novo*, of prokaryotes *and* nucleated cells, specifically eukaryotes of the protista realm<sup>[1]</sup> - going as far as claiming the same process of spontaneous heterogenic generation for the production of cancer cells.

The central observation was the equation of sarcinoid packets of prokaryotes to Reich's notion of 'packet amoeba', which buttressed the view that protozoa were naturally assembled from the aggregation of PA vesicles or bions, with one or more of these bions becoming a nucleus or nuclei and the rest remaining as cytoplasmic inclusions. Again <sup>[1]</sup>, Reich was here a prisoner of optical appearances. The fact that cocci can form packets, even geometrically regular ones, or form colonies, and that some such cocci packets or colonies can surround themselves with a thick, microscopically visible, colonial "array envelope" (as is seen in the regular sarcina of the coccus *Lampropedia hyalina* <sup>[45]</sup>) or with a capsule (eg *Sarcina ventriculi*), or even display unity of motion when the entire packet moves in coordination, does *not* result in the formation of amoebae, genuine nucleated protozoa, or even proto-

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protozoa. Thus, to regard the PA bions as strictly pre-cellular precursors that are able to form a protozoon, makes the dual mistake of ignoring the prokaryotic nature of the PA bion, including its potential formation from a spore, and of mistaking colonial prokaryotes, or their packets, for amoebae, which are protozoa.

What is interesting about these very basic errors is that they contain a grain of intuition and truth, but they contain very different time scales or time horizons - not the direct, short-span processes es involved in the sporulation, germination, budding and fission of colonial prokaryotes; *but* the long-term, neo-Lamarckian (involutionary) and evolutionary processes that (1) first abiopoietically generated proto-prokaryotes in various prebiotic soups, (2) later permitted the varied expression and selection of prokaryotes (now including algae in this term) and (3) that permitted, still later, emergence of the philogenetic lines of transformation of diverse prokaryotes by symbiosis and endosymbiosis, and resulted in the creation of a variety of proto-protozoa (methanogenic, photosynthetic, aerobic).

Undoubtedly, primordial cells or proto-cells did not emerge from other cells, but arose de novo through abiogenic processes. Indeed, there is today in modern biology a substantial current of thought <sup>[46]</sup> that holds that the most archaic, non-nucleated, single strand DNA (or even RNA) cells emerged abiopoietically from the metabolic articulation of *two distinct fluxes* of formed materials (nucleic acids and peptides) which, together, managed to intercept a flux of energy (physical and chemical flux), while being trapped within micellar-proteinaceous membrane constructs that are best described as protomicroplasts or protocytoplasts . A variety of double-articulations between nucleic acid and peptide polymers capable of diverting and converting energy will likely have occurred in niches of prebiotic soups long before being trapped in protocytoplasts.

One of the critical biophysical problems of biopoiesis is to determine at which point a globular vesicle ceases being a prebiotic vesicle and becomes a protocell, a living vesicle. The concept of a unity of metabolism (a physiology) is critical for this determination, since only when it is present can one state that the system in question is capable of autonomously deriving its own energy from the environment. Biologists can recognize integral metabolism by microbiological techniques that identify its type (aerobic or anaerobic, etc) and biochemical studies that identify the precise variants; the study of motility, however, has not been considered a reliable tool to identify living cells or metabolic units. Amongst the prebiotic vesicles, only those which qualified as metabolizing constructs were the true protobionts which, if a successful or stable 'metabolizing double articulation' was struck, might develop into the first living cells - possibly the most primitive *Archaeobacteria*, or even simpler bacterial cells with deformable membranes, such as mycoplasma or other *Mollicutes*. There is no way to reliably estimate how far back in terrestrial time such archaic bacteria first appeared.

One often hears about the spore theory of life, which holds that cosmic vessels, such as

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comets, may disperse archaic forms of life across the cosmos by carrying the hardy spores of prokaryotes (theory of panspermia). But even acceptance of this unproven contention raises the question of how did the first spore arise - it subtends a cell, and pushes the question back to how did the first cell arise. Thus the biopoietic soup hypothesis is the only hypothesis that puts a stop to this bad infinite regression that led to the cellular dogma: cellular life emerges abiopoietically *from* molecular life, from the articulation of molecular substrates that successfully captures and harnesses a flux of energy. Accordingly, the abiogenic theory of Reich may actually be correct in its general lines - given the right mix of molecules (amino acids, nucleic acids, mineral salts, sugars and lipids) in colloidal suspension and a high-energy injection - prebiotic soups may gather all the necessary conditions for the production of proto-cellular constructs. During the 1970's, Manfred Eigen showed that in an artificially created laboratory prebiotic soup formed by proteins and polynucleotides, the "final state was characterized by a genetic code and a remarkable stability" <sup>[47]</sup>.

Interestingly, however, if one were to hold on to the spore theory of life, but were to speculate instead that it is not so much spores that evolutionarily developed from cells, but cells that developed from 'spores' or proto-spores (to distinguish them from the complex endospores that have a exosporium, spore coat, cortex and core) one might arrive at the possibility that what Reich was actually tracking is something still different than what may be explained either by spontaneous generation, as he did, or by contamination with endospores. Indeed, in this context, it would suffice to suppose that the abiogenic event occurs more frequently within mineral and proteinaceous (aminated) "shells" capable of swelling by trapping water, carbon and salts, and regularly formed in geological processes, than it does within micellar or lipoprotein globules in ample prebiotic soups. As soon as that step is taken, one is now in a position to consider the possibility of an ubiquitous existence of 'cellular' foci of potential protospore-formation dispersed over all geological layers - within volcanic rocks, silica crystals, carbonaceous and ferruginous minerals, etc - that could abiogenically seed archaic, and effectively immortal, prokaryotes. In this scenario, the true prebiotic vesicles would actually be protospores formed in the geological processes, and not complex membrane vesicles that were eventual precursors of prokaryotes such as Archaeobacteria or Mollicutes. They would be distinct from the evolutionary endospores and be the real seeds of Reich's PA bions. The obvious objections to such a scenario is that there is no evidence for the trapping of fully polymerized nucleic acid sequences within such 'cell foci', and thus the scenario entirely bypasses the biological necessity of a conjugation of oligonucleic acid and polypeptide sequences.

Perhaps the most important set of parallels between evolutionary microbiology and Reich's theory of the PA bions relates to how current biology explains the emergence of the first eukaryotic cell - how an originally prokaryotic cell acquired one or more nuclei, and just as well, how it acquired

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organelles, in particular bacteria-like and algae-like organelles - the precursors of the endosymbionts of protozoa and metazoa, most important being the chloroplasts in plants and the mitochondria in both plants and animals. The most profound insights are that single cell eukaryotes developed, not from coalescent colonial prokaryotes (eg packet cocci), but by the incomplete phagocytosis of a eubacterium by a more primitive (Gram-positive) bacterium that lacked a cell wall. L. Sagan's theory (1967) proposed that eukaryotes first formed from the symbiosis of two different types of prokaryotes, with an eubacterium being ingested but not digested by an heterotrophic bacterium capable of photosynthesis <sup>[48]</sup>. Subsequently, and according to Sagan, formed eukaryotes became, in turn, capable of acquiring - by a similar process - photosynthetic bacteria that gave rise to the chloroplasts of eukaryotic algae and green plants.

Both the nucleus - with its double plasma-membrane characteristic - and the double plasmamembrane of chloroplasts and mitochondria would be major residual indicators, along a long list of other lines of circumstantial biological evidence, of this process of symbiosis that interrupted phagocytosis, thus suggesting that incomplete phagocytosis of eubacteria generated both the eukaryotic nucleus (or nuclei) and the power-generating cytoplasmic organelles of the first protozoa. Lynn Margulis continued Sagan's work into what has become the endosymbiotic theory of the origin of eukaryotes. The theory proposes that primitive eukaryotes acquired their mitochondria or chloroplasts in the form of heterotrophic and phototropic symbionts derived from aerobic bacteria which took up residency in the cytoplasm of those eukaryotes - ie in a protective environment with a ready supply of nutrients. Margulis and co-workers suggested that the nucleus emerged inside the symbiotic eubacterium when the need arose to "join the genes of disparate partners and segregate them to offspring" <sup>[49-50]</sup>.

Yet, despite the acceptance now gained by the endosymbiosis theory of the emergence of eukaryotes, one still wonders whether the model subjacent to Reich's interpretation of his microscopic bion observations has some value in this regard. The coalescence of identical bacteria in colonial packets may not be a source of diversity or heterogeneity - as is needed to explain the emergence of photosynthetic and aerobic eukaryotes - yet it could well have played a role in selecting the eubacteria most inclined towards symbiosis. The cell-to-cell partial coalescence observed among chemoorgan-otrophs like the *Lampropedia hyalina* cells composing an envelope-surrounded sarcina or tablet <sup>[32]</sup> - and which is thought to be due to incomplete cytokinesis - may well have provided the pathway for the initial junction of a group of such cells with a prospective endosymbiont. In this hypothetical scenario, however, it would be the 'eubacterial syncytium' of a packet, and not an *Archaeobacterium*, that would swallow up photosynthetic and respiratory bacteria.

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## 7. SAPA bions and sarcina

A similar correlation with known prokaryotic species might be attempted for what Reich termed SAPA ('Sand Packet Amoeba') bions or cocci obtained by taking pulverized ocean sand to incandescence. Based on his work, we can list their basic characteristics as being identical to PA bions - bacterial cell wall, OD of 2 to 5 µm, aerobic metabolism, Gram-positive - *with the differences* that (1) their packets were always regular, sarcinoid or sarcina, (2) carried a yellowish-orange pigment (yellow-orange color of the agar colonies that Reich considered to be axenic or pure) and (3) were bioluminescent, whether spontaneously or upon stimulation.

Reich presents two photomicrographs of SAPA bions (figures 48a and 48b of "The Cancer Biopathy"), one taken from culture in bouillon, the other from an axenic (supposedly clonal) culture in agar. An obvious observation from both plates is that the cell packets are surrounded by a strong refractile halo, and this is evidence for the existence of a common capsule surrounding these SAPA bions. Thus we can add one more characteristic to SAPA bions. Now note that the clumps are more irregular in liquid culture (bouillon) than in semi-solid culture (agar), forming very regular cubic packets and aggregates of packets in agar. In Fig. 11, we compare Reich's agar SAPA bions with a plate of Sarcina ventriculi shot in 1960 by Canale-Perola and Wolfe [51] - who discovered and isolated S. ventriculi and established the anaerobic genus Sarcina. Visually, these appear to show the same prokaryote, but one cannot legitimately jump from this resemblance to conclude that they are one and the same entity - even if both share the same size and stain Gram-positive. In fact, S. ventriculi is a strict anaerobe isolated from soil, mud, diseased human stomach and the gut of rodents, whereas SAPA bions are (supposedly) aerobic, like the genus Micrococcus they carry a carotenoid pigment, and presumably - if Reich's theory of prokaryotic abiogenesis is correct - are common in marine environments. S. ventriculi is also considered to be nonmotile, whereas SAPA bions were motile in liquid culture. Yet, notably, both S. ventriculi and SAPA bions share a highly refractile capsule (cellulose-rich in S. ventriculi) surrounding the cell wall of all cocci in a packet (up to 64 cells in S. ventriculi), and if this capsule is included (as Reich might well have done, since it is our view that in darkfield he mistook the refractile capsule as evidence for a proximal energy field radiating from the SAPA bions) in the measurement of each coccus, then an apparent OD up to 3.5-4 µm easily results. Just as noteworthy is the fact that, despite being classified among the non-spore forming Gram-positive cocci, S. ventriculi has been reported to form spherical spores [52], a fact that should reclassify it as a sporosarcina. Virtually nothing is known about the heat-resistance of these spores.

Perhaps even more challenging than the closeness between SAPA bions and *S. ventriculi* is the even greater closeness of the former with the oxygenic photosynthetic bacteria (cyanobacteria or bluegreen algae related to plant chloroplasts, and which are abundant in the oceans, fresh water and soil)

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Fig. 11A - Fig. 48b of Reich W (1947) "The Cancer Biopathy", showing live, unstained, sarcina colonies of SAPA bions in agar. Each coccoidal cell has a 2 -2.5µm OD. Brightfield, apochromatic lenses. Original magnification:400x. View magnification:ca 1,000x.

Fig. 11B - Plate from reference 51 (Canale-Perola E & Wolfe RS (1960) "Studies on *Sarcina ventriculi*", *J Bact*, 79:887) showing live, unstained sarcina packets of *Sarcina ventriculi* in agar. Each coccoidal cell has a 1.8 - 2.4µm OD. Original magnification of 540x, brightfield. View magnification: -500x.

Fig. 11C - Time course of the development on a mineral medium agar of a *Myxosarcina baeocyte* into an encapsulated cubic sarcina packet microscopically analogous, in all respects, to Reich's SAPA bions (from Waterbury JB & Stanier RY (1978) *Microbiol Rev*, 42:2, © American Society for Microbiology). The last plate, at 554 hours, shows the cycle being completed by the profuse release of new baeocytes. *Myxosarcina* (eg stains PCC 7312, PCC 7325) is an ubiquitous marine packet-forming coccus. Brightfield, timelapse photography. Bar:10µm.

of the poorly characterized *Myxosarcina* <sup>[53-54]</sup> and *Chroococcidiopsis* genera (see Fig. 11C for their growth in agar, an in particular note the cubical form of the clonal packet at 385 hours, ie d17). Both form large, clonal sarcina packets in agar virtually identical to the packets of Reich's SAPA bions, and they are found in marine environments and in soil (*Myxosarcina concinna* is the terrestrial botanical type of the marine *Myxosarcina*). The packets are surrounded by a sheath (and hence their edge is very refractile) and each cell by a fibrous outer cell wall. Oceanic *Myxosarcina* have elevated salt requirements. Both types of cyanobacteria also release small, non-metabolizing cells, the baeocytes (it is actually not proven that baeocytes are non-vegetative cells). Each baeocyte looks like a coccus and measures on the order of 1 to 3  $\mu$ m - and from a single one, an entire clonal packet of vegetative cells grows by repeated binary fission in three consecutively alternating planes (see Fig. 11C, and compare with Fig.s 11 A and B). Baeocytes form by multiple fission of the vegetative cells, and in these two

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genera of cyanobacteria, the processes are highly synchronous. Each cell produces between 4 to 16 baeocytes, depending on the strain of *Myxosarcina* (*Chroococcidiopsis* strains produce 4 baeocytes per cell). The vegetative cells are a bit larger than the baeocytes, with OD's of 4-10 µm for a vegetative cell at the time of the first division (thereafter cell size is either maintained or reduced). Most frequently, vegetative cells measure 4 to 5 µm in diameter. The Gram reaction of these genera is unknown for both vegetative and non-vegetative cells. The quiescent baeocytes must be related to spore-formation in some unknown way and, once again, nothing is known about the heat resistance of these baeocytes. The term 'baeocyte' was coined by Waterbury and Stanier (1978) <sup>[55]</sup> to avoid assimilation to the concept of endospores in the realm of the prokaryotes, or to the "nannocytes" of botany. Baeocytes may actually possess metabolic states, rather than be vegetative cells. Also note that whereas the *Myxosarcina* baeocytes (or single cocci) *are* motile (but apparently not flagellated), the *Chroococcidiopsis* baeocytes are not motile because, like vegetative cells, they are also surrounded by a fibrous outer cell wall.

Significantly, other genera of cyanobacteria are also barely discernible from *Myxosarcina*. The *Gloecapsa* group tentatively placed in the order of the *Chroococcales* (rather than in the order of the *Pleurocapsales*, where *Myxosarcina* and *Chroococcidiopsis* are still placed) possesses an extracellular capsule and also divides by binary fission, forming packets in agar that resemble those of the *Pleurocapsales*. The *Pleurocapsa* group (eg strain PCC 7322) - tentatively placed in the *Pleurocapsales* - also closely resembles *Myxosarcina* when grown in agar heterophototrophically with sucrose. This group presents medium- and metabolism-dependent pleomorphism, forming more irregular packets without cuboidal structure when grown photoautotrophically on agar in a mineral medium. The irregularity of the packets and size of cells is mostly due to the non-consecutive order of the successive planes of cell division. When grown photoautotrophically, the packet may be so irregular as to microscopically appear like an amoeba with extruding pseudopods.

These facts are highly suggestive of a possible taxonomical identification of SAPA bions with marine *Myxosarcina*. In Reich's language, the encapsulated sarcina is a packet amoeba, and the formation and release of baeocytes (this release is shown in **Fig. 11C**, last plate at 554h), a bionous vesiculation and decomposition of the packet amoeba. But even though he was able to grow these SAPA sarcina in agar in 5 out of 8 attempts, he did not observe the clonal growth from a baeocyte or "single vesicle".

The proposed identification of SAPA bions with marine *Myxosarcina* suggests a novel explanation for the production of SAPA bions by the incandescence of marine sand. It is entirely plausible that non-vegetative transitional cells - the baeocytes of these marine sarcina - may be incrusted and enveloped by the shells of marine animals and protozoal diatoms (in fact, penetration of calcareous

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shells and substrates has been shown to be a result of a symbiosis between acid-producing chemoheterotrophic bacteria and the cyanobacteria of the *Pleurocapsa* group <sup>[56-57]</sup>), as well as by quartz released from the erosion of igneous and metamorphic rocks, all of which are the main sources of marine sand. Incandescence of these crystalline structures (geologically and biologically formed) would release these baeocytes or 'spores' from their trapped state. If these nonvegetative cells were capable of enduring tremendous temperatures for a short time (the situation in incandescence, it being understood that the crystalline structures also insulated these trapped baeocytes or 'spores' from the full heat of incandescence) then, given the right conditions for growth, they would give rise to metabolizing photosynthetic sarcina. Such ability would have permitted these genera to populate nearly all the geomarine strata, from the surface of the seas down to volcanic hydrothermal vents. Conceivably lava and igneous rock could be peppered with sarcina baeocyte inclusions.

If nonsporing anaerobic *Sarcina* can produce spores like aerobic *Sporosarcina* does, and the marine aerobic 'plant' *Myxosarcina* produce baeocytes, and if - under the microscope and in agar plates - they can all produce analogic packets, it seems that the only difference that biologically matters is that of metabolism and its functional adaptation. Thus, given the fact that the now accepted nomenclature of sarcina splits them between anaerobic sarcina (genera *Sarcina* and *Methanosarcina*) and aerobic sarcina - which may be nonspore formers (*Micrococcus* and *Halococcus*) or spore formers (*Sporosarcina*) - one is left wondering whether strains from these different genera do not simply constitute fundamental adaptations of the same sarcinoid precursor (or phylum), likely marine, to different environments, in particular with respect to exposure to sunlight (pigments), oxygen (difference in aerobic and fermentative metabolism), salt (halophiles vs salt intolerants), heat (spore formers), etc. Such a consideration casts doubts on the validity of the accepted taxonomic distinctions based on spore-formation, ribosomal RNA affinities and the notion that, evolutionarily, *Sporosarcina* are closer to *Bacilli* than to *Micrococcus*.

In light of these considerations, Reich's notion that PA bions could switch between sizes and forms (were pleomorphic) - being blue cocci when large (> $2\mu$ m), and blackish cocci when small (0.5 to 1.5  $\mu$ m) - according to whether their metabolism was aerobic or anaerobic, with rod forms being prevalent in anaerobic conditions, has a very interesting resonance with the problem of the taxonomic and evolutionary distribution of sarcina, suggesting that a parallel morphic variation could underlie the various genera of sarcina, with the aerobic sarcina also being larger, more oval and pigmented, and the anaerobic sarcina (like *S. ventriculi*) smaller and non-pigmented. Such a model necessarily underscores that these are different adaptations of the same microbiological phylum to distinct environments.

Lastly, there is one stunning aspect of this correlation of SAPA bions with Myxosarcina that

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implies and confirms the requirement for extreme heat resistance on the part of either vegetative or nonvegetative cells - or maybe on the part of both. We have in our laboratory replicated this astonishing finding - that the SAPA bion packets withstand multiple standard autoclavations in liquid culture, with growth starting anew each time. Thus, if we consider how Reich obtained SAPA bions - under the extreme conditions of heating and moist-heat sterilization - and how these SAPA bion packets withstand multiple re-autoclavations, one is forced to conclude that, if SAPA bions are identical to *Myxosarcina* or a closely related genus, these must be extremely heat-resistant marine forms of true sporosarcina. Accordingly, there would be, therefore, no real basis to consider SAPA bions as being microbes that were created *de novo* in the laboratory by an abiogenic process.

## 8. The question of autonomous motility vs Brownian motion

Whereas the presence of a metabolic unity and the capacity to replicate or proliferate are primary defining characteristics of a living system (at the limit, a cell), the property of locomotion or motility has not been considered a reliable tool to identify living cells. Many prokaryotes are nonmotile life forms. Motility, in the sense of locomotion, is exhibited in three main forms: screwmotion, gliding and swimming. Screw-motion, as observed with spirochaetes, is caused by contractile flexions of intracellular axial fibers; gliding, as observed with mycoplasma, is made possible when the bacteria have a flexible cell wall that permits bending by attachment of fibrils that pass through the cell envelope and cell wall; and the most common locomotion, the swimming motion, is the result of the beating of flagella (single or multiple), specialized submicroscopic helical filaments whose waving propels the bacterium.

Reich claimed that his PA bions were capable of a variety of motions, some locomotor and others not - such as vibratory and rotary movements. He suggested that all these motions were the product of inner impulses related to the time-varying "internal charge" of the vesicles. Whether the PA bions exhibited locomotor motion or not - and this type of motility was observed to constantly appear and disappear - the other pulsatile motions were always present. SAPA bions presented similar forms of pulsatile activity, their locomotion being described as "slightly motile" (in the first, 1947 translation of "*The Cancer Biopathy*") or "scarcely motile" (in the 1952 and 1979 editions of the same book) <sup>[58]</sup>.

As anyone can confirm with *in vivo* microscopic observations, most ordinary cocci - though not all - also display pulsatile activity in liquid media: they constantly quiver, vibrate and rotate. But coccal locomotion is considered to occur only when these cocci are flagellated. We should note that some bacteriologists or microbiologists contend that micrococci, for the most part, are not motile, and that motility is alone the property of the strictly aerobic, sporing and packet-forming (tetrads or

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cubical packets) cocci of the genus *Sporosarcina* <sup>[26]</sup>. In the absence of flagella, any external displacement of cocci are considered to be the result of external forces, and fall under the category of random 'Brownian motion'. Once canonized by Einstein for the motion of submicroscopic particles, the theory of Brownian motion became accepted in microbiology to explain the 'apparent motility' of 'dead' or inert particles of submicron size in suspension, as well as the 'random motion' of immotile cocci, once they are suspended in aqueous phase. The theory argues that the motion of these living but immotile cocci is caused by the random impact of thermally diffusing molecules in solution acting upon particulate or vesicular bodies, whether living or nonliving, to confer them haphazard movement and transfer momentum.

It is obvious that the concept of Brownian motion cannot account for the presence of pulsatile forms of motility lacking overt external displacement, such as quivering or rotating motions. These are most likely the mark of living prokaryotes (but see below what Fox reported for his prebiotic vesicles). However, having been reproached in a few occasions for claiming for his PA bions genuine locomotor motility that was deemed instead to be the effect of Brownian motion, Reich made an effort <sup>[59]</sup> to prove that the observed displacements were the result of forces internal to the bion vesicles, and not due to Brownian motion.

In our view, this attempt did not succeed, largely because Reich failed to devise an experimental protocol that would unarguably prove that the observed external displacements of his PA cocci were autonomous movements. However, independently of the performance of such a protocol, there are a number of problems that immediately arise to block the interpretation of Brownian motion as applied to Reich's PA bions.

The most obvious one is that, despite Reich's good microscopic optics, his techniques, even the darkfield method, did not lend themselves easily to the detection of bacterial flagella in his PA bions. Any demonstration of the presence of flagella in his bions would have immediately nullified the charge of Brownian motion.

Aside from this, however, the first problem that arises with respect to the Brownian explanation of the apparent motility of any so-called "immotile cocci" in aqueous solution is that there is a theoretical upper limit - at 1  $\mu$ m - to the size of objects which will respond by motion to the molecular impact, and while this includes indeed many cocci, including staphylococci, it does not include either the single PA bions (in the 1.5 to 2  $\mu$ m range), let alone the large clumps they form, of >4 $\mu$ m in any axial direction.

The second problem with the interpretation of Brownian motion is what Fox himself enunciated for his own prebiotic microspheres as: "The possibility exists that very small particles of certain composition move randomly whereas those of others move nonrandomly" <sup>[60]</sup>. Fox conducted time-

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lapse studies of his microsphere preparations once they were activated by the addition of zinc and ATP. He concluded that whereas most proteinoid microspheres are engaged in Brownian jiggling, he could find coupled microspheres that reversed their rotation and moved nonrandomly throughout the field. Fox's argument is that such microspheres 'look' alive even though he knows they are not - because they have acquired a basic simulation of energy metabolism and autonomous performance. Nonrandom motion could then be seen as a property of some prebiotic vesicles expressed in the course of the abiogenic processes of cellular biopoiesis. It would not be a marker of cellular life, not per se, but would nevertheless raise the doubt of whether the motion of "normally immotile" prokary-otes can be explained at all by Brownian motion.

This brings us to the third problem with the theory of Brownian motion as applied indiscriminately - be it to deny the existence of internal forces in microscopic and submicroscopic living systems, cytoplasts or 'prebiotic vesicles', or to explain the motility of so-called 'immotile cocci'. It has been suggested, in this sense, that it is the heat of the microscope lamp aborbed by the solution molecules which, during *in vivo* light microscopy observations, indirectly causes the Brownian motion of the particles. If the particles are microspheres, Fox himself admits that heat accounts for the random motion of most microspheres, but not so for the nonrandom motions that he also observed. However, if the particles exposed to heat are living cocci, or PA bions, for that matter, the question is far from being susceptible to such a simplistic reduction. Indeed, as we ourselves have observed countless times, the erratic or oscillatory locomotion in aqueous solution of large or small cocci, and of PA and SAPA bions obtained by replication of Reich's protocols, often stops after a period of exposure, despite the continuation of the "thermal agitation" of the medium, or the continued application of microscopic illumination.

Fourthly, there is the critical problem of bioelectricity, and how it affects the motility of living micrococci, PA and SAPA bions, and Fox's microspheres. One may in fact consider that the distinctive trait of living systems is the presence of action potentials on their membrane structures. Essentially, cellular plasma membranes are capable of becoming electrically polarized, acquiring either net negative or positive charges. Prokaryotes and unicellular eukaryotes exhibit galvanotaxis as a function of this polarization. For example, *Paramecia* are negative galvanotropic if the direct current through a solution is low, but if increased beyond a threshold, then their cillia reverse motion and they swim back to the anode <sup>[61]</sup>. Jahn demonstrated that ciliary reversal is brought about by currentinduced depletion of calcium ions <sup>[62]</sup>. Reich, in his PA bion preparations observed that, while the 'fresh bion mixtures' migrated towards the anode and were thus positively galvanotactic, second generation cultures in agar ("yellow packet amoebae") were negatively galvanotactic or cationic <sup>[20]</sup>. He further stated that he was convinced that the electrical charge of the mixture was "an essential condi-

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tion for culturability", since preparations "that yielded no cultures were electrically neutral". In this very context of the electric charge of colloids, whether cellular or prebiotic, Reich proposed that the jiggling, quivering and dancing motions of bions was entirely "connected with the force effects of electrical fields" <sup>[63]</sup>, rather than with the impact of thermally agitated solution molecules. About the source of this bioelectricity, he added: "it is assumed that the charge is produced by the buildup of ions from the electrolyte at the surface of the particles" <sup>[64]</sup>.

It is curious how Fox never examined the galavanotactic properties of proteinoid microspheres. Instead, in some of the worst anthropomorphic pages he wrote, he spoke of the attraction responsible for 'the dating and mating' of microspheres as being the result of the latter forming an heterogenous population containing both negatively (acidic) and positively (basic) charged groups, while repulsion would be observed between microspheres carrying either the same negatively charged carboxyl groups, or positively charged lysine and arginine groups. There is little doubt that Fox observed in situ attractions and repulsions between microspheres, nor that the electrochemical explanation which he provided is analogous to that which Reich provided for his own observations of attraction and repulsion between bionic vesicles: "Forces of attraction tend to combine the particles; (...) On the other hand, electrical charges of the same sign give rise to repelling electrical forces" [64]. It is easy to observe these events of attraction and repulsion, for example, even in a preparation of Pasteurized milk that has been refrigerated - amongst the quivering and jiggling motion of the proteinaceous lipid globules and monococci of various sizes, one can observe prolonged attractions and repeated repulsions between two or more cocci, that can only be explained by monopolar electrical interactions. Independently from whether one chooses to employ the theory of Brownian motion or not as the explanation for the constant jiggling of "immotile" cocci in aqueous solution, there is no doubt that both Fox and Reich are correct in proposing that specific changes in position manifested by repeated attractions and repulsions between microspheres and bions or cocci, cannot be explained away by thermally mediated Brownian motion, and that they raise instead the question of bioelectricity and electric field effects. This was already what Reich was reaching for when he concluded in a veiled fashion that "our experiments would seem to indicate that the particles themselves are electrically charged as vesicles", to mark his notion that the charge arose as the vesicles formed and 'became alive'. Only vesicles formed with charge would be viable, since the very condition to maintain a colloid in suspension was that it had to be electrically charged.

However, Fox's arguments that his populations of prebiotic microspheres are heterogenous with respect to charge, but effectively carry charge of one polarity or the other, suggests that, even if electric charge is a precondition for living systems, it is not an exclusive property of them. Formation of proteinoid microspheres itself can be seen as the result of electric processes that separate charge.

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Accordingly, what characterizes more properly speaking cellular systems is not so much their capacity for electric polarization or galvanotaxis, as it is the regular variation in electric potential which they undergo synchronously with their pulsation or pulsatile motion, and which ultimately depends upon their unity of metabolism, or upon an autonomous process that increases their *internal energy content*.

# 9. Experimental replication of the SAPA bion experiments

We've proposed above an hypothesis regarding the presence of baeocytes or 'spores' of Myxosarcina in sand crystals that suggests these inclusions are the reason why the sand incandescence experiments of Reich yielded Myxosarcina-like SAPA bions. We've shown that agar growth, irrespective of differences in metabolism, yields microscopically identical growths of S. ventriculi (see Fig 11B) and Myxosarcina (see Fig, 11C). Now we are interested in correlating the results of our replication of Reich's SAPA protocol and the hypothesis of an ubiquitous presence of Myxosarcina baeocytes in marine sand. We performed the experiment 45 times with a total of 21 controls, with dry-sterilized (180°C, 2hrs) sand taken from 6 ocean beaches on either side of the Northern Atlantic. 5 of the 6 sources yielded burst grains and single motile cocci after incandescence, and 4 out of the 6 presented quiveringly motile packets. The duration of incandescence in the oxidizing portion of an oxygen/natural gas flame mixture was investigated - with periods between 20 seconds and 3 minutes. Best results were obtained with 1 minute incandescence. Experiments employing a reducing flame (red hot sand) yielded negative results. Results were also negative when pure silica sand controls were employed. Immediately after incandescence, the sand was plunged into a 50:50 solution of 0.1N KCl and beef broth, with or without 3% NaCl (addition of NaCl gave best results). All liquid media were autoclaved (15 lbs, 30 min.s) and 0.45µm filtered prior to inoculation. After inoculation, liquid cultures were incubated at 25, 30 and 37°C. Best results were obtained at 30 and 37°C.



**Fig. 12** - Nomarski contrast interference of fully formed sarcina and sand crystals in 0.45µm filtered beef bouillon liquid culture right after (15 min.s) incandescence of sand in an oxidizing flame. Each coccus or coccoidal inclusion in a sand crystal is 2.5 - 3µm long and 1.5 -2µm wide. Marine sand (Oura beach, Portugal). 100x neofluar oel, 2x projective. Hitachi VK-C2000 CMOS camera.

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Fig. 13 - High power darkfield view of the 'burst' or 'popcorn' appearance of a sand crystal brought to incandescence and plunged into  $0.45\mu$ m filtered liquid beef bouillon medium. Vesicles vary between 1 -  $5\mu$ m OD. The vesicular nature of the extruded crystals is evident. Marine sand (Oura beach). 100x oel apochromat, 1.4 NA supercondenser, 4x projective, 50 W mercury lamp, Hitachi VK-C2000 CMOS camera. View magnification: ca. 1,700x.



Fig. 14 - Low power darkfield view of the typical outcome of a SAPA experiment, taken right after plunging incandescent sterile sea sand into 0.45µm-filtered beef bouillon. Virtually all sand particles have a vesicular "burst-like" structure. Same optics, illumination and video as in Fig. 13, except 2x projective employed. View magnification: ~400x.



**Fig. 15** - Low power darkfield view of the vesicular inclusions in a grain of marine sand taken to incandescence but which did not burst. Same conditions as for **Fig. 14**.View magnification: ~630x.

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**Fig. 16A** - Fully formed *Myxosarcina*-like, double-cuboidal (end-to-end) packet identical to SAPA formations obtained right after marine sand (Oura Beach) was taken to incandescence and plunged into 0.45µm, 3% NaCl, 50:50 beef broth and 0.1N KCL. Each vesicle ranges between 1.5 and 3µm in OD. Darkfield, 1.4 NA ultra-condenser, 100x oel apochromat. Hitachi VK-C2000 CMOS camera.

**Fig. 16B** - The two double cuboidal (end-to-end) packets obtained by Reich in agar (day of culture unknown; see **Fig. 11A**), compared to the double cuboidal packets we obtained after incandescence (inverted darkfield of **Fig. 16A**) and compared to a d12 (288h) single cuboidal packet of *Myxosarcina* grown in agar and mineral medium (see **Fig. 16C**). Adjusted to the same magnification of 1,000x.

The most notable result is the microscopic observation of 'spawning cocci' and even fully formed irregular and quasi-regular packets in liquid cultures of sterilized marine sand taken to incandescence (see Fig.s 12 and 13). This is far from being an odd occurrence, since field after field (see Fig. 14) taken right after incandescence shows large numbers of such coccoidal packets, highly refractile and motile in twitching and quivering fashions. Likewise, sand crystals are microscopically peppered with vesicular inclusions (see Fig. 15). In some instances, and only when 3% NaCl was added to the broth, *Myxosarcina*-like packets were obtained immediately after incandescence (see Fig. 16A, and compare Fig. 16B with Fig.s 11A-11C).

In 7 out of 18 broth agar plates tested (variously employing either simple broth, peptoneenriched broth and tryptose-enriched broth), the SAPA vesicles grew only in simple broth-agar (without mineral medium), and formed sarcina and cuboidal packets (see Fig. 17) that were apparent by 48h after the incandescence procedure. Similar formations can be observed in lightly teased smears of d7 agar colonies (see Fig. 18). Some of the teased apart d7 agar cultures presented packets more evocative of the *Gloeocapsa*-group than *Myxosarcina* (see Fig. 19). Since we employed a passively humidified incubator, agar cultures on Petri dishes did not usually last beyond 12-15 days. We were forced therefore to transfer the agar cultures and reclone the colonies. Fig. 20 shows a phase-contrast section through a recloned large colony of SAPA sarcina . The cross-section through the central near-cuboidal

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Fig. 17 - Scooped and lightly teased-apart, live d2 broth-agar culture of SAPA vesicles inoculated on d0, a few hours after marine sand(Oura beach) incandescence and immersion in liquid medium. Each coccus is  $1.3 - 1.5\mu m$  in OD. Diplococci, tetrads and cuboidal packets are observed. Brightfield, oil immersion, 100x apochromat. Hitachi VK-C2000 CMOS camera.

Fig. 18 (inset lower right) - Day 7 lightly teasedapart live colonies of SAPA bions in broth-agar obtained by the incandescence of marine sand (Myrtle Beach). Brightfield, same optics and video as in Fig. 16. Culture was pigmented yellow. Each coccus is 1 - 1.15µm OD.



Fig. 19 - Gloecapsa-like sheath-enveloped sarcina and tetrad from d7 live colony of SAPA bions in broth-agar, obtained by the incandescence of marine sand (Oura Beach). Culture was also pigmented yellow. Each coccoidal cell is  $1.5 - 3\mu m$  in OD. Some look hemispherical. Phase contrast, oil immersion (100x Ph3). Hitachi VK-C2000 CMOS camera.

**Fig. 20** - Phase contrast of a re-cloned d15 SAPA sarcina colony in broth-agar, showing a cross-section of eight cells through the central cuboidal packet. Phase contrast (63x Ph3 dry mount objective), Hitachi VK-C2000 CMOS camera.



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packet (estimated 32 cells) buried in agar shows it consists of an eight-cell tablet. Whether the observed sarcina in agar were adaptations of *Myxosarcina*, or still other baeocyte-forming marine sarcina could not be resolved, likely because we did not test any agar plates with 3% NaCl. Lastly, we should note that, while it is expected that cyanobacterial sarcina should stain Gram-negative, our Gram stain tests indicated ambivalent results, with the peripheral cocci of a packet staining blue (Gram-positive) and the inner cocci staining red (Gram-negative), whether the samples were taken from liquid cultures or from agar colonies.

These findings, therefore, confirm the results obtained by Reich with his SAPA protocol. But, as already discussed, instead of confirming Reich's interpretation of abiogenesis, our findings suggest that the presence of spores of marine sarcina in ocean sand, in the form of crystal inclusions, is sufficient to explain the emergence of SAPA bions in sterile liquid culture immediately following immersion of incandescent sand.

# 10. Institutional science and the suppression of microbiological facts

In January 1937, Reich sent to the Paris Academy of Sciences and to Roger du Teil (University Centre of Nice) various bion cultures and a preliminary report of his findings with PA bions (not including his SAPA results, which date from 1939 onwards). Roger du Teil repeated these experiments in three sets <sup>[11]</sup>.

In various vessels built to serve as controls for Pasteur's own vessels (which were designed to ensure sterility), du Teil shows that Gram-positive cocci can be obtained in egg-lecithin preparations, with and without milk, even when the entire apparatus was autoclaved for 130°C for one hour. Similarly, he obtains these cocci from gelatinized blood mixed with pulverized coke taken to incandescence and autoclaved at 130°C for 45 minutes, or 134°C for 30 minutes. The last experiment was also the most disconcerting and - in our view - most illuminating (see below). Since it was employment of KCl that was common to all preparations, du Teil wanted to check whether it alone could be the source of the bion cultures. So he devised an experiment that only employed the addition of autoclaved (130°C up to 1 hour) KCl solution to broth - and in all instances he again obtained similar cocci.

The Paris Academy then sent the material received from Reich and du Teil to Louis Lapicque (1886-1952), a physiologist at the Sorbonne University known for his work in studying nerve excitation. Lapicque was also a long-standing member of the Order of the Grand Orient of France, and later, as of 1945, "Conseiller" of the same masonic order. Lapicque confirmed the observations on the living cultures that Reich sent to the Paris Academy of Sciences, and in a letter of January 25, 1938, offered publication if Reich accepted that it be followed by Lapicque's own "physico-chemical inter-

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pretation" (sic). This interpretation was not, in his communication with Reich, elaborated upon, nor does Lapicque return to it later. Lapicque then advises Reich to "leave aside your electrical theory which has nothing to do with experience" <sup>[65-66]</sup>.

It is remarkable that Lapicque refers to "experience" and "interpretation" but not to experimentation or experimental facts; and that he entirely glosses over the unmentioned spontaneous generation, an issue which somehow seems to be subsumed under the heading "electrical theory". Reich refused this abusive request. Thus his findings with bion preparations were consigned never to receive the approval of Official Science, being not even deserving of a peer review.

This episode is important because it contains three distinct problems that concern science, its content and its practice, and what happens to these when science becomes reduced to institutionally manufactured consensus: science is suppressed - and in the best of cases, delayed or postponed to another epoch.

First, this episode elucidates (once again) how mainstream peer-review - ensconced in academies and journals - operates to the total detriment of science: access to publication and dissemination of facts is hostage to the interests of scientist bureaucrats that seek their own advancement while acting in the name of the collective institution they "serve", and that most frequently also ply the interests of extra-academic social forces which have a stake in the outcome of research.

Secondly, this episode gives us the dimension of how much Reich's insights were beyond his own epoch - irrespective of whether his science was right or not. Reich's observations might have erroneously led him to think that eukaryotes emerge from colonial prokaryotes and that this is a regular occurrence; yet his theory has profound parallels with the endosymbiotic theory of proto-eukaryote formation; simply, he enunciated it 3 to 4 decades before Sagan and Margulis did, and the timeframe of his theory is much shorter in duration (rather than being a slow phylogenetic process, Reich believed it happened under his very eyes, compressed by laboratory techniques). Likewise, in the experiments that he sent the Paris Academy, Reich was showing directly how Pasteur had *not* established for a fact what are the conditions for sterilization. His bion cultures remained alive even after 5 cycles of autoclavation at 130°C in closed vessels. The problem was real. The difficulty confronting the science of biology was real, even if Reich's theory did not satisfactorily address it. Yet, that is not what Lapicque focuses on. In fact, Lapicque does not even seem to care one bit about the central problem of the experiments that Reich sent to the Academy.

Thirdly, there is indeed the real problem that arises from these experiments of Reich and confronts both microbiology and the theory of sterilization; and it needs to be addressed both experimentally and analytically. Let's return to du Teil's conclusions about his three experiments. He concludes that there are only two possible interpretations - one according to Pasteur, and the other

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according to Reich's theory of the spontaneous ion-electric self-ordering of material substances into living cells, or their prototypical forms or precursors (the bions). In favor of the former interpretation, there is the assumption that KCl contains very hardy germs or spores which contaminate Reich's preparations. Du Teil explicitly accepts that this is a possibility and, if confirmed, "a matter of some interest". In favor of Reich's interpretation, he contends that the broth preparation has all the necessary precursor building blocks required to assemble living cells.

Now, this is of great interest to us because of two aspects of the problem that so far no one has recognized. First, there is the fact that broth does *not* have all the required building blocks needed for the assemblage of a cell. It has nearly all the nutrients required for most cells - and not even all cells. But, secondly and far more importantly than the argument regarding completeness of broth, is the fact that Reich and du Teil report that broth controls remained clear, and that this ruled out broth as the source of the cocci or their germs. However, the fact that no growth was observed in broth without KCl could hardly be proof that broth itself was not the source of bacterial spores. The addition of KCl might simply promote the swelling and thus the germination of spores present in broth that, otherwise, will remain quiescent.

Indeed, we ourselves came to discover that broth *is* the source of bacterial spores, in the course of our own investigation of the source of the cocci in our first replications of Reich's SAPA experiments. We had come to suspect KCl and NaCl of being sources of bacterial spores, only to find out that the source itself was the broth in the absence of any added salts. Filtering broth through 0.45 µm did not eliminate the problem, nor did repeated autoclavations up to 140°C and 30 minutes! Only after 45 minutes at 141.5°C were we able to generate filtered broth free of "spontaneous" bacterial growth.

This fact then suggests that spores with sizes of less than half a micron are contaminants present in broth and capable of surviving multiple cycles of hyper-autoclavation, defying what is still the accepted view in microbiology with respect to the requirements for sterilization with moist heat. According to further research of ours, it seems that in many cases some of these contaminants are Mollicutes. Irrespective of their identification - and too buttress our case regarding the existence of hyperthermally-resistant prokaryotes or their 'spores' (in a wide sense) - we should mention that an unpublished study at the washing facility of the now defunct Department of Anatomy and Cell Biology (where Dr. Correa worked for 10 years) at the Faculty of Medicine of the University of Toronto, and which employed *Bacillus stearothermophilus* (the most heat resistant member of the endospore-forming *Bacillus* genus, and whose spore size may be as small as 0.4 µm) as sterility indicator, found that at the standard 121°C, 151bs of pressure, autoclavation time increased with the volume of liquid (1L of tapwater was sterile after 30 minutes, but 3L of tapwater required 60 minutes),

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and that agar cultures in a polypropylene bag also required 60 minutes before sterilization.

It is apparent that spore-forming bacteria, and not just members of *Bacillus* genus, can and do survive the standard techniques of autoclavation thought to be sufficient for sterilization. Maybe even some nonspore-formers can do the same. This should be of some concern to both microbiology and medicine, even as these facts remain obscure and obfuscated to this day.

# REFERENCES

1. Correa PN & Correa AN (2010) "Wilhelm Reich's Claim of the Heterogenesis of Eukaryotic Amoebae", *J Biophys Hematol Oncol* 1(1):1.

2. Reich W (1938) "The Bion Experiments", 1979 ed., Farrar Straus Giroux, NY, NY, p. 62.

3. Idem, p. 63.

4. For the treatment of the co-acervate as a concentrated colloidal sol, see Oparin, AI (1938) "The origin of life", reprinted in 1965 by Dover Publications, NY, pp. 148-162. The coacervate had a triple ability - to absorb water (solvate), and to absorb and separate hydrophobic and hydrophilic molecules. Reich was well aware of the importance of the work of A.I. Oparin and B. de Jong in the 1930's, see Hahn, A (1945) "The history of the conceptions of the origin of organic life since the 17th century" in "Bions, on the genesis of vegetative life", reprinted in 1977 by Freedom Press, LA, pp. 174-175.

5. Fox SW (1964) "Thermal polymerization of amino acids and production of formed microparticles of lava", *Nature*, 201:336.

6. Fox SW (1974) "Coacervate droplets, proteinoid microspheres and the genetic apparatus", in "The origin of life and evolutionary chemistry", Ed. by S.W. Fox et al, pp.119-130.

7. We have experimentally addressed the problems raised by the applicability of Brownian motion to the movement of very small non-flagellated cocci, cell-free mitochondria and mycoplasmas in another report.

8. A formal answer to this question can only be experimentally determined: assuming the possibility that agar cultures - which are second generation cultures - could be the result of systemic contamination of the original liquid cultures, one should prepare RNAse-free DNA extracts from first and second generation cultures and determine whether DNA is present and whether, by sequencing, it is identical, as well as homologous or not to any of the known and sequenced bacterial genotypes.

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9. Prigogine I (1972) "La thermodynamique de la vie", *La Recherche*, Juin, reprinted in "La recherche en biologie moléculaire", Éd. du Seuil, Paris, 1975, p. 225.

10. Reich (1938) op. cit., pp. 78-83.

11. du Teil R (1937) "Three series of experiments based on the tension-charge principle", Appendix to Reich W (1938) "The Bion Experiments", reprinted in 1979, Farrar Straus Giroux, NY, NY, pp. 179-192.

12. Reich (1938) op. cit., pp. 107-110.

13. See - for testing, analysis and discussion - the forthcoming report: Correa P (2010) "Cellfree growth of highly thermoresistant mitochondria isolated from human peripheral blood leukocytes", J Biophys Hematol Oncol 1(11):1.

14. Reich W (1939) "Bion experiments on the cancer problem", Oslo, Norway, p. 11.

15. Reich W (1948) "The cancer biopathy", republished by Farrar Straus Giroux, NY, NY, 1973 ed., pp. 31-32.

16. Reich (1938) op. cit., p. 82.\_

17. Reich (1948) op. cit., p. 19.

18. du Teil (1937) op. cit., Appendix, p. 185.

19. Reich (1938) op. cit., p. 72.

20. Idem, p. 73.

21. Idem, p. 79.

22. Sapp J (2007) "Mitochondria and their hosts: morphology to molecular phylogeny", in "Origin of mitochondria and hydrogenosomes", ed. by W.F. Martin and M. Müller, Springer-Verlag, Berlin, pp. 57-58.

23. Hahn (1945) op. cit., p. 195.

24. Bryant AH & Bryan CG (1953) "Bacteriology- Principles and Practice", Barnes & Noble, NY, pp. 21 and 80-81.

25. Hawker LE & Linton AH (eds) (1971) "Micro-organisms: function, form and environment", 1979 ed., University Park Press, London, UK, pp. 96-98.

26. Wilson GS & Miles AA (1975) "Principles of bacteriology, virology and immunology", Vol. I, Williams and Wilkins, Baltimore, MD.

27. Nicholson WL et al (2000) "Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments", *Microbiol Mol Biol Rev*, 64:548.

28. Brock TD, Smith DW & Madigan MT (1979) "Biology of micro-organisms", 1984 ed., Prentice-Hall, Englewood Cliffs, NJ, p. 702.

29. "Bergey's manual of determinative bacteriology", 8th ed., 1974, Williams & Wilkins,

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Baltimore, MD, p. 574.

30. Reich (1938) op. cit., p. 71.

31. Idem, p. 91.

32. See Figures 2 and 3 of Plate 10.3, p. 444 of "Bergey's manual of determinative bacteriology", 8th ed., 1974.

33. Idem, p. 440.

34. "Bergey's manual of systematic bacteriology", 1984, Vol. 1, Williams & Wilkins, Baltimore, MD, pp. 404-405.

35. Clark JB (1979) "Sphere-rod transitions in *Arthrobacter*", pp. 73-92, in "Developmental biology of prokaryotes", JH Parish Ed., U of California Press, CA, p.77.

36. Ensign JC & Wolfe RS (1964) "Nutritional control of morphogenesis in Arthrobacter crystallopoietes", J Bact, 87:924.

37. Reich (1948) op. cit., Preface, pp. xvi-xvii.

38. Hawker & Linton (eds) (1971) op. cit., p. 93.

39. See Fig. 45 of Reich (1938) op. cit., pp. 70-71.

40. "Bergey's manual of determinative bacteriology", 8th ed., 1974, p. 270.

41. Idem, p. 488.

42. "Bergey's manual of systematic bacteriology", 1986, Vol. 2, Williams & Wilkins, Baltimore, MD, p. 1003.

43. Stanier RY, Adelberg EA & Ingraham J (1976) "The Microbial World", Prentice-Hall, NJ, p. 164.

44. "Bergey's manual of determinative bacteriology", 8th ed., 1974, pp. 273, 527, 573.

45. Idem, see Figure 1 of Plate 10.3.

46. See, in this sense, Lehninger's 'balanced' assessment of the proponents of proteins and the proponents of nucleic acids as alternative determinants of the origins of the first cell in the context of a discussion of the structural correspondence between amino acids and codons, in Lehninger A (1970) "Biochemistry", Worth Publishers, NY, 1978 second ed., in particular p. 1052.

47. Prigogine (1972) op. cit., p. 241.

48. Sagan L (1967) "On the origin of mitosing cells", J Theor Biol, 14:255.

49. Margulis L, Dolan MF & Guerrero R (2000) "The chimeric eukaryote: origin of the nucleus from the karyomastigont in amitochondriate protists", *Proc Natl Acad Sci USA*, 97:6954.

50. Dolan MF et al (2002) "Motility proteins and the origin of the nucleus", *The Anatomical Record*, 268:290.

51. Canale-Perola E & Wolfe RS (1960) "Studies in Sarcina ventriculi", J Bacteriol, 79:887.

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52. Knöll H (1965) "Zur Biologie der Gärungssarcinen", Dtsch Akad Wiss Berl, 7:475.

53. Printz H (1921) "Subaerial algae from South Africa", K Nor Vidensk Selsk Skr, 1:35.

54. Geitler L (1932) "*Cyanophyceae*" reprinted 1971, in "Kryptogamenflora von Deutschland, Österreich und der Schweiz", Vol. XIV, Akademische Verlags, Leipzig.

55. Waterbury JB & Stanier RY (1978) "Patterns of growth and development in Pleurocapsalean cyanobacteria", *Microbiol Rev*, 42:2.

56. "Bergey's manual of systematic bacteriology", 1989, Vol. 3, Williams & Wilkins, Baltimore, MD, p. 1785.

57. Golubic S (1973) "The relationship between blue-green algae and carbonate deposits", in Carr & Whitton Ed.s, "The biology of blue-green algae", U. of California Press, CA, pp. 434-472.

58. Note that R. Dadoun is totally mistaken in describing SAPA bions as "presenting a vigor and intensity of motion (...) clearly superior [to PA bions]", in Dadoun R (1975) "Cent fleurs pour Wilhelm Reich", Payot, Paris, p. 73.

59. Reich (1948) op. cit., pp. 28-30.

60. Fox S (1988) "The emergence of life - Darwinian evolution from the inside", Basic Books, NY, p. 98.

61. Jahn TL, Bovee EC & Jahn FF (1949) "How to know the protozoa", reprinted 1979, Wm. C. Brown Co. Publishers, Dubuque, IA, pp. 239-240.

62. Jahn TL (1962) "The mechanism of ciliary movement. II. Ion antagonism and ciliary reversal", J Cell & Compar Physiol, 60:217.

63. Reich (1938) op. cit., p. 129.

64. Idem, p. 128.

65. Reich (1948) op. cit., pp. 24-25.

66. Dadoun (1975) op. cit., pp. 69-70.

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