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"!...Comment...?"

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Most of us feel, from time to time, that other authors have not acknowledged the work of our own or other groups or have omitted to interpret important aspects of their own data. Perhaps we have observations that, although not sufficient to merit a full paper, add a further dimension to one published by others. In other instances we may have a useful piece of methodology that we would like to share.

The Editors hope that readers will take full advantage of this section and use it to raise matters that hitherto have been confined to a limited audience.

***Jon Saunders*, Editor-in-Chief**

Nanobacteria and associated 'elementary bodies' in human disease and cancer

Reports showing that very small bacteria can be isolated from environmental samples and human blood have recently caused considerable excitement and controversy. Called nanobacteria (or nannobacteria), these very small bacteria appear as spheres and ellipses of a diameter between 0·03 and 0·2 μm, often occurring in chains or groups of similar-sized forms (1). Nanobacteria have been isolated from blood as clusters of coccoid cell-walled organisms (0·08–0·5 μm) and associated 'elementary particles' (0·005–1 μm) which together produce biofilms containing carbonate or hydroxyapatite. Recent data from 16S rRNA gene sequences have positioned blood-borne nanobacteria in the α-2 subgroup of the *Proteobacteria* (2). Such isolates are extremely resistant to heat and certain antibiotics, and exhibit a 'bizarre morphology' (i.e. extreme pleomorphism).

Although nanobacteria are usually portrayed as being novel, very small bacteria have frequently been reported in the past and have been associated with a wide variety of diseases, notably cancer (3, 4, 5). Very small entities, similar to elementary particles of nanobacteria or the 'elementary bodies' (6) of species of *Chlamydia* and mycoplasmas (mollicutes) have also frequently been mentioned in the historical literature under a confusing variety of names, including elementary forms, gonidia, granules, inclusion bodies, infrabacteria, arthrobacteria and antebacterial forms.

Ultra-small bacteria and elementary bodies were for a long time regarded as part of the bacterial life cycle and associated with extreme bacterial pleomorphism (4, 5). For example, Bechamp, a contemporary and rival of Pasteur, claimed to have found so-called 'microzymas' in the body, i.e. very small entities, capable of independent existence). In 1873, Lister found minute granules in urine which grew by dividing into four units (so-called 'fissiparous generation') and which he termed 'Granuligera' (8). Belief in the existence of such elementary bodies continued with Enderlein who claimed that blood cells contain primitive life forms which he termed 'protits'. Such protits were seen under dark-field illumination and were of the order of 0·01 μm in diameter. Gaston Naessens continued this tradition with the 'somatid', an elementary particle that apparently survived the death of the infected organism and then regenerated into bacteria, often via a complex life cycle (7).

During the early part of this century a number of microbiologists claimed that bacteria could pass through ultra-fine filters and then be regenerated as normal-sized bacteria on cell-free media. Kendall and Hadley, the main advocates of bacterial filterability claimed that disease-causing bacteria, or a phase of their growth cycle, could pass through filters (7). Filter-passing bacteria were originally referred to as 'viruses', a confusing term first used to refer to any infective agent, then to filter-passing bacteria, finally achieving its modern definition following the appearance in 1928 of Rivers' seminal book *Filterable Viruses* (9). Gruner (10), quoting Lipshutz, commented that filterable bacteria are part of the bacterial life cycle and are unable to grow except when in symbiotic association with a septic organism. Such 'filterable bodies' (0·2 μm and smaller) have frequently been isolated from patients suffering from the a wide variety of diseases, including the common cold, herpes, influenza, meningitis and smallpox (11).

It could be argued that this historical literature can be ignored because it was based on microscopy and isolation techniques that have been superseded by modern molecular approaches. Some of these studies were subjected to a variety of contemporary criticism; so-called 'bacterial life cycles' were, for example, thought to result from contamination and the fanciful linking of individual forms into a non-existent cycle. Other critics suggested that pleomorphic bacterial forms, including ultra-small bacteria, resulted from staining or chemically induced artefacts; many of these criticisms were, however, countered by the claimants (4). Despite such criticism, evidence supporting the existence of ultra-small bacteria has been accumulating for over a century, and has been backed up by the recent application of modern molecular techniques.

The historical literature on filterable bacteria and elementary bodies finds its modern equivalent in the work of Domingue & Schlegel (12) who found that when filter-passing bacteria (0·2 μm) were grown on laboratory media they reverted to normal-sized bacteria. They also noted the appearance of what they called 'small dense bodies' which were observed microscopically, but disappeared when ordinary bacteria grew; a few of these bodies were shown to revert to normal bacteria. Domingue & Woody (13) have also reported what they term as

▶ *GUIDELINES*

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elementary reproductive units (ERU) which are apparently part of a complex pleomorphic life cycle of cell-wall-deficient bacteria. Similarly, Kajander *et al*. have reported (2) the presence of elementary particles (0·05–0·1 μm) in filtered samples of human blood. These authors claim that larger nanobacteria cells that can replicate might release smaller particles which cannot multiply on their own. Itoh *et al*. (14) also isolated granular bodies after passing *Spiroplasma mirum* (the suckling mouse cataract agent) through a 0·2 μm filter. When cultured, these gave rise to helical filaments on which small granular bodies appeared. As the culture aged, larger 'spherical bodies' containing 'granular bodies' appeared; the nature of these latter bodies was not determined. So-called filterable 'granular cocci' have also been isolated from commercially prepared BCG vaccines (15). Although the smallest of these may be incapable of independent existence, they can apparently aggregate together to form viable units.

A considerable literature exists implicating highly pleomorphic bacteria in cancer (16, 17, 18, 19). Extremely small particles, approximating to elementary bodies, have also frequently been reported as part of the life cycle of 'cancer germs' (5, 10, 17). Elementary bodies apparently bud off from the parent cells (the process of so-called 'gemmulation') and can divide into even smaller particles, all of which can then transform back into the original cell type.

Spherical forms (0·2–1·0 μm) and acid-fast, pleomorphic bacteria were isolated in culture, after long incubation periods, from human and animal neoplasms by Allen (19). Gregory (20) also isolated an organism from a human breast cancer which, after passage through a Berkefeld filter, could be grown on agar. Similar cultureable 'fine granules' have also found in highly invasive Hodgkin's disease and in mycobacteria from human carcinomas, sarcomas and melanoma; these were said to evolve into larger globoid forms which in turn could break down to form new granules (21).

Wuethele-Caspe Livingston & Alexander Jackson isolated a highly pleomorphic organism (named *Progenitor cryptocides*) from the blood of hundreds of cancer patients which also apparently has a virus-like phase and elementary bodies (0·2 μm) visible as small dots under oil immersion (22). Such bodies were found in both the tumours and culture medium, and after 1 or 2 months were said to evolve into larger mycoplasma-like L forms and then into frank bacterial rods and filaments. Unidentified, antibiotic-resistant, pleomorphic bacteria have also been seen in the blood of patients with various chronic diseases such as lymphomas and cardiovascular disease (23).

Diller & Donelly (24) isolated a pleomorphic bacterium from rat and mouse tumours, the filterable phase of which could pass through 0·1 μm Millipore filters and could be grown on a synthetic medium. Similar pleomorphic bacteria, varying in size from 0·1–8 μm (when grown on medium) and possessing 'inclusion bodies' (0·01–0·05 μm), have been isolated from human tumours and leukaemic blood. Two of these isolates were filterable, but reverted to the original, normal-sized, bacteria when transferred to growth medium (25).

Some proponents of the role of pleomorphic bacteria in cancer claim to have obtained evidence in support of the ultimate heresy, namely that a number of viruses (e.g. the Bittner mouse milk virus, the Rous chicken sarcoma and the Shope rabbit cancer agent) are filterable stages of the bacterial life cycle. Crofton, for example, concluded that parasitic micro-organisms break up into minute infective cancer-causing granules, and that the Rous sarcoma agent is cultureable as a pleomorphic bacillus (26). Alexander Jackson even claims to have prepared a vaccine from a mycoplasma isolated from the Rous sarcoma virus which apparently protected healthy chickens from infection by this virus (22, 27).

From the historical and more recent literature, we can predict that a cancer-causing bacterium would be expected to exhibit (a) extreme pleomorphism and possess nanobacteria, as well as filaments and cocci, and (b) an L-form-type life cycle, including small elementary bodies. It comes as no surprise therefore to find that the most recent 'cancer germ', *Helicobacter pylori*, possesses a coccal form, and possibly also an L-form-type life cycle (28), which includes very small bacteria and elementary bodies. It is also noteworthy that both *Chlamydia* species (29) and mycoplasmas (30), both of which exhibit ultra-small forms, have been linked with oncogenesis. The fact that nanobacteria are often resistant to antibiotics (2) helps explain why, assuming they play a role in oncogenesis, the incidence of cancer has not declined following the widespread use of antibiotics. Finally, ultra-small bacteria have a bearing on the validation of sterile filtration processes, since *Brevundimonas diminuta* and even smaller, so-called 'diminutive bacteria', can pass through 0·2 or 0·22 μm filters (31).

Unfortunately, the evidence supporting the role of nanobacteria, and elementary bodies, as disease-causing agents continues to be ignored, or more depressingly, suppressed. Kajander *et al*. (2), for example, recently complained that it is difficult to publish work on nanobacteria in relation to human infection simply because journal reviewers do not believe that such organisms exist. Hopefully, by putting the existence of nanobacteria in a historical context, this short article will encourage readers to keep an open mind regarding the potentially important role of nanobacteria in human disease and cancer.

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