site sexes. Since one of the sexes may predominate in sporangial growth, the writer has found it a surer method to pick out with fine needles young zygospores free from sporangial spores and to plant them in Petri dishes on nutrient agar. One or both suspensors are likely to grow into mycelia which can be tested out as suggested above.

Inoculation of many sporangial spores causes a dense growth of small sporangia and a reduction of the mycelial growth at the point of inoculation. It is therefore advisable to inoculate only a small number of spores when desiring zygospore production or better yet, to make transfers of the mycelia from fresh tubes of the fungus before they have produced sporangia. In either way the opposite sexes may be sown together or slightly separated so as to cause a somewhat indefinite mass of zygospores where the opposing growths meet. If the nutrient requirements are satisfied and the atmosphere is kept saturated, zygospores may be thus obtained in abundance and nearly free from sporangia.

To teachers on my regular exchange list I am planning to send out dried male and female spore material of *Rhizopus* for use with their classes, together with reprints of the present article. I should also be glad to supply any other teachers with this material who may request it. Cultures should be started from this dried material within a month's time. The male and female cultures may be kept running by transfers to fresh nutrient about every three or four months.

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Notes on the Factors Involved in the Germicidal Effect of Freezing and Low Temperatures

Many interpretations and conclusions on the germicidal activity of low temperatures and freezing have been given by earlier investigators. Cold was formerly considered a powerful disinfecting agent, but now there is a tendency to emphasize other factors than cold itself as potent. In fact we know that cold may act as a preserver of germ life, as the high bacterial content of frozen food stuffs after weeks and months of refrigeration indicates. Ice, on the other hand, tends to purify itself upon storage.

There are numerous variables which may have an important bearing upon the experiments. A partial list of these includes: (1) The species or strain of bacteria used, (2) the history and cultural manipulation of the organism prior to freezing, (3) the physical and chemical composition of the medium in which the organism is frozen, (4) the temperature of the frozen mixture, (5) the duration of the freezing, (6) the abruptness of temperature changes, (7) the cultivation of the organism subsequent to freezing. This list includes those factors which we took special pains to control.

The bacteria may be killed by the mere fact of low temperature interfering with metabolism; by freezing of the cell contents and rupture of the membrane by internal pressure; by external pressure or grinding developed during crystallization, or by expansion of the frozen medium within the receptacle; or by more or less prolonged suspension of metabolic activities, leading to slow death from old age or starvation.

We shall not take the space to give more than a summary of our preliminary results.

I. The comparative germicidal potency of freezing on different species and strains of bacteria.

B. coli and *B. subtilis* (twenty-four hour old cultures, the latter presumably practically spore-free), showed the former species to be much more susceptible to freezing. Ninety-nine per cent. and over of the *B. coli* succumbed to freezing in tap water in three hours, while with *B. subtilis* the reduction was not at all uniform, but seldom exceeded eighty per cent. Three strains of *B. coli* tested showed no appreciable variability in relation to the disinfecting influence of cold and freezing.[1]

[1] The remainder of our experiments were performed with *B. coli*.

II. The influence of intermittent freezing and thawing upon *B. coli.*

If crystallization is in some way effective in destroying germ life, then alternate freezing and thawing should bring about a greater reduction than prolonged freezing. Table I. shows a part of one of our protocols. It will be noted that intermittent freezing has but slightly greater germicidal value than has sustained freezing for the same period of time.

III. The effect of the degree of cold used in the freezing mixture.

Tubes containing the bacteria were frozen and held for three hours for comparison at approximately —15 degrees C. and —2 degrees C. The colder temperature was considerably more fatal. Tubes kept at +.5 degree C., used as controls in most of the experiments, showed marked variation, but seldom showed over 30 per cent. to 40 per cent. of the bacteria to be killed.

IV. The composition of the media and its influence upon germ survival in freezing mixtures.

It was with the object of studying this feature of the work that we began our experiments. They are still very deficient, but what we have found is worthy of consideration.

Distilled water and Boston tap water give very uniform and comparable results.

Using cream containing 30 per cent. of butter fat, we found very striking protection afforded the bacteria when frozen, whether the freezing be continuous or intermittent. Held at just above the freezing temperature, we find about the same percentage reduction to occur as in water, though the results are very erratic, occasionally showing an increase during the course of a few hours. Freezing and thawing at intervals is considerably more fatal than continuous freezing. A few typical results of freezing *B. coli* in cream are given in the second table.

It is premature to suggest conclusions but our results lead us to infer that the degree of cold, time of freezing, crystallization and external pressure, and the composition of the media in which the freezing occurs all have an influence upon the germicidal potency exhibited by cold. Probably all of the explanations for the mode of destruction, suggested in the early part of these notes, must be considered as important.

TABLE I

A Comparison of the Percentage Reduction of B. coli held at .5° C., —15° C., and Frozen Intermittently for a Three-hour Period

Initial Count	First Freezing	Second Freezing	Third Freezing	Fourth Freezing	Freezing 3 Hours	Cold 3 Hours
2130	82.2%	99.9%	99.9%	99.9%	99.9%	23.0%
1610	92.8	96.1	99.8	99.9	99.7	29.0
1320	93.8	98.7	99.9	99.9	99.4	47.0
3015	97.6	99.6	99.5	99.9	99.8	31.3
4800	98.6	99.4	99.8	99.8	99.8	32.0
1370	98.6	99.5	99.8	99.9	99.7	8.1
1070	97.9	99.5	99.5	99.9	99.9	97.3

TABLE II

Percentage Reduction Obtained with B. coli in Cream at Freezing Temperatures

Initial Count	First Freezing	Second Freezing	Third Freezing	Fourth Freezing	Freezing 3 Hrs. 0° C.	Cold 3 Hrs. .5° C.
4350	4.8%	39.3%	45. %	48.9%	61.3%	18.6%
4740	40.5	45.5	71.5	75.9	67.7	42.7
5275	43.1	46.7	71.9	81.2	44.2	16.4
5284	33.4	48.2	60.2	—	26.4	20.8
5028	32.2	36.2	48.4	71.7	34.8	20.6
3732	35.2	20.9	42.3	50.1	33.6	38.9
4030	71.0	67.1	78.6	83.1	67.6	3.9
5085	21.1	51.6	53.3	75.4	65.3	23.8
4725	16.1	36.5	52.2	72.6	58.0	16.8
4560	34.8	47.1	67.4	63.8	54.2	19.7

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SOCIETIES AND ACADEMIES

THE AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE—SECTION OF EDUCATION

THE special summer session of Section L, Education, of the American Association for the Advancement of Science met at the University of California on Tuesday, August 3, and at Stanford University on the following day. The morning meeting on Tuesday was a joint meeting with