EFFECT OF NUTRITION ON THE RESPIRATORY VIRULENCE OF *LISTERIA MONOCYTOGENES*

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ABSTRACT

FRIEDMAN, MISCHA E. (U. S. Army Chemical Corps, Frederick, Md.) AND DONALD A. KAUTTER, Effect of nutrition on the respiratory virulence of *Listeria monocytogenes*. J. Bacteriol. **83**:456–462. 1962.—The respiratory virulence for the mouse and guinea pig of two strains of *Listeria monocytogenes* grown in a rich nonsynthetic medium may be increased four to ninefold by decreasing the glucose concentration from 1.0 to 0.6% or by including 0.1% L-cysteine in the medium. This effect, not observed in intraperitoneal challenge of mice, is not due to selection of a more virulent population. Cultures demonstrating lowered virulence at 16 hr increase in virulence if incubated beyond 50 hr. The presence of 1.0% fructose or cellobiose results in the same growth and virulence characteristics as when glucose is used; however, cultures in media with 1.0% maltose are of decreased cell yield but increased virulence. Strain A4413 grown in media of low glucose concentration or in the presence of cysteine has greater oxidative activity than when grown in a medium of high glucose content. Other strains of lesser respiratory virulence, when grown in a low concentration of glucose, contain glucose dehydrogenase systems equal in activity with that of strain A4413.

The virulence of microorganisms can be affected by physical or nutritional factors. In most cases, however, the final determining cause of a change in this characteristic is genetic.

Cole and Braun (1950), Goodlow, Braun, and Mika (1951), and Waring and coworkers (1953) found that Mn^{++} or Mg^{++}, D-alanine, and iron stimulated the dissociation of smooth *Brucella* species. In addition, Sanders and Huddleson (1956) and Altenbern et al. (1957) reported that oxygenation reduced population changes in *B. abortus* cultures.

Fukui and coworkers (1957) observed a reduction in virulence for the mouse of *Pasteurella pestis* grown in aerated broth at 37 C, and regarded this as due to a selection of an avirulent mutant. Wessman, Miller, and Surgalla (1958) demonstrated that an observed toxic effect of glucose on virulent *P. pestis* resulted in a selection of avirulent mutants. Kupferberg and Higuchi (1958) added Ca^{++} to a defined medium and were able to control this selection at 37 C by providing optimal growth conditions for the virulent form.

Garber (1954) reported that the virulence of nutritional mutants of *Erwinia aroideae* for certain plant tissues was related to the availability of required nutrients at the surface of the tissue. Hart and Rees (1956) induced a persistent virulence in *Mycobacterium tuberculosis* H4Ra, an avirulent strain, by nonionic surface-active agents. Growth of *Coccidioides immitis* in the parasitic spherule form was stimulated by Na^{+}, Ca^{++}, sodium bicarbonate, glutathione, and fatty acids (Converse and Besemer, 1959). Lincoln and coworkers (1946) reported that the respiratory virulence for guinea pigs of *Bacillus anthracis* grown in a corn-steep liquor medium was decreased, compared with that observed for cultures in a Pepticase-yeast medium; subcutaneous virulence, however, was enhanced. The authors stated that the decrease in virulence was not hereditary.

The present report presents evidence that the carbohydrate nutrition of *Listeria monocytogenes* influences the respiratory virulence of this organism for the mouse and guinea pig.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Most of the studies to be reported were done with *L. monocytogenes* strain A4413. Data were also collected with the less virulent strains JHH, AT-14,

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and Cornell, and an avirulent rough strain, 9037-7. Stock cultures were maintained on tryptose agar (Difco), stored at 4 C, and transferred every 6 months. Cells for the preparation of inocula were grown at 37 C on a medium composed of 2.6% tryptose broth (Difco), 0.4% glucose, 0.4% yeast extract (Difco), and 1.5% Bacto agar (Difco). Growth was removed from the slants with sterile deionized water and adjusted to an approximate optical density of 0.301 (655 mμ). This represented 1.2×10^9 cells/ml. Into 30 ml of medium in a 250-ml Erlenmeyer flask, equipped with an optically standardized 18 by 150 mm test-tube side arm, were inoculated 0.2 ml of this cell suspension, so that at zero time there were approximately 8×10^6 cells/ml in the flask. Unless stated otherwise, cultures were incubated for 16 hr at 37 C with agitation on a shaker set at 100 excursions/min. At the end of the incubation period, duplicate cultures were pooled for use in virulence assays.

Media routinely used were Difco's brain heart infusion (BHI) and the NS-4CA, NS-3, and NS-4CA plus 0.1% L-cysteine media developed in these laboratories. Components of these media are presented in Table 1. Under the cultural conditions described above, the average 16-hr viable count of all strains in BHI was 3.3×10^9 cells/ml; this compared with counts in the other media of 10×10^9 cells/ml, except for strain 9037-7 which grew at a slower rate. All viable-count assays were made on tryptose agar (Difco) incubated at 37 C for 18 to 24 hr.

Virulence assays. The desired respiratory doses for virulence assays were prepared by dilution of cultures with sterile BHI. Swiss-Webster mice (16 to 18 g) or guinea pigs (350 g) were challenged in a modified Henderson (1952) apparatus. A titration of five doses, ten animals per dose, was performed. The aerosol was sampled with a liquid impinger simultaneously with exposure of the animals. Aerosol particles collected were considered to consist of single cells (Harper, Hood, and Martin, 1958). The dose quantitation was calculated from the concentration of cells collected in the impinger fluid (Roessler and Kautter, 1962), using the respiratory rate and volume as established by Guyton (1947). Cells to be used for intraperitoneal challenge were diluted with 1% tryptose, and the same number of animals and doses were used as in respiratory challenge. Exposed animals were observed for 15 days. All animals that succumbed were autopsied and examined for gross pathological changes. The organism was readily isolated from the lungs, spleen, and liver. The LD_{50} values and the 95% confidence limits were calculated by the method of Litchfield and Wilcoxon (1949); in some cases, LD_{50} values were determined by the method of Reed and Muench (1938).

TABLE 1. *Composition of liquid media**

Component	Medium NS-4CA	Medium NS-3
	%	%
Peptone "C"†	1.5	1.5
Yeast extract	0.6	0.6
K_2HPO_4	1.8	0.5
Sodium citrate	0.5	
Glucose‡	1.0	0.6

* Final pH adjusted to 7.5.
† Pancreatic digest of casein, Albimi Labs.
‡ Glucose sterilized separately by filtration.

Analytical methods. Total carbohydrate in growth filtrates was determined by a modification of the anthrone method of Gary and Klausmeier (1954), in which the reactant tubes of the samples as well as the glucose standard were heated in a boiling-water bath for 10 min and read at 620 mμ in a model 14 Coleman Universal spectrophotometer. Lactate was determined by the method of Barker and Summerson as outlined by Umbreit, Burris, and Stauffer (1949), pyruvate by the method of Friedemann and Haugen (1943), and acetoin by the method of Westerfield (1945). Sulfhydryl content of media was measured by the method of Grunert and Phillips (1951) and confirmed by the method of Alexander (1958).

Dehydrogenase assay. Resting cells for dehydrogenase assays were harvested from 16-hr liquid cultures, washed three times with distilled water, and resuspended in water at a concentration of 0.8 mg of cell nitrogen/ml. The usual Thunberg technique, with methylene blue as indicator and glucose as substrate, was used (Umbreit et al., 1949).

RESULTS

Respiratory virulence and aerosol survival of *Listeria* were assayed routinely with cells grown in BHI. In these laboratories several rich nonsynthetic media were developed which ultimately

gave rise to a threefold increase in cell yield. However, the respiratory virulence of strain JHH grown in the medium supporting the most growth was found to be one-half to one-tenth that of cells from BHI. Subsequent studies revealed that strain A4413 was approximately tenfold more virulent than JHH, and further investigations were carried out with this strain.

Effect of medium. Statistical analysis of data from 28 experiments showed that the respiratory virulence of strain A4413 for the mouse differed according to the growth medium from which the cells were harvested. NS-4CA cultures were significantly less virulent than cells from either BHI, NS-3, or NS-4CA plus cysteine (geometric means of LD_{50} values $\times 10^3$ were 22.8, 6.0, 2.61, and 6.1, respectively).

TABLE 2. *Effect of subculture on the respiratory virulence of Listeria monocytogenes A4413 for the mouse*

Medium	Inoculum	$LD_{50} \times 10^3$	95% Confidence limits $\times 10^3$
	I		
NS-4CA	Agar slant	60.0	33.3–108.0
BHI	Agar slant	6.2	3.6–10.5
BHI	Primary NS-4CA culture	5.6	2.5–12.3
BHI	4th Subculture in NS-4CA	4.7	2.5–8.7
	II		
BHI	Agar slant	7.2	4.5–11.5
NS-4CA	Agar slant	22.5	11.3–45.0
NS-4CA	1st Subculture in BHI	80.0	40.0–160.0
	III		
BHI	Agar slant	4.5	2.9–7.0
NS-4CA	Primary BHI culture	22.0	11.6–41.8
NS-4CA + cysteine	Primary BHI culture	4.8	1.9–12.5
NS-4CA + cysteine	6th Subculture in NS-4CA + cysteine	2.9	1.8–4.6
NS-4CA	7th Subculture in NS-4CA + cysteine	62.0	41.3–93.0

TABLE 3. *Analysis of variance of log LD_{50} values for the mouse of cultures from four media*

Effect	Degrees of freedom	Sum of squares	Mean square
Between media	3	5.970404	1.990135
Between experiments in media	36	5.684334	0.157898
Between transfers	13	0.470905	0.036223
Total	52	12.125643	

TABLE 4. *Effect of age of culture on respiratory virulence for the mouse of Listeria monocytogenes A4413 grown in NS-4CA medium*

Hours	LD_{50} (with 95% confidence limits) $\times 10^3$		
	Expt. I	Expt. II	Expt. III
8		2.9	3.8 (1.8–8.2)
16	94.0	13.0 (5.9–28.6)	8.5
24	>113.0		
30		18.0	13.0 (7.6–22.1)
37		8.5	11.2 (7.1–17.7)
48	13.0 (6.8–24.7)		
56		2.4	3.1
62			4.7 (2.8–8.0)

Transfer experiments. The possibility that a selective process was responsible for the observed differences in LD_{50} values was tested in a series of transfer experiments. Progeny of cells which had been subcultured four times in NS-4CA medium (primary culture plus four subcultures) and then inoculated into BHI had the LD_{50} value of a BHI culture which had arisen from an agar-slant inoculum (Table 2). These data also exclude the selective process upon subculture in BHI and subsequent transfer to NS-4CA. In addition, cysteine did not bring about a population change to more virulent cells in the NS-4CA medium, since subcultures in its presence with subsequent inoculation into NS-4CA gave rise to a culture whose LD_{50} was increased 16-fold.

An analysis of variance of the LD_{50} values from the 28 experiments, including the transfer experiments, may be seen in Table 3.

Effect of age of culture. When incubation was extended beyond 30 hr, the cells in the NS-4CA medium increased in virulence. Data from three experiments (Table 4) indicate a real effect due to age of culture. These values form a pattern (Fig. 1) whose chance occurence under the hypothesis of equivalent LD_{50} values is obviously very unlikely.

Effect of glucose. Since the NS-3 medium differed from the NS-4CA medium in the concentrations of glucose and K_2HPO_4 and in the absence of sodium citrate, the effect of these components on virulence was tested. Earlier studies had indicated that the presence or absence of citrate had no effect on the virulence of strain JHH. Citrate

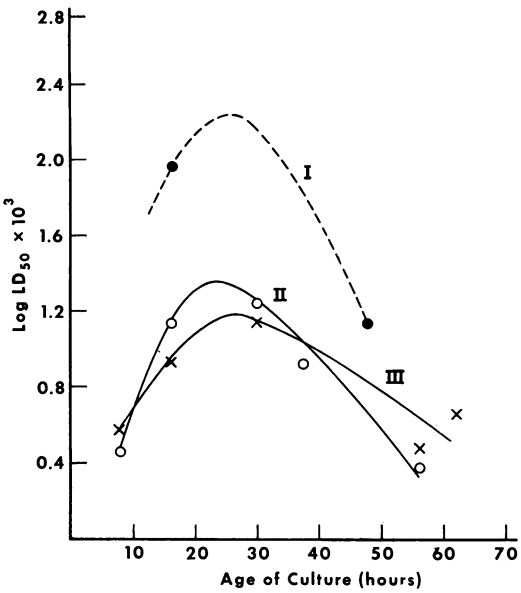

FIG. 1. *Effect of the age of the culture on the respiratory virulence for the mouse of Listeria monocytogenes strain A4413 grown in NS-4CA medium. Roman numerals refer to replicate experiments.*

TABLE 5. *Effect of glucose and K_2HPO_4 in peptone media* on the respiratory virulence of Listeria monocytogenes A4413 for the mouse*

Glucose	K_2HPO_4	$LD_{50} \times 10^3$	95 % Confidence limits $\times 10^3$
%	%		
1.0	1.8	26.0	14.4–46.8
		29.0	14.5–58.0
0.6†	0.5	3.7	2.5–5.6
0.6	1.8	5.5	
1.0	0.5	48.0	22.9–100.8
0.2	0.5	4.5	
1.5	0.5	58.7	
0.6	1.5	4.3	3.3–5.7

* The media contained 1.5% Peptone C as a nitrogen source.

† Sodium citrate was omitted from this medium.

is not utilized as a carbon souce, but is included in the medium as a chelating agent. The glucose concentration was responsible for the variation in the respiratory virulence of the *Listeria* cultures (Table 5). Also, the addition of 300 mg of glucose to 30 ml of growing NS-3 culture after 7½ hr incubation increased the LD_{50} of the 16-hr cells eightfold. Subsequent study showed that a significant increase in the LD_{50} value had occurred within 95 min after the addition of this amount of glucose. At the end of the 95-min period, there was no difference in the viable count between the culture to which the glucose had been added and the control culture which received no added glucose. On the other hand, the addition of glucose to BHI (final concentration 1.7%) did not cause a decrease in virulence. BHI normally contains 0.2% glucose. The LD_{50} value of cells from such a medium was 2×10^3 compared with 1.4×10^3 for the normal BHI culture.

Effect of other carbohydrates. Fructose or cellobiose (1%), which supported a cell yield equal to glucose in the NS-4CA medium, affected virulence in the same manner as glucose. When 1% maltose was used, the yield of cells was approximately one-half that shown with the same concentration of glucose; however, the maltose-grown cells were two to seven times more virulent. In contrast to the utilization of 90% of the glucose in NS-4CA or NS-3 medium during the 16-hr incubation period, the organism utilized only 34% of the maltose. On the other hand, although the 16-hr viable count of strain A4413 grown in the presence of 1% trehalose was one-third the count of that with 1% glucose, cultures grown in the presence of trehalose demonstrated the same virulence characteristics as glucose-grown cells.

Stationary vs. shaken cultures. The aerosol virulence of cells grown in 30 ml of NS-4CA medium containing 1.5% glucose in a stationary 20 by 265 mm tube was compared with that of cells grown in the same volume of medium in a 250-ml Erlenmeyer flask on a shaker. The 16-hr viable count of the stationary culture was one-half the count of the shaken culture, but the virulence was sevenfold greater than that of the shaken culture (LD_{50} values were 2.6×10^3 and 18.8×10^3, respectively).

Intraperitoneal challenge. A comparison of

TABLE 6. *Effect of medium on respiratory virulence of Listeria monocytogenes JHH for the mouse*

Medium	$LD_{50} \times 10^3$	95% Confidence limits $\times 10^3$
NS-4CA	150.0	71.4–315.0
NS-3	18.0	10.0–32.0
BHI	53.0	
NS-4CA + cysteine	60.0	52.4–111.0

TABLE 7. *Effect of medium on respiratory virulence of Listeria monocytogenes A4413 for the guinea pig*

Medium	LD_{50} (with 95 % confidence limits) $\times 10^4$		
	Expt. I	Expt. II	Mean*
NS-4CA	22.5 (9.0–56.3)	21.0 (9.1–48.3)	21.74[a]
NS-3	10.3	13.0 (6.2–27.3)	11.57[b]
BHI	4.3 (2.1–9.0)	3.9 (1.6–9.4)	4.10[c]
NS-4CA + cysteine	5.6 (3.1–10.1)	8.0 (3.3–19.6)	6.69[c]

* Means with dissimilar superscript are significantly different.

TABLE 8. *Analysis of variance of log LD_{50} values for the guinea pig of cultures from four media*

Source	Degrees of freedom	Sum of squares	Mean square	F
Experiment	1	0.0043	0.0043	
Media	3	0.5839	0.1946	41.40**
Error	3	0.0141	0.0047	

** Highly significant $P < 0.01$.

strain A4413 grown in BHI and NS-4CA revealed no differences in virulence when assayed via the intraperitoneal route. In a typical experiment, LD_{50} values for the mouse were 24 cells (BHI) and 21 cells (NS-4CA).

Strain JHH. Results of respiratory virulence assays of strain JHH grown in the four media are shown in Table 6. The virulence of the NS-4CA culture was significantly lower than that of the other three cultures.

Guinea pig challenge. The LD_{50} for the guinea pig of strain JHH grown in BHI was approximately tenfold higher than the LD_{50} of this culture for mice. The LD_{50} values of strain A4413 for guinea pigs and mice showed the same relationship, but in general were one-tenth the JHH values. The effects on strain A4413 in mice were related to medium differences and were observed generally for guinea pigs, the differences (Table 7) being in the same direction if somewhat less in magnitude. An analysis of variance of the data from the guinea pig experiments (Table 8) established that the differences between the LD_{50} values of the various cultures were highly significant.

Dehydrogenase experiments. With glucose as substrate, resting cells of strain A4413 grown in BHI, NS-3, or NS-4CA plus cysteine decolorized methylene blue in 4 min, whereas the NS-4CA cells took 25 min. On the other hand, strains which are approximately 20-fold less virulent than A4413, such as Cornell and AT-14 as well as avirulent 9037-7 grown in BHI, reduced the methylene blue at the same fast rate as A4413 grown in BHI.

DISCUSSION

The effect of glucose concentration on the respiratory virulence for mice and guinea pigs of several strains of *L. monocytogenes* is not a selection effect, as evidenced by the results of the transfer experiments. In addition, the decrease in virulence in the presence of a high glucose concentration is not due to increasing acidity of the cultures. The buffering capacity of the high concentration of K_2HPO_4 in the NS-4CA medium kept the pH from dropping below 6.3 during the 16-hr growth period. Further, media containing 0.6% glucose and a low concentration of K_2HPO_4, in which the pH dropped to approximately 6.1, and BHI, which decreased to a pH of 5.6, yielded cells of high virulence.

The results of the dehydrogenase studies suggest a possible correlation between respiratory virulence and the oxidative ability of a culture. The lack of such a correlation observed in strains Cornell, AT-14, and 9037-7 grown in BHI suggests that the relationship is phenotypic and not genotypic, since strain lines were not crossed. A direct correlation between pyruvate oxidation and virulence of *P. tularensis* has been reported by Weinstein, Guss, and Altenbern (1959, 1960). On the other hand, differences in enzyme activity may reflect the coincidental control glucose exercises over the synthesis of some enzymes, including dehydrogenases (Gale, 1943; Neidhardt and Magasanik, 1956).

The role of cysteine is not clear. However, it does not act as a selective agent for the more virulent cells. Despite the fact that cells growing in the presence or absence of cysteine in the NS-4CA medium utilize glucose and accumulate end-products such as lactate, pyruvate, and acetoin to the same extent, the presence of cysteine does stimulate the production of cells that are more oxidative in the resting state. It is possible that the excess cysteine assimilated by the cells maintains the cells in a reduced state, thereby causing them to become more oxidative in order to metabolize the glucose efficiently and

to become more virulent. BHI, which supports the growth of cells of greater virulence, contains three times more sulfhydryl groups than the NS-4CA medium, and this may account for the failure of an eightfold increase in glucose concentration in BHI to decrease the virulence of cells from such a culture. The increased virulence of cells grown under stationary conditions, compared with the virulence of the shaken culture, may be similar to the effect on virulence of the addition of cysteine to the NS-4CA medium.

The decreased growth of *Listeria* in the presence of 1.0% maltose reflects a weak maltase system in that organism. Since these cells metabolize maltose only to a limited extent and are still highly virulent via the respiratory route, they may be similar to the cells which are produced in media containing only 0.2 to 0.6% glucose.

Another possible role of glucose may be its effect on cellular constituents which may be related to the virulence of the cell. Preliminary antigenic studies with antisera to BHI- and NS-4CA-grown cells have shown no differences in antigen makeup of cells from various cultures. Other experiments have shown the NS-4CA cells to contain slightly less total nitrogen on a dry wt basis than cells from the other three media.

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LITERATURE CITED

ALEXANDER, N. M. 1958. Spectrophotometric assay for sulfhydryl groups using N-ethylmaleimide. Anal. Chem. **30**:1292–1294.

ALTENBERN, R. A., D. R. WILLIAMS, J. M. KELSH, AND W. L. MAUZY. 1957. Metabolism and population changes in *Brucella abortus*. II. Terminal oxidation and oxygen tension in population changes. J. Bacteriol. **73**:697–702.

COLE, L. J., AND W. BRAUN. 1950. The effect of ionic Mn and Mg on the variation of *Brucella abortus*. J. Bacteriol. **60**:283–289.

CONVERSE, J. L., AND A. R. BESEMER. 1959. Nutrition of the parasitic phase of *Coccidioides immitis* in a chemically defined liquid medium. J. Bacteriol. **78**: 231–239.

FRIEDEMANN, T. E., AND G. E. HAUGEN. 1943. Pyruvic acid. II. The determination of keto acids in blood and urine. J. Biol. Chem. **147**:415–442.

FUKUI, G. M., J. E. OGG, G. E. WESSMAN, AND M. J. SURGALLA. 1957. Studies on the relation of cultural conditions and virulence of *Pasteurella pestis*. J. Bacteriol. **74**:714–717.

GALE, E. F. 1943. Factors influencing the enzymic activities of bacteria. Bacteriol. Rev. **7**:139–173.

GARBER, E. D. 1954. The role of nutrition in the host-parasite relationship. Proc. Natl. Acad. Sci. U. S. **40**: 1112–1118.

GARY, N. D., AND R. E. KLAUSMEIER. 1954. Colorimetric determination of ribose, deoxyribose, and nucleic acids with anthrone. Anal. Chem. **26**:1958–1960.

GOODLOW, R. J., W. BRAUN, AND L. A. MIKA. 1951. The role of D-alanine in the growth and variation of *Brucella abortus*. Arch. Biochem. **30**:402–406.

GRUNERT, R. R., AND P. H. PHILLIPS. 1951. A modification of the nitroprusside method of analysis for glutathione. Arch. Biochem. **30**:217–225.

GUYTON, A. C. 1947. Measurement of the respiratory volumes of laboratory animals. Am. J. Physiol. **150**:70–77.

HARPER, G. J., A. M. HOOD, AND J. D. MARTIN. 1958. Airborne micro-organisms: A technique for studying their survival. J. Hyg. **56**:364–370.

HART, P. D., AND R. J. REES. 1956. Induction of virulence in an avirulent strain of *Mycobacterium tuberculosis* by certain non-ionic surface-active agents. Brit. J. Exptl. Pathol. **37**:372–384.

HENDERSON, D. W. 1952. An apparatus for the study of airborne infection. J. Hyg. **50**:53–68.

KUPFERBERG, L. L., AND K. HIGUCHI. 1958. Role of calcium ions in the stimulation of growth of virulent strains of *Pasteurella pestis*. J. Bacteriol. **76**:120–121.

LINCOLN, R. E., M. R. ZELLE, C. I. RANDLES, J. L. ROBERTS, AND G. A. YOUNG, JR. 1946. Respiratory pathogenicity of *Bacillus anthracis* spores. III. Changes in pathogenicity due to nutritional modifications. J. Infectious Diseases **79**: 254–265.

LITCHFIELD, J. T., JR., AND T. W. WILCOXON. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exptl. Therap. **96**:99–113.

NEIDHARDT, F. C., AND B. MAGASANIK. 1956. The

effect of glucose on the induced biosynthesis of bacterial enzymes in the presence and absence of inducing agents. Biochim. et Biophys. Acta **21**:324–334.

REED, L. J., AND H. MUENCH. 1938. A simple method for estimating fifty per cent endpoints. Am. J. Hyg. **27**:493–497.

ROESSLER, W. G., AND D. A. KAUTTER. 1962. Modifications to the Henderson apparatus for studying air-borne infections. Evaluations using aerosols of *Listeria monocytogenes*. J. Infectious Diseases **110**:17–22.

SANDERS, E., AND I. F. HUDDLESON. 1956. The influence of environmental conditions on the growth and dissociation of *Brucella abortus*. Am. J. Vet. Research **17**:324–330.

UMBREIT, W. W., R. H. BURRIS, AND J. F. STAUFFER. 1949. Manometric techniques and tissue metabolism. Burgess Publishing Co., Minneapolis.

WARING, W. S., S. S. ELBERG, P. SCHNEIDER, AND W. GREEN. 1953. The role of iron in the biology of *Brucella suis*. I. Growth and virulence. J. Bacteriol. **66**:82–91.

WEINSTEIN, I., M. L. GUSS, AND R. A. ALTENBERN. 1959. Pyruvate oxidation by *Pasteurella tularensis*. Bacteriol. Proc., p. 109.

WEINSTEIN, I., M. L. GUSS, AND R. A. ALTENBERN. 1960. Pyruvate oxidation by *Pasteurella tularensis*. Bacteriol. Proc., p. 155.

WESSMAN, G. E., D. J. MILLER, AND M. J. SURGALLA. 1958. Toxic effect of glucose on virulent *Pasteurella pestis* in chemically defined media. J. Bacteriol. **76**:368–375.

WESTERFIELD, W. W. 1945. A colorimetric determination of blood acetoin. J. Biol. Chem. **161**:495–502.