

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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MAY, 1971 William Choney of the Anglish department for checking my rough draft,

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ACKNOWLEDGMENTS

I gratefully acknowledge the privilege extended to me by Brother George Pahl, F.S.C. and Brother Charles Severin, F.S.C. of allowing me to complete the research requirements for my M. S. degree under the advisement of Sister Mary Odile Cahoon, O.S.B., at the College of St. Scholastica, Duluth. I am also appreclative of financial support provided by the National Science Foundation through its faculty research participation program for equipment and materials required for the research reported here.

Particular recognition belongs to my advisor, Sister Mary Odile Cahoon, O.S.B., whose comments, suggestions and encouragement made this venture possible and profitable. Appreciation is also due to David Johnson, clinical chemist at St. Mary's hospital in Duluth, for programming assistance in the processing of my data and to Dr. Chandra Mehrotra of the psychology department of the College of St. Scholastica for helpful suggestions on the organization of the results section. Special thanks must go to Sr. Agnes Fleck, O.S.B., and Mr. William Cheney of the English department for checking my rough draft, to the business manager, Mr. Harold Sultberg, for allowing the utilization of the faculty typists in producing the final draft, and to the typists, Lois McCorkell and Diane Wickline, for their careful

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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 mitric 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 104 ergs/cm2 ultraviolet light of 2537 A 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 hunidity 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.

The nature of the resistance of Santerial spores to may advorse environmental conditions in the subject of much spaculation and of extensive research. Investigation of these capabilities suggests some fundamental electrostives; at these there are separate exchanisms for these various capacities for resistance or there are only one or much meshanisms which account for all of them. Further, these abilities are due to capacities demon to all spores, or some of them are the result of the elaboration of species specific restations acceptance already present in the vegetative call.

lines of percent. Experimental evidence expects that heat and rediction recisences are at a different nature. This evidence includes the findings case the heat and rediction resistance of a species do not always above correlation. Durgar and Wollamber (1934) demonstrated that in Section are reversed. Endotion resistance appears before heat resistance are reversed. Endotion resistance appears before heat resistance in species such as Section of the parties and Vyas, 1959). Additionally, in experiments in which approximate and sixtuad to heat by resistance, the reciprocal experiments do not give the same results (Keeps, 1955). This evidence, however, does not eliginate the possibility the prove than one machantem contrib-

utes to one or both of these resistance capacities.

Some interesting models have been derived from attempts to explain the spore's shifty to INTRODUCTION at and chamical attempts.

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. Durgar and Hollander (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 were than one mechanism contrib-

utes to one or both of these resistance capacities.

by active transport of calcius and other divalent less through the Some interesting models have been derived from attempts to mashrane. This explains the description is described issertance of high releius explain the spore's ability to withstand heat and chemical stresses. ion concept in the meintenance of heat resistance in amores One of these models ascribes the resistance capabilities of bacterial This design is very attractive in that it is also concatible spores to a combination of impermeability to water and an anhydrous with some of the proposed packagines of radiation registance interior region, the protoplast. This is an attractive model in view isclude the existence of protective compounds and machanisms for the of the fact that heat resistance has long been linked with low water rapair of radiation decayed Dia content as evidenced by the killing efficiency of moist heat and the Webb (1965) suggests that it is the removal of bound water heat resistance of lyophilized vegetative cells. However well this distorting the structure of vital aggresolocules, that causes the model accounts for many spore characteristics, the permeability studultraviolet radiation damage in the vegetative cells of 3, margaments ies of Gerhardt and Black (1961; Black and Gerhardt, 1962) and water and H. coli. He further proposes that the pretective effect of a exchange studies of Murrell and Scott (Murell, 1961, 1967) indicate compound such as institul is don to its replacion bound water in the that neither of its basic assumptions can be valid. Spore water is structure and thereby preserving its integrity. If the bound water essentially in equilibrium with the phase outside (Murrell and Scott, does play this important atructural role, and spore water to in fact 1966), and spores, though less hygroscopic than vegetative cells, in equilibrium with the entarnal environment, relative bunidity with show a significant affinity for water (Waldham and Halvorson, 1954). be expected to have an impact on the radiation scoultivity of bacter-In addition, water sorption curves for crushed and for intact Bactal spores as it does in verstative calls. As was previously mentionillus subtilis spores are similar, contraindicating significant ed, the nature of the heat and redistion recistance of basterial perseability barriers (Neibof, Thompson, and Deitz, 1967). spores does not now to be identical. In order to expense the possi-

These facts are consistent with the contractile cortex theory postulated by Lewis, Smell and Burr (1960) (Alderton and Smell, 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, of to allow for agreesive speculation. The distorting the structure of vital macromolecules, that causes the ultraviolet radiation damage in the vegetative cells of S. merceacens worked totoe in storile Storilled waand 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 d remesended in water to give a conbe expected to have an impact on the radiation sensitivity of bacterial spores as it does in vegetative cells. As was previously mentionof the spores were prepared by the ed, 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.

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spores in 0.02 M calcius acerate at ph 5.7 and incubating at 20%C. a

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distilled water. Thee, three force of Paciline ambtilis spores were Organism Preparation

available for experime

The organism selected for use throughout the experimentation was the Narburg 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 X 10° spores/ml. Subsequently, dilutions were made from this stock suspension.

method of Alderton, Thompson and Snell (1964). Spores were suspended in mitric 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

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 windstered was 2 % 10 ergs/cm. The UV done was measured by means

Standard Millipore filter techniques employing CS type filters of 0.22µ mean pore size and a dismeter 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 two all aliquots of declaracrosopth matrical broks. These were then

box and placed on matricest pade, each of which had been sealed with

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 2537A 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 bumidity: 35.3% NaOH and P2Os

60% relative humidity: 24.7% NaOH

90% relative humidity: water

Pluctuation 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 × 10⁴ ergs/cm². The UV dose was measured by means of a Blak-Ray ultraviolet intensity meter, model # J-225.

than at leaves to Post-irradiation Incubation These results are

box and placed on nutrient pads, each of which had been socked with two al aliquots of double-strength nutrient broth. These were then incubated for eighteen hours at 30°C.

following the suggested Millipore technique (1969). Visible colonies were counted with the sid of a stereoscope at a magnification of 20x.

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parishtics of management form date as 30% was quite large. This

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TABLE I

Probability of Relative Bunidity Pains a Significant Factor in the Z Corminally of Chirocaed, Bydrogen Form and Calcium Form Famillus subtilts Spores

Effect of Humidities

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.

982 .492 .037 .550 .033 .543 .034

The 50% to 90% interval was significantly different only in

the calebon Term.

TABLE I

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

Relative Humidities	Probability					
Compared	Untreated	Hydrogen form		Calcium form		
10% - 30%	Da B .	10 4 8		n.s.		
10% - 60%	.025	.001	ı	.005		
102 - 902	.025	.001	ı	.001		
30% - 60%	.005	.05		n.s.		
307 - 907	.005	.025		n.s.		
60Z - 90Z	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		Treatment					
www.craraylout	Unt	Untreated		gen form 60	Calcium form		
10%	Mean .372	St.Error .012	Mean .313	St.Error	Mean .358	St.Error	
30%	.338	.015	.404	.042	.437	.049	
60%	.460	.030	.535	FIGURE	.460	.016	
90%	.492	.037	.550	.033	.543	.014	

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

Probability of Relative Equidity Reluc a Significent Pactor in the L Carmination of Universed, Bedragen Your and Calaius Form Facilius subtills Spores

	Probability		Pelative Westdiales Coppored
		Untreated	
.0.0	.9.3	+3-2	IOX - NOI
200.	200.	250.	101 - 601
100.	100.	250.	102 - 902
. 10 . 11	.03	200.	30X - 50%
-8.0	ero.	800.	308 - 308
₹60.	-6.0	PICURE I	

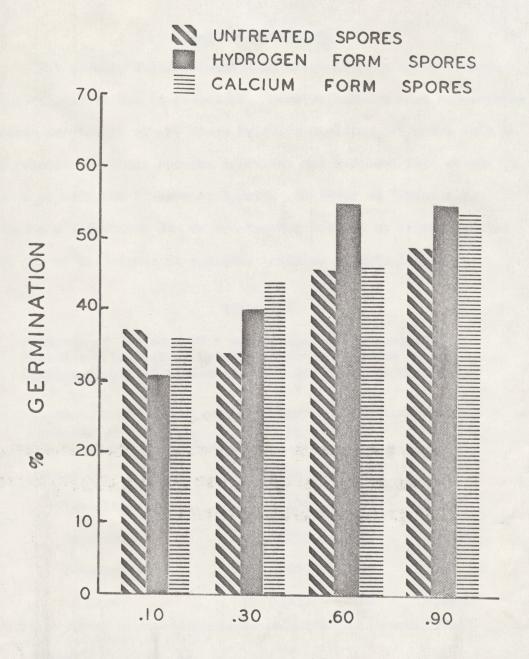
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the par see 90% interval was significently different only in



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

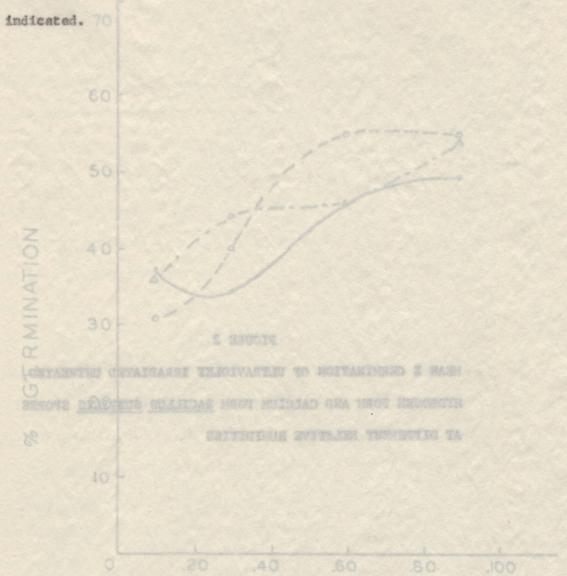
Sources of Variation	55	d£	213
Total	.8307	76	546
Humidities	.4194	3	.1398*
Treatments	.0311	2	.0255**
Interaction	.0449	6	.0074
Error	.3153	65	.0048

*Significant at .12 **Significant at 12

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 HYDROGEN FORM SPORES

Interaction between treatments and humidities is contra-

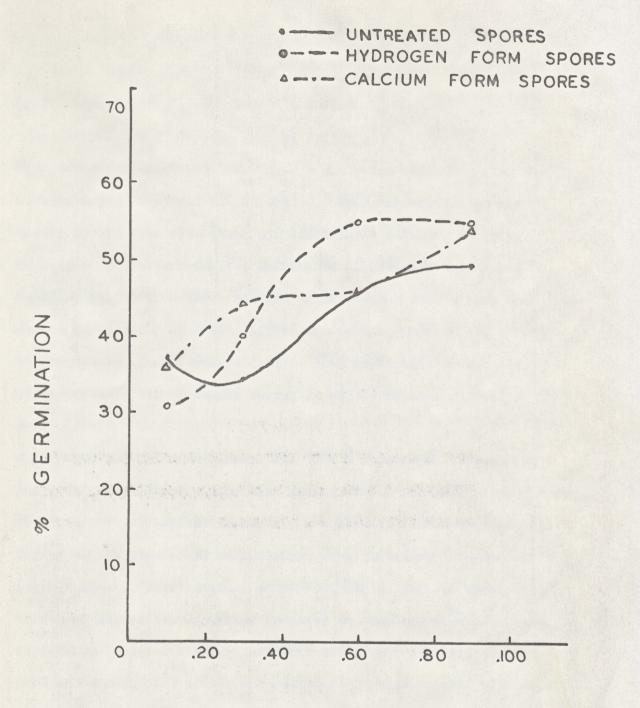


RELATIVE HUMIDITY

FIGURE 2

FIGURE 2

MEAN & CERMINATION OF ULTRAVIOLET IRRADIATED UNTREATED,
HYDROCEN FORM AND CALCIUM FORM BACILLUS SUBTILIS SPORES
AT DIFFERENT RELATIVE HUMIDITIES



RELATIVE HUMIDITY

FIGURE 2

cames of the photograduots depends upon what to formed under a given

photochemical lesions. Photygodpiscussion to be formed under the

conditions of the experie Effects of Humidity

The introduction establishes a basis for believing that the vater 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.

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-

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-DMA crosslinks (Smith end Hanawalt, 1969).

Water addition products include hydrates of cytosine and uracil. These may occur in single stranded nuclaic acid so that during replication and transcription the formation of pyrimidine hydrates may assume significant biological importance, possibly causing autations (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 disers by Beukers and Berends Sively less brireted then the A fore found in appeal vegetative colle in 1960. Evidence indicates that though dimers are of major biolo-(Suith and Henmait, 1965). The assiste diner is also found efter sical importance to cells under many experimental conditions, this irradiation of DMA in frozen solution. Procesing also affects the is not always true. Presumably the increased sensitivity of DNA macromolecular configuration. Falk, markets and lard (1983), in ea with a high thymine-adenine content (Haynes, 1964) is related to infrared study of the brigation of DNA found that a transition from a thymine dimerization. It is notable that the type of thymine dimer formed in spores is unlike that formed in vegetative cells upon and 75% r.h. The study indicated that the attentues of 1884 Gozo which most of the research has been done. The spore photoproduct rely upon the correct positioning of bound water maderales, and that is an azetane thymine dimer while the vegetative cell yields a cyclothe macromolecular sites byerated do depend upon the level of relabutane type dimer. According to Donnellan and Setlow (1965), in tive hundity. The first molecules to be adsorbed are build most thysine photoproducts, the difference between vegetative cells and cightly and the later molecules are held with a hydrogen bond spore DNA is dependent upon the physical state of the DNA. The DNA strength shout equivalent to the of liquid water. The west rapidly of the vegetative cell is like that of DNA in solution, while DNA is hydrated groups are PO2- Ne . O-C-B and F-G-F. The C-C groups and ring 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 sensitichamical behavior above 532 r.b. possibles that of DMA in dolution vity. Betimates indicate that the killing efficiency of the cyclowith few if any spore photoproducts being formed. This is consisbutane type of dimer is about eleven times that of the azetane tent with Pola's finding that the transition from low r.h. to high variety (Smith and Hanawalt, 1969), which suggests that either the r.b. helical etructure occurs between 60 and 75% r.b. Wood and azetane dimer is in some way less damaging or is more efficiently Descrie (1963) suggest that the conformational changes in Tild at repaired. According to some research (Setlow, Swenson and Cerrier, 1963), the cyclobutane type dimers are evidently able to halt DNA addition products of neighboring bases. In view of the evidence that bydration affects the configuration of DNA and that its confi-

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

photoproducts associated with the IMA of irradiated apores. tively less hydrated than the B form found in normal vegetative cells (Swith 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 macrosolecular 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 PO2- Na+, O-C-O and P-O-P. The C-O groups and ring nitrogens become hydrated above 65%. Rabn and Hoaszu (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 Palk'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-0 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 probability that a proper peoplemention of water aplocales to relight at low humidities, provided that the lower r.h. causes a crove functional configuration can not occur. It comes feasible departure from the A configuration of the DNA as usually found in that the relative availability of water velocular will affect the spores to a more distorted configuration. DSA - DNA crosslinks are degree of recovery schieved. more apt to occur where DMA is tightly packed, under conditions not Bacause the action spectrum of pitraviolat light and the favoring the formation of the cyclobutane type of dimer. Glisin and manista and absorption spectrus are similar, many ranger Doty found that more crosslinks are produced in partly denstured DNA assign the major role of biological inactivation by altravialer unless denaturation has proceeded to the point where strand separadiation to DMA damage, However, McLaron and Shugar (1964) note ration begins (Mosely, 1968). Under ordinary circumstances this type that the quentum yields for the inscrivation of suclete selds are of photochemical lesion is not likely to be of biological signifiless than for the inactivation of protein. In fact, the product of solar absorptivity and the quantum violes for proteins at 2537 A to

Ultraviolet radiation has a denaturing effect on protein. approximately equal to that for mucleic soids. This suggests that 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. soid incorrection. Additionally, protein denaturation may affect in-Ultraviolet radiation can break hydrogen bonds and cause the expodirectly the radioresistance of a biological system by its sessetsaure of hydrophobic groups. Part of the protein hydration is due tions with modinic stid. Perdes and Prontings (1967) note that the to the "squeezing" of water between hydrophobic groups. The breakage of hydrogen bonds and the repositioning of hydrophobic repair systems for INA. The close meanciarion of histones with groups can be expected to affect the state of hydration of the pro-Wit, possibly to erosalisking TWA helicos, would also make the contein molecule, its structure and its reactivity with neighboring jugated matromolecules more wilnerable if protein were subject to molecules. Recovery will then be dependent upon the proper orientation of water molecules during rehydration. The hydrogen bonding In wegetative cells, our biologically significant affect of after water loss may determine whether or not rehydration will be

auccassful. 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 relacules 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 altraviolet radiation to DWA damage. However, McLaren and Shugar (1964) note that the quantum yields for the inscrivation of sucleic scids are less than for the inactivation of protein. In fact, the product of molar absorptivity and the quantum yields for proteins at 2537 A 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 Prostidge (1967) note that the cytoplasmic damage is quite important because this entails injury to repair systems for DEA. 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). Tory. Bateman et al. (1961) suggest

In vegatative cells, one biologically significant effect of

2537 A wavelength is the ferestion of DNA - protein cross-links nell Wabb's data for 5; marroscens and E. colf was (Smith, Hodgkins and O'Leary, 1966). The pertinence of this to spores is indicated by the fact that freezing which reduces the loans increase in acqualivity to altroviolet radiation as r.h. was cyclobutane thymine dimer yield, causes an increase in the rate of weredy Bis differences in survivel between low and high rib, conformation and yield of protein - DNA cross-linkage (Smith and Hanawalt, 1969). This is accompanied by increased lathality. If divious were on the order of three angulandes. It is apprent that nucleic acid and protein structures in spores are distorted by dehytabb's comeston, experiments using massimyers of calls on dration in such a way as to increase their proximity, such crossfilters yielded results comparable to those of severols. Work by linkings should be enhanced. and Manny (1933) indicates that the encount of Sound Water

In addition to specific molecules whose sensitivity to UV in apprec is brice that of wagetative bulls although their free water demage may be influenced by relative humidity levels, low r.h. may content to wary low. If intracellular cubecades antiety the hydro be effective in reducing the number of water molecules available for gon bonding requirements resulting from the departure of enter makeenergy migrations. The dissipation of energy through an H bonded water latrice is an attractive possibility for the protection of lvely rehydration takes place. If at least part of the opera contents key macromolecules. Webb (1965) suggests that this is an important part of the mechanism involved in the relative humidity effects on taly to rescore functional matresolocator configurations. UV sensitivity. Water might provide effective protection by damping passible mechanism for padic-resistance to secret world or altering the direction of excitation energy and/or by functioning med of anarres which are north realistant to ally, then those as structural pillars preventing the distortion of nucleoproteins. extating in vegetative esits; Tyne 41989) found evidence for this, Webb also suggests a similar mode of action for radio-protective sub-Ley mid However (1964) found that some aporal decrees are like stances such as inositol and thioures. even more sensitive than their commercants to vegetative colle / This

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 acrosol. Rebb found a nignificant 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 such less subject to w.h. influence. According to Mebb's research, experiments using monolayers of cells on the filters visided results comparable to those of aerosols. Nork by Priedman and Henry (1938) indicates that the amount of bound water in shores is twice that of wegetative cells although their free water content is very low. If intracellular substances satisfy the hydrogon bonding requirements resulting from the departure of water molecules, the emount 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 colecules, rehydration vill be more likely to restore functional macromolecular configurations. Another possible mechanism for radio-resistence in spores would be the presence of ensures which are more registrat to q.v. than those existing in vegetative cells. Wynn (1959) found evidence for this, but Rowley and Mowcomb (1964) found that some sporal assymes are even sore sensitive than their counterparts in vegetative calls. 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

The higher concentrations of bound water and protective sub-

might be radioprotective, provided that they involve reversibile bonding and that they stabilize molecular configuration, protecting This
active sites. To look subject to the UV newsitizing effects of low

Several possible ways in which relative bunfdity say influence UV radiation damage have been suggested. The actual observed effect on spores is rather small in the light of these mechanises 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 way stabilize the configurations of agcresolecules as water is withdrawn. Sussman and Description of Treatments Halverson (1966) suggest this role for DPA which is found in high The spore treatments used in this experiment had been shown concentrations in spores. The concentration of intracellular sugars by didexion and Inell (1970) to affect the operal sonsitivity to may also be important in radioresistance of spores as they have been heat. Evidence that there are separate mechanisms for heat and shown to be in such non-spore formers as E. coli (Woodside and Kochoradiation resistance was mentioned in the introduction. However this laty, 1964). The denaturing effects of UV could be expected to be does not preclude the possibility that some mechanisms say extend reduced by substances able to form hydrogen bonds which stabilize the their protective effects to both kinds of stress. Although the structure of macromolecules. Sokolowski et al. (1969) found evidence analysis of variance revealed the treatments did affect radiosensithat suggests inositol does not replace water as Webb suggests, but tivity, the test indicated a significant difference between only the may bring about a conformation change in macromolecules by binding untreated and hydrogen form spores. This is interesting in the light at positions adjacent to the site of reaction. This inference arises of Lobmoon's work (1965) on metal-water-ligand complexes in radiofrom the finding that inceited lowers the temperature at which assoprotection. He suggests that these substances modulate the energyciation takes place, rather than raising it. Additionally, the lower charge transfer to the satire sites of macrosolecules. If notal ions metabolism of spores may reduce the number of non-reversible bends participate in the water lattice surrounding ancrocolecules in such 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 mecromolecular stabilizers and efficiency of repair of the key molecules.

Although there walffects of Treatments un. the small offect of

by Alderton and Smell (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 radio-protection. He suggests that these substances modulate the energy-charge transfer to the active sites of macromolecules. If metal ions participate in the water lettice surrounding macromolecules in such

them should increase sensitivity. One would also expect the protective effect to decrease at low relative humidities, where vater 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 untrented 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.

length light of temeratures of 50°C. At 195 p.b. the hydrogen form

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.

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W. CONCLUSIONS

Remove 5 to 2 1956 to be transfer topped aget us of restories decrees at

Marburg atrain <u>Bacillus</u> 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 ferming shility occurring as a result of exposure to 2 X 10⁴ ergs/cm² of 2537 Å wavelength light at temperatures of 30°C. At 10% r.h. the hydrogen form appore 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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