THE EFFECTS OF RELATIVE HUMIDITY ON THE ULTRAVIOLET SENSITIVITY OF UNTREATED, HYDROGEN FORM AND CALCIUM FORM *BACILLUS SUBTILIS* SPORES

Sister Donna Schroeder, O.S.B.

May, 1971

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by

SISTER DONNA SCHROEDER

B.A. COLLEGE OF ST. SCHOLASTICA, DULUTH, MINN., 1961

A THESIS

PRESENTED TO THE FACULTY OF

SAINT MARY'S COLLEGE IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN THE GRADUATE PROGRAM IN BIOLOGY

WINONA, MINNESOTA
MAY, 1971

Approved: S. Mary Odile Cahoon Accepted: _____
Advisor Dean of the Graduate Program

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ABSTRACT

Spores of the Marburg strain of Bacillus subtilis were freed of vegetative cell components by lysozyme digestion, washed and suspended in distilled water. They were partitioned into three experimental groups: untreated, hydrogen form and calcium form. Hydrogen form spores were prepared by equilibration in nitric acid adjusted to a pH of 4.0 followed by resuspension in distilled water. Some of the hydrogen form spores were suspended for several hours in 0.02 M calcium acetate at pH 5.7 and resuspended in distilled water to produce calcium form spores. Millipore filter techniques were utilized to prepare the samples which were then equilibrated at relative humidities of 10, 30, 60, and 90% at a temperature of 30° C and subsequently subjected to 2 X 10^4 ergs/cm^2 ultraviolet light of 2537 Å wavelength. Analysis of variance indicated a significant effect of relative humidity on the UV inhibition of the colony-forming ability of the spores. Lowering the relative humidity increased the UV sensitivity but to a much smaller extent than is evident in the vegetative cells of many bacterial species. Chemical treatments had no apparent effect except at 10% r.h. where the t test indicated that the hydrogen form was more sensitive than the untreated form.

This sensitivity can be interpreted readily if metal-water-

ligand complexes are radioprotective. The humidity effect supports the conclusions of other workers that spore water is in equilibrium with the environment and that bound water is important in the structural integrity of key macromolecules.

I. INTRODUCTION

The nature of the resistance of bacterial spores to many adverse environmental conditions is the subject of much speculation and of extensive research. Investigation of these capabilities suggests some fundamental alternatives: either there are separate mechanisms for these various capacities for resistance or there are only one or two mechanisms which account for all of them. Further, these abilities are due to characteristics common to all spores, or some of them are the result of the elaboration of species specific resistance mechanisms already present in the vegetative cell.

A partial answer to these questions is provided via several lines of research. Experimental evidence suggests that heat and radiation resistance are of a different nature. This evidence includes the findings that the heat and radiation resistance of a species do not always show correlation. Duggar and Hollaender (1934) demonstrated that in Bacillus megaterium and Bacillus cereus, thermo- and radiation resistance are reversed. Radiation resistance appears before heat resistance in species such as Bacillus cereus (McDonald and Wyss, 1959). Additionally, in experiments in which spores are sensitized to heat by radiation, the reciprocal experiments do not give the same results (Kempe, 1955). This evidence, however, does not eliminate the possibility that more than one mechanism contrib-

utes to one or both of these resistance capacities.

Some interesting models have been derived from attempts to explain the spore's ability to withstand heat and chemical stresses. One of these models ascribes the resistance capabilities of bacterial spores to a combination of impermeability to water and an anhydrous interior region, the protoplast. This is an attractive model in view of the fact that heat resistance has long been linked with low water content as evidenced by the killing efficiency of moist heat and the heat resistance of lyophilized vegetative cells. However well this model accounts for many spore characteristics, the permeability studies of Gerhardt and Black (1961; Black and Gerhardt, 1962) and water exchange studies of Murrell and Scott (Murell, 1961, 1967) indicate that neither of its basic assumptions can be valid. Spore water is essentially in equilibrium with the phase outside (Murrell and Scott, 1966), and spores, though less hygroscopic than vegetative cells, show a significant affinity for water (Waldham and Halvorson, 1954). In addition, water sorption curves for crushed and for intact Bacillus subtilis spores are similar, contraindicating significant permeability barriers (Heihof, Thompson, and Deitz, 1967).

These facts are consistent with the contractile cortex theory postulated by Lewis, Snell and Burr (1960) (Alderton and Snell, 1963). According to this model, the mechanical pressure exerted by a contracted cortex accounts for a low free water content in both spore protoplast and cortex. Further, the contracted condition can be initiated

by active transport of calcium and other divalent ions through the membrane. This explains the demonstrated importance of high calcium ion content in the maintenance of heat resistance in spores.

This design is very attractive in that it is also compatible with some of the proposed mechanisms of radiation resistance. These include the existence of protective compounds and mechanisms for the repair of radiation damaged DNA.

Webb (1965) suggests that it is the removal of bound water, distorting the structure of vital macromolecules, that causes the ultraviolet radiation damage in the vegetative cells of S. marcescens and E. coli. He further proposes that the protective effect of a compound such as inositol is due to its replacing bound water in the structure and thereby preserving its integrity. If the bound water does play this important structural role, and spore water is in fact in equilibrium with the external environment, relative humidity might be expected to have an impact on the radiation sensitivity of bacterial spores as it does in vegetative cells. As was previously mentioned, the nature of the heat and radiation resistance of bacterial spores does not seem to be identical. In order to examine the possible effect of relative humidity on sensitivity of spores to ultraviolet light and to determine if chemical treatment designed to alter heat sensitivity of spores might be in some way involved, the following experiment was undertaken.

II. METHODS AND MATERIALS

Organism Preparation

The organism selected for use throughout the experimentation was the Marburg strain of Bacillus subtilis (ATCC # 6051). Stock cultures on Difco nutrient agar were prepared from the original freeze-dried preparation. Subcultures were made on nutrient agar plates and incubated at 30°C for one week to allow for extensive sporulation. The harvested cells and spores were washed twice in sterile distilled water. Trypsin and lysozyme were added to the bacterial suspension to obtain concentrations of 100 μg/ml and 200 μg/ml respectively. The mixture was then incubated at 20°C overnight for digestion of the vegetative cells. The resulting spore suspension was washed twice with sterile distilled water and resuspended in water to give a concentration of 5×10^9 spores/ml. Subsequently, dilutions were made from this stock suspension.

Hydrogen and calcium forms of the spores were prepared by the method of Alderton, Thompson and Snell (1964). Spores were suspended in nitric acid adjusted to a pH of 4.0. The spores were resuspended at intervals in fresh acid until there was no further rise in pH, indicating that spores were no longer taking up more hydrogen. Spores were then resuspended in water; these are referred to as the hydrogen form. Calcium form spores were obtained by placing hydrogen form

spores in 0.02 M calcium acetate at pH 5.7 and incubating at 20°C overnight. This treatment was followed by resuspension in sterile distilled water. Thus, three forms of Bacillus subtilis spores were available for experimentation: untreated, hydrogen form, and calcium form.

Preparation for Incubation

Standard Millipore filter techniques employing GS type filters of 0.22μ mean pore size and a diameter of 47 mm were carried out. Each filtration utilized a ten milliliter aliquot of a 50 spore/ml dilution of the suspension.

Incubation and Irradiation

A Germfree benchtop model dry box equipped with heater, thermostatic control, air circulator and a GE 15 watt germicidal UV lamp with peak output at 2537Å was used for incubation and ultraviolet irradiation. The dry box and contents were sterilized by means of the UV lamp.

Humidities were controlled by inclusion of the following in the dry box:

10% relative humidity: phosphorus pentoxide

30% relative humidity: 35.3% NaOH and P_2O_5

60% relative humidity: 24.7% NaOH

90% relative humidity: water

Fluctuation of humidity was within 2% r.h., and the dry box temper-

ature was maintained at 30°C. Relative humidity was measured with a Springfield humidity gauge.

Filters with spores were equilibrated in the dry box at specified humidities for 48 hours. Following the equilibration period, the spores were irradiated on the filters for eight seconds while being retained in the dry box at the specified humidity. The total dose administered was 2×10^4 ergs/cm^2. The UV dose was measured by means of a Blak-Ray ultraviolet intensity meter, model # J-225.

Post-irradiation Incubation

Following irradiation, the filters were removed from the dry box and placed on nutrient pads, each of which had been soaked with two ml aliquots of double-strength nutrient broth. These were then incubated for eighteen hours at 30°C.

Filters were then placed on pads saturated with methylene blue, following the suggested Millipore technique (1969). Visible colonies were counted with the aid of a stereoscope at a magnification of 20X.

III. RESULTS

Effect of Humidities

All results are given in terms of percent germination of ultraviolet irradiated spores as compared to non-irradiated spores of the same chemical treatment. Percent germination is determined by ability to form colonies.

Colony formation at higher relative humidities was greater than at lower humidities for all treatments. These results are summarized in Figure 1. In only one instance (untreated spores at 30% relative humidity) was the mean percent germination as indicated by colony formation smaller for higher humidity. In this case, the test indicated that the difference was not significant (Table I).

In addition, it may be noted that the same interval in the other treatments also yielded a non-significant difference.

Comparisons of germination at 10% relative humidity with that at 60% and 90% demonstrated significant differences in all cases. The differences for the intervals between 30% and both 60 and 90% relative humidity were significant except in the calcium form. The variability of the calcium form data at 30% was quite large. This is readily evident in Table II.

				.037	.460	.016
90%	.492	.037	.350	.033	.349	.014

The 60% to 90% interval was significantly different only in the calcium form.

TABLE I

Probability of Relative Humidity Being a Significant Factor in the % Germination of Untreated, Hydrogen Form and Calcium Form Bacillus subtilis Spores

Relative Humidities Compared	Probability		
	Untreated	Hydrogen form	Calcium form
10% - 30%	n.s.	n.s.	n.s.
10% - 60%	.025	.001	.005
10% - 90%	.025	.001	.001
30% - 60%	.005	.05	n.s.
30% - 90%	.005	.025	n.s.
60% - 90%	n.s.	n.s.	.005

TABLE II

Mean Survival of Ultraviolet Irradiated Untreated, Hydrogen Form and Calcium Form Bacillus subtilis Spores at Different Relative Humidities

Relative Humidity	Treatment					
	Untreated		Hydrogen form		Calcium form	
	Mean	St.Error	Mean	St.Error	Mean	St.Error
10%	.372	.012	.313	.017	.358	.017
30%	.338	.015	.404	.042	.437	.049
60%	.460	.030	.535	.037	.460	.016
90%	.492	.037	.550	.033	.543	.014

The 60% to 90% interval was significantly different only in the calcium form.

FIGURE I

MEAN % GERMINATION OF ULTRAVIOLET IRRADIATED UNTREATED, HYDROGEN FORM AND CALCIUM FORM *BACILLUS SUBTILIS* SPORES AT DIFFERENT RELATIVE HUMIDITIES

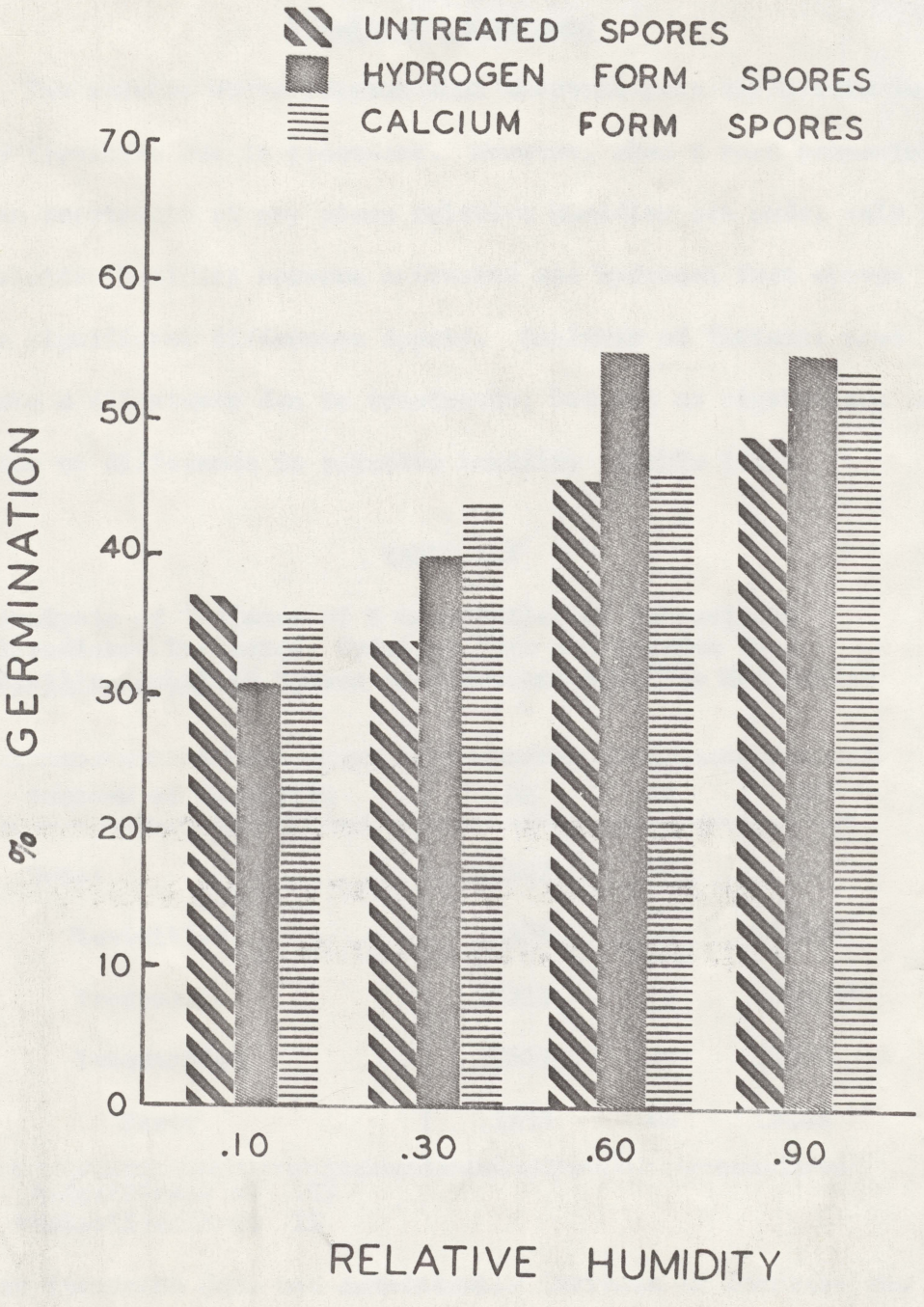
UNTREATED SPORES
HYDROGEN FORM SPORES
CALCIUM FORM SPORES
% GERMINATION
70
60
50
40
30
20
10
0
.10
.30
.60
.90
RELATIVE HUMIDITY

FIGURE 1

Effect of Treatments

The results above suggest that there is some difference in colony formation due to treatment. However, when t test comparisons between treatments at any given relative humidity are made, only at 10% relative humidity between untreated and hydrogen form spores does a significant difference appear. Analysis of Variance does indicate a difference due to treatments, but not as significant as that due to difference in relative humidity (Table III).

TABLE III

Analysis of Variance of % Germination of Ultraviolet Irradiated Untreated, Hydrogen Form and Calcium Form *Bacillus subtilis* Spores at Different Relative Humidities

Sources of Variation	SS	df	MS
Total	.8307	76	-
Humidities	.4194	3	.1398*
Treatments	.0511	2	.0255**
Interaction	.0449	6	.0074
Error	.3153	65	.0048

*Significant at .1%
**Significant at 1%

A given treatment does not consistently increase or decrease the % germination over that of the untreated at all relative humidities. This can be seen readily in Figure 2.

Interaction

Interaction between treatments and humidities is contra-indicated.

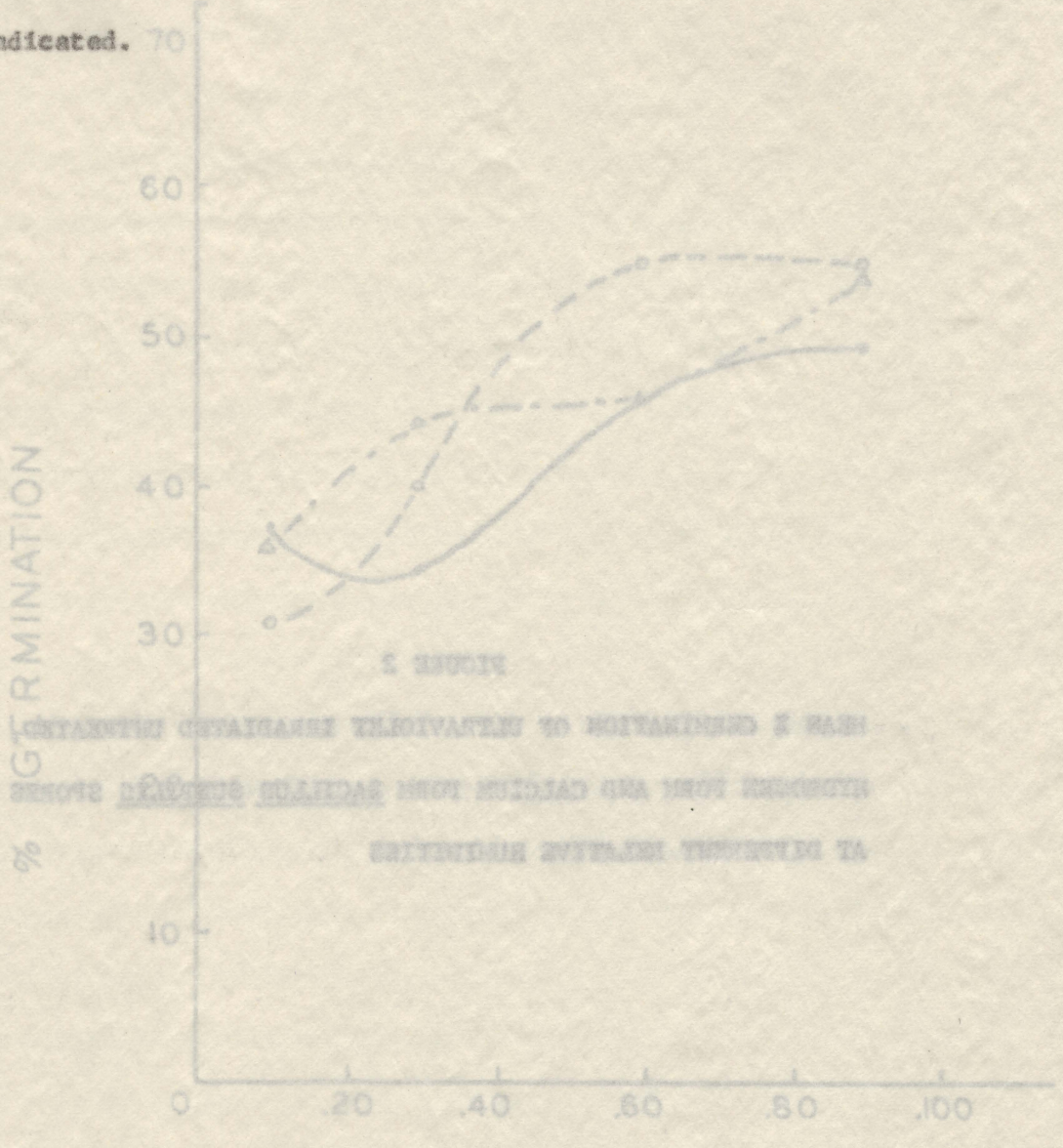

FIGURE 2

FIGURE 2

MEAN % GERMINATION OF ULTRAVIOLET IRRADIATED UNTREATED, HYDROGEN FORM AND CALCIUM FORM *BACILLUS SUBTILIS* SPORES AT DIFFERENT RELATIVE HUMIDITIES

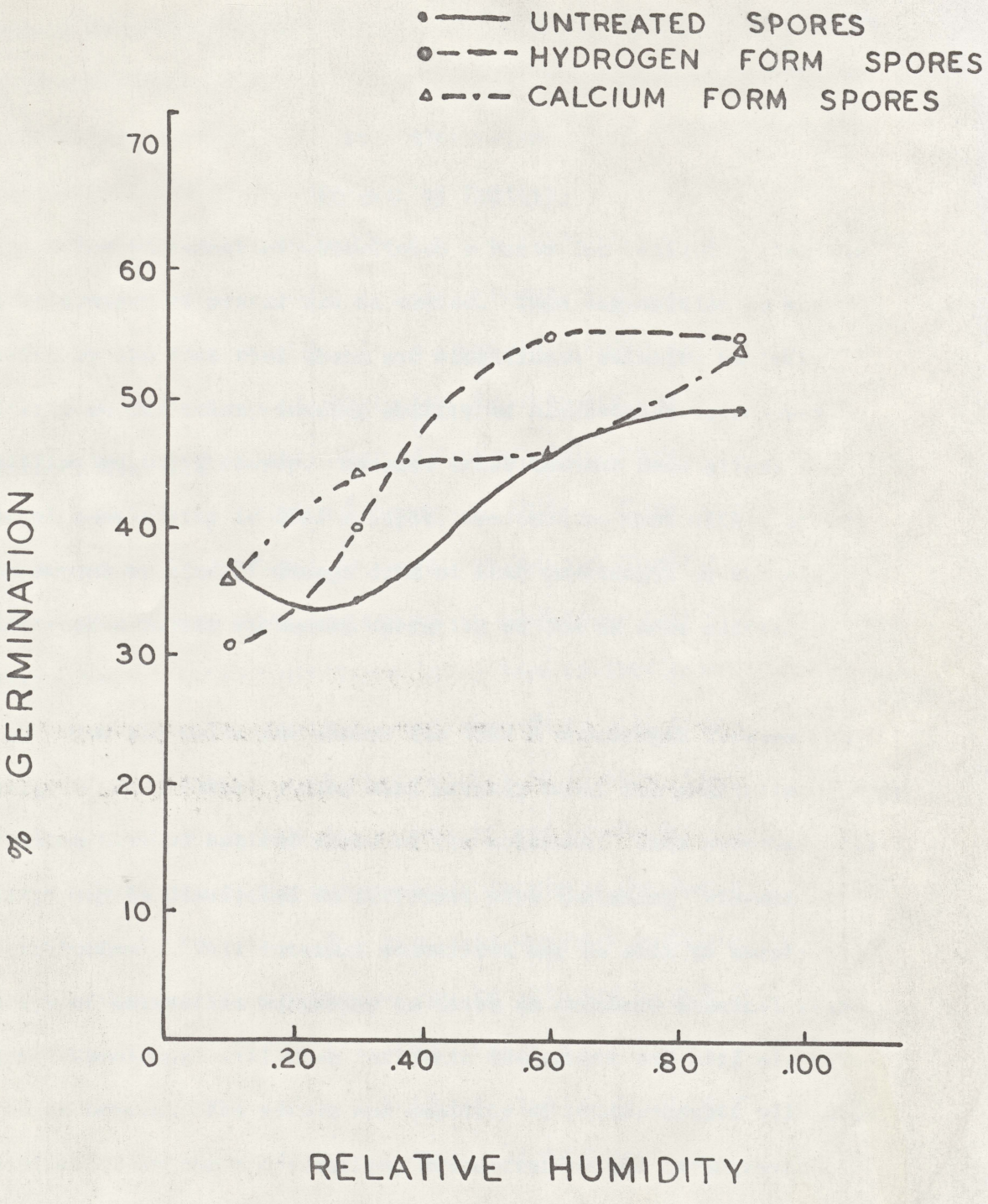
UNTREATED SPORES
HYDROGEN FORM SPORES
CALCIUM FORM SPORES
% GERMINATION
70
60
50
40
30
20
10
0
.20
.40
.60
.80
.100
RELATIVE HUMIDITY

FIGURE 2

IV. DISCUSSION

Effects of Humidity

The introduction establishes a basis for believing that the water content of spores can be varied. This supposition is supported by the fact that there are significant relative humidity effects on the colony-forming ability of ultraviolet irradiated Bacillus subtilis spores. Because water content does affect the sporal sensitivity to 2537 Å light, desiccation must either affect the amount or kind of damage done at that wavelength or the efficiency of a repair mechanism operating on one or more classes of damage.

Not all molecules absorb the 2537 Å wavelength because the energy of an absorbed photon must exactly equal the excitation energy for some allowed excited state of the molecule. Once absorbed, the energy may be dissipated in different ways including "vibrational deexcitation". This internal conversion may be able to supply the energy of activation necessary to drive an ordinary chemical reaction. Wavelength will then influence the amount and kind of photoproduct formed. The nature and quantity of photoproducts will also be affected by the conformation, concentration and arrangement of the "target" molecules, and therefore by the physical condition of the environment. The physical environment also affects the ability of the photoproducts to revert spontaneously. The biological impor-

tance of the photoproducts depends upon what is formed under a given set of conditions, and upon the ability of the system to repair the photochemical lesions. Photoproducts likely to be formed under the conditions of the experiment include water addition products, thymine dimers, and protein-DNA crosslinks (Smith and Hanawalt, 1969).

Water addition products include hydrates of cytosine and uracil. These may occur in single stranded nucleic acid so that during replication and transcription the formation of pyrimidine hydrates may assume significant biological importance, possibly cause mutations (Smith and Hanawalt, 1969). These hydrates are also more readily produced in denatured DNA and therefore may assume greater importance under environmental conditions promoting distortion of macromolecular configuration. Some workers dismiss water addition products as important contributors to cell inactivation because these products lack thermostability (Setlow, 1966). Webb (1965) suggests that this is an unwarranted conclusion in view of the possibility that the temperatures necessary for the reversal may be damaging to the cell. The fact that chemical toxicity, including that of water addition products, can be affected by relative humidity is pertinent. It is conceivable that this effect could contribute to the observed relative humidity influences on colony formation, but it is not necessarily among the most important of the possible mechanisms involved.

The importance of the role of thymine dimerization in cell inactivation by ultraviolet light has been emphasized by a number

of researchers since the discovery of dimers by Beukers and Berends in 1960. Evidence indicates that though dimers are of major biological importance to cells under many experimental conditions, this is not always true. Presumably the increased sensitivity of DNA with a high thymine-adenine content (Haynes, 1964) is related to thymine dimerization. It is notable that the type of thymine dimer formed in spores is unlike that formed in vegetative cells upon which most of the research has been done. The spore photoproduct is an azetane thymine dimer while the vegetative cell yields a cyclobutane type dimer. According to Donnellan and Setlow (1965), in thymine photoproducts, the difference between vegetative cells and spore DNA is dependent upon the physical state of the DNA. The DNA of the vegetative cell is like that of DNA in solution, while DNA is found in a different form in the spore. During spore germination there is an increase in the amount of the cyclobutane type dimer formed and this correlates with an increasing ultraviolet sensitivity. Estimates indicate that the killing efficiency of the cyclobutane type of dimer is about eleven times that of the azetane variety (Smith and Hanawalt, 1969), which suggests that either the azetane dimer is in some way less damaging or is more efficiently repaired. According to some research (Setlow, Svenson and Carrier, 1963), the cyclobutane type dimers are evidently able to halt DNA synthesis.

The preferential formation of the azetane type dimer in spores is associated with the A configuration of DNA which is rela-

tively less hydrated than the B form found in normal vegetative cells (Smith and Hanawalt, 1969). The azetane dimer is also found after irradiation of DNA in frozen solution. Freezing also affects the macromolecular configuration. Falk, Hartman and Lord (1963), in an infrared study of the hydration of DNA found that a transition from a low r.h. to a high r.h. helical form of DNA occurs between about 60 and 75% r.h. The study indicated that the structure of DNA does rely upon the correct positioning of bound water molecules, and that the macromolecular sites hydrated do depend upon the level of relative humidity. The first molecules to be adsorbed are held most tightly and the later molecules are held with a hydrogen bond strength about equivalent to that of liquid water. The most rapidly hydrated groups are PO_2^- Na^+, O-C-O and P-O-P. The C-O groups and ring nitrogens become hydrated above 65%. Rahn and Hosszu (1969), in studies of the photochemistry of DNA films found that the photochemical behavior above 65% r.h. resembles that of DNA in solution with few if any spore photoproducts being formed. This is consistent with Falk's finding that the transition from low r.h. to high r.h. helical structure occurs between 60 and 75% r.h. Webb and Dumasia (1968) suggest that the conformational changes in DNA at lower r.h. would promote water absorbed to P-O groups to form water addition products of neighboring bases. In view of the evidence that hydration affects the configuration of DNA and that its configuration determines the nature of photoproducts formed, it is plausible that relative humidity variation may cause some change in

photoproducts associated with the DNA of irradiated spores. This could contribute to an increased sensitivity of DNA to ultraviolet light at low humidities, provided that the lower r.h. causes a departure from the A configuration of the DNA as usually found in spores to a more distorted configuration. DNA - DNA crosslinks are more apt to occur where DNA is tightly packed, under conditions not favoring the formation of the cyclobutane type of dimer. Glisin and Doty found that more crosslinks are produced in partly denatured DNA unless denaturation has proceeded to the point where strand separation begins (Mosely, 1968). Under ordinary circumstances this type of photochemical lesion is not likely to be of biological significance.

Ultraviolet radiation has a denaturing effect on protein. Bound water is attached to protein molecules by hydrogen bonds and thus forms a part of the structure. The term bound water also includes that which is attached more loosely by dipole attraction. Ultraviolet radiation can break hydrogen bonds and cause the exposure of hydrophobic groups. Part of the protein hydration is due to the "squeezing" of water between hydrophobic groups. The breakage of hydrogen bonds and the repositioning of hydrophobic groups can be expected to affect the state of hydration of the protein molecule, its structure and its reactivity with neighboring molecules. Recovery will then be dependent upon the proper orientation of water molecules during rehydration. The hydrogen bonding after water loss may determine whether or not rehydration will be

successful. The greater the degree of dehydration, the higher the probability that a proper reorientation of water molecules to restore functional configuration can not occur. It seems feasible that the relative availability of water molecules will affect the degree of recovery achieved.

Because the action spectrum of ultraviolet light and the nucleic acid absorption spectrum are similar, many researchers assign the major role of biological inactivation by ultraviolet radiation to DNA damage. However, McLaren and Shugar (1964) note that the quantum yields for the inactivation of nucleic acids are less than for the inactivation of protein. In fact, the product of molar absorptivity and the quantum yields for proteins at 2537 Å is approximately equal to that for nucleic acids. This suggests that protein inactivation plays a larger role in the biological effects of UV than is implied by the predominance of research on UV-nucleic acid interaction. Additionally, protein denaturation may affect indirectly the radioresistance of a biological system by its associations with nucleic acid. Pardee and Prestidge (1967) note that the cytoplasmic damage is quite important because this entails injury to repair systems for DNA. The close association of histones with DNA, possibly in crosslinking DNA helices, would also make the conjugated macromolecules more vulnerable if protein were subject to significant damage (Webb, 1965).

In vegatative cells, one biologically significant effect of

2537 Å wavelength is the formation of DNA - protein cross-links (Smith, Hodgkins and O'Leary, 1966). The pertinence of this to spores is indicated by the fact that freezing which reduces the cyclobutane thymine dimer yield, causes an increase in the rate of formation and yield of protein - DNA cross-linkage (Smith and Hanawalt, 1969). This is accompanied by increased lethality. If nucleic acid and protein structures in spores are distorted by dehydration in such a way as to increase their proximity, such cross-linkings should be enhanced.

In addition to specific molecules whose sensitivity to UV damage may be influenced by relative humidity levels, low r.h. may be effective in reducing the number of water molecules available for energy migrations. The dissipation of energy through an H bonded water lattice is an attractive possibility for the protection of key macromolecules. Webb (1965) suggests that this is an important part of the mechanism involved in the relative humidity effects on UV sensitivity. Water might provide effective protection by damping or altering the direction of excitation energy and/or by functioning as structural pillars preventing the distortion of nucleoproteins. Webb also suggests a similar mode of action for radio-protective substances such as inositol and thiourea.

Studies on the effect of relative humidity on vegetative cell sensitivity have been contradictory. Bateman et al. (1961) suggest this disparity may be due to a lack of sufficient control of the

chemical environment. Webb's data for S. marcescens and E. coli was obtained by using well washed cells in aerosol. Webb found a significant increase in sensitivity to ultraviolet radiation as r.h. was lowered. His differences in survival between low and high r.h. conditions were on the order of three magnitudes. It is apparent that sporal sensitivity is much less subject to r.h. influence. According to Webb's research, experiments using monolayers of cells on filters yielded results comparable to those of aerosols. Work by Friedman and Henry (1938) indicates that the amount of bound water in spores is twice that of vegetative cells although their free water content is very low. If intracellular substances satisfy the hydrogen bonding requirements resulting from the departure of water molecules, the amount of damage done will also depend upon how effectively rehydration takes place. If at least part of the spore contents are substances which stabilize the key molecules, rehydration will be more likely to restore functional macromolecular configurations.

Another possible mechanism for radio-resistance in spores would be the presence of enzymes which are more resistant to u.v. than those existing in vegetative cells. Wynn (1959) found evidence for this, but Rowley and Newcomb (1964) found that some sporal enzymes are even more sensitive than their counterparts in vegetative cells. This suggests that for these enzymes, resistance stems from the complex sporal milieu rather than from an intrinsic resistance. Many enzymes do not function within the spore. Perhaps inhibiting mechanisms

might be radioprotective, provided that they involve reversible bonding and that they stabilize molecular configuration, protecting active sites.

Several possible ways in which relative humidity may influence UV radiation damage have been suggested. The actual observed effect on spores is rather small in the light of these mechanisms and in comparison to effects on vegatative cells. Spores then must have mechanisms for mitigating the effects of dehydration. One possibility is the presence of substances which may stabilize the configurations of macromolecules as water is withdrawn. Sussman and Halvorson (1966) suggest this role for DPA which is found in high concentrations in spores. The concentration of intracellular sugars may also be important in radioresistance of spores as they have been shown to be in such non-spore formers as E. coli (Woodside and Kochoraty, 1964). The denaturing effects of UV could be expected to be reduced by substances able to form hydrogen bonds which stabilize the structure of macromolecules. Sokolowski et al. (1969) found evidence that suggests inositol does not replace water as Webb suggests, but may bring about a conformation change in macromolecules by binding at positions adjacent to the site of reaction. This inference arises from the finding that inositol lowers the temperature at which association takes place, rather than raising it. Additionally, the lower metabolism of spores may reduce the number of non-reversible bonds formed during irradiation, thereby reducing inactivation.

The higher concentrations of bound water and protective sub-

stances could be expected to alter the photoproducts formed and this may be the most important part of the spores resistance to u.v. This system would be less subject to the UV sensitizing effects of low r.h. than would that of vegetative cells. The overall effectiveness of the system depends on conditions of sporulation, radiation and postirradiation treatment as well as genetic differences between species and strains within species. This latter might be operative in determining concentrations of macromolecular stabilizers and efficiency of repair of the key molecules.

Effects of Treatments

The spore treatments used in this experiment had been shown by Alderton and Snell (1970) to affect the sporal sensitivity to heat. Evidence that there are separate mechanisms for heat and radiation resistance was mentioned in the introduction. However this does not preclude the possibility that some mechanisms may extend their protective effects to both kinds of stress. Although the analysis of variance revealed the treatments did affect radiosensitivity, the test indicated a significant difference between only the untreated and hydrogen form spores. This is interesting in the light of Lohmann's work (1965) on metal-water-ligand complexes in radioprotection. He suggests that these substances modulate the energy-charge transfer to the active sites of macromolecules. If metal ions participate in the water lattice surrounding macromolecules in such

a way as to enhance the protective effect of the lattice, removing them should increase sensitivity. One would also expect the protective effect to decrease at low relative humidities, where water also is removed from the lattice. At the same time it is difficult to explain why the calcium form did not show increased resistance at low r.h. unless untreated spores already have an optimal quantity of metal ions to perform this function. Possible effects of the chemical treatments might be more apparent at different u.v. dosages.

Interaction

Although there was no evident interaction, the small effect of chemical treatments found, and the relatively small range of the humidity effects would tend to mask the presence of slight interaction.

V. CONCLUSIONS

The sensitivity of untreated, hydrogen form and calcium form of Marburg strain Bacillus subtilis spores to UV light is subject to significant influence by relative humidity. At low relative humidity, there is an increase in the inhibition of colony forming ability occurring as a result of exposure to 2×10^4 ergs/cm^2 of 2537 Å wavelength light at temperatures of 30°C. At 10% r.h. the hydrogen form spore is more sensitive to UV effects than the untreated form, but increased sensitivity of this form is not evident at other relative humidities. There is no apparent interaction between chemical treatments and relative humidity effects.

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