FACTORS INFLUENCING THE MORPHOLOGY OF BLASTOMYCES DERMATITIDIS

SEYMOUR LEVINE AND Z. JOHN ORDAL[1]

Department of Bacteriology and Public Health, University of Illinois, College of Medicine, Chicago 12, Illinois

Received for publication August 16, 1946

Blastomyces dermatitidis possesses two morphological forms, a yeastlike form in tissue and a mycelial form on Saboraud's medium at room temperature. A yeastlike form similar to the form appearing in tissue may be obtained if the organism is grown on blood agar at 37 C.

An extensive literature has developed around blastomycosis as a clinical entity, but only a small proportion of the papers have been concerned with the biological nature of the etiological agent, *Blastomyces dermatitidis*. Ricketts (1901) described the two morphological forms. Hamburger (1907) concluded that temperature was the most important factor influencing morphology, and that room temperature favored mycelial formation, but incubator temperatures favored the yeastlike form. He did not determine the effect of intermediate temperature or of hydrogen ion concentration. Michelson (1928) studied the effect of higher temperatures and noted that temperatures of 37, 41, and 45 C favored the growth of the yeastlike form. Moore (1933) studied the organism for the purpose of determining its position in botanical classification. He studied its growth in media of different hydrogen ion concentrations, but each hydrogen ion concentration was represented by a different medium.

The purpose of the present study was to determine the effect of various controlled changes in the environment, such as nutrition, hydrogen ion concentration, temperature, and type of inoculum on the two morphological forms of *Blastomyces dermatitidis*.

METHODS

Media. Solid media were used for all the studies, since preliminary work indicated that the organism grew best under purely aerobic conditions, and that the yeast form grew very poorly in liquid media.

Preliminary nutritional studies showed that the organism grew as well as or better on a peptone glucose medium than it did on extract blood agar. The organism grew equally well on a medium consisting of salts, glucose, and vitamin-free casein hydrolyzate, indicating that it does not require the addition of any of the ordinary accessory factors. Growth also occurred on a medium consisting of salts, glucose, and ammonium sulfate, but never equalled that occurring on the more complete peptone glucose medium. This indicates the stimulatory action of amino acids.

[1] Present address: Process and Product Research Division, Owens Illinois Glass Company, Toledo, Ohio.

Two different media were employed: (1) a complete medium represented by the peptone glucose medium; and (2) a deficient medium represented by the ammonium sulfate glucose medium.

Peptone glucose medium		*Ammonium sulfate glucose medium*	
Peptone	0.5 g	$(NH_4)_2SO_4$	0.5 g
Glucose	1.0 g	Glucose	1.0 g
K_2HPO_4	0.5 g	K_2HPO_4	0.5 g
KH_2PO_4	0.5 g	KH_2PO_4	0.5 g
$MgSO_4 \cdot 7H_2O$	0.2 g	$MgSO_4 \cdot 7H_2O$	0.2 g
NaCl	0.01 g	NaCl	0.01 g
$FeSO_4 \cdot 7H_2O$	0.01 g	$FeSO_4 \cdot 7H_2O$	0.01 g
$MnSO_4 \cdot 2H_2O$	0.0065 g	$MnSO_4 \cdot 2H_2O$	0.0065 g
Agar	1.5 g	Agar	1.5 g
H_2O	100 ml	H_2O	100 ml

Portions of each of these media were adjusted to hydrogen ion concentrations of pH 3.5, 4.5, 5.5, 6.5, 7.5, 8.5, and 9.5 and dispensed into tubes. All tubes were autoclaved at 15 pounds' pressure for 15 to 20 minutes and slanted.

Inoculum. Two types of inocula were used, a suspension of the yeastlike cells and a spore suspension. A 3- to 5-day blood agar culture of the yeast phase was suspended in sterile physiological saline and washed. The suspension of the washed cells was brought to a standard turbidity by means of a lumetron model 400 G photoelectric colorimeter (60 per cent transmission, blue filter wave length 420 A°). One drop of the standard suspension was delivered from a Wright pipette into each tube and then spread over the surface of the agar slant with a sterile wire loop.

The spore suspension was prepared in sterile distilled water by gently scraping the surface of a 2- to 4-week-old culture grown at room temperature. The suspension consisted primarily of blastospores with a minimum amount of mycelial fragments. A drop of this suspension was delivered into each tube and spread with a wire loop. All tubes were inoculated in duplicate.

The inoculated tubes were incubated at room temperature and in water baths at 31, 33, 35, and 37 C. The water baths were capable of maintaining a constant temperature within 0.5 C. Observations were made at 3, 5, 8, 11, and 15 days. The amount of growth was estimated visually and the type of growth was also determined by microscopic examination. No attempt was made to follow the pH during the course of growth, since the medium was well buffered and any changes that might have occurred would have been common to all the tubes. The validity of the observations was confirmed by means of repeat experiments.

Organism. The strain of *Blastomyces dermatitidis* used for the study was originally from the culture collection at Duke University. A duplicate series of experiments with two other strains of *Blastomyces dermatitidis*[2] confirmed the results obtained with the original strain.

[2] The other strains of *Blastomyces dermatitidis* used were as follows: a strain originally obtained from the culture collection of the late Dr. A. T. Henrici and a recently isolated strain obtained from a patient at the Research and Educational Hospital of the University of Illinois.

RESULTS

Peptone glucose medium: Yeast inoculum. Growth on this medium was maximal. Using the yeast cell inoculum, growth was visible in 2 to 3 days, regardless of the resulting morphology. From room temperature through 33 C the organism grew as the mycelial form. At 35 and 37 C the organism grew as the yeastlike form. This occurred irrespective of the hydrogen ion concentration.

Each form of the organism possessed an optimum temperature at which growth

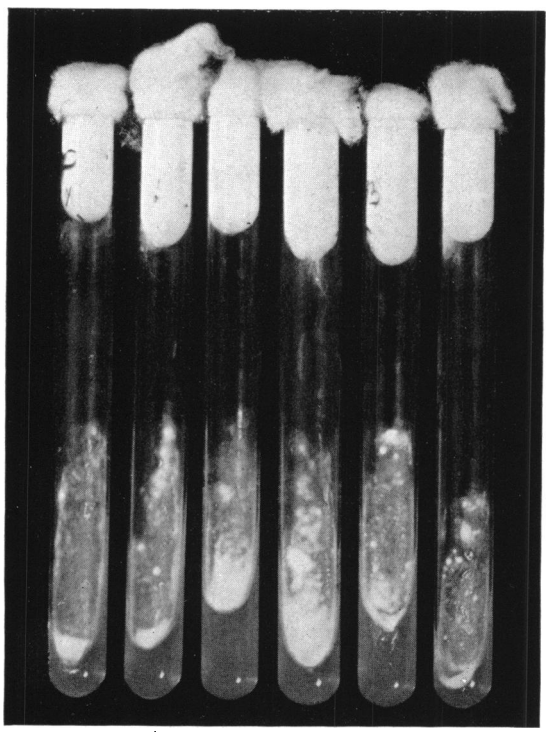

FIG. 1. SHOWING THE EFFECT OF TEMPERATURE ON THE GROWTH OF BLASTOMYCES DERMATITIDIS ON A PEPTONE GLUCOSE MEDIUM AFTER FIVE DAYS

From left to right, the tubes are arranged as follows: 2 tubes incubated at room temperature, 2 tubes incubated at 31 C, and 2 tubes incubated at 33 C.

proceeded at the most rapid rate. For the mycelial form this temperature was 31 C, but for the yeastlike form it was 35 C.

At its optimum temperature (31 C) the mycelial form grew at all hydrogen ion concentrations used. Its growth was retarded at pH 3.5, 8.5, and 9.5. The growth at pH 8.5 and 9.5 eventually reached a maximum very close to that attained at the more optimum hydrogen ion concentrations, pH 4.5 to 7.5. The formation of aerial mycelia was restricted at pH 4.5 and below. At room temperature, the organism grew well at all hydrogen ion concentrations with the exception of pH 3.5. Growth was retarded at pH 8.5 and 9.5, but the amount

of growth eventually equalled that attained at the more optimum hydrogen ion concentrations. The formation of aerial mycelia was somewhat restricted at the greater hydrogen ion concentrations. Although growth proceeded at a slower rate, the maximum growth attained eventually equalled that occurring at 31 C. Growth at 33 C was not so good as at the lower temperatures. The amount of growth was restricted and the rate of growth was retarded at pH 4.5, 5.5, 8.5, and 9.5. The best growth occurred at pH 5.6 to 7.5. The formation of aerial mycelia was restricted at all hydrogen ion concentrations. This effect, however, was particularly marked at the greater hydrogen ion concentrations. There was never any growth at pH 3.5.

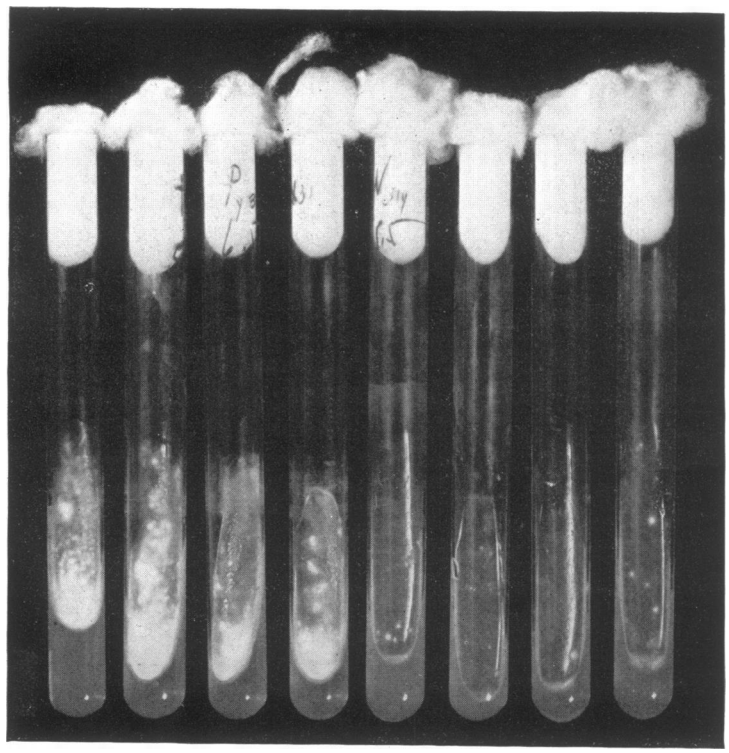

FIG. 2. SHOWING THE EFFECT OF NUTRITION ON THE GROWTH OF BLASTOMYCES DERMATITIDIS INCUBATED AT 31 C FOR 5 DAYS

The tubes are arranged in groups of 4 for each medium and from left to right are as follows: the peptone glucose medium and the ammonium sulfate glucose medium.

At its optimum temperature (35 C) the yeastlike form grew at all hydrogen ion concentrations except pH 3.5. The optimum range of hydrogen ion concentrations for growth was pH 5.5 to 8.5. Growth was greatly restricted at pH 4.5 and retarded at pH 9.5. At 37 C no growth occurred at pH 4.5 or below. The growth at the other hydrogen ion concentrations was slower in starting than at the corresponding hydrogen ion concentrations at 35 C, but the maximum growth attained was the same.

Spore inoculum. The results obtained when the spore suspension was used to inoculate the medium were essentially the same as those observed with the the yeast phase inoculum. The temperature at which the transition between the yeast and mycelial phases occured was the same, i.e., between 33 and 35 C. The optimum temperatures for the two forms were also the same, 31 C for the mycelial form and 35 C for the yeastlike form. It takes longer for growth to get started when this type of inoculum is used. Whereas growth was apparent at 2 to 3 days when yeast cells were used as the inoculum, it took 3 to 5 days to get comparable growth with the spore inoculum. This was primarily due to the length of time necessary for germination of the spore. Therefore, at any one

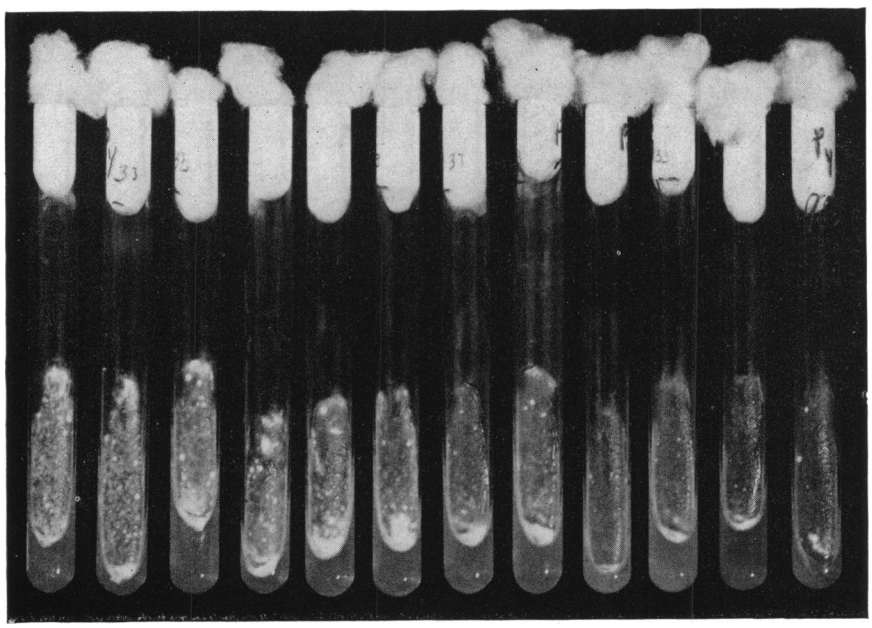

FIG. 3. SHOWING THE EFFECT OF HYDROGEN ION CONCENTRATION ON THE GROWTH OF BLASTOMYCES DERMATITIDIS INCUBATED AT 33 C ON A PEPTONE GLUCOSE MEDIUM FOR 5 DAYS

The tubes are arranged in pairs for each hydrogen ion concentration, as follows, from left to right: pH 4.5, 5.5, 6.5, 7.5, 8.5, and 9.5.

time when this inoculum was used, the amount of growth was always less than when the yeast phase was used. The conditions for germination are apparently more rigid than for cell multiplication, as evidenced by the fact that the pH limits for growth were more restricted. Growth at pH 4.5 was delayed for more than 5 days and then never approached the growth observed at the lower hydrogen ion concentration. Growth was also restricted at pH 8.5 and 9.5. No growth occurred at pH 4.5 or below at 37 C.

In general, it would seem as if the range of hydrogen ion concentrations at which good growth will occur becomes more restricted as the temperature increases.

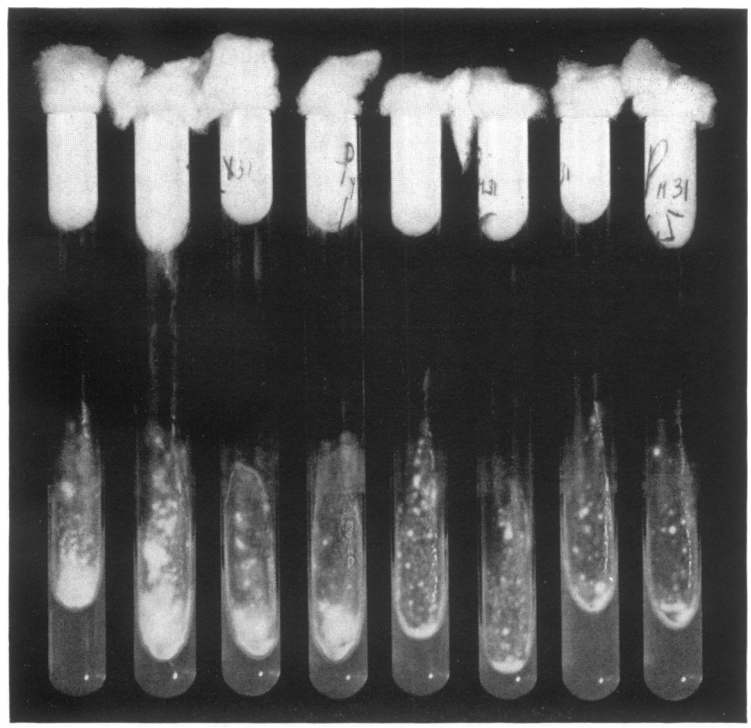

FIG. 4. SHOWING THE EFFECT OF THE TYPE OF INOCULUM ON THE GROWTH OF BLASTOMYCES DERMATITIDIS INCUBATED AT 31 C ON A PEPTONE GLUCOSE MEDIUM FOR 5 DAYS

The tubes are arranged in groups of 4 for each type of inoculum, as follows from left to right: yeast phase cell suspension and spore suspension.

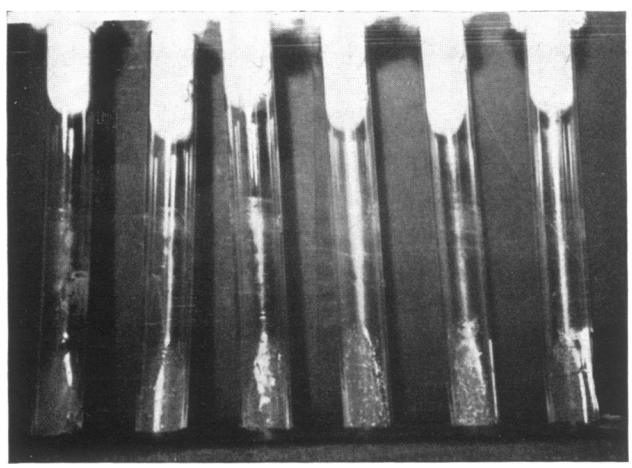

FIG. 5. SHOWING THE EFFECT OF HYDROGEN ION CONCENTRATION ON THE GROWTH OF BLASTOMYCES DERMATITIDIS INCUBATED AT 37 C ON THE AMMONIUM SULFATE GLUCOSE MEDIUM FOR 5 DAYS

From left to right the tubes are as follows: pH 4.5, 5.5, 6.5, 7.5, 8.5, and 9.5.

Ammonium sulfate glucose medium. Growth on this deficient medium assumes a different pattern from that occurring on the complete peptone glucose medium. The amount of growth never equalled that on the peptone medium. The effect of hydrogen ion concentration is different. On the peptone glucose medium there occurred an optimum hydrogen ion concentration somewhere between pH 5.5 and 7.5 to 8.5 with growth falling off on either side. On this medium, regardless of the temperature and therefore of the morphological form assumed by the organism, the quantity of growth increased as the hydrogen ion concentration decreased. When the pH reached 9.5, there occurred a slight decrease in the quantity of growth. The effect of temperature was also different. Although the transition between the mycelial and yeastlike forms took place at the same temperatures, that is between 33 and 35 C, the optimum temperature for each form was different. In general, the higher the temperature, the greater the amount of growth. This effect was not so apparent with the mycelial phase (i.e., from 31 to 33 C) as it was with the yeast phase (35 to 37 C). The amount of growth occurring at 37 C was very much greater than that occurring at 35 C. These effects were observed with both the yeast and spore inocula.

There was a tremendous difference in the amount of growth occurring when the different inocula were used. Although, as stated above, growth on this medium never approached that occurring on the peptone glucose medium, the tubes inoculated with the yeastlike phase exhibited a great deal more growth than those inoculated with the spore suspension. When the spore suspension was used for inoculation, no growth occurred at room temperature and practically none occurred at 31 and 33 C. Practically no growth occurred even at the lower hydrogen ion concentrations. This inability of the spore suspension to initiate growth to any extent on this deficient medium again demonstrates the more rigorous conditions necessary for spore germination.

ACKNOWLEDGMENT

The authors are indebted to Dr. Esther Meyer for supplying the cultures of *Blastomyces dermatitidis* used in this study.

CONCLUSIONS

The most important factor affecting the change in morphology of *Blastomyces dermatitidis* is temperature.

On a complete medium, each morphological form possesses an optimum temperature for growth, 31 C for the mycelial form and 35 C for the yeastlike form.

On the complete medium, both forms tend to grow at all hydrogen ion concentrations used, except pH 3.5. At 37 C, however, there is no growth at pH 4.5 and below.

On the deficient medium, the opimum temperature and the effect of hydrogen ion concentration are different. In general, the higher the temperature, the greater the amount of growth exhibited by each form. Therefore, for the mycelial form the optimum temperature is 33 C, and for the yeastlike form the

optimum temperature is 37 C. As the hydrogen ion concentration decreases, the amount and rate of growth increases to a maximum at pH 8.5 for both forms. The amount of growth is somewhat decreased at pH 9.5.

The tendency to form aerial mycelia decreases as the hydrogen ion concentration and the temperature increase.

Blastomyces dermatitidis does not require any of the commonly known accessory factors for growth. Amino acids have a stimulatory role.

REFERENCES

HAMBURGER, W. W. 1907 A comparative study of four strains of organisms isolated from four cases of generalized blastomycosis. J. Infectious Diseases, **4**, 201–209.

MICHELSON, I. D. 1928 Blastomycosis. Pathological and bacteriological studies. J. Am. Med. Assoc., **91**, 1871–1876.

MOORE, M. 1933 Blastomycosis: Reports of a case, with a study of an etiological factor and a classification of the organism. Ann. Missouri Botan. Garden, **20**, 79–118.

RICKETTS, H. T. 1901 Oidiomycosis of skin. J. Med. Research, **6**, 373–546.