JOURNAL OF BACTERIOLOGY, May 1970, p. 514-520 Vol. 102, No. 2
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Recovery of Viability and Radiation Resistance by Heat-Injured Conidia of *Penicillium expansum* Lk. ex Thom

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Received for publication 21 February 1970

Spores heated in water at 54 C for up to 1 hr were plated on nutrient agar immediately or held for 3 days in aerated water at 23 C and then plated. Under these conditions, holding was optimal for recovery, increasing survival percentage up to 20-fold over values for immediate plating. Recovery was prevented partially or completely, however, when spores were held in any of the following solutions: glucose, potassium phosphate, ammonium or sodium acetate, sodium azide, or 2,4-dinitrophenol, or in the sodium or potassium salts of pyruvate, and tricarboxylic acid cycle acids. Both anaerobiosis and incubation at 0 C prevented recovery. Survivors of a heat treatment were more sensitive to gamma radiation than were unheated spores. Conditions which affected the recovery of viability had the same effect on restoration of radiation resistance. Thus, many of the processes for restoration of radiation resistance seem involved also in recovery of viability after heating. After a 99% inactivating treatment (about 30 min at 54 C), heated spores respired as fast as unheated spores, or faster. Malate, citrate, succinate, and acetate stimulated respiration in unheated spores and inhibited it in heated spores.

While testing the effectiveness of combined heat and radiation in inactivating fungal pathogens of fruit (i.e., rendering them incapable of growth), Sommer et al. (11) heated fungal spores sublethally, sensitizing them to radiation. Sommer et al. (8) later recognized that the effectiveness of combining the treatments diminished with time between treatments. Wood (13) recognized a similar loss of sensitivity to irradiation by heated yeast.

Since sublethally heated spores can recover their resistance to radiation, we thought they might be able to recover their viability after a potentially lethal heat treatment.

We chose to investigate recovery of *Penicillium expansum* Lk. ex Thom spores because our initial studies showed they had a large capacity for recovery. Further, this fungus is a prolific spore producer. The spores are unicellular and uninucleate; the colonies are well defined and can be counted after they reach macroscopic size.

Our preliminary experiments showed that heated *P. expansum* spores could be reactivated. We then sought as our objectives answers to these questions. (i) Is spore recovery affected by factors, namely temperature, anaerobiosis, respiratory poisons, and exogenous energy sources, which are known to affect cell energy production? (ii) Is spore recovery affected by inhibitors of protein and ribonucleic acid synthesis? (iii) Are recovery of viability and recovery of radiation resistance similarly affected by the same test conditions?

MATERIALS AND METHODS

Stock cultures of *P. expansum* isolated from a blue mold lesion in a pear fruit stored at 0 C, were maintained on potato-dextrose agar (PDA). Cotton-stoppered 300-ml Erlenmeyer flasks with 75 ml of PDA were inoculated with 2 ml of water containing 10^4 to 10^7 spores/ml to provide cultures from which test spores were harvested. The flasks were incubated either on a laboratory shelf in an air-conditioned room (22 to 26 C) or in a covered cardboard box at 22.5 C. Test spores were harvested with a Tween 80 solution (2 drops/100 ml) from 6- to 20-day-old cultures by introducing 20 to 30 ml of solution into the flasks and swirling the flasks to dislodge conidia. The suspension of spores was then washed six times with fresh Tween solution by centrifuging to form a pellet of spores and pouring off the liquid. Since *Penicillium* spores are produced in chains, the con-

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nectors between spores were severed by shaking the suspension 1 to 2 min in a Braun model MSK mechanical cell homogenizer without glass beads. Spores were counted with a hemocytometer to establish the spore concentration.

To determine viability, 0.1 ml of spore suspension was spread over PDA in petri dishes. According to the severity of the treatment, we adjusted spore concentration to have approximately 100 viable spores on each plate. Colonies were counted after they reached macroscopic size. Unheated spores grew uniformly and were countable 2 days after plating, whereas with heated spores some colonies were countable after 2 days but others required 5 days to become macroscopically visible. Counts from 5 to 10 replicate dishes per treatment were averaged and the standard deviation was calculated.

Used in tests were PDA (Difco), ammonium acetate agar, glucose-ammonium minimal agar, and yeast hydrolysate-neopeptone agar. The composition of ammonium acetate medium (in g/liter) was ammonium acetate, 4; KH_2PO_4, 5; $MgSO_4 \cdot 7H_2O$, 1; agar, 20. The *p*H was brought to 6.9 before addition of agar and autoclaving. Glucose-NH_4 minimal medium (in g/liter) consisted of glucose monohydrate, 10; NH_4Cl, 1; KH_2PO_4, 5; $MgSO_4 \cdot 7H_2O$, 1; agar, 20. The *p*H was brought to 6.0 before addition of agar and autoclaving. Yeast hydrolysate-neopeptone medium contained the following (in g/liter): glucose monohydrate, 20; neopeptone (Difco), 2; yeast hydrolysate (Nutritional Biochemicals Corp., Cleveland, Ohio), 0.5; agar, 20. The *p*H was 6.7 before addition of agar and autoclaving. Media were autoclaved for 12 min at 124 C.

The most concentrated solutions prepared from the sodium or potassium salts of pyruvate, citrate, α-ketoglutarate, succinate, or malate were adjusted to *p*H 6.9 to 7.1 with KOH. Other *p*H values were: ammonium acetate, 7.0; 2,4-dinitrophenol, 6.1; sodium azide, 5.6 to 5.8; glucose, 5.6 to 5.8; water, 5.6 to 5.8. Only water that had been distilled, deionized, and redistilled in glass was used in experiments.

In experiments involving radiation, 20-ml spore suspensions in 125-ml Erlenmeyer flasks were irradiated at ambient temperatures in a ^{60}Co source described previously (6). Dosimetry was determined by the Fricke ferric sulfate method. In all irradiation treatments, a 20-krad dose was given at 4.6 krad/min. The flasks were flushed with air during irradiation to prevent accumulation of ozone.

All heat treatments were in a water bath maintained at 54 $\pm$ 0.05 C. At this temperature, relatively long periods of heating (up to 1 hr) were required for inactivation. Such lengthy heating periods minimized problems of temperature equilibration. Heat treatments were begun by adding a 5- to 10-ml suspension of spores to a 100-ml volumetric flask containing about 80 ml of water equilibrated to the temperature of the water bath. To terminate a treatment, the flask was plunged into an ice bath and the spore suspension was swirled to expedite cooling. When respiration readings were taken, a 40-ml, capped centrifuge tube containing the spore suspension at room temperature was put directly into the water bath without prior equilibration. This procedure was used initially to avoid diluting the suspension, but was continued later when, for reasons given below, a centrifugation step was incorporated into the procedure. After a spore suspension to be used for respiration studies was heated, a sample was taken to check viability and the capacity to recover from heat injury. Spores were heated the same day they were harvested except for the experiments in which they were incubated 11 to 17 hr in cycloheximide before heating.

Respiration was measured with a Gilson Oxygraph equipped with a Clark oxygen electrode and a 1.6-cc reaction chamber. Readings were made at 25 C on a suspension usually containing 0.5×10^8 to 2×10^8 spores/ml. When readings were to be taken 1 or more days after heating, spores were held at 23 C on a shaker in 135 ml of water or test solution in a cotton-stoppered 300-ml Erlenmeyer flask. The spores were held at a concentration too dilute for respiration readings because at 10^7 spores/ml a few spores will germinate even in water after 2 to 3 days. At lower concentrations, this problem was not encountered. Before respiration was measured, spores were concentrated by centrifugation. Since some samples had to be centrifuged, we decided to centrifuge them all, even those whose respiration was measured on the day of harvest. After respiration was measured, the spores from the respiration chamber were counted to verify spore concentration.

During the period for spore recovery, conidia were held in 20 ml of water or test solution in cotton-stoppered 125-ml Erlenmeyer flasks. When recovery was determined at 0 C, the suspension was stirred with a magnetic-bar stirrer. The same stirring method was used when spores were held in Brewer jars under anoxia at 23 C. Except in these two experiments, the flasks were kept on a shaker at 23 C. The recovery period was 3 days unless the time course for recovery was being determined.

To achieve anoxia, the 125-ml Erlenmeyer flask was set in a Brewer jar, the jar was sealed, and its contents were subjected to a vacuum until there was bubbling of the spore-suspending fluid. The jars were then filled with hydrogen, again subjected to a vacuum, and refilled with hydrogen. This step was repeated three more times. With the jars full of hydrogen, the tubing to the hydrogen tank was then sealed and the jar lids containing the platinum catalyst were heated to expedite the catalytic combustion of remaining oxygen.

To screen inhibitors of RNA and protein synthesis for their effect on *P. expansum* spores, unheated spores were held for 3 days on a shaker at 23 C in 20-ml suspensions of the inhibitors at several concentrations. A 1-ml sample from each suspension was then removed to determine viability and hence, the concentrations at which the inhibitors were toxic. A 2-ml amount of a fivefold concentrate of the glucose-NH_4 minimal medium was then added to the suspensions to obtain normal concentrations of the medium. After an additional 5 days on the shaker, the suspensions were examined for mycelial growth. Absence of mycelial growth was considered evidence that ribonucleic

acid or protein synthesis was blocked. The inhibitors used were those that prevented mycelial growth at concentrations that did not affect the number of colonies from unheated conidia held 3 days in the inhibitor, then removed and plated.

RESULTS

The first tests were of the hypothesis that spores with potentially lethal injuries could be reactivated. Sommer et al. (9) found that a 1- to 2-day postirradiation holding in aerated water at room temperature permitted *Rhizopus stolonifer* sporangiospores to repair damage that was lethal to other spores that were not held but immediately stimulated to grow on nutrient agar. The first experiment held heated *P. expansum* spores under similar conditions to assess recovery from heat injury. Figure 1 shows the percentage of colonies which grew from spores placed on PDA either immediately after heating or first held for 3 days in aerated water at 23 C. The survival curves clearly show that a fraction of the spores with potentially lethal injuries were rescued during the holding period. There was no effect on survival when comparisons were made of PDA, ammonium acetate minimal medium, glucose-NH_4 minimal medium, and a yeast hydrolysate-neopeptone medium. PDA was used in all other experiments.

To define the time-course for recovery, the percentages of colonies from heated spores were compared after holding for 0, 24, 48, 72, or 96 hr at 23 C. In the same experiment, rate of recovery of viability (reactivation) was compared with rate of recovery of radiation resistance (desensitization). The heated spores were therefore portioned into twin sets of suspensions held for the various periods, with one set irradiated (20 krad) before plating. Samples allowed no recovery period were plated within 1 hr of heating. Both reactivation and desensitization were complete within 96 hr (Fig. 2).

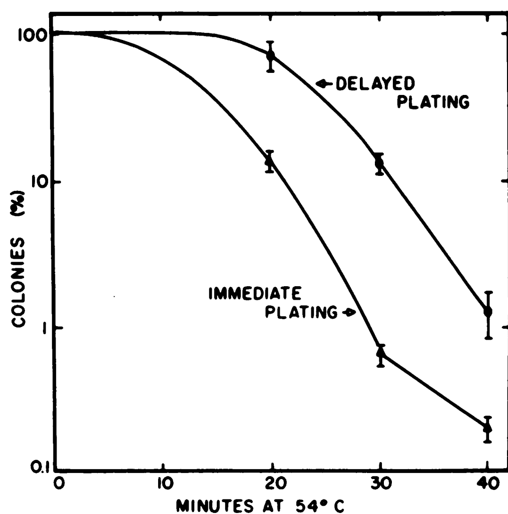

FIG. 1. *Effect on viability of holding spores after heating. Spores were either plated immediately after heating or held first for 3 days at 23 C in aerated water. Vertical lines indicate 1 standard deviation above and below the mean.*

The occurrence of recovery in water suggested that a suppression of metabolic activity promotes recovery. Tests were therefore made to determine whether recovery could be improved by holding spores at a suboptimal temperature. The 23 C used is near the optimum (25 C) for growth of *P. expansum*, whereas growth is very slow at 0 C. Table 1 shows that recovery is retarded at 0 C. It also shows that anaerobiosis and the respiratory poisons 2,4-dinitrophenol and sodium azide retarded recovery. The results clearly indicate that at least some metabolic activity is essential for recovery.

Sommer et al. (10) found that anaerobiosis also suppressed recovery of irradiated *R. stolonifer* sporangiospores unless they were held in 2% glucose while under anoxia, in which case they recovered as well as in aerated water. The glucose analogue, 2-deoxy-D-glucose, had no effect (12). Apparently, the energy necessary for recovery could be provided by the fermentation of glucose. However, recovery of heated *P. expansum* spores held under anoxia was not promoted by 0.1 M glucose (Fig. 3). With this species, therefore, aerobic respiration appeared necessary for recovery.

To determine whether breakdown products

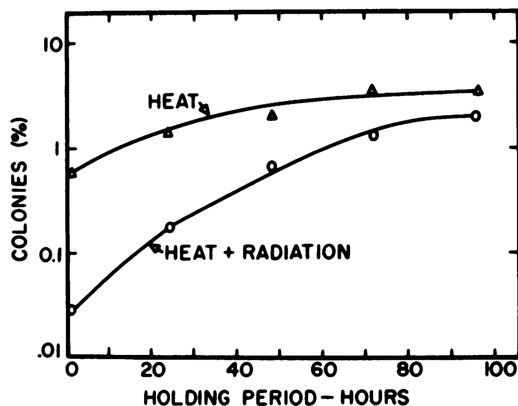

FIG. 2. *Effect of holding period after heating on recovery of viability and restoration of radiation resistance. After heating (54 C for 30 min), spores were held at 23 C in aerated water for the indicated periods. Then spores were either plated (△) or irradiated and plated (○).*

of glucose inhibit recovery, tests were made of the effects of pyruvate, ammonium acetate, and tricarboxylic acid cycle acids. Potassium phosphate was also tested. Although phosphate is not an energy source, it is important in intermediary metabolism. Results of these tests are included in Table 1. The results with ammonium acetate are presented, but sodium or potassium acetate also suppressed recovery. However, ammonium chloride did not affect recovery. Table 2 shows the concentrations at which phosphate, glucose, pyruvate, ammonium acetate, and tricarboxylic acid cycle acids suppressed recovery.

Finding that these substrates (especially phosphate) suppress recovery caused doubt that they stimulate respiration. Respiration of heated and unheated spores was therefore measured both in water and in substrates. Figure 4 shows the respiratory response elicited by introducing ammonium acetate into suspensions of heated and unheated spores. Recorder tracings for both samples are superimposed. Notice that the endogenous respiration in water was nearly identical for heated and unheated spores, although 99% of the heated spores failed to form colonies when plated immediately after heating.

TABLE 1. *Effect of holding conditions on the recovery of viability and restoration of radiation resistance of heated spores*[a]

Holding conditions	Treatments[b]: Heat (54 C, 30 min)	Heat (54 C, 30 min) plus delayed irradiation (20 krad)
	%	%
Water	7.7 ± 0.4	2.7 ± 0.7
Water, 0 C	0.41 ± 0.15	0.09 ± 0.05
Water, anoxia	0.43 ± 0.10	0.08 ± 0.02
0.1 M Glucose	1.4 ± 0.2	1.0 ± 0.2
3.7×10^{-4} M 2,4-Dinitrophenol	1.3 ± 0.1	0.52 ± 0.07
5×10^{-6} M Sodium azide	0.52 ± 0.13	0.14 ± 0.07
0.1 M Ammonium acetate	0.24 ± 0.05	0.15 ± 0.04
0.02 M Potassium citrate	1.8 ± 0.2	0.79 ± 0.13
0.1 M Sodium succinate	0.50 ± 0.13	0.25 ± 0.06
0.1 M Sodium fumarate	0.35 ± 0.19	0.22 ± 0.08
0.1 M Potassium malate	0.23 ± 0.07	0.13 ± 0.03
0.1 M PO_4 (K)	1.6 ± 0.2	0.52 ± 0.10

[a] Data are from a representative experiment. All conditions were tested with spores from one heat treatment. Heated spores and unheated control spores were held 3 days at 23 C in aerated test solutions, except in tests with 0 C or anoxia. Values are means of colony counts ±1 standard deviation, expressed as per cent of control.

[b] Values for: heated spores plated immediately after heating, 0.59 ± 0.07%; spores irradiated and plated immediately after heating, 0.09 ± 0.02%; unheated spores either not held but irradiated or held and then irradiated, 38 to 78% of unirradiated controls.

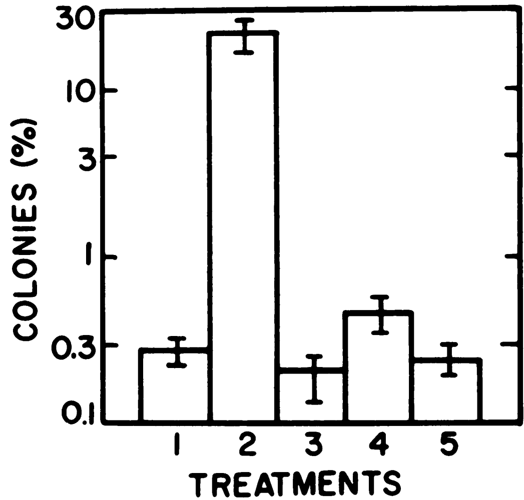

FIG. 3. *Effect on recovery of viability of holding heated spores in glucose under anoxia. Heating was for 30 min at 54 C. (1) Plated within 1 hr of heating. (2) Held 3 days at 23 C in aerated water and then plated. (3) Held 3 days at 23 C in water under anoxia and then plated. (4) Held 3 days at 23 C in aerated 0.1* M *glucose and then plated. (5) Held 3 days at 23 C in 0.1* M *glucose under anoxia and then plated. Vertical lines indicate 1 standard deviation above and below the mean.*

When ammonium acetate was added, respiration rate immediately increased in unheated spores, but did not change in heated spores. Patterns were the same with added citrate or succinate, though stimulation was not as great.

To determine the long-term effects of holding solutions on the respiration of heated and unheated spores, measurements were taken after a 2-day holding period in water, acetate, phosphate, glucose, malate, or citrate (Table 3). The respiration of heated spores held 2 days was less in acetate, citrate, or malate than in water. Unheated spores, in contrast, respired faster in acetate, citrate, or malate than in water. Heated spores held 2 days respired somewhat more slowly in phosphate than in water, whereas spores respired faster in glucose than in water whether heated or not, although unheated spores were stimulated more. The respiration rates of both heated and unheated spores, in either water or substrates nearly always declined from the 1st to the 2nd day, but varied from experiment to experiment. The variations could not be correlated with age of culture or any other variable except capacity to recover (a linear correlation with a coefficient of 0.77).

The capacity of spores to recover, as well as their sensitivity to heat, varied with the maturity

TABLE 2. *Effect of holding-solution concentration on recovery of viability by heated spores*[a]

Expt no.	Holding solution	10^{-1} M	10^{-2} M	10^{-3} M
		%	%	%
1	PO_4 (K)	2.1 ± 0.5	2.6 ± 0.5	6.2 ± 0.7
2	Ammonium acetate	0.25 ± 0.04	0.21 ± 0.04	0.33 ± 0.04
2	Potassium malate	0.21 ± 0.04	0.50 ± 0.04	0.80 ± 0.10
2	Potassium citrate		0.30 ± 0.04	0.82 ± 0.10
2	Sodium pyruvate	0.43 ± 0.12	0.84 ± 0.08	
2	Glucose	0.34 ± 0.03	0.60 ± 0.28	0.77 ± 0.15
3	Potassium α-ketoglutarate	0.28 ± 0.08	9.6 ± 1.4	12.2 ± 1.0
3	Potassium succinate	1.4 ± 0.2	2.7 ± 0.1	5.7 ± 0.7
3	Potassium fumarate	0.66 ± 0.2[b]	4.9 ± 0.4[c]	8.2 ± 1.0[d]

[a] Heated spores (54 C, 30 min) and unheated control spores were held 3 days in aerated water or test solutions. Values are means of colony counts ± 1 standard deviation, expressed as per cent of control. Without a holding period values were: experiment 1, 0.89 ± 0.11; 2, 0.25 ± 0.02; 3, 0.39 ± 0.03. Values for water-held spores were: experiment 1, 16 ± 2; 2, 1.1 ± 0.4; 3, 9.6 ± 0.6.

[b] At a concentration of 6×10^{-2} M.

[c] At a concentration of 6×10^{-3} M.

[d] At a concentration of 6×10^{-4} M.

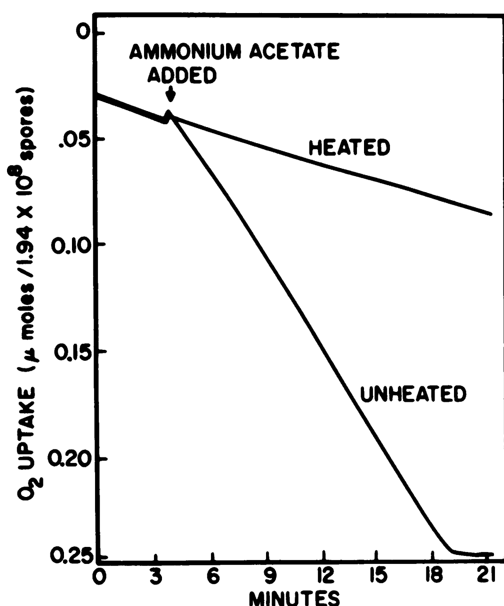

FIG. 4. *Effect of 0.1 M ammonium acetate on respiration rate of heated and unheated spores. The reading for the heated spores was taken immediately after the reading for the unheated spores. The recorder tracings are superimposed. The 1.94 × 10^8 spores/ml were in water before injecting 10 μliters/ml of 10 M ammonium acetate into the respiration chamber. The reading of the unheated spores was started 2 hr after the beginning of harvest (1.5 hr after the end of heating).*

of the culture from which spores were harvested. Spores from older cultures were more resistant to heat and produced many more colonies that appeared only after 5 days than did spores from younger cultures. Some of these spores that produced colonies only after 5 days after heating and immediate plating were probably recovering on the plates, raising the value for initial survival, and hence lessening the difference between initial survival and survival after recovery in water.

The next tests sought to ascertain whether recovery depended on protein or ribonucleic acid synthesis. This study involved specific inhibitors. Screenings were first made to determine the effectiveness on *P. expansum* spores of actinomycin D, 5-bromouracil, chloramphenicol, 6-chloropurine, cycloheximide, 5-fluorouracil, and puromycin. On the bases described in Materials and Methods, 5-fluorouracil (5×10^{-4} M) and cycloheximide (0.36×10^{-3} to 1.0×10^{-3} M) were chosen for testing. Neither compound affected the level or the time course of recovery.

TABLE 3. *Effect of holding solution on the respiration of heated and unheated spores*[a]

Expt. no.	Holding solution	O_2 uptake (nmoles/10^6 spores/hr)	
		Unheated spores	Heated spores (54 C, 30 min)
1	Water	0.25	0.69
1	0.1 M Potassium malate	0.45	0.16
1	0.02 M Potassium citrate	0.47	0.14
2	Water	0.25	0.54
2	0.1 M PO_4 (K)	0.30	0.34
2	0.1 M Glucose	1.15	0.72
2	0.1 M NH_4 acetate	1.60	0.10

[a] Unheated and heated spores were suspended in the aerated test solutions for 2 days before readings were taken in the same solutions.

DISCUSSION

These results indicate that the same heat injuries that sensitize *P. expansum* spores to irradiation are included in the injuries that inactivate spores. This conclusion is based on our finding that desensitization and recovery of viability occur at similar rates. It is also based on our finding that any condition so far examined which affects reactivation also affects desensitization. This study indicates that desensitization and reactivation of *P. expansum* spores are respiration dependent. The recovery-suppressing effects of acetate, phosphate, tricarboxylic acid cycle acids, sodium azide, 2,4-dinitrophenol, low temperature, and anoxia seem at least partially attributable to respiratory suppression.

One speculative explanation for the suppressing effect on recovery and respiration by acetate, phosphate, and tricarboxylic acid cycle acids is that they lead to faster adenosine triphosphate (ATP) production. The fact that heat-injured spores do not even germinate indicates that at least some synthetic processes are disrupted. Therefore, since ATP is not utilized by the normal number of ATP-requiring processes, it must accumulate. When it reaches high enough levels it may inhibit phosphofructokinase of the glycolytic pathway and citrate synthase of the tricarboxylic acid cycle. This inhibition would contribute to the suppression of oxygen uptake. Moreover, ATP, by blocking tricarboxylic acid and glycolytic pathways, would block the sources of a number of simple compounds that might be necessary for repair. Of course this hypothesis is only one of several that could be suggested to explain the effect of phosphate, acetate, and tricarboxylic acid cycle acids. It does not adequately explain the results with glucose. Heated spores respired somewhat faster in glucose than in water.

Bluhm and Ordal (1) found that succinate and ribose inhibited the respiration of heated and recovered *Staphylococcus aureus* cells. However, succinate and ribose were not included in the recovery media, so their effect on recovery is unknown.

Most studies of heat inactivation of lower organisms have been made with bacteria. We believe that the recovery from heat injury by *P. expansum* spores may be a different phenomenon from that occurring in heat-injured bacteria. The two types of recovery are distinct in that heat-injured bacteria recover from injuries that do not prevent growth but only restrict conditions under which growth is possible (2, 4, 7). *P. expansum* spores, on the other hand, recover from injuries that prevent growth even under favorable conditions. The plating media influence the estimation of surviving bacteria (3, 5) but not the estimation of surviving *P. expansum* spores.

We do not know which cellular sites in *P. expansum* spores are damaged first during heating. Sogin and Ordal (7) showed that heat-induced sensitivity of *S. aureus* to high NaCl concentrations is associated with a breakdown in ribosomes. Ribosomal ribonucleic acid is synthesized during recovery of salt tolerance. But the finding that *P. expansum* recovery is not inhibited by 5-fluorouracil suggests RNA need not be synthesized during fungal spore recovery.

Heated, in contrast to unheated, *S. aureus* cells have lower lactate dehydrogenase and fructose diphosphate aldolase activities (1). If essential enzymes or structural proteins were denatured by heating *P. expansum* spores, one would think these proteins would be resynthesized during spore recovery. We found, however, that cycloheximide and 5-fluorouracil have no effect upon recovery. Also, heated spores form as many colonies on minimal media as on complex media. Apparently, then, the enzymes for synthesizing the compounds present in neopeptone and yeast hydrolysate but missing in glucose-NH_4 minimal medium are not inactivated by the heat treatment.

Wood (14) found that diploid yeast cells were more heat resistant than haploid. This suggests that some of the effects of heating are due to disruption of the genetic mechanism. Since the nuclear material is a major site of radiation injury, it seems reasonable that the interaction between heat and radiation occurs in the nucleus. But differences in spore recovery from heat or irradiation suggests there are differences in the injuries these two treatments produce. *R. stolonifer* sporangiospores are capable of about a 60-fold recovery from irradiation (9) but only a 5- to 10-fold recovery from heat injury (Baldy and Sommer, *unpublished data*). On the other hand, *P. expansum* spores are capable of a 20-fold recovery from heat injury but only a 2- to 3-fold recovery from radiation, whereas *R. stolonifer* spores are relatively sensitive to heat and resistant to radiation. The responses of heated and irradiated spores also differ in that heated spores of both *R. stolonifer* and *P. expansum* either completely fail to germinate, or germinate and continue to grow. Irradiated spores, however, often cease growth only after the development of germ tubes which may be deformed (9).

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of M. Dally, M. Miranda, and K. Shelton.

This investigation was supported by U.S. Atomic Energy Commission contract AT(11-1)-34 PA73, report no. UCD-34P73-31.

LITERATURE CITED

1. Bluhm, L., and Z. J. Ordal. 1969. Effect of sublethal heat on the metabolic activity of *Staphylococcus aureus*. J. Bacteriol. 97:140-150.
2. Edwards, J. L., Jr., F. F. Busta, and M. L. Speck. 1965. Heat injury of *Bacillus subtilis* spores at ultrahigh temperatures. Appl. Microbiol. 13:858-864.
3. Harris, N. D. 1963. The influence of the recovery medium and the incubation temperature on the survival of damaged bacteria. J. Appl. Bacteriol. 26:387-397.
4. Iandolo, J. J., and Z. J. Ordal. 1966. Repair of thermal injury of *Staphylococcus aureus*. J. Bacteriol. 91:134-142.
5. Nelson, F. E. 1943. Factors which influence the growth of heat-treated bacteria. I. A comparison of four agar media. J. Bacteriol. 45:395-403.
6. Romani, R. J., E. C. Maxie, C. O. Hesse, and N. F. Sommer. 1962. Cobalt-60 gamma-ray irradiator. Calif. Agr. 16:2-4.
7. Sogin, S. J., and Z. J. Ordal. 1967. Regeneration of ribosomes and ribosomal ribonucleic acid during repair of thermal injury to *Staphylococcus aureus*. J. Bacteriol. 94:1082-1087.
8. Sommer, N. F., P. M. Buckley, R. J. Fortlage, D. A. Coon, E. C. Maxie, and F. G. Mitchell. 1968. Heat sensitization for control of grey mold of strawberry fruits by irradiation. Radiat. Bot. 8:441-448.
9. Sommer, N. F., M. Creasy, R. J. Romani, and E. C. Maxie. 1963. Recovery of gamma irradiated *Rhizopus stolonifer* sporangiospores during autoinhibition of germination. J. Cell. Comp. Physiol. 61:93-98.
10. Sommer, N. F., M. Creasy, R. J. Romani, and E. C. Maxie. 1964. An oxygen-dependent postirradiation restoration of *Rhizopus stolonifer* sporangiospores. Radiat. Res. 22: 21-28.
11. Sommer, N. F., R. J. Fortlage, P. M. Buckley, and E. C. Maxie. 1967. Radiation-heat synergism for inactivation of market disease fungi of stone fruits. Phytopathology 57:428-433.
12. Sommer, N. F., J. H. Görtz, and E. C. Maxie. 1965. Prevention of repair in irradiated *Rhizopus stolonifer* sporangiospores by inhibitors of protein synthesis. Radiat. Res. 24: 390-397.
13. Wood, T. H. 1954. Influence of temperature and phase state on X-ray sensitivity of yeast. Arch. Biochem. Biophys. 52:157-174.
14. Wood, T. H. 1956. Lethal effects of high and low temperatures on unicellular organisms. Advan. Biol. Med. Phys. 4: 119-165.