MiSAC matters
MICROBIOLOGY IN SCHOOLS ADVISORY COMMITTEE
50th Anniversary Articles

Bacterial endospores: their roles in resolving the spontaneous generation controversy and understanding cell development

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The function of bacterial endospores is survival. They are dormant structures which enable the small number of genera which produce them tolerate extreme conditions of temperature, radiation, acidity, chemical disinfection, desiccation and starvation for long periods. Some have been recovered from samples that are several thousand years old. The best-known genera of such bacteria are *Bacillus* and *Clostridium* which are widely distributed in soil and associated environments†. Some species cause disease, *e.g*. anthrax (*B. anthracis*), tetanus (*Cl. tetani*) and gangrene (*Cl. perfringens*), and food poisoning (*B. cereus* and *Cl. botulinum*); others are beneficial in making products such as antibiotics (*e.g*. *B. polymyxa*) and insecticides (*e.g*. *B. thuringiensis*).

Figure 1. A stained preparation of species of an endospore-forming bacterial genus with endospores (unstained) that are central or sub-terminal and not wider than the vegetative cell.

Endospores are so-called because they are formed *within* the vegetative cell. Unlike the many (exo)spores that fungi produce which enhance their dispersal, only one endospore is formed in a vegetative cell. They are readily visible microscopically as refractile structures in a wet mount and unstained areas in a simple stain (**Figure 1**). Differences in shape, position and width are characteristic of different species of the genera, *e.g*. *Cl. tetani* has circular, terminal endospores wider than the vegetative cell (**Figure 2**).

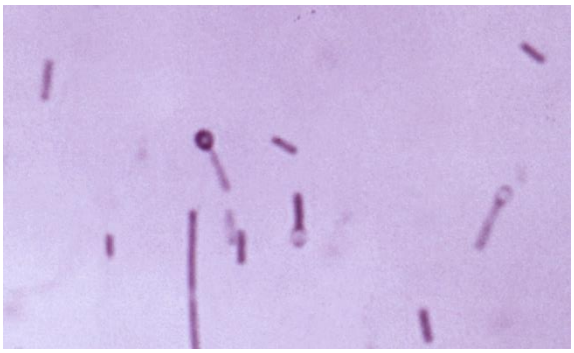

Figure 2. A stained preparation of *Clostridium tetani*, the causative organism of tetanus (lockjaw), with endospores (unstained) that are terminal and wider than the vegetative cell. A stained immature endospore is also present.

The spontaneous generation controversy

Although Francesco Redi (1626-1697) disproved the idea that organisms arise spontaneously from non-living matter, such as maggots from meat, the notion of protozoa and bacteria being generated spontaneously from vegetable and animal infusions persisted. It took another 200 years before the theory of spontaneous generation was finally dismissed by a variety of additional observations in the late 19th century. All these observations arose from relatively simply designed experiments which indicated that some forms of life have an ability to survive heat which was shown to be by the formation of endospores. Many workers entered the debate and made significant contributions. For example, Lazarro Spallanzani (1729-1799) in subjecting vegetable and cereal seeds to different conditions of heat in

open and sealed vessels showed that open vessels yielded many more 'animalcula' than sealed ones and some types were more sensitive to heat than others. His observations were confirmed much later by Theodore Schwann (1810-1882) who demonstrated that boiled meat infusions did not putrefy when air fed into the vessel was heated before entry. Louis Pasteur (1822-1895) also improved Spallanzani's procedure with his famous 'swan neck' flasks (**Figure 3**).

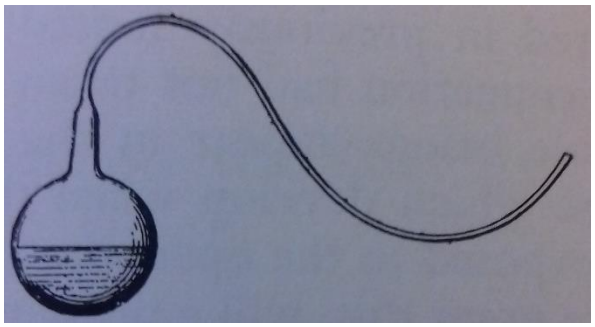

Figure 3. A Pasteur flask from 'Annales des sciences naturelles' 1861.

The special feature of the flasks was to have the neck drawn out sideways to form a narrow, curved tube. Air could enter a flask but any dust carried with it became deposited within the curved tube and therefore did not contaminate the sterilised contents, variously yeast, urine, sugar beet juice, pepper water and milk. The flasks were easy for others to make and use and thereby accept his conclusions which became one of the turning points in the controversy. The features responsible for heat resistance became known as 'spores' (see Cohn and Koch below) and the controversy seemed to be coming to its end. However, it lingered because some attempts to repeat these experiments failed because of contamination, largely caused by careless technique in general as well as because the standard way of achieving sterilisation, *i.e*. boiling, was not always effective. John Tyndall (1820-1893) was one who had found great difficulty in achieving reliable sterility which led to his results being highly variable but he was so confident in the theoretical basis of his research that he looked for inadequacies in his own technique. Hitherto experiments had largely been done with meat and yeast but Tyndall was working with hay infusions. Inspired by the work of Joseph Lister (1827-1812) on antisepsis in surgery, Tyndall demonstrated that hay infusions†† contained very large numbers of heat-resistant forms of bacteria that survived a single exposure to boiling. However, sterilisation could be achieved by boiling on successive days if the material was maintained at room temperature between treatments. The reason is that as well as destroying heat-sensitive vegetative forms and some endospores, boiling activates surviving endospores to return to the heat-sensitive vegetative state during the period at room temperature and are killed by the next boiling treatment. The procedure became known as 'fractional sterilisation' or Tyndallisation which is now rarely needed with the introduction of the autoclave.

Tyndall's demonstration of the significance of the heat resistance of endospores having not been fully taken into account led to the final demise of the theory of spontaneous generation. Meanwhile, Pasteur was lucky that his close association with fermentation had led him to work mainly with fruit juices which did not contain the vast numbers of endospores found in hay infusions!

Towards understanding cell development

Research into the complex changes in the biochemistry and genetics of the life cycle of endospore formation and regeneration to the vegetative state has provided a model for wider studies on cell development and differentiation, gene expression, intercellular communication and cell morphogenesis.

Understanding the cycle began with Ferdinand Cohn's (1828-1898) observation that refractile bodies in boiled hay infusions 'germinated' into vegetative cells again when added to sterile hay infusion. He called the bodies 'spores' and named the bacterium *Bacillus subtilis*. His observations were confirmed by Robert Koch (1843-1910) with *B. anthracis* inoculated into laboratory animals during his famous proof that a specific micro-organism could cause a specific disease. Collaboration with Cohn made Koch aware of the inferiority of his own microscopes, his improvements to which were part of his other major contribution to biology by encouraging the

use of oil immersion lenses, the Abbe condenser and photomicrography, and by developing new staining techniques.

More recent research shows that endospore formation is induced by depletion of an essential nutrient for growth, not by unfavourable conditions in general. The process is very complex, taking several hours for the production of a mature endospore during which period new structures are formed and new substances synthesised in a precise sequence of 7 genetically directed stages. Transformation of dormant endospores into vegetative cells is also a complex but quicker process of 3 stages, *i.e*. activation, germination and outgrowth. Activation can be promoted by sub-lethal heat treatment which is relevant to the success of Tyndall's investigations mentioned above. Germination is triggered by normal metabolites or provision of nutrients. Reasons for the remarkable resistance of mature endospores are less well understood.

† see www.misac.org.uk/activities.html (Activity 2) for a practical investigation on bacterial endospores in soil

†† see www.misac.org.uk/PDFs/MiSAC_helps.pdf

AUTHOR PROFILE

John Grainger gained a BSc in Bacteriology at the University of Birmingham and a PhD in Microbiology at the University of Reading where he made his academic career becoming Head of the Department of Microbiology and then a Visiting Research Fellow. Alongside research interests in environmental microbiology he developed a parallel career promoting microbiology and biotechnology in schools, mainly through MiSAC of which he is Chairman and as Founder and former Director of the National Centre for Biotechnology Education. Work overseas included membership of the management board of the European Initiative for Biotechnology Education. He is a Chartered Biologist, a Fellow of the Royal Society of Biology (RSB) and has awards from the RSB and the Microbiology Society.