

PHYSICAL SURFACE FEATURES AND CHEMICAL DENSITY
OF DRY BACTERIAL SPORES

ABSTRACT

BERLIN, E. (U.S. Department of Agriculture, Washington, D.C.), H. R. CURRAN, AND M. J. PALLANSCH. Physical surface features and chemical density of dry bacterial spores. *J. Bacteriol.* **86**:1030-1036. 1963.—Gas-displacement and gas-adsorption techniques were used to determine the chemical density and physical surface properties of the spores of *Bacillus subtilis* 15U, *B. cereus* 720, and *B. stearothermophilus* 1518 held in the dry state. Neither the observed densities nor the specific surface areas measured could be correlated with the heat resistance of the spores studied. Analysis of data obtained from a study of the adsorption of nitrogen by spores held at -195°C led to the postulate that the surface of the dry spore is relatively smooth and impervious, and is characterized by the presence of a few pores having radii of approximately 300 Å. The presence of such orifices could account for the permeability of the spore form reported by other workers.

Past consensus, drawn from observations of gross properties, has been that dormant bacterial spores are relatively dense and impervious biological structures. These characteristics were thought to account, in large part, for the observed inertness and heat resistance of the spore. Recent work has thrown considerable doubt on the validity of these inferences.

Studies of the relationship between the density of the bacterial spore and its heat resistance are conflicting (Yesair and Cameron, 1936; Sugiyama, 1951; Anand, 1961). Gerhardt and his associates demonstrated that a large part of the spore structure is permeable, not only to water, but to a large variety of low molecular weight solutes (Black and Gerhardt, 1961, 1962*a*, *b*; Gerhardt and Black 1961*a*, *b*). Gerhardt and Black (1961*a*, *b*) concluded, from their data, that

the spore surface is a heteroporous structure with pore sizes ranging from 10 to 100 Å in radius.

All cited studies were carried out with spores suspended in an aqueous phase with intercellular water complicating the interpretation of the results. This apparently accounts for the lack of agreement in the reported densities of bacterial spores (Black and Gerhardt, 1962*b*; McIntosh and Selbie, 1937; Lamanna, 1952; Murrell, 1961).

This paper reports the density and the physical characteristics of the surfaces of lyophilized spores of known and graded heat resistance. Gas-displacement and gas-adsorption techniques were used to obtain the reported data which described the density and surface structure of dry spores.

MATERIALS AND METHODS

Spores used were obtained by culturing *Bacillus subtilis* 15U, obtained from the American Can Co., *B. cereus* 720, obtained from N. R. Smith, and *B. stearothermophilus* 1518, obtained from National Canners Association, on the surface of a solid medium containing 0.3% beef extract and 1.0% peptone (Difco), supplemented with 1.0% starch and 3 ppm of manganese (MnSO_4). The organisms were incubated at 37, 30, and 55 C, respectively. When sporulation was complete, the growth was collected by washing the surface of the media with sterile distilled water. The spore suspensions were filtered through cotton and centrifuged. After decanting the supernatant, the spores were resuspended in distilled water and held at 4 C to promote autolysis of the residual vegetative cells. The spores were finally washed eight times with distilled water and lyophilized. The dried materials were stored at 4 C until used. On examination with a microscope, a few vegetative cells (estimated 6%) could be found in *B. cereus* and a few vegetative

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ghosts (estimated 3%) in *B. stearothersophilus*. The *B. subtilis* preparation was 100% spores.

Viable-spore counts were obtained by suspending weighed quantities of spores in distilled water and heating *B. cereus* for 15 min at 75 C, *B. subtilis* for 10 min at 95 C, and *B. stearothersophilus* for 20 min at 95 C before subculturing on glucose nutrient agar.

The thermostability of the spores was assessed by heating dormant spores suspended in 0.067 M neutral phosphate buffer (2×10^5 spores per ml). Approximate thermal death times at 115 C were 76 min for *B. stearothersophilus*, 11 min for *B. subtilis*, and 3 min for *B. cereus*.

Before density or surface-area determinations were made, occluded gas and water vapor were removed from the spores by holding samples at room temperature under a high vacuum. Degassing was considered complete when the pressure in the system held constant at 0.01 to 0.005 μ of Hg. Studies of degassing performed at temperatures up to 70 C indicated that the lower temperatures routinely employed were sufficient to clear the spore surfaces of volatiles. Gas-adsorption studies required 1.5 to 2.0 g of dry spores. Samples of 7 to 8 g were used in gas-displacement studies. The amount of nitrogen adsorbed by a weighed quantity of degassed spores held at -195 C under various nitrogen pressures, as well as the volume of helium displaced by these spores at 26.2 C, was measured by use of an all-glass volumetric adsorption apparatus, custom-built along lines suggested by Barr and Anhorn (1959). An electronic contact indicator (Mills, 1941) was installed to ensure precision in setting the mercury level of the manometer.

Nitrogen-adsorption data were converted to specific surface areas in accordance with the Brunauer, Emmett, and Teller (BET; 1938) multilayer adsorption theory. The average pore size of the surfaces was calculated according to the method of Emmett and DeWitt (1941). The pore size distribution was obtained by use of the method of Barrett, Joyner, and Halenda (1951) as modified by Pierce (1953). Spore densities were calculated by dividing the weight of the spore sample by the volume of helium it displaced.

In exploratory experiments, the volumes of nitrogen and hydrogen displaced at 26.2 C by the spores were also determined.

All gases used were purchased from the Southern Oxygen Co., Washington, D.C. (The use of

trade names is for the purpose of identification only, and does not imply endorsement of the product or its manufacturer by the U.S. Department of Agriculture.) The helium was purified by passing it through a charcoal-filled trap held at -195 C. Electrolytic hydrogen was prepared for use by passing it successively through a "Deoxo" catalytic hydrogen purifier (Engelhard Industries, Inc., Newark, N.J.) and a charcoal-filled trap held at -195 C. Prepurified nitrogen was used after moisture removal by passage through a low-temperature trap.

RESULTS

The densities of lyophilized spores, as measured by the helium-displacement technique, were as follows: *B. subtilis*, 1.409 ± 0.011 g/cm³; *B. stearothersophilus*, 1.273 ± 0.018 g/cm³; *B. cereus*, 1.399 ± 0.003 g/cm³. Identical results, within the limits, were obtained by measuring hydrogen displacement. Slightly higher values were obtained with nitrogen. These high values indicated slight nitrogen adsorption by the spore surface. The close agreement of the density values obtained by using different gases as displacement media shows that the spore surfaces are devoid of pores of such dimensions that they could discriminate between the gases used, on the basis of molecular radii.

A comparison of the densities of the dry spores and their heat resistance showed no correlation between the observed values.

The adsorption of nitrogen by spore surfaces at -195 C was reversible and exhibited no hysteresis. The complete adsorption isotherm for nitrogen adsorbed on the surfaces of *B. subtilis* 15U is shown in Fig. 1. The shape of this curve

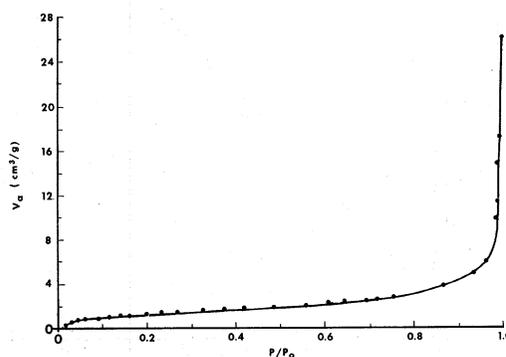


FIG. 1. Adsorption isotherm for nitrogen on *Bacillus subtilis* 15U at -195 C.

indicates adsorption on relatively smooth surfaces with little or no fine porosity and capable of unlimited multilayer adsorption. Isotherms having this form are classified as type II by Brunauer et al. (1940), and can be described by the limited form of the BET equation (Brunauer et al., 1938):

$$\frac{X}{V_a(1 - X)} = \frac{1}{V_m C} + \frac{(C - 1)X}{V_m C}$$

where X is the relative pressure (P/P_0) for the adsorbate; V_a is the volume of gas, measured at standard temperature and pressure (STP), adsorbed at relative pressure X ; V_m is the volume of adsorbate in a monomolecular layer on the surface of the adsorbent; and C is a constant related to the heat of adsorption. In case of type II adsorption, a standard BET plot of $X/V_a(1 - X)$ versus X should produce a straight line in the linear portion of the isotherm, $0.05 < X < 0.35$. Evidence for the linearity of this type of plot is presented in Fig. 2. Each spore sample studied adsorbed nitrogen in a similar manner. A summary of data pertaining to the surface area and porosity of the three species of spores is presented in Table 1.

The specific surface area of each type of spore

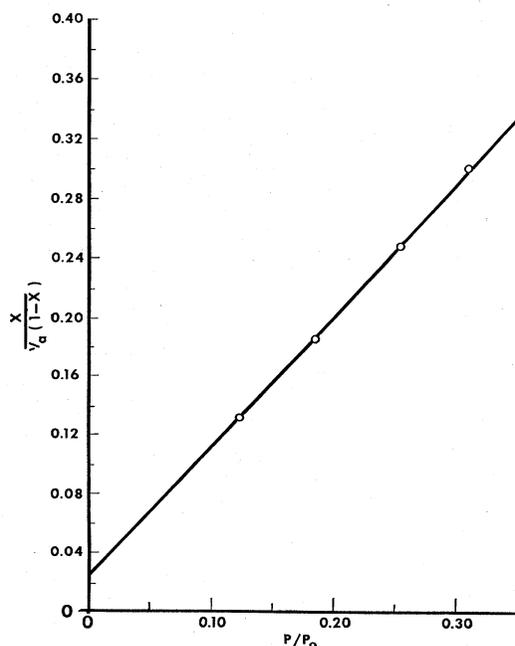


FIG. 2. BET plot for nitrogen adsorbed on *Bacillus subtilis* 15U spores.

TABLE 1. Porosity and surface data for bacterial spores

Organism	Surface area	V_a at P/P_0^*	V_p^\dagger	Porosity (θ)	ρ_a	\bar{r}_p
	m^2/g			%	g/cm^3	\AA
<i>Bacillus subtilis</i> 15U.....	5.04	26.2	0.041	5.5	1.332	163
<i>B. stearothermophilus</i> 1518..	5.20	16.3	0.025	3.1	1.234	96
<i>B. cereus</i> 720.....	2.97	22.7	0.035	4.7	1.334	236

* Equal to 1.0 cm^3 per g at STP.

† Equal to $(0.001558)V_a$ and expressed as cm^3 (liquid) per g.

studied was calculated from the volume of adsorbed nitrogen necessary to form a monomolecular film on the spore surface and the cross-sectional area of the nitrogen molecule. The calculated values were typical of nonporous solids.

Total pore volume of surface pores (V_p) was estimated from the observed adsorption at $P/P_0 = 1$. At this point, according to an adsorption theory (Ries, 1954), all pores should be completely filled with the condensed or liquified adsorbed portion. Conversion of the volume of gas adsorbed (expressed in STP terms) to liquid nitrogen volume, which is the true volume of such a condensed phase, was accomplished by use of the standard conversion factor $M/M_v\rho$, where M is the molecular weight, M_v the molar volume, and ρ the density of the adsorbed phase. By use of Lange's (1961) value of 0.808 for the specific gravity of N_2 at its normal boiling point, the value $M/M_v\rho = 0.001558$ was obtained. The possible total volume of pores on the surface of the spores was then less than 0.041 cm^3/g of spores.

The apparent density (ρ_a) of the spores investigated was derived from the equation

$$\frac{1}{\rho_a} - \frac{1}{\rho_t} = V_p$$

where ρ_t is the true chemical density as measured by helium displacement.

The percentage of spore volume occupied by pores (θ) was calculated from the equation

$$\theta = (V_p/\rho_a)$$

All calculations showed that less than 5.5% of the total spore volume was occupied by possible pores.

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TABLE 2. Calculation of pore size distribution on *Bacillus subtilis* 15U*

$\frac{P}{P_0}$	V	ΔV	n	Δn	ΔV_f	ΔV_k	R	V_{p-g}	r_p	Δr_p	\bar{r}_p	A_p	ΣA_p	V_p	$\frac{\Delta V_p}{\Delta r_p} \times \frac{l}{(0.001558)}$
	cm^3/g	cm^3/g			cm^3/g	cm^3/g		cm^3/g	\AA	\AA	\AA	m^2/g		cm^3/g	cm^3/g
1.000	26.2		19.0				1.00								
0.975	8.1	18.1	13.0	6.0	0	18.1	1.24	22.4	433	136	(500)	1.39	1.39	.03490	.1647
0.950	5.6	2.5	8.8	4.2	1.34	1.16	1.36	1.58	227	206	330	0.15	1.54	.00246	.00767
0.925	4.7	0.9	6.7	2.1	0.74	0.16	1.43	0.23	148	79	188	0.004	1.544	.00036	.00291
0.900	4.2	0.5	5.6	1.1	0.89	0.11	1.49	0.163	111	37	130	0.004	1.548	.00025	.00441
0.850	3.53	0.67	4.4	1.2	0.43	0.24	1.60	0.384	75	36	93	0.13	1.678	.00060	.01067
0.800	3.11	0.42	3.5	0.9	0.35	0.07	1.66	0.116	56	19	66	0.054	1.732	.00018	.00611
0.700	2.58	0.53	2.50	1.0	0.40	0.13	1.78	0.231	36	20	46	0.156	1.888	.00036	.01155
0.600	2.24	0.34	2.08	0.42	0.18	0.16	1.95	0.312	26	10	31	0.312	2.200	.00049	.0312
0.500	1.99	0.25	1.78	0.30	0.15	0.10	2.15	0.215	20	6	23	0.290	2.490	.00033	.0358

* Symbols: P/P_0 = relative pressure; V = volume adsorbed at STP; ΔV = increment in volume adsorbed at STP for increment in P/P_0 ; n = number of molecular layers adsorbed on surface of pore walls; ΔV_f = increment in volume of adsorbed film at STP on pore wall equal to $(\Sigma A_p) (\Delta n) (0.23)$; $\Delta V_k = \Delta V - \Delta V_f$ equal to inner capillary volume; $R = \bar{r}_p^2/\bar{r}_k^2$ equal to the ratio of pore radius to inner capillary radius; $V_{p-g} = RV_k$ equal to pore volume in units of gas volume at STP; r_p = pore radius; Δr_p = increment in pore radius; \bar{r}_p = average pore radius over given increment; A_p = surface area of pores equal to $31V_{p-g}/\bar{r}_p$; ΣA_p = summation of pore-wall surface area; V_p = pore volume based on liquid volume of adsorbed portion equal to $V_{p-g} (0.001558)$; $\frac{V_p}{\bar{r}_p} \times \frac{1}{(0.001558)}$ = differential pore volume function for pore size distribution shown in units of gas volume to expand scale on pore size distribution plot.

The average pore radius, \bar{r}_p , was calculated by use of the equation,

$$\bar{r}_p = 2V_p/\text{surface area}$$

as derived by Emmett and DeWitt (1941). The average pore radii ranged from a high of 236 \AA , found on the surface of *B. cereus* spores, to a low of 96 \AA , found on the surface of *B. stearothermophilus* spores.

In general, all the spores studied had a low order of porosity, and the pores present had relatively large average radii (Table 1). None of the observed physical features of the spore surfaces could be correlated with their known heat resistance.

Data pertaining to the calculation of the distribution of the size of pores on the surface of *B. subtilis* spores are presented in Table 2. The actual distribution is presented graphically in Fig. 3. Data and distribution curves for *B. cereus* and *B. stearothermophilus* were found to be similar and are not reported. The plot shows that the major contribution of pore volume is in the region of interparticle condensation.

Table 3 presents a comparison of the volume and surface area of the *B. subtilis* spore as derived

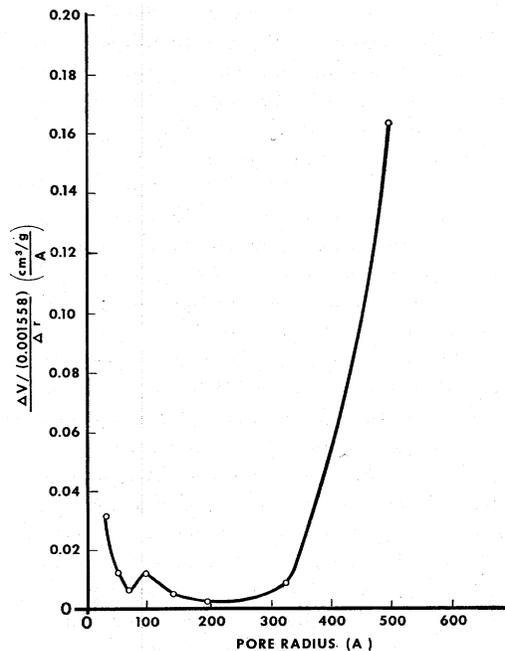


FIG. 3. Pore volume distribution on surface of *Bacillus subtilis* 15U spores.

TABLE 3. *Dimensions of Bacillus subtilis*
15U spores

Determined from	Spore size	Volume of spore	Surface area per spore
	μ	μ^3	μ^2
Direct microscopic examination.....	$1.1 \times .7-.8$	0.324*	2.33†
Measured surface areas, densities, and spore count.....	—	0.38	2.65

* Based on assumption that the spore is a perfect prolate ellipsoid of revolution (Knaysi, 1946) and calculated from the equation $V = 4\pi ab^2/3$ where a and b are the semimajor and semiminor axes, respectively.

† Calculated from the equation surface area = $2\pi b^2 + \frac{2\pi a^2 b}{\sqrt{a^2 - b^2}} \sin^{-1} \frac{\sqrt{a^2 - b^2}}{a}$.

from direct microscopy and gas-displacement and gas-adsorption techniques. Rather close agreement in the figures was noted with a roughness factor of 1.14.

DISCUSSION

The lack of correlation between the density of the bacterial spores studied and their known heat resistance tends to substantiate prior similar claims (Anand, 1961). However, direct comparison of our data with that obtained by other investigators is unwarranted, since previous investigations concerned themselves with the relationship between the variations in density and heat resistance observed with a group of spores representing a single species. Our studies concern only the observed interspecies differences in density and heat resistance.

More interesting than the lack of correlation between interspecies density differences and heat resistance is the low density value reported for *B. stearothermophilus*. This comparatively low value demonstrates a marked compositional difference between this most heat-resistant spore form and the other spores which were studied, as all three displayed similar structural properties.

The summation of all data we obtained during the course of studying nitrogen adsorption by spores held at low temperature, leads to the conclusion that lyophilized spores possess outer surfaces that are relatively smooth and free from a large number of pores. The shape of the ad-

sorption isotherm and the observed lack of hysteresis on the reversal of adsorption give good evidence of this possible low order of porosity of the spore surface. When the major portion of the surface of a solid material lies in pores, the shape of the adsorption isotherm indicates the disappearance of surface as the pores fill with liquid condensate. If the pores are small, the isotherm is type I. Filling of large pores leads to type IV or type V isotherms. If, however, a sample has some micropores that fill at low relative pressure and extensive open surface outside of these pores, the isotherm may be type II.

Contributions due to capillary condensation in small pores and to adsorption on the free surface may be separated by use of an equation suggested independently by Frenkel (1946), Halsey (1948), and Hill (1952). This equation has the form: $-\ln P/P_o = k/n^s$, where k is a constant related to the energy of adsorption in the first layer; $n = V_a/V_m$ is the number of statistical layers, and the exponent s is related to the fall-off of the van der Waals forces of the surface with successive layers in the adsorbed film. Pierce (1959, 1960) and Pierce and Ewing (1962) have shown that the ideal N_2 isotherm fits a modification of the multilayer equation, in the form

$$n^{2.75} = \left(\frac{V_a}{V_m} \right)^{2.75} = \frac{k}{\log P_o/P} = \frac{1.305}{\log P_o/P}$$

and have tabulated values of n at selected relative pressures.

Analysis of adsorption data by n values permits computation of both the volume held in small pores and the free surface area not in these pores. When there is adsorption both in small pores and on free surface outside of these pores, we have the relation $V_a = V_o + nV_m'$ where V_o is the gas volume held in the pores and V_m' is the volume to form a monolayer on the free surface. Simultaneous solution of this equation with our data for V_a at different relative pressures and Pierce's n values showed no capillary condensation in micropores.

The n values of Pierce's equation may be used to calculate V_m independently of a BET plot. This is done by determining the ratio V_a/n for different relative pressures and taking the average values as V_m . Determinations of V_m by this method together with BET values for V_m and the BET constant C are shown in Table 4. The V_a/n ratios, designated at V'_m , are averages

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TABLE 4. Computation of V_m

$\frac{P}{P_0}$	n	$\frac{V_a}{n}$		
		<i>Bacillus subtilis</i>	<i>B. cereus</i>	<i>B. stearo-thermophilus</i>
0.20	1.25	1.13	0.65	1.06
0.30	1.39	1.16	0.68	1.13
0.40	1.54	1.18	0.71	1.17
0.50	1.70	1.24	0.71	1.18
0.60	1.90	1.26	0.72	1.19
0.70	2.17	1.26	0.72	1.20
V_m' (cm ³ N ₂)		1.21	0.70	1.16
BET value for V_m (cm ³ N ₂)		1.15	0.66	1.19
C value		38	45	31

for all relative pressures up to the start of interparticle condensation.

Deviations of type II isotherms from the normal n values at relative pressures above 0.7 may be due either to condensation between particles or to condensation in large macropores within particles. Usually when the latter effect is present, the isotherm is type IV or type V, but interparticle condensation may occur when the isotherm appears to be type II. The normal n values may, however, be used to show the existence of this effect. Isotherm values below 0.7 P_0 were used to compute V_m' from the average V_a/n values as in Table 4. The product nV_m' was then compared with the experimental values of V_a at selected relative pressures (Table 5). These results together with the deviation from linearity of the log-log plot of the Frenkel-Halsey-Hill isotherm at high relative pressures (Fig. 4) clearly show that interparticle condensation is occurring after $P/P_0 \approx 0.9$.

Hence, further analysis of our data, when considered with roughness factor of only 1.14 leads us to the conclusion that the surfaces of lyophilized dormant spores held at -195 C are relatively smooth and impervious with few if any pores present.

Examination of the log-log plot of V_a against $\log P_0/P$ (Fig. 4) for bacterial spores shows a slightly less steep slope than for Pierce's theoretical plot (Fig. 4) for the number of statistical layers (n). Smaller slopes for such plots are characteristic of adsorbents with low C values as exhibited by all three species of spores studied. Similar low C values and slopes for Frenkel-

Halsey-Hill isotherms were obtained recently by Emmett and Hightower (J. Polymer Sci., *in press*) for N₂ adsorption on several types of high polymers. This is interesting when one considers Black and Gerhardt's (1962b) hypothesis: "the core of the dormant spore exists as an insoluble and heat-stable gel, in which cross-linking between macromolecules occurs through stable but reversible bonds so as to form a high-polymer matrix with entrapped free water."

Although our observations on spore porosity are in apparent conflict with those reported by Gerhardt's group, there may in fact be no valid basis for comparing the data in the two studies.

TABLE 5. Demonstration of interparticle condensation

$\frac{P}{P_0}$	V_a (cm ³ of N ₂)					
	<i>Bacillus subtilis</i>		<i>B. cereus</i>		<i>B. stearo-thermophilus</i>	
	Observed	1.21 n	Observed	0.70 n	Observed	1.16 n
0.20	1.41	1.51	0.81	0.88	1.32	1.45
0.30	1.61	1.68	0.95	0.97	1.57	1.61
0.40	1.82	1.86	1.09	1.08	1.80	1.79
0.50	2.11	2.05	1.21	1.19	2.01	1.97
0.60	2.40	2.30	1.36	1.33	2.26	2.20
0.70	2.74	2.62	1.57	1.52	2.60	2.52
0.75	2.93	2.83	1.72	1.64	2.81	2.71
0.80	3.16	3.12	1.87	1.80	3.10	2.99
0.85	3.53	3.51	2.08	2.03	3.44	3.36
0.90	4.21	4.05	2.45	2.34	3.89	3.89
0.925	4.75	4.60	2.75	2.66	4.20	4.41
0.950	5.75	5.32	3.44	3.08	4.80	5.10
0.975	8.88	6.90	5.71	4.00	7.12	6.61

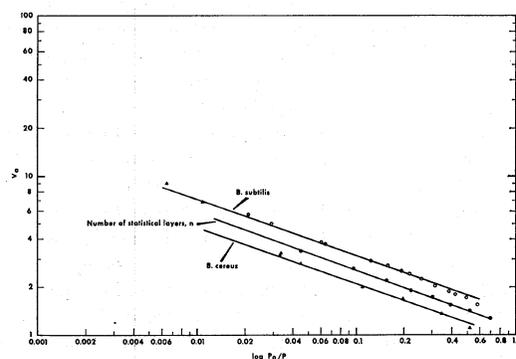


FIG. 4. Log-log plots for adsorption in the multi-layer region for *Bacillus subtilis* 15U, for *B. cereus* 720, and for the number of statistical layers with n values tabulated by Pierce (1960).

They demonstrated the permeability of bacterial spores to low molecular weight solutes in an aqueous substrate, from which they postulated a heteroporous spore coat. Our data were obtained with dry spores studied in systems in which the water content was reduced to an absolute minimum. The effect of adsorbed or ingested water on the spore surface structure can only be conjectured. There may also be contraction of the surface when the spores are kept at -195°C . Quite apart from this, however, the apparent discrepancies can be reconciled by the entirely plausible assumption that the surface of the bacterial spore is comparatively smooth and impervious, except for the presence of a few large pores which allow access of water and solutes to the interior of the spore, a possibility which Gerhardt and Black (1961*a*) have, in fact, considered. Since orifices of this nature are not unknown in the design of living systems, our investigation is now being extended to establish their existence by direct observation with an electron microscope.

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LITERATURE CITED

- ANAND, J. C. 1961. Density of bacterial spores and their destruction rate by heat. *J. Sci. Ind. Res. (India)* **20C**:353-355.
- BARR, W. E., AND V. J. ANHORN. 1959. Scientific and industrial glass blowing, p. 257-283. Instruments Publishing Co., Pittsburgh.
- BARRETT, E. P., L. G. JOYNER, AND P. P. HALLEND. 1951. The determination of pore volume and area distributions in porous substances. I. Computations from nitrogen isotherms. *J. Am. Chem. Soc.* **73**:373-380.
- BLACK, S. H., AND P. GERHARDT. 1961. Permeability of bacterial spores. I. Characterization of glucose uptake. *J. Bacteriol.* **82**:743-749.
- BLACK, S. H., AND P. GERHARDT. 1962*a*. Permeability of bacterial spores. III. Permeation relative to germination. *J. Bacteriol.* **83**:301-308.
- BLACK, S. H., AND P. GERHARDT. 1962*b*. Permeability of bacterial spores. IV. Water content, uptake, and distribution. *J. Bacteriol.* **83**:960-967.
- BRUNAUER, S., L. S. DEMING, W. E. DEMING, AND E. TELLER. 1940. On a theory of the van der Waals adsorption of gases. *J. Am. Chem. Soc.* **62**:1723-1732.
- BRUNAUER, S., P. H. EMMETT, AND E. TELLER. 1938. Adsorption of gases in multimolecular layers. *J. Am. Chem. Soc.* **60**:309-319.
- EMMETT, P. H., AND T. DEWITT. 1941. Determination of surface areas—pigments, carbon blacks, cement, and miscellaneous finely divided or porous materials. *Ind. Eng. Chem. Anal. Ed.* **13**:28-33.
- FRENKEL, J. 1946. Kinetic theory of liquids. Oxford University Press, London, England.
- GERHARDT, P., AND S. H. BLACK. 1961*a*. Permeability of bacterial spores. II. Molecular variables affecting solute permeation. *J. Bacteriol.* **82**:750-760.
- GERHARDT, P., AND S. H. BLACK. 1961*b*. Permeability of bacterial spores, p. 218-228. *In* H. O. Halvorson [ed.], *Spores II*. Burgess Publishing Co., Minneapolis.
- HALSEY, G. 1948. Physical adsorption on non-uniform surfaces. *J. Chem. Phys.* **16**:931-937.
- HILL, T. L. 1952. Theory of physical adsorption. *Advan. Catalysis* **4**:212-258.
- KNAYSY, G. 1946. On the process of sporulation in a strain of *Bacillus cereus*. *J. Bacteriol.* **51**:187-197.
- LAMANNA, C. 1952. Biological role of spores. *Bacteriol. Rev.* **16**:90-93.
- LANGE, N. A. 1961. Handbook of chemistry. McGraw-Hill Book Co. Inc., New York.
- MCINTOSH, J., AND F. R. SELBIE. 1937. The measurement of the size of viruses by high-speed centrifugalization. *Brit. J. Exptl. Pathol.* **18**:162-174.
- MILLS, B. 1941. A sensitive contact indicator. *Rev. Sci. Instr.* **12**:105.
- MURRELL, W. G. 1961. Discussion, p. 229-235. *In* H. O. Halvorson [ed.] *Spores II*. Burgess Publishing Co., Minneapolis.
- PIERCE, C. 1953. Computation of pore sizes from physical adsorption data. *J. Phys. Chem.* **57**:149-152.
- PIERCE, C. 1959. Effects of interparticle condensation on heats of adsorption and isotherms of powder samples. *J. Phys. Chem.* **63**:1076-1079.
- PIERCE, C. 1960. The Frenkel-Halsey-Hill adsorption isotherm and capillary condensation. *J. Phys. Chem.* **64**:1184-1187.
- PIERCE, C., AND B. EWING. 1962. Physical adsorption in the multilayer region on heterogeneous and homogeneous surfaces. *J. Am. Chem. Soc.* **84**:4070-4075.
- RIES, H. E., JR. 1954. Physical adsorption. *Catalysis* **1**:1-30.
- SUGIYAMA, H. 1951. Studies on factors affecting the heat resistance of spores of *Clostridium botulinum*. *J. Bacteriol.* **62**:81-96.
- YESAIR, J., AND E. J. CAMERON. 1936. Centrifugal fractionation of heat resistance in a spore crop. *J. Bacteriol.* **31**:2-3.