

~~R56-1~~
R56-2

CORRELATION OF RESPIRATORY ACTIVITY WITH PHASES OF SPORE
GERMINATION AND GROWTH IN *BACILLUS MEGATERIUM* AS
INFLUENCED BY MANGANESE AND L-ALANINE

HILLEL S. LEVINSON AND MILDRED T. HYATT

*Pioneering Research Division, U.S. Army Quartermaster Research and Development Center,
Natick, Massachusetts*

Reprinted from JOURNAL OF BACTERIOLOGY
Vol. 72, No. 2, pp. 176-183, August, 1956
Printed in U.S.A.

CORRELATION OF RESPIRATORY ACTIVITY WITH PHASES OF SPORE GERMINATION AND GROWTH IN *BACILLUS MEGATERIUM* AS INFLUENCED BY MANGANESE AND L-ALANINE

HILLEL S. LEVINSON AND MILDRED T. HYATT

Pioneering Research Division, U.S. Army Quartermaster Research and Development Center, Natick, Massachusetts

Received for publication December 29, 1955

Respiration during and following germination of *Bacillus megaterium* spores in a complex medium containing glucose, peptone, yeast extract, and phosphate is characterized by three phases coinciding with germination, swelling and elongation (Mandels *et al.*, 1956). Germination occurs within the first 5 to 10 min of incubation, i. e., the spores become stainable, heat labile (Powell, 1950), appear dark under phase microscopy (Pulvertaft and Haynes, 1951), and suspensions become less turbid (Powell, 1950; Hachisuka *et al.*, 1955). Accompanying these changes is an undefined increase in the rate of oxygen consumption. Following this, a period of swelling marked by a phase of linear increase in rate of respiration ensues. This phase changes to another linear phase of increase in respiratory rate while the cells elongate, the point of transition between the two linear phases corresponding to emergence. The elongated cells eventually divide, and the rate of respiration falls after one or two divisions, due, probably, to exhaustion of nutrient factor(s).

Spores incubated in a simple medium containing acetate-buffered glucose and L-alanine germinate rapidly (Levinson and Hyatt, 1955). Spore germination in acetate-buffered glucose and manganese is somewhat delayed, but the rate of oxygen consumption eventually reaches a level as high as, or higher than, that in L-alanine. No division was noted in any of these experiments using acetate-buffered glucose.

The present study constitutes an attempt to determine the relationships between respiratory activity and morphological stages in spore germination and development in simple media. In the course of these investigations, we have made some observations on the effect of pH on germination and on post-germinative development, and on the failure of germinated spores to develop in glucose solution with L-alanine.

MATERIALS AND METHODS

Spores of *B. megaterium* (strain QM B1551) were from the same pool of lyophilized spores used previously (Levinson and Sevag, 1953). Briefly, they were harvested from phosphate-buffered liver broth, pH 6.8 (Foster and Heiligman, 1949), washed by centrifugation at 4 C, and dried from the frozen state.

Spore germination was estimated by a modification (Levinson and Sevag, 1953) of the method of Powell (1950), based on the fact that germinated spores stain with methylene blue, while ungerminated spores do not. Germination data were recorded from microscopic examination of spores incubated in Warburg vessels under the same conditions as for respiration studies. For the purposes of this report, development subsequent to germination was separated into stages of swelling, emergence, elongation, and cell division.

Oxygen consumption was measured by conventional Warburg techniques, using 0.3 ml substrate in the sidearms, 1.5 mg of spores (*ca.* 6.3×10^8 spores) in 1.2 ml of buffer in the main chamber, and 0.2 ml of 10 per cent KOH in the center wells. The sidearm was tipped after 15 min equilibration at 30 C. Methods for double sidearm vessels are described where used (Results (d)).

Acetate buffer contained potassium and ammonium acetates, 50 mM each, adjusted with dilute acetic acid to an initial pH of 6.8-6.9. Phosphate buffer, adjusted to pH 6.0 or to pH 7.0 with 5.5 N KOH, contained KH_2PO_4 , 50 mM, and 20 mM each of ammonium acetate and NaCl. The concentrations indicated are final, after tipping.

Glucose was included in all Warburg experiments at a final concentration of 25 mM. L-alanine (Nutritional Biochemicals Corp., Cleveland,

TABLE 1
Spore germination in glucose with and without added manganese or L-alanine

Buffer* (pH change)	Treatment	Additive†	Germination			Post-germinative Changes‡				
			Initiation		Maximum min	Sw	Em	El	CD	Deg
			min	per cent						
Acetate (pH 6.8-5.5)	Unheated	L-alanine	5	85	50	++	0	0	0	++240
		Mn	50	80	90	++	0	0	0	0
		—	60	30	ca. 200	+	0	0	0	0
	Heated	L-alanine	5	92	40	++	0	0	0	++240
		Mn	30	97	45	++	0	0	0	0
		—	40	80	120	+	0	0	0	0
Phosphate (pH 7.0-6.8)	Unheated	L-alanine	5	51	75	+++	0	0	0	+280
		Mn	85	18	140	140	240	240	300	0
		—	110	22	240	++	10%	0	0	0
	Heated	L-alanine	5	82	45	+++200	0	0	0	++280
		Mn	20-40	45	90	180	180	180	280	0
		—	90	30	160	++	10%	0	0	0
Phosphate (pH 6.0-5.8)	Unheated	L-alanine	10	84	85	+	0	0	0	+
		Mn	30	59	110	+	0	0	0	0
		—	80	38	ca. 150					
	Heated	L-alanine	5	89	ca. 60	+	0	0	0	+
		Mn	20	72	ca. 80	+	0	0	0	0
		—	60	44	ca. 105					

* pH change after 6-7 hr of incubation.

† Mn = manganese; — = glucose alone.

‡ Sw = swelling; Em = emergence; El = elongation; CD = cell division; Deg = "degeneration."
Results in degree (0 to +++) of change, or as time (min) at which the change begins. Unless indicated by percentage, all germinated spores undergo the change.

Ohio) was used at 2.0 mM, and CP MnSO_4 at 0.2 mM final concentration.

Heat shock was accomplished by a 15-min immersion of from 10 to 15 ml of a suspension containing 1.5 mg of spores per 1.2 ml of buffer in a water bath at 60 C. The buffer concentration during heating was such that the addition of 0.3 ml substrate to 1.2 ml buffered spore suspension gave the final buffer concentrations indicated above.

The influence of L-alanine and manganese on the viability of spores was tested by shaking spores (4.2×10^8 per ml) with these substances (6.0 ml of reaction mixture per 50-ml Erlenmeyer flask) at 30 C, and plating aliquots of appropriate water dilutions of the reaction mixtures on nutrient agar after 2 and after 4 hr. Colonies on petri dishes were counted after 48 hr at 30 C, only those dilutions having between 100 and 200 colonies being counted.

RESULTS

(a) *Acetate buffer.* Unheated spores germinate rapidly in L-alanine, reaching a maximum in 50 min (table 1). There is slight swelling, but no emergence. Generally, after about 4 hr of incubation in L-alanine, spores lose the definiteness of their outline, stain rather poorly, appear in many cases to have leaked protoplasm, and have a "degenerate" appearance. Germination of unheated spores in manganese proceeds rapidly after a 40 to 50-min lag to a maximum at about 90 min. The germinated spores swell slightly, but as in L-alanine, they do not emerge. No "degeneracy" was observed with manganese-incubated spores. Suspensions of unheated spores with glucose alone begin to germinate in 60 to 90 min, and the maximum germination is reached only after 3+ hr.

Heated spores reach their maximum percent-

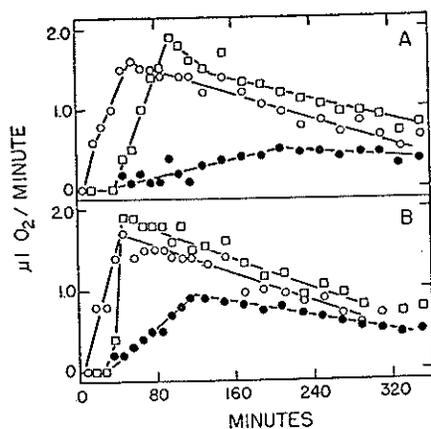


Figure 1. Rate of oxygen consumption by *Bacillus megaterium* spores in acetate buffer. A, unheated spores; B, heated spores; open circles, L-alanine + glucose; squares, manganese + glucose; closed circles, glucose.

age of germination (table 1) earlier than do unheated spores, and spores in L-alanine start germinating earlier than do those in manganese. In glucose alone, germination of heated spores begins in the first 40 min and reaches about 80 per cent by 120 min. The greatest effect of heating appears to lie in the shortening of the lag preceding germination and respiration.

In all of the above media, there is a rapid initial rise in rate of oxygen consumption (figure 1) corresponding to the initial rise in germination. Without further development of the spores, other than the slight swelling noted above, there is no further increase in the rate of oxygen consumption. In fact, a post-maximal decrease in respiratory rate is noted.

In acetate medium, the pH falls rapidly to about 5.5 with manganese, 5.6 with L-alanine, and 5.8 with glucose alone. In view of the poor buffer capacity of acetate, phosphate buffer was used in most of the following experiments.

(b) *Neutral phosphate buffer.* Higher levels of germination in L-alanine are reached by acetate-buffered than by phosphate-buffered spores, but maximum germination is reached comparatively early in both buffers (table 1). Neither heated nor unheated spores incubated with L-alanine in neutral phosphate emerge after germination, but appear instead to be disintegrating after about 280 min.

Heated and unheated spores undergo similar germination changes, but the initiation of each change is earlier with the former (table 1).

The rate of respiration of spores in L-alanine appears to increase linearly during the period of germination, but does not increase thereafter (figure 2). In glucose alone, and in manganese + glucose (figure 2), the initial respiratory response is much slower than in acetate buffer (figure 1). Coinciding with the initiation of each of the phases of morphological development in manganese (table 1), there is a change in the respiratory rate (figure 2).

The turbidity of heated spore suspensions, containing 0.05 mg spores per ml, initially at 33 (Klett-Summerson colorimeter, No. 56 filter), rises to 167 after 20 hr incubation with manganese + glucose, and to 208 after incubation with L-alanine + glucose, or with glucose alone.

(c) *Low pH phosphate buffer.* Heated and unheated spores germinate more completely in phosphate buffer at pH 6.0 than at pH 7.0 (table 1). After germination, the spores swell, but there is no further development over a 7-hr period. The pH falls to 5.8, as compared to 5.5 in acetate and 6.8 in neutral phosphate.

Oxygen consumption with L-alanine begins at about the same time in acetate and in neutral phosphate as in low pH phosphate. While the post-maximal fall in rate of oxygen consumption is not quite as marked in low pH phosphate as in acetate, it is of a higher order than in neutral phosphate.

The lag in commencement of oxygen uptake by manganese incubated spores in neutral phosphate (figure 2) is lacking or considerably reduced with low pH phosphate (figure 3). Corresponding to the failure of spores in low pH phosphate to develop after germination, there is no increase in the respiratory rate such as occurs with manganese in neutral phosphate (figure 2).

(d) *Preincubation with L-alanine.* In the above experiments, spores germinated in L-alanine and glucose swell, but do not emerge even under the same conditions as prevail when manganese-incubated spores germinate and divide. Incubation of spores with L-alanine often produces swollen and degenerate germinated spores.

Spores in neutral phosphate buffer were shaken on the Warburg apparatus with L-alanine + glucose, or with glucose alone. After 2 hr, manga-

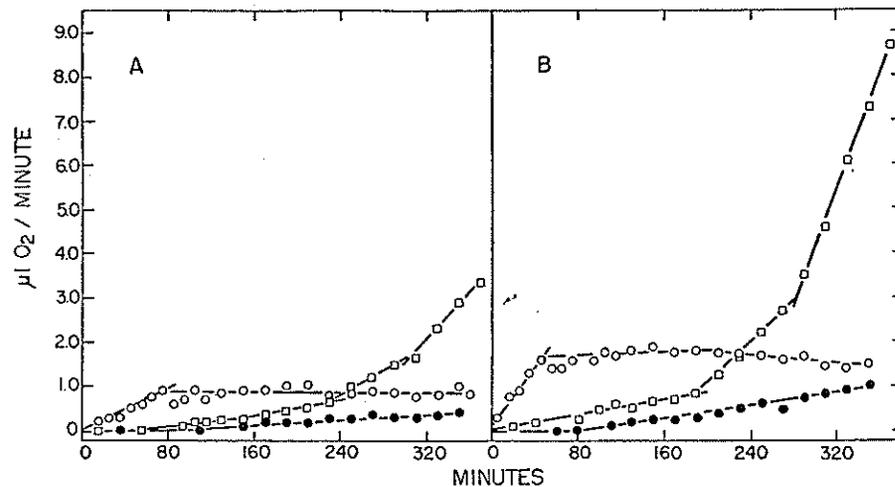


Figure 2. Rate of oxygen consumption of *Bacillus megaterium* spores in phosphate buffer, pH 7.0. Same symbols as for figure 1.

nese was added to the former and L-alanine + manganese was added to the latter from the second sidearm of double sidearm vessels. Other vessels, containing spores in the complete medium—L-alanine, manganese, and glucose—from zero time were also included.

Unheated spores incubated from zero time in the complete medium germinate, and by 190 min have begun to divide. Heated spores behave similarly, but all phases of spore development occur earlier (table 2). When the spores are shaken for 2 hr in glucose before the addition of L-alanine and manganese, emergence is delayed for almost exactly this 2-hr period. Elongation appears to be normal, but cell division is greatly delayed. Emergence of spores preincubated with L-alanine + glucose occurs at about the same time as with glucose-preincubated spores, but the germinated spores in the former case are greatly swollen and have a degenerate, "vacuolated" appearance. Some elongation of the emerged cells occurs, but these elongated cells, too, are quite abnormal in appearance. The L-alanine-preincubated spores do not divide at all in the time of these experiments.

Changes in slopes of the rate curves (figure 4) of spores treated in this manner coincide very well with the morphological post-germinative changes (table 2). Several points, we believe, bear emphasis. We refer now only to unheated

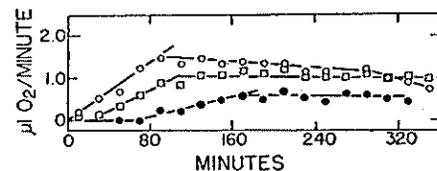


Figure 3. Rate of oxygen consumption of unheated spores of *Bacillus megaterium* in phosphate buffer, pH 6.0. Same symbols as for figure 1.

spores, but these remarks, with different time values, apply equally well to heated spores. The oxygen uptake rate curve for glucose-preincubated spores is parallel after 230 min with the portion of the rate curve, from 110 to 190 min, of spores in the complete medium from zero time. This portion of the respiratory rate curves corresponds to the morphological phase of elongation. These glucose-preincubated spores do not exhibit the change in respiratory rate, corresponding to cell division, which spores incubated from zero time in the complete medium manifest at 190 min. This phenomenon corresponds to the long delay in inception of cell division of the glucose-preincubated spores. While there is a change in slope of the rate curve (figure 4) of L-alanine-preincubated spores at the time of emergence, the rate curve during elongation of these spores is much flatter than

TABLE 2

Germination of spores preincubated in L-alanine and in glucose

Substrate Added at		Treatment	Post-Germinative Changes		
0 min	120 min		Emergence	Elongation	Cell division
			min	min	min
Mn + L-alanine + glucose	—	Unheated	100	110	190
		Heated	80	90	150
L-Alanine + glucose	Mn	Unheated	230	240 Cells abnormal	
		Heated	200	210 (see text)	
Glucose	L-Alanine + Mn	Unheated	230	240	360+
		Heated	200	210	340+

Mn = manganese. The time of commencement of the post-germinative change is indicated, and is subject to an error of 5 min in either direction.

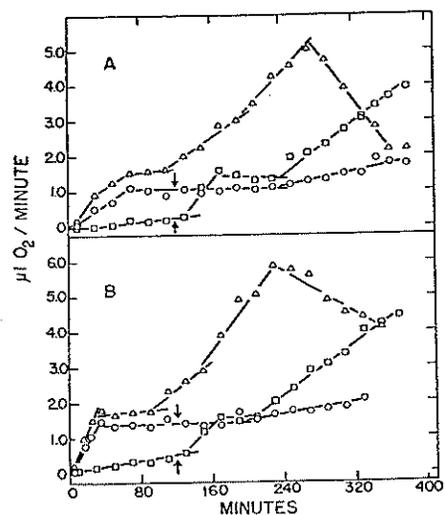


Figure 4. The effect of preincubation with glucose and with L-alanine on the subsequent rate of oxygen consumption by *Bacillus megaterium* spores in manganese and L-alanine. A, unheated spores; B, heated spores; triangles, L-alanine + manganese + glucose at 0 time; circles, L-alanine + glucose at 0 time, manganese at 120 min; squares, glucose at 0 time, L-alanine + manganese at 120 min. Arrows indicate time of tipping of sidearm 2.

that during elongation of glucose-preincubated spores, reflecting, perhaps, the microscopically observable degeneracy of the emerged bacilli. Spores retain their viability (table 3) after 4

TABLE 3
Spore viability after incubation with manganese, L-alanine, and glucose

Additive	Viability After	
	2 hr per cent	4 hr per cent
1. L-Alanine.....	102	93
2. L-Alanine + glucose.....	76	59
3. Mn.....	100	107
4. Mn + glucose.....	102	102
5. Mn + L-alanine.....	93	98
6. Mn + L-alanine + glucose.....	102	69
7. Glucose.....	102	107
8. None (buffer).....	112	107

Mn = manganese. Viability given as percentage of initial count (4.2×10^8 spores per ml). For other conditions see Methods.

hr in manganese, manganese + glucose, L-alanine, glucose alone, or buffer alone. However, there is a significant drop in the number of colonies recoverable on nutrient agar (Difco) after 2 hr in L-alanine + glucose. Spores exposed to a mixture of manganese, L-alanine, and glucose also suffer a loss in viability, but only after 4 hr, after which a sharp drop in rate of oxygen uptake (figure 4) is noted. This loss in viability may be explainable on the basis of exhaustion of nutrient(s) or accumulation of toxic product(s) accompanying a rapid rate of oxygen consumption (figure 4).

DISCUSSION

Spores, buffered with phosphate at pH 7.0, germinate in manganese + glucose, and the

germinated spores swell, emerge, elongate, and divide. On the other hand, in phosphate buffer at pH 6.0, the spores do not develop beyond a slight post-germinative swelling and have not undergone degenerative changes. The arresting of the development of germinated spores at pH 6.0, and their continuing growth at pH 7.0, may provide a tool useful in the separation of the different stages of development.

Initiation of germination of phosphate-buffered spores, at both pH levels, is quite rapid in L-alanine + glucose, with a consistently higher maximum percentage of germination at the lower pH. Initiation of germination in manganese + glucose or in glucose alone is slower than in L-alanine. Germination in manganese and in glucose also begins earlier and attains higher maximal values at the lower pH. This may be connected with a manganese requirement for germination, since, in neutral solution, manganese is bound by phosphate. It does not appear that the depressing effect of phosphate, as an anion, on spore germination (Levinson and Sevag, 1953) is operative in the experiments reported here, since the concentration of univalent anions (chloride, acetate) should be adequate to neutralize this phosphate effect. In contrast, spore germination in *Bacillus terminalis* (Church *et al.*, 1954)¹ is at least twice as rapid at pH 7.0 as at pH 6.0, and indeed appears to be optimal at a pH near 8.0 (Harrell and Halvorson, 1955).

Germination data with acetate buffer supplement the observations with phosphate buffers. A direct comparison is, of course, not intended because of the different ionic concentrations, buffer capacities, etc., of these mixtures. Acetate buffered spores germinate rapidly, and, like the spores in low pH phosphate, do not continue to develop past germination. Although this phenomenon may be a function of the anions, *per se*, rather than of pH, it is interesting that while there is no post-germinative development at the H ion concentrations of acetate and low pH phosphate buffers, there is much development at the pH of neutral phosphate buffer. Germination of spores of *B. megaterium* may then be favored by a relatively low pH, but a pH nearer to neutrality may be required for vegetation.

B. megaterium spores contain from 1.3 per

¹ Gordon (*personal communication*, 1956) states that this strain of *B. terminalis* should be termed *Bacillus cereus*.

cent to 2.2 per cent phosphorus (our unpublished data and Curran *et al.*, 1943). Since vegetative cells contain about twice as much phosphorus, it appears that phosphorus deficiency may be a significant factor in the failure of development of the spores in acetate buffer.

Although our respiration rate data are subject to the errors inherent in the Warburg apparatus, the sequence of germination changes is followed closely by changes in the slopes of these curves. Thus, the slopes during swelling, and during elongation, are distinct from each other, and the commencement of division is marked by yet another change in slope of the rate of oxygen consumption curve. These curves are linear, or close to being so, and confirm, in large measure, results obtained in complex media (Mandels *et al.*, 1956). Furthermore, the initial increase in respiratory rate accompanying germination may also be linear. This initial increase was previously described as being of an undefined nature, since germination and increase in respiration in the complex medium were too rapid to permit precise characterization.

The rate curves for spores incubated in neutral phosphate with manganese + glucose do not show an exponential increase even after division has begun. Only one or two divisions take place in the time of the experiments, and it is possible that on further incubation these rates would increase exponentially.

The respiratory rate of heated spores incubated from zero time with manganese + L-alanine declines after 230 min (figure 4), whereas in the absence of L-alanine, the rate is still increasing at 340 min (figure 2). Since a high respiratory rate is reached much earlier with manganese + L-alanine than without L-alanine, the fall in rate of oxygen consumption by spores in the former case may have no more significance than the exhaustion of available growth factor(s) or nutrient(s).

The failure of spores to continue their development in solutions of glucose + L-alanine is of interest. Spores germinated in such solutions have a "degenerate" appearance after several hours of incubation. Our viability and growth measurements, and Murrell's (1955) observations on *Bacillus subtilis* spores germinated in L-alanine without glucose, suggest that such spores may be dead. However, when manganese is also present, our spores germinate rapidly, and within 3 hr have begun to divide, although

many of the original spores are no longer viable after 4 hr. Indeed, the increase in turbidity of spore suspensions after 20 hr in manganese + glucose, and the lack of such an increase in the absence of manganese, indicates that this element meets a requirement for the post-germinative growth of *B. megaterium*.

A 2-hr preincubation with L-alanine, before addition of manganese, greatly delays and reduces subsequent emergence of germinated spores.² We have not determined whether more extended incubation of the L-alanine-preincubated spores would result in a more complete emergence and elongation. The inhibitory effect of L-alanine cannot be attributed solely to shaking, since spores shaken in glucose for 2 hr before the addition of L-alanine and manganese emerge and elongate normally, although their division is delayed. A stimulatory rather than an inhibitory effect by L-alanine would have been less surprising. Possibly comparable is the observation (Pulvertaft and Haynes, 1951) that adenosine, while necessary for germination of *Bacillus cereus* spores, is inhibitory to vegetative growth in concentrations higher than 22.5 mM. L-Alanine might be toxic in the sense of being inhibitory to post-germinative development, but in view of the good division of germinated spores incubated with L-alanine and manganese from zero time, this does not appear to be the case. The presence of certain amino acids is essential for phage development (Gots and Hunt, 1953) in a lysogenic strain of *Escherichia coli*. The possibility that we are dealing with a lysogenic *B. megaterium* requiring L-alanine for maturation of its phage cannot be discounted. This might explain the abnormal morphology of spores incubated with L-alanine and the loss in viability of some of the spores incubated with manganese and L-alanine from zero time. We have not gone further in the determination of the nature of the L-alanine inhibition. In future investigations, the possibility of reversible or irreversible injury of the spores on exposure to L-alanine must be a consideration.

Previously (Levinson and Sevag, 1953; Levinson and Hyatt, 1955), we considered germination to be the entire transition from spore to

² The work of Fitz-James (1955), brought to our attention after submission of this manuscript, also indicates failure of spores germinated in L-alanine to develop into vegetative cells.

vegetative cell. The initial change, including stainability with methylene blue, was termed "pre-germination." However, demonstration (Mandels *et al.*, 1956) of the rapid increase in respiratory activity coincident with inception of stainability now leads us to accept the validity of the latter as a criterion for spore germination. We refer to the stages subsequent to germination as swelling, emergence, elongation, and cell division. In view of the changes in respiratory activity occurring during these events, we feel that such a terminology has a sound physiological as well as a morphological basis.

ACKNOWLEDGMENTS

The authors are indebted to Dr. G. R. Mandels of our laboratories for criticizing the manuscript, and to Dr. Ruth E. Gordon of the Institute of Microbiology, Rutgers University, for confirming the identity of our strain of *B. megaterium*.

SUMMARY

The morphological stages of spore germination and subsequent growth in a chemically defined medium coincide with changes in respiratory activity, the respiratory rate curves being characterized by phases of different linear increases in rate corresponding to the phases of germination, swelling, emergence, elongation and cell division.

A higher percentage of spores of *Bacillus megaterium* germinate in phosphate buffer at pH 6.0 than at pH 7.0. Germinated spores vegetate in manganese + glucose at the higher, but not at the lower pH. Data obtained from acetate buffer supplement these observations.

Spores germinate rapidly in L-alanine + glucose, but no post-germinative development takes place even after 7 hr of incubation. Exposure of spores to L-alanine + glucose for 2 hr greatly decreases subsequent emergence in manganese; the spores appear to be disintegrating, and their viability is much reduced.

REFERENCES

- CHURCH, B. D., HALVORSON, H., AND HALVORSON, H. O. 1954 Studies on spore germination: its independence from alanine racemase activity. *J. Bacteriol.*, **68**, 393-399.
- CURRAN, H. R., BRUNSTETTER, B. C., AND MYERS, A. T. 1943 Spectrochemical analysis of vegetative cells and spores of bacteria. *J. Bacteriol.*, **45**, 435-494.

- FITZ-JAMES, P. C. 1955 The phosphorus fractions of *Bacillus cereus* and *Bacillus megaterium*. II. A correlation of the chemical with the cytological changes occurring during spore germination. *Can. J. Microbiol.*, **1**, 525-548.
- FOSTER, J. W. AND HEILIGMAN, F. 1949 Mineral deficiencies in complex organic media as limiting factors in the sporulation of aerobic bacilli. *J. Bacteriol.*, **57**, 613-615.
- GOTS, J. S. AND HUNT, G. R., JR. 1953 Amino acid requirements for the maturation of bacteriophage in lysogenic *Escherichia coli*. *J. Bacteriol.*, **66**, 353-361.
- HACHISUKA, Y., ASANO, N., KATO, N., OKAJIMA, M., KITAORI, M., AND KUNO, T. 1955 Studies on spore germination. I. Effect of nitrogen sources on spore germination. *J. Bacteriol.*, **69**, 399-406.
- HARRELL, W. K. AND HALVORSON, H. 1955 Studies on the role of L-alanine in the germination of spores of *Bacillus terminalis*. *J. Bacteriol.*, **69**, 275-279.
- LEVINSON, H. S. AND HYATT, M. T. 1955 The stimulation of germination and respiration of *Bacillus megaterium* spores by manganese, L-alanine, and heat. *J. Bacteriol.*, **70**, 368-374.
- LEVINSON, H. S. AND SEVAG, M. G. 1953 Stimulation of germination and respiration of the spores of *Bacillus megaterium* by manganese and monovalent anions. *J. Gen. Physiol.*, **36**, 617-629.
- MANDELS, G. R., LEVINSON, H. S., AND HYATT, M. T. 1956 Analysis of respiration during germination and enlargement of spores of *Bacillus megaterium* and of the fungus *Myrothecium verrucaria*. *J. Gen. Physiol.*, **39**, 301-309.
- MURRELL, W. G. 1955 The bacterial endospore. Four lectures delivered as Thomas Lawrence Pawlett Scholar of the Faculty of Agriculture, University of Sydney, Australia, in April, 1953 (mimeographed).
- POWELL, J. F. 1950 Factors affecting the germination of thick suspensions of *Bacillus subtilis* spores in L-alanine solution. *J. Gen. Microbiol.*, **4**, 330-338.
- PULVERTAFT, R. J. V. AND HAYNES, J. A. 1951 Adenosine and spore germination; phase contrast studies. *J. Gen. Microbiol.*, **5**, 657-663.