Reports

Water Permeability of Bacterial Spores and the Concept of a Contractile Cortex

Abstract. Assumption of a water-impermeable coat on bacterial spores is inconsistent with known permeabilities of organic materials. A low water content may arise through compressive contraction of the cortex during spore maturation.

Hypotheses for the heat stability of bacterial spores usually assume a low water content maintained by a water-impermeable coat. Such a coat, tenths of a micron thick, implies properties not approached in plastics technology.

A rough approximation establishes the range of maximum magnitude of time required for water equilibration within a spore immersed in water. Assume an initially dry sphere 1 μ in diameter, with moisture sorption properties of wool keratin, surrounded by a coat (0.1-μ thick) of material, such as Saran, with moisture permeability 0.0005×10^{-8} gm/cm sec cm-Hg (water vapor pressure difference). If this composite particle is immersed in water at 25°C, it can be calculated that the center will become 90 percent saturated in about 200 seconds. Since the spore coat is dominantly protein (*1*), perhaps 10^6 times as permeable as Saran, this time is probably high by several orders of magnitude. It seems clear that a water-impermeable layer on a spore requires an organic material many orders of magnitude less permeable than the best available technological materials.

Thus, hypotheses for stabilization of essential spore constituents should be compatible with rapid equilibration of all constituents with the water vapor pressure of the external environment—usually liquid water. Murrell and Scott (*2*) found the spread in heat resistance between species diminished greatly at external relative vapor pressures ("activities") below 0.9. This implies water equilibration of the sensitive volumes within the experimental periods of 1 to 6 weeks. They also showed that all the water of a moist spore cake is freely exchangeable with D_2O.

There are good reasons to think that some sort of barrier is involved in maintenance of a low water content. We have observed that spores in sparse aqueous suspension pressed under a cover slip on a microscope slide become less refractile more or less rapidly, depending upon the pressure. With moderate pressure contents are extruded. When a phase objective was racked down with just the right pressure, spores changed from light to dark (without visible change of shape) by the time the microscope could be refocused —that is, within a few seconds. *Bacillus cereus, B. coagulans, B. megaterium,* and *B. subtilis* have been studied. We believe that the phenomenon is essentially that of "mechanical germination" —simulation of physiological germination achieved through abrasion by Rode and Foster (*3*).

The hypothesis that the bare essentials of a vegetative cell—deoxyribonucleic acid and indispensable enzymes—are rendered hydrophobic or otherwise stabilized on a molecular level lacks simplicity and elegance in our view. Electron micrographs of sections of germinating spores (*4*) show that the region within the innermost membrane remains intact (and develops into the vegetative cell) while the outer coats rupture or disintegrate. This apparent integrity of a minute vegetative cell within the spore and the speed and trigger-like action of physiological and mechanical germination seem inconsistent with a stripping of covalently-bonded hydrophobic groups not only from hydrophilic materials of the outer portions, but also from the vital core. We propose an alternative hypothesis that we owe directly to a recent opportunity to hear Werner Kuhn lecture at the University of California on the reversible interconversions of chemical potential energy and mechanical work in synthetic copolymers (*5*).

We visualize an essentially familiar but minute vegetative cell within the spore, dehydrated and shrunken to about one-half the diameter of the intact spore. It is surrounded by the cortex—dense, refractile, of unusual biochemical composition (*6*), and with a wall thickness about one-fourth the diameter of the spore. The cortex is surrounded by a thin coat of protein, admixed with cell membrane material (*1*). The entire system is in water vapor equilibrium, but the water content within the interior vegetative cell is reduced and accounts for heat stability. The lowered water content is maintained by mechanical pressure upon the interior vegetative cell since a hydrated cell would have a larger volume than a dehydrated cell. Pressure is exerted by a slow contraction of the cortex during spore maturation, which serves to dehydrate both the interior vegetative-cell and the cortex itself.

The spore cortex may contract under a slowly changing chemical potential in the latter stage of sporogenesis. Concentration of calcium (and other divalent cations) in or about the cortex by active transport across a membrane surrounding the immature cortex provides an attractive possibility for a controlling chemical potential. Sections of *Clostridium sporogenes* show that the initially wrinkled (*7*) outer membranes of the spore primordium assume a tightly stretched appearance as the spore develops. The typically ridged mature spore coat may be a device for taking up space vacated by the contracting cortex, thus enhancing the concentration of solutes of the immature cortex.

The contractile cortex, with low molecular weight components apparently not held by covalent bonds, should be a fascinating subject for experimentation. Its ready disintegration seems particularly well suited for germination. Its integrity is dependent upon the spore coat. Although the notion of water impermeability must be abandoned, it seems quite possible that the rate limitation in mechanical or physiological germination could be diffusion through the ruptured or otherwise altered coat of calcium or other small solutes. If the coat became permeable, their diffusion could be well-advanced in a small fraction of a second. Indeed, the cortex resembles a system reversibly precipitated by a high calcium ion concentration. Dipicolinate release was accelerated at 65° compared with 4°C in

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

abraded but not in normal spores (*3*), an effect consistent with the usual influence of temperature upon solubility product. Spore sections previously floated on 10 percent acetone in water were relatively electron transparent in the cortical region; when floated on a dilute aqueous solution of lanthanum, the cortical region was much more dense (*4*), showing a strong adsorption of the trivalent cation.

The notion of a contractile cortex unifies the problems of development and release of a low-water condition. On the other hand, the stability of the vital cores of dormant spores to heat and the development of germinated spores now do not appear more mysterious than the behavior of lyophilized cells.

The hypothesis is not grossly inconsistent with free energy requirements for dehydration and with known strengths of materials. Wool keratin may again be taken as representing the spore core. The free energy required in the change of wool with 36 percent water (equilibrated by vapor transfer with pure water) to 15 percent water (equilibrated against a salt solution with water activity 0.5) is about 1 cal/gm of dry keratin (*8*), and for complete dehydration about 10 cal. A relatively small amount of substrate should suffice for the observed dehydration of the spore even with considerable inefficiency in the contraction.

To approximate the strength required for compressive dehydration of wool to 15 percent water, we equate the pressure energy (resilience) to the free energy of dehydration: 1 cal/cm^3 of keratin = 43 kg/cm^2. (This ignores density and osmotic pressure. The pressure obtained is well above measured osmotic pressures of vegetative cells; that of the specialized spore core may be very low.) A 1-μ sphere of keratin held by a tensile coat 0.1-μ thick requires a strength of about 90 kg/cm^2; a 0.5-μ sphere with a 0.25-μ coat requires about 35 kg/cm^2. Muscle and Kuhn's copolymers (both wet) have tensile strengths of 5 to 20 kg/cm^2; rubber and plastics have strengths to 700 kg/cm^2.

We present this speculation in hope that a simple definitive test will be suggested, and also for the insights to be expected from a novel view. If sporulation involves the walling off of a minute vegetative cell *within* the cytoplasm of the parent cell (the newly germinated spore is osmotically stable), some factors involved in cell wall formation should be essentially similar in sporulation and in vegetative growth. Thus in Collier's (*9*) elegant system with *Clostridium roseum*, penicillin (an inhibitor of cell wall synthesis) inhibits an early stage of sporulation but not the conversion of dipicolinate-rich immature spores to heat-stable spores, a stage that might involve primarily the active-transport of divalent cations across the previously-formed external membrane (*10*).

J. C. LEWIS
N. S. SNELL
H. K. BURR

Western Utilization Research and Development Division, U.S. Agricultural Research Service, Albany, California

References and Notes

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10. We thank the many persons who have discussed this concept with us.

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Transmissible Agent Associated with 26 Types of Experimental Mouse Neoplasms

Abstract. A transmissible agent or factor has been found to be associated with all transplanted and spontaneous experimental tumors examined. This observation was made possible by utilization of a biochemical response of normal animals when inoculated with plasma or organ extracts from tumor-bearing hosts. This transmissible enzymic "lesion" is expressed by a five- to tenfold increase in the plasma lactic dehydrogenase activity of the injected normal test animals. The factor is heat labile, passes through bacteria-retaining filters, but is nondialyzable. It is partially sedimented by centrifugation at 100,000*g* for 1 hour.

With the aid of a new technique, a transmissible factor has been detected in the blood of all animals bearing transplanted and spontaneous tumors so far examined. This includes a spectrum of 25 standard transplanted mouse tumors, and one variety of a "spontaneous" neoplasm of uncertain etiology.

The agent is detected by its induction of an abnormal enzyme response in the peripheral blood of normal test animals after injection of plasma from a tumor-bearing donor. This enzyme indication of the presence of an otherwise "silent" agent is in the form of a five- to tenfold increase of lactic dehydrogenase (LDH) activity in the recipient blood plasma. This usually appears within 48 hours and can be transmitted serially to other normal recipients apparently indefinitely.

The factor is transmitted through injection of plasma, whole blood, tumor extracts, or organ extracts from a tumor-bearing or leukemic animal. No detectable enzyme alteration or transmission occurs with similar materials from analogous normal animals or with heated materials from tumor-bearing animals. The factor may be transmitted by intraperitoneal, subcutaneous, intramuscular, or intravenous injections, or by dermal application.

Plasma, or organ extracts, from the tumor-bearing host may be employed in crude form or they may be clarified by centrifugation at relatively high speed, or passed through bacteria-impervious Selas filters of 03 porosity. Some agent was still present in the centrifuged supernatant after 1 hour at 100,000*g*, but the highest activity was associated with the resuspended pellet. The factor did not pass through standard Visking cellophane dialysis tubing which retains particulates exceeding a molecular weight of approximately 20,000.

With this simple enzymic technique, the presence and the transmissibility of the factor can be followed even though no immediate overt pathological or other physical manifestations are readily detectable when the factor is injected into adult animals.

In general, Swiss ICR male mice were used as recipient test animals for testing mouse tumors, although appropriate strains matching the requirements for tumor transplantation were also employed. However, the transmissibility of the factor crosses all mouse strain lines so far tested, so that Swiss

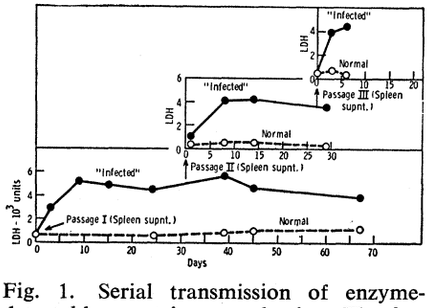

Fig. 1. Serial transmission of enzyme-detectable agent in normal mice. The factor originated in an Ehrlich carcinoma–bearing mouse and was transmitted by way of spleen extract supernatants.