THE FACTORS INFLUENCING THE LONGEVITY OF MICROORGANISMS WHEN SUBJECTED TO DESICCATION

THESIS FOR DEGREE OF M. S.

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THESIS

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1. Properties of the organism which probably depend on species differences.

(a) Spore-formation.

(b) Capsule formation.

(c) Peculiarities of cell composition.

2. Physiological differences in organisms resulting from treatment before drying.

(a) Temperature of cultivation.

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PART I.

The Factors Influencing the Longevity of Microorganisms When Subjected to Desiccation.

(With special reference to the factors influencing longevity of bacteria in soil)

A general discussion of the factors influencing the longevity of microorganisms should involve all the unfavorable conditions to which they may naturally be subjected, in the course of their existence, as lack of available food material, accumulation of their own bi-products or those of organisms with which they are growing, the injurious effect of temperature, direct sunlight and insufficient moisture. The sum total of these represents the machinery by which nature's defensive activity is exercised, but since the importance of each of these is controlled by a great number of lesser factors, this discussion has been restricted to the subject of desiccation with the idea of giving it a more detailed consideration than would be possible with the more general topic.

The factors influencing the length of time an organism will live, when subjected to drying, are here divided into five main groups, with sub-divisions as indicated in outline on page

1. Properties of the organism which probably depend on species differences.

2. Physiological differences in organisms resulting from treatment before drying.

3. Nature of medium in which organism is suspended before drying.

4. Physical structure of sub-stratum on which drying occurs.

5. Effect of such physical influences as light, temperature, and variations in humidity.

1.

Properties of the organism itself, which may have an effect upon its resistance, such as (a) spore-formation, (b) capsule formation, (c) peculiarities of cell composition, to which resistance of certain non-spore-bearing species is attributed.

(a) Such spore-forming organisms as B. mesentericus, and the bacilli of anthrax and tetanus, have been known to live in the air dry condition for many years, tetanus spores having been known to produce the disease after eleven years or more of drying in soil. [1]This fact is of especial significance since the disease is commonly acquired by introduction into wounds of such dry materials as garden soil and street dust. Chapin[2] states that tetanus spores may retain their vitality for sixteen years, so that it is not surprising that lands have been known to remain infected for several years. The spores of B. anthracis remain alive in dry garden soil at least fifteen years.[3] Briscoe[4] in regard to the resistance of certain

bacterial spores, says: "We have found that the spores of B. subtilis dried on are agar slant and remaining in this state for eight years, gave growth when seeded into broth". Conn[5] states that spore-bearing bacteria may be dried without injury, for their spores protect them from destruction. "According to Swan", states Lafar[6], "spores of B. megatherium dried on a coverglass retained their vitality and germinating power for more than three years. The seat of this high resistance has already formed the object of numerous researches. One school looks for it in a peculiar modification of the spore-plasma, for instance in the presumably low water content thereof, as suggested by Lewith. Others again attribute to the spore membrane an exceptionally low heat-conducting power, and a very slight degree of permeability to noxious substances". Jordan[7] says the spore is a resting stage which serves to tide the species over a period of dryness, famine, or unsuitable temperature. In this resting state the living matter of the spore may remain dormant for years or even for decades. Fraenkel[8] states that the continued or temporary influence of dryness and moisture,heat and cold, is well borne by the spore. Sternberg[9] makes the statement that spores in a desiccated condition preserve their vitality for a great length of time. Löhnis[10] states that the resistance of spores to all unfavorable external influences is extremely great, and that drying and high temperatures which cannot ordinarily be endured by vegitive forms are of practically no effect upon the spores.

(b) Certain bacteria possess gelatinous walls or

capsules. Considerable evidence is offered in the literature in support of the belief that such a structure makes the organism less readily affected by heat and chemicals, and that it also retards the removal of moisture. Since the protection offered by such a structure is by no means comparable to that of the spore, the relation of capsules to nonspore-bearers is here considered, exclusively.

Jordan[7] states that most of the vegetative forms of bacteria are rather quickly killed by ordinary air drying "if the actual body substance is not protected by a gelatinous capsule". Lafar[6] says of Streptococcus mesenterioides (sometimes referred to as Leuconostoc mesenterioides), "Liesenberg and Zopf were unable to discover any spores, and in any case their presence would be unimportant, since the organism already possesses, in its mucinous envelope, an excellent means of protection against adverse circumstances". Owing to this envelope it was able (according to Liesenberg and Zopf), to withstand three and one-half years desiccation in the air, whereas the naked modification, i.e. the form developed on a medium containing no sugar, and having no capsule, succumbed after a much shorter exposure. Löhnis[11] describes a fluorescent organism which produces slime when growing on certain plants, which slime makes it possible for it to combat successfully destruction through drying. Revis[12], in discussion of results with cultivation of organisms of the coli type in soil for a period ranging between twelve and sixteen months, remarks: "The two types of organisms which developed a mucilaginous type of growth were the ones which survived longest". In another article[13] he suggests that the slime formed by organisms of the coli type may add to the water-absorbing and

retaining capacity of the soil, and therefore promote the longevity of that organism. Löhnis[10] says, "not only the spores, but also the bacteria with slimy walls endure the effects of desiccation very well".

Lafar[6] emphasizes the importance of making distinction between organisms like Str. mesenterioides, which surrounds itself with a gelatinous envelope, and organisms which carry on a slimy fermentation, i.e., conversion of sugar outside the cell into mucinous matter without themselves being enclosed in capsules. Jensen[14] uses the terms capsule-formation and slimy fermentation interchangeably and regards the process as protecting the organism against desiccation.

Buchanan's article[15] on the gum of Ps. radicicola offers a very comprehensive review of the literature on the nature and morphological origin of bacterial slimes. Certain investigators, as he says, describe gum formation as the result of a true fermentation of carbohydrates, by bacteria. Of these, some call it an extracellular synthesis, while others contend that the gum formation is a true synthetic process, but not necessarily due to an extra cellular ferment. But, to quote directly, "In the majority of cases, the slimes and gums have been determined to be the result of a swelling or solution of the cell wall or bacterial capsules". He suggests that there is a continuous movement outward of substances elaborated by the protoplast, and a growth in thickness and area of the capsule and wall by interpolation. If this material deposited is capable of swelling in water a definite capsule is developed; and if soluble in water, either immediately or gradually, a viscid solution

may develop.

Most of the bacterial gums reported in the literature are described as carbohydrates of the formula $(C_6H_{10}O_5)n$. Bacterial slimes classed as dextrans are described by Bräutigam, Kramer, Ritsert, Scheibler and many others[15]. Lipman, Greig-Smith, Maassen, and Laxa[15] found levulan to be the specific gum of several slime-forming bacteria. Schmidt-Mühlheim, Hueppe, Emmerling, Greig-Smith, Laurent, Ward and Seiler[15] describe bacterial gums having the characteristics of galactans. A few nitrogenous bacterial gums are mentioned, but they appear to be less common than those of a carbohydrate nature.

The protective action of these gums has been ascribed to their water retaining capacity.

(c) Exclusive of organisms with such special protective structures as spores or capsules, it appears to be true that certain species are more resistant then others. To quote Chester[16], "Neisser found that the organisms of typhoid and diphtheria were the most resistant; cholera, influenza, bubonic plague and gonococci, the least, and the pus-forming cocci, meningococcus and tubercle bacillus of intermediate resistance. "Briscoe[4] credits the tubercle bacillus with a greater resistance than most non-spore-bearing organisms, and says: "As regards desiccation tubercle bacilli appear to take an intermediate position between spore and nonspore-bearrers". To quote further, "This power of resistance is no doubt due, in part at least, to the content of the waxy or fatty substance found largely in the outer layer of the tubercle bacillus. The presence

of this waxy material gives them their well-known character of "acid-proof" power when stained".

There are doubtless other characteristics of a cell, in addition to those mentioned, spores, capsules or high fat content, which may render it less readily injured by desiccation, but with our present insufficient knowledge of the bacterial cell it is difficult to explain why B. typhous or Bact. diphtherial, with none of the previously mentioned faculties, should exhibit so much greater longevity than Bact. pestis, the gonococcus and many other nonspore-formers.

2.

Physiological differences in organisms, resulting from treatment before drying as (a) temperature of cultivation, (b) nutrition, (c) age of culture and (d) virulence.

(a) Although but little work has been done to demonstrate that organisms grown on a favorable medium are more resistant to desiccation than those developed on a less favorable nutrient material, the nutrition has been shown to affect the resistance of the cell to moist and dry heat, and it might reasonably be inferred that the same would be true of its resistance to drying. In certain cases the medium is undoubtedly important. Streptococcus mesenterioides[6], as previously stated, has been found to resist desiccation for a much longer period if developed on a saccharine medium, than on one which contains no sugar; in this case the effect of the medium is only indirect, as it is the capsule developed by the organism in the presence of proper sugars, which makes it resistant.

(b) Ficker's experiments[18] with the cholera vibrio deal with the influence of the temperature of growth upon the ability of the organism to endure desiccation. He says: "Doubtless, the temperature at which the organisms are cultivated and their ability to resist drying at different temperatures, stand in a certain relation. Contrary to expectation, the drying at a higher temperature does not always produce a more rapid, and the drying at a lower temperature a more gradual effect. Experiments show that the organisms cultivated at 37° and occurring on the dryer agar surface are better prepared for the rapid removal of moisture occurring in the desiccator at 37° than are the organisms grown at lower temperatures. They suffer no such sudden removal of water if dried at a temperature closely approaching that at which they were cultivated. "However, the cultures grown at 15° or 22° and dried at 15° were found to live considerably longer than the cultures developed at 37°, the conclusion, therefore, being that cultivation at a temperature below the optimum produces the individual with the greatest resistance to desiccation.

Copy of Ficker's table, showing the relation of temperature of growth to the temperature at which the organism is desiccated.

		Desiccator kept at 37°	Desiccator kept at 22°	Desiccator kept at 15°
Cholera Culture grown 2 days at	37°	Removal after 18 hrs. +	Removal after 2 days +	Removal after 4 days 0
		2 days 0	3 days +	6 days 0
		3 days 0	4 days 0	
			6 days 0	
	22°	18 hrs. 0	2 days +	4 days +
		2 days 0	3 days +	6 days +
		3 days 0	4 days +	9 days 0
			6 days 0	
	15°	18 hrs. 0		4 days +
		2 days 0		6 days +
		3 days 0		9 days 0

As the method by which these results were secured is not apparent from the table, Ficker's explanation of methods employed is quoted here directly: "From agar plates of the cholera organism kept two days at 15°, 22° and 37°, equal quantities were smeared directly in thin streaks on cover-glasses, which were kept in the desiccator at 15°, 22° and 37°. Since the cultures grown at different temperatures did not contain comparable masses of bacterial growth, for proof of ability of the organism to develop, two cover-glasses were transferred at intervals to each of four tubes of peptone solution".

Ficker suggests that the differences in the protoplasm of cultures grown at a lower temperature and the cell substance of the form developing in least time at the optimum temperature of growth, may hold true in other respects; that there may be imperceptible differences which possess nevertheless, a far-reaching significance. To quote directly, "It has never been proven that the vegetation developing at the optimum temperature and with the greatest rate of growth is also more advantageously situated in the struggle with life-menacing influences, or that it displays all the capacities of its species any more than with plants the development is best in all physiological respects even when the most luxuriant vegetative development occurs".

(c) The literature offers quite contradictory information as to the effect of the age of the culture upon its vitality. Von Wahl[17] in discussing resistance of spores from cultures of different age, says the age of the culture is immaterial so long as mature spores are present in abundance.

Ficker's results[19] with the drying of cholera vibrio cultures of different age indicate that cultures one or two days old endure desiccation better than older cultures, but of these two the forty-eight hour culture is less sensitive to drying at 37° than is the twenty-four hour culture. The results of Kitasato and Berckholtz, quoted in the same article, show about the same resistance in cultures from one to five days old. Cultures older than these showed a marked decrease in resistance. This, as Ficker[19] claims to have demonstrated, was due not only to the fact that there were fewer living organisms present in the same mass of an old culture, but these surviving organisms possessed in themselves less vitality than did the vibrios from younger cultures. Chapin[2] states that old cultures die sooner than fresh ones, and that different strains have different powers of resistance. Chester[16] states that fresh cultures of Ps. radicicola, by containing a larger number of active organisms are better for inoculating cotton than old cultures. By "fresh" cultures are meant those which have just attained their maximum growth.

(d) Ficker[19] also demonstrated, in the case of cholera vibrio that a virulent strain was more resistant than an avirulent strain.

3.

The nature of the medium in which the organism is suspended before drying, in regard to (a) its possible plasmolyzing effect and (b) its content of protective or water-retaining substances.

It has been demonstrated both experimentally and

practically that the medium with which the organisms are surrounded, before being subjected to desiccation, has an influence upon their resistance.

(a) Ficker's experiments[18] showed that transfers of old cholera vibrios from the surface of agar to distilled water resulted in a disturbance of the turgor of the cell which was so injurious as to make its death, when desiccated, occur much sooner than it did if suspended in physiological salt solution before drying. With young cultures the reverse was true. Suspension in tap water or distilled water before drying appeared to have the same effect, but desiccation after suspension in physiological salt solution was quickly injurious. He explains this on the basis that as the air drying process resulted in increase of concentration of the salt solution, the cell was subjected to both plasmolysis and desiccation. The explanation is not complete, however, for a broth of the same salt content as the physiological salt solution was favorable to both young and old cultures.

(b) Ficker[18] found the cholera vibrio to retain its vitality longer when dried after suspension in milk or broth than in distilled water, tap water, physiological salt solution, serum or saliva. He considers it remarkable that the bacteria died out so quickly, dried in saliva, since,as he writes, one might expect the slime of the saliva to protect the enclosed bacteria for some time. The saliva was filtered for this experiment, however, so that there is a possibility that the "large cell elements" otherwise offering sure protection against drying, were excluded. These experiments imply a certain protection gained by presence of

nitrogenous or albuminous constituents. Ficker also showed that a greater longevity resulted when the organisms were cultivated on a solid medium and suspended in fresh broth or milk than when they were grown in those liquids and dried on cover glass films prepared directly from the cultures in which they developed. No doubt the presence of their own biproducts was injurious, in the latter case. (A portion of these, at least, was filtered out in the case of the suspension in fresh liquid). This injury must have been an appreciable one, to outweigh the shock, to the organisms of transferance from the medium upon which they grew, into a fresh solution.

Numerous examples are cited of long preservation of organisms, in a dry state, when surrounded by nitrogenous or albuminous material. Chapin[2] says, "The thicker the layer of infectious material, the longer is its virulence likely to be maintained. This thickness depends largely upon the native of the medium. In a dried watery medium, bacteria may die quickly, while they may survive long in sputum or foeces.

Heim[20] found the pneumococcus, concerning whose resistance there is much dispute, to live as much as one and one-third years, and retain its virulence dried in blood or pus on silk threads. His experiments show that this method will practically guarantee the virulence of this organism for at least six months. This method, tested out on other pathogenic organisms, was found practical, although not to the same extent with all as with the pneumococcus. Burger[2] recovered pneumococci from a handkerchief seven days after it had been in use. Wood[2] found that sputum containing

pneumococci might, under favorable conditions, preserve their virulence for thirty-five days.

Many of the earlier writers claim a considerable longevity for the tubercle bacillus in dried sputum. Fischer[21] found that the organisms lived from one hundred twenty-six to one hundred eighty-six days in tuberculous sputum dried on glass. Sormani[22] found the bacilli to live two months in dried tuberculous sputum. Cadeac[23] obtained evidence of tubercle bacilli living eighty to one hundred fifty days in a dried tuberculous lung. Villemin, Koch, DeThoma and Moffuci credit that organism with a life of from one to nine months in dry sputum. Briscoe[4], in discussing resistance of tubercle bacilli, says: "When exposed to desiccation, pure cultures of the germs, in thin layers are found to be dead in a few days. In sputum and other foul material they appear to live longer than other nonspore-bearers". In another place he says: "In the presence of foul material tubercle bacilli live from a month to a year or more. . . . It would be expected that tubercle bacilli protected by the mucoid material, as found in sputum and diseased tissues, in which these germs more frequently occur, and also by their abundance of naturally waxy constituents would be protected against drying and injuries from the presence of foul material". As Briscoe's conclusions are based not only on his individual research, but a most comprehensive survey of previous investigations, they may be regarded as well founded.

Diphtheria bacilli remain virulent for months dried in the false membrane!

Chapin[2] says: "Vaccine virus when dried in the crust which forms from the vesicle retains its virulence for

a considerable time. A thin layer of the same lymph on a quill does not remain active, when exposed to the air, for more than a week or ten days. The ivory points covered with vaccine matter, which were so much used a few years ago, were usually guaranteed to keep three weeks and often did remain virulent a month or more. But there was usually more than one layer and the thickness of the material was further increased by the presence of blood and leucocytes".

Whether the protective action of these albuminous and water retaining substances is, as Chapin[2] suggests due merely to the greater thickness of the layer of dry substance, or to its water retaining capacity or nitrogenous constituents, has not been definitely determined. From the results of my own experiments on drying Ps. radicicola in quartz sand, after suspension in different solutions, it would seem that the protective power of the material is not due merely to its increasing the thickness of the film.

4.

Physical structure of thesubstratum upon which drying occurs, showing comparisons between longevity of organisms on (a) smooth, non-absorbent surfaces, (b) textile fibres or fabrics and (c) soil.

Organisms have been found to die out much more rapidly dried on glass, marble, porcelain or other smooth, had surfaces than when dried on threads, cloth, cotton or in soil.

To quote from Marshall[24], "Hansen found that yeast cells dried on cotton were still alive after two to three years, while if dried on platinum wire some died in five days and others lived as long as one hundred days. Compressed

beer yeast mixed and dried with powdered charcoal kept as long as ten years; Ps. radicicola dried on a cover-glass or filter paper died within twenty-four hours; on seeds this same organism was still alive after fourteen days, and in the dried nodules of legumes a few cells were able to reproduce after more than two years".

Chester[16] says that Ps. radicicola when dried in thin films on glass perishes very rapidly, but that it may live eleven to sixteen days on cotton.

Harding and Prucha[25] have shown that Bact. compestris may live for as much as thirteen months on cabbage seeds, but dried on cover slips is dead at the end of ten days. Briscoe[4] says that this difference is no doubt largely due to the difference in the hygroscopic moisture retained by these substances. He found tubercle bacilli to live only eight to twelve days when dried in thin smears glazed paper slips. B. coli, B. vilaceus and B. prodigiosus, according to his experiments, were even more sensitive, dried under thoseconditions.

Billings and Peekham[26] found that B. typhosus, B. coli, and Staph. aureus endured desiccation on silk threads for as much as five months without loss of vitality. Other investigators found those organisms to have a much lower resistance, on the same material.

Abel[2] observed of the plague bacillus that it lived fourteen days on a cover-glass and thirty days on threads, pieces of linen and parts of organs. Germano[2] working with the same organism observed a longevity of twenty-five to thirty days, when it was dried on pieces of wool or silk.

Rosenau[1] claims that the bacillus of bubonic plague may be harbored in bedding and clothing, hence the necessity, in case of such diseases for destruction or at least thorough disinfection of garments, carpets or rugs possibly contaminated.

Koch and Gaffky[18] say that the cholera vibrio may live only a few hours, dried on glass, but four days on fabrics.

Tubercle bacilli have been known to retain their virulence thirty-nine to seventy days in a folded handkerchief, or carpet, or woolen cloth kept at room temperature in diffused light[2]. Noetel[27] experimented to determine the longevity of tubercle bacilli on clothing. The organisms were conclusively demonstrated on:

Coat and vest worn daily,

Jacket and hose worn daily,

An old coat,

Coat, hose and plush vest not worn for three weeks,

Wool jacket and old hose not worn for five weeks.

Chapin[2] although minimizing the importance of infection by fomites, cites a few authentic cases in which disease has resulted from cloth, and other absorbent materials, infected a considerable time previous. The two spore-formers most important as disease-producers, tetanus and anthrax have frequently been shown to be transferred on such materials as lamp wick, wool, hair and dust. Chapin also mentions a case in which typhoid fever was proved to have been caused by use of army blankets, infected at least seven months previously. Living bacilli were demonstrated on several of the blankets at that time. In another instance[2] an

individual acquired diphtheria by contact with a garment on which a laboratory assistant had spilled a portion of a bouillon culture of that organism, two days before.

Lehmann and Neumann[28] state that Strep. pyogenes has been known to retain its virulence one and one-third years, dried on silk. This organism is usually regarded as sensitive.

Von Wahl[17] claims that spores dried on metallic or other hard, smooth surfaces are less resistant than spores dried on wadding or silk.

(c) The evidence obtainable from the literature in regard to the length of time an organism may live in air-dry soil is neither definite nor complete. To quote from Marshall's Microbiology[24], "Under air-dry conditions each soil grain is surrounded by a very thin film of moisture designated as hygroscopic water..... According to Hall this film is about .75 microns in thickness. Nevertheless it will be seen that the moisture even in air-dry material is deep enough to allow the bacteria a reasonable amount of protection. This will account for the survival of nonspore-bearing bacteria in dry soil for a long time. Indeed instances are on record of the isolation of Azotobacter and Nitrosomonas from soils that had been kept in the laboratory for several years".

Löhnis[10] says, "Vegetative cells can better endure drying when they are in soil. With spores also this is true. The resistance of spores dried in earth is usually found to be higher than that of spores dried on cotton, silk, glass, etc."

Duggar and Prucha[29] found that after rapid drying out of soil cultures there remained a large number of living organisms whose vitality would extend over a considerable period.

Nestler[30] investigated an old herbarium and found that even after twenty-three years, ninety-thousand colonies could be obtained from one gram of soil.

Azotobacter[31] remain alive in soil samples if these samples are kept for one hundred sixty days in a desicator and then one hundred forty-eight days in an air-tight condition.

Germano's results seemed to indicate that the organisms of typhoid and diphtheria did not live as long in soil as on fabrics, although the diphtheria bacillus averaged twenty to forty days longevity in soil, in all trials. Firth and Horocks[2] found that the typhoid bacillus would live for twenty-three days in dry sand. Phuhl[33] found the byphoid bacillus to live twenty-eight days in dry sand, and eighty-eight days in moist garden earth. The bacillus of dysentery, on which he experimented at the same time, lived only twelve days in sand, and one-hundred one days in moist garden earth.

Briscoe[4] found the tubercle bacillus to live two hundred thirteen days in garden soil.

But little work has been done to determine the effect of different soil types on the longevity of organisms dried in them. The data offered in the literature on this point is not only scanty but far from recent.

Modern texts hold that dust does not offer protection to many pathogenic organisms, the dangers due to

ordinary dust being much exaggerated according to Rosenau[1] and Chapin[2]. They do not attempt to deny that street dust may carry pus-forming cocci and occasionally tubercle bacilli. The latter are commonly found in the dust of rooms occupied by careless tuberculous patients. Washbourn and Eyre[2] claim to have found the pneumoccus in dust from a ward and laboratory at Guy's hospital, but failed to find it in street dust.

Dempster[34] found that the cholera vibrio lived only a short time in perfectly dry soil, but survived for a prolonged period in a soil containing a small amount of moisture. The typhoid bacillus showed a greater tenacity of life in soil than did the cholera vibrio, but entire desiccation proved quickly fatal to it also. Comparison of longevity of these organisms in white sand, grey sand, garden mould and peat showed that with the exception of peat, which apparently contained substances toxic to the organisms, the nature of the soil did not have a direct influence. The vitality of the organisms appeared to depend rather on the moisture content of the soil than its composition. My own experiments, given in Part II, on the longevity of soil organisms in different types of soil, have led me to similar conclusions.

The longevity of vegetative cells in air-dry soil is probably, as Lipman[24] suggests, due mainly to the presence of moisture in the hygroscopic form. Undoubtedly the presence of organic colloidal substances with a tendency to retain moisture, is of importance also in this connection although as the amount of such substances would be apt to vary with different soils it can hardly be designated as the important factor.

Van Suchtelen[35] in speaking of analysis of

soil solution in the report of the bacteriologist of this college, in 1913, makes certain statements, which, on account of their immediate bearing on this subject deserve direct quotation: "In many cases there was found in the soil solution a slime. This must be regarded as the first experimental proof of the presence of this substance in soil and it is not impossible that much of the irregular behavior of the life in soil can be explained, to some extent, with a knowledge of this slime. If I may be permitted I should like to call your attention to the possibility of this substance having an effect on desiccation, diffusion and other processes".

It has been suggested that the presence of this substance in soil may be a factor influencing longevity and a few experiments have been started with a view to securing some information as to its value in the soil. It is hardly necessary to add that with a problem which involves such a number of factors, none of which are very completely understood, results come very slowly.

5.

Effect of such physical agencies as (a) light, (b) temperature, and (c) variations in humidity.

(a) Chapin[2] considers light one of the most important factors to be taken into consideration in regard to the effect of drying upon bacteria. He says: "Germs that are killed in a few minutes in direct sunlight may live for weeks in a dark place or even in diffused light. Twicheel[2] found that tubercle bacilli lived from one to two months in diffused light, but died in a few hours if exposed to direct sunlight. Migneco[2] found that when dried

on a cloth in the sun tubercle bacilli lived from twenty to thirty hours. More recent observers ascribe considerably less resistance to this organism. Weinzirl[2] claims that it can stand drying in direct sunlight for only a few minutes. The consensus of opinion seems to be that the less the duration and intensity of illumination, the greater the endurance of desiccation.

(b) With regard to the temperature of desiccation, Chapin[2] says, "The higher the temperature, the sooner the germs perish. Ficker[19] found the cholera vibrio to live six days when dried at 15°, but no more than eighteen hours dried at 37°. Verjbitski[2] found plague bacilli to live one hundred thirty days at 4° - 5° or thirty-five days at room temperature. Tidswell[2] says that with plague bacilli the colder the climate the greater the persistence of infection.

(c) As to relative merits of desiccation in room air and desiccator some fairly positive statements have been obtained. Chapin[2] says, "As a rule bacteria live much longer when dried in a desiccator than when dried in the open air under natural conditions". Ficker[19] showed that rapid drying of organisms in a desiccator over $CaCl_2$ or H_2SO_4 was preferable to drying in ordinary room air. This is probably due to the fact that variations are continually occurring in the humidity of the room atmosphere which may have the effect of drying and remoistening the organisms. This process is known to have a very destructive effect, as shown by Ficker's experiment[19], in which the organisms were placed alternately in a desiccator and moist chamber for a couple of hours at a time. The organisms so

treated died out much more rapidly than did those which were left in the desiccator continuously for the same length of time. Löhnis[10] states that frequent changes between drying and remoistening are most injurious, but that rapid drying in a space with "rarefied atmosphere", (i.e. in a desiccator), is comparatively favorable.

Unpublished experiments of J. Simon have shown that repeated drying and moistening of the soil is much more detrimental to nodule bacteria than keeping the soil constantly dry. Chester[16] in his experiments with Ps. radicicola found that an important condition for successful preservation of the organism in a dry state was to keep the culture sealed from the air and in a dark, cool place.

Malassez and Vignal[36] state that tuberculous sputum, alternately dried and moistened eight times lived only twelve days, as compared to a longevity of over one hundred days when continuously dry.

PART II.

Experimental Work.

The experimental work, which covers a portion only, of the subject as outlined in Part I was undertaken with the particular aim of determining some of the factors which may have an influence upon the longevity of micro-organisms in soil. As a foundation for this it was of great importance to secure all possible data relating the general subject of desiccation, not only to obtain suggestions for further work but to make evident the multiplicity of factors involved and the necessity for their control in carrying on such experiments.

So far as can be discovered from the literature, nobody has tried to give a full explanation of the fact that organisms live longer in air-dry soil than when dried on any other material and for the present this is not possible, considering our rather incomplete knowledge of the real nature of soil. However, from the review of previous investigations, given in Part I, it is seen that two principal factors are suggested; first, the physical structure of soil which makes possible the retention of moisture in the hygroscopic form, and second, the presence of organic colloids, with a tendency to retain moisture.

If the factor first named is the one to which

most importance should be attached, then differences in structure of the soil or other substrata which influence the hygroscopicity would have also an effect upon the survival of organisms dried therein. A portion of the experiments, therefore, have been devoted to the drying of organisms in soils of different types which display different capacities for retaining moisture, and also on textels which vary in hygroscopicity.

Under the second heading are considered the role of the slime found in soil solution by Van Suchtelen and also the possible protective effect of any similar colloidal substances formed as a result of bacterial growth. Before it could successfully be demonstrated that such slimy or mucoid substances were among the compounds important in protecting the bacteria in soil, it was necessary to secure evidence that slime or gelatinous materials exert a decided protection against desiccation, in soil or out. This may be considered as an explanation for the introduction of experiments apparently irrelevant to the problem in hand.

The experiments bearing upon these points are arranged in the sequence given their subjects, in Part I, such topics of the complete outline as are not pertinent to the plan being omitted.

1.

(a)

Experiment 1.

Object of experiment: To demonstrate the resistance of spore-bearing organisms when dried in thin films on glass.

Plan: Cultures of four spore-bearing bacilli, B. ramosus, B. subtilis, B. mycoides, and a spore-bearer similar to B. mesentericus vulgatus, isolated from slimy bread, were used for this experiment. The organisms were grown five days on nutrient agar at room temperature, an abundant production of spores, in that length of time, being a certainty. By means of a sterile platinum loop approximately equal amounts of bacterial growth were transferred from these cultures to sterile coverslips (broken in halves to facilitate dropping them in tubes); this material was spread out in a thin layer with the platinum needle and the pieces replaced in sterile petridishes which were then placed in the temperature room (22°-25°C.) in darkness.at At intervals a cover-slip of each organism was transferred to a tube of nutrient broth. Appearance of characteristic growth in the tube of broth was interpreted as proof that the dry spores were still capable of germination.

TABLE 1.

Days desiccated	7	39	74	91	100
B. ramosus	+	+	+	+	+
B. subtilis	+	+	+	+	+
B. mycoides	+	+	+	+	+
Bacillus from slimy bread .	+	+	+	+	+

Results: It is apparent from the data above tabulated that bacterial spores are not readily destroyed when exposed to desiccation in thin films on glass in a dark, well-ventilated place. (This experiment should be continued for years.)

(b)

Experiment 1.

Object of experiment: To compare the effect of desiccation upon slime-forming and nonslime-forming bacteria, (all of which are spore-free) when they are dried in thin films on cover-slips.

Plan: The cultures used were:

Slime-formers	Nonslime-formers
Milk bacterium A.	B. violaceus.
" " B.	B. prodigiosus.
" " C.	B. coli.
	Bact. aerogenes.
	Ps. campestris.
	Sarc. lutea.
	M. varians.

The above organisms were cultivated on nutrient agar for 48 hours, at room temperature. Material from the surface of these agar slants was transferred to clean sterile cover-slips, method of Expt. (a) 1, which were then replaced in the clean petri dishes in which they had been sterilized and stored in a dark, well ventilated place at room temperature. Forty-eight hour cultures were preferred, because with a few of the organisms that was the minimum time in which an appreciable amount of growth would develop. Cultures older than that are supposed to be less vigorous. Since the desiccation was to be carried on at room temperature, it was thought best to cultivate all the organisms at that temperature, even those whose optimum was 37° (see Part I, 2 c).

For test of longevity cover-slips of each organism were transferred at intervals to tubes of sterile nutrient broth, this being done with all precautions to avoid contamination. Such tubes were kept at room temperature. The development, within ten days, of characteristic growth in the broth as regards to slime, pigment or other features specific for the particular organism was regarded as proof of its viability. If in any case the growth in broth was not distinctly characteristic, transfers were made to agar slants to demonstrate the identy of the organism causing the growth in broth with that which was dried on the cover-slip. Up to the time of the first negative test but one slip of each organism was transferred to broth at the different trials; thereafter, for at least three times in succession, a slip was transferred to each of two tubes of broth.

Data given in Table 2.

Results: It cannot be concluded from the results as shown in Table 2, that a slime-forming organism is invariably more resistant than one which forms no slime, None of the three milk bacteria tested in this experiment showed a resistance equal to that of Bact. aerogenes, M. varians, or Sarcina lutea, but transfers of these three did give growth, at irregular intervals, long after B. violaceus, B. prodigiosus, Ps. campestris and B. coli had given positive and final proof of inability to develop. They may, therefore, be said to occupy an intermediate position, being neither the most sensitive nor the most resistant

TABLE 2.

The effect of desiccation upon spore-free bacteria.

Days desiccated	3	6	9	13	16	19	22	28	35	42	49	53	63	71	81	85	92	104	114	119	127	144
Milk bact.A	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
Milk bact.B	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	−	+	+	+	−	−	−
Milk bact.C	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	−	−	−	+	+	−	−
B.violaceus	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
B.prodigiosus	+	+	+	+	+	+	+	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−
B.coli	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
Bact.aerogenes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Ps.campestris	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
S.lutea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
M.vavians	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−

among the nonspore-forming species, with regard to desiccation, so far as indicated by this experiment.

In certain cases thick smears of the slime-formers proved viable, when thin smears did not. It may be that the influence of slime is, as Chapin[2] suggests, only indirect, and that its "protection" is merely the result of its increasing the thickness of the surface film. In that case the irregularity in the longevity of slime-formers on different cover-slips may be explained entirely on differences in the thickness of the smears, it being difficult to distribute mucilaginous material evenly over a small piece of glass.

It is not easy to find an explanation for the unusual longevity of Bact. aerogenes, M. varians, and Sarcina lutea as evidenced by the results of this experiment. As none of them are "acid-fast" this cannot be due to presence of "waxy or fatty constituents"[4] of the cell. In the case of Sarcina lutea it is not impossible that the peculiar arrangement of groups of individuals in thick packets, may tend toward preservation of at least the innermost cells, from complete and rapid desiccation. The validity of this assumption can only be established by extensive experimentation and comparison of the resistance of sarcines with cocci of other arrangement. As to the great longevity of the other two organisms, Bact. aerogenes, and M. varians no explanation has been suggested, or attempted. Resistance to desiccation, in certain nonspore-forming species may be an inherent quality, the basis for

which cannot be exactly determined.

(b)

Experiment 2.

Object of experiment: To compare the effect of desiccation upon slime-forming and nonslime-forming strains of a single organism, Ps. radicicola, when dried in thin films on glass.

Plan: For this work were used two strains of Ps. radicicola (variety from red-clover nodules) which had been cultivated five days at room temperature on slants of nitrogen-free ash agar. One of these was the transfer from a culture which had been kept growing on nitrogen-free ash agar for over five months. The growth was vigorous but not slimy. The other was the transfer from a plate colony of this same organism, which plate had been inoculated with soil in which the organism had been kept for two and a half months. This strain was decidedly mucilaginous. Material from these cultures was smeared on pieces of sterile coverglass, method of Experiment (a) 1, placed in petri dishes and stored in a dark place at a temperature of 22° - 25°C. Coverslips of these in duplicate were placed, at intervals, in Ashby's solution. The development of typical growth was regarded as proof of viability.

Table 3.

Effect of desiccation upon slimy and nonslimy cultures of the same organism.

		24 hours	14 days
Slimy culture	(a)	+	-
" "	(b)	-	-
Nonslimy culture	(a)	+	-
" "	(b)	+	-

Results: The results of the preceding table demonstrate plainly that Ps. radicicola is more sensitive to desiccation when exposed in thin films on glass than are most of the nonspore-bearing organisms tested in Experiment (b) 1, but they do not indicate that a slimy culture is any more resistant than one which is not slimy. Failure to make more frequent tests of the dry smears in Ashby's solution may be accountable in part for this result. As the organism grew so slowly in that medium, failure of the first transfers to develop cloudiness, inside of five days, was mistakenly interpreted as an indication that the smears placed in those tubes contained no living organisms; consequently no further transfers were made until after the appearance of growth in the first four tubes. This was at a time beyond the limit of longevity of this organism. Unfortunately there was not time in which to repeat this experiment, but it seems reasonable to infer that even if transfers between 1 and 14 days had shown growth, the difference could not have been such as to give the mucilaginous culture any decided advantage over the other, so far as resistance to desiccation was concerned.

(b)

Experiment 3.

Object of experiment: To compare the longevity of three slime-forming, and one nonslime-forming organism, (all spore-free) when these are kept in soil which is permitted to dry out gradually.

Plan: The cultures used were:

Slime-forming	Nonslime-forming
Milk bacterium A.	B. violaceus.
" " A and C.	
Ps. radicicola.	

The two slime-formers from milk, and B. violaceus were grown 48 hours at room temperature on nutrient agar. Ps. radicicola, as it requires a special medium and a longer period in which to develop even moderate growth, was cultivated five days at room temperature on nitrogen-free ash agar. The growth from six slant cultures of each organism was washed off and suspended in 300 c.c. sterile physiological salt solution. This suspension was thoroughly shaken in an attempt to separate clumps of organisms. (That this method of separation is not effectual may be seen from data given later. Filtering through sterile glass wool would have been preferable, had that method been recommended earlier).

For the purpose of determining the approximate number of bacteria placed in soil, 1 c.c. of the suspension of each organism was diluted in physiological salt solution and plated on an appropriate medium. Ten c.c. of the suspension of each organism was then added to each of twelve flasks of sterile garden soil which had been prepared in the following manner: The soil was mixed, air-dried, sifted, and placed in 100 c.c. Erlenmeyers, 50 g. to a flask. Each flask was fitted tightly with a one-holed rubber stopper, through which was passed glass tubing 1-1/2 inches long, and

plugged with cotton at the upper end. The flasks of soil were sterilized by heating them in the autoclav 45 minutes under 15 pounds pressure.

The inoculated flasks were kept on a shelf in the laboratory exposed to diffused light. A cheese-cloth curtain suspended from the shelf above diminished somewhat the intensity of the light without interfering with ventilation.

At intervals of three to five weeks quantitative plates were made to determine the number of organisms per gram of soil, samples being taken from two flasks of each organism at all platings. The procedure was as follows: By means of a flamed spatula the contents of the flask were thoroughly mixed and a 1 g. sample weighed out on sterile paper. This was added to 100 c.c. of sterile physiological salt solution and shaken with a rotary motion at least fifty times, to insure thorough mixing. One c.c. of this suspension was then further diluted to 100 c.c., making a strength such that each cubic centimeter represented 1/10,000 g. of soil. Further dilutions of 1/100,000, 1/1,000,000 or higher were obtained by diluting this latter suspension ten, a hundred, and in some cases a thousand times. Three plates were made from each sample, these representing two, or more often three dilution. In all cases 1 c.c. of the proper suspension was used for plating and mixed with the medium in the petri dish. For plating, the media were employed on which the organisms had previously been grown.

The plates were kept one week at a temperature of 22° - 25°C. before counting. The figures given in the table represent the average of six counts, three from each sample,

except in cases where one of the plates of a set has been discarded on account of contamination or improper dilution.

It should be explained here that while the three slime-formers, Milk bacteria A and C and Ps. radicicola, were placed in the soil in April,1914, comparative tests with the nonslime-former, B. violaceus, were not successfully started until December 17, 1914. Thre reason for this was that the culture of Ps. radicicola, used for inoculating the soil at the beginning of the experiment was not noticeably mucilaginous; in fact the intention, at that time, was to compare the longevity of Ps. radicicola, as a nonslime-former, with the two milk bacteria. As the work was carried on by some one else during July and August, 1914, it was not discovered until September that the colonies of Ps. radicicola, appearing on plates at that time were of a decidedly viscous consistency. This being the case it was necessary to inoculate a set of soil flasks with some spore-free, nonslime-forming organisms for comparison with the three on which a series of counts had already been made. It was desirable not only that this organism be one which would under no circumstances develop a slimy type of growth, but that it form colonies of such characteristic appearance that contamination might readily be detected. B. prodigiosus was tried and proved not to be adaptable as its colonies were so spreading and irregular that quantitative determinations were impossible. After two more failures, resulting from use of incompletely sterilized soil, a set of soil flasks were inoculated with B. violaceus, which proved well chosen for

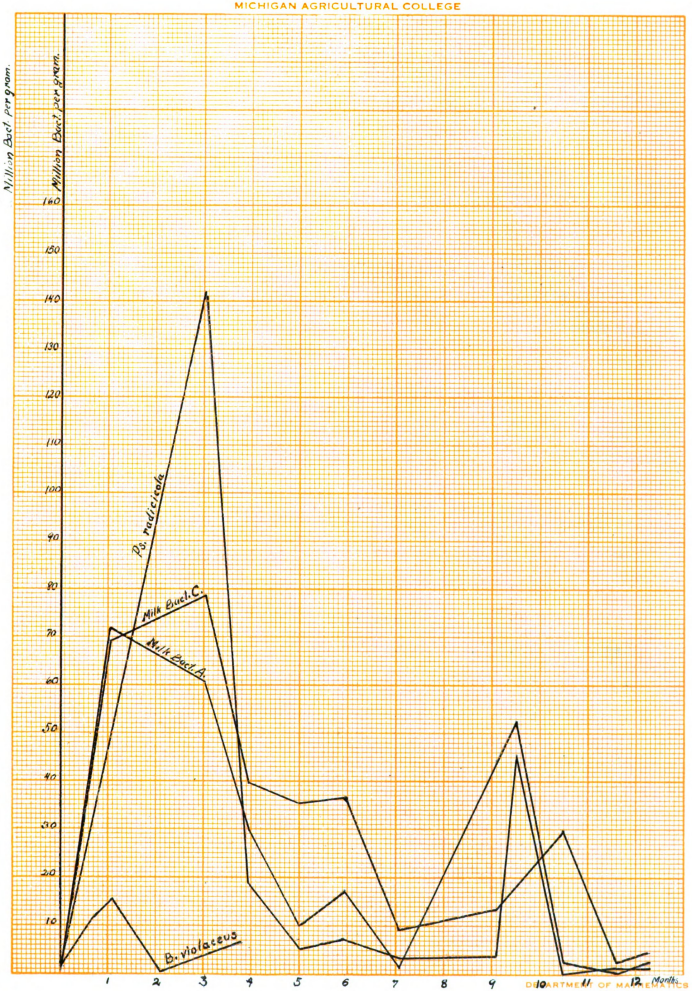
this purpose as it forms distinct colonies which are easily recognizable. The table gives the bacterial counts of the different dates, but offers no ready comparison between the numbers of the four organisms. The curve makes more evident the comparison between the increase and decrease in numbers of the different organisms, from the time the soil was inoculated up to the last plating. The first two counts on Ps. radicicola were discarded as the culture used proved to be contaminated. The flasks were reinoculated in June and counted with the others thereafter.

Table 4.

Longevity of organisms in soil which is allowed to dry gradually.

Date	Milk bact.A.	Milk bact.C.	Ps.radicicola	B.violaceus
Apr. 2,'14	719,333	1,685,000	Contaminated	
May 6,'14	70,400,000	69,259,000	Contaminated	
July 5,'14	60,881,666	78,215,000	141,008,000	
Aug. 1,'14	29,910,000	39,850,000	18,800,000	
Sept.3,'14	10,067,000	35,500,000	5,346,000	
Oct. 1,'14	17,440,000	36,200,000	7,300,000	
Nov.10,'14	1,445,000	9,322,000	3,600,000	
Dec.17,'14	No count made	No count made	No count made	2,956,000
Jan. 9,'15	44,500,000	13,316,000	3,907,000	11,650,000
Jan.21,'15	52,700,000	Contaminated	45,666,000	15,460,000
Feb.18,'15	2,500,000	29,800,000	10,000	743,000?
Mar.22,'15	290,000	2,767,000	1,570,000	4,183,000
Apr.13,'15	2,155,000	4,750,000	1,433,000	6,377,000

Results: On account of the extreme fluctuation in numbers, which is always a feature of quantitative determination on soil, by the plate method, it is hardly permissible to form positive conclusions from the results of a single such experiment. One condition is apparent, viz., that certain



organisms, which exhibit a mucilaginous type of growth, if placed in sterile garden soil, may be found in that soil in large numbers even twelve months from the date of inoculation. As the nonslime-forming organism, B. violaceus, was placed in the soil only four months before the date of the last plating it is not known whether or not that organism, if remaining in the soil for twelve months would show an appreciably lower number of living cells than did the other three. However, it appears to be true, at least with the species tested, that the rate of decrease, during the same length of time is more rapid with the nonslime-former than with the slime-formers. The continuation of this experiment for at least two more years, might yield more complete and satisfactory data.

As an improvement on this method of keeping soil cultures it is recommended that cotton plugs be employed, instead of rubber stoppers. The evaporation through the glass tubing took place so gradually that the soils did not reach the air-dry condition until at least ten months after inoculation. As these stoppers could not be flamed, when samples were taken for plating, the opportunity for contamination at such times was much increased. That bacterial and mold contaminations did actually occur, was frequently evidenced by quantitative determinations made during the last few months when it was necessary to reopen a few flasks on account of loss of certain numbers of the series.

In all experiments which involve quantitative

plating from soils, at intervals, throughout an extended period, the choice of plug for the culture flask and the manner of its preparation is fundamentally important as contaminations may be such as to utterly ruin all quantitative results.

(b)

Experiment 4.

Object of experiment: To compare the longevity of three slime-forming and one nonslime-forming organism (all spore-free) when these are kept in soil from which the evaporation of moisture is retarded.

Plan: This experiment was carried on with the same organisms and under the same methods as Experiment 3, the only difference being that the Erlenmeyer flasks, containing the soil, were supplied with a device intended to retard the removal of moisture. This consisted of a small, glass, bucket-like tube containing 3 to 5 c.c. of distilled water which was suspended in each flask above the soil. The glass tubing passing through the rubber stopper was curved so as to have the shape of the letter J, this curve not only being an aid in preventing rapid loss of moisture, but serving as a hook on which to hang the glass tubing.

The soil was inoculated with Milk bacteria A and C, and Ps. radicicola in June, 1914, plating for this experiment and the preceding one being done on the same day or on successive days, thereafter. As in experiment three the non-slime former was not placed in soil until December 17, 1914.

Erlenmeyer Flask with Device for Retarding Evaporation of Moisture.

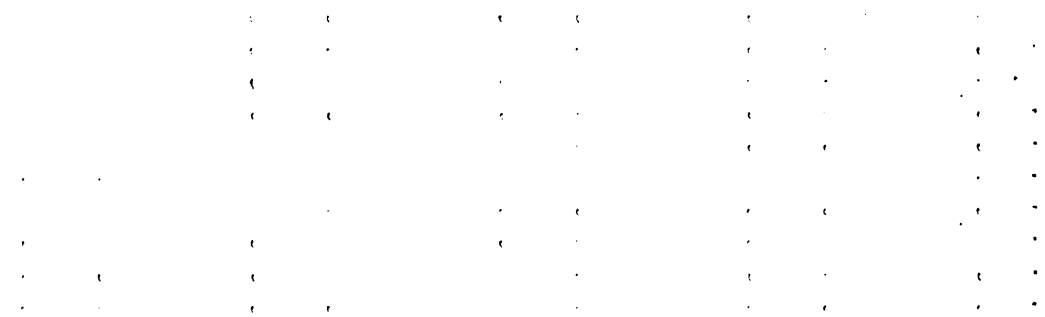
Table 5.

Longevity of organisms in soil whose moisture is retained.

Date	Milk bact.A.	Milkbact.C.	Ps.radicicola	B.violaceus
July 5,'14	171,883,000	85,150,000	75,150,000	
Aug. 1,'14	128,000,000	59,625,000	47,300,000	
Sept.4,'14	56,950,000	23,110,000	58,800,000	
Oct. 1,'14	34,775,000	28,014,000	24,500,000	
Nov.10,'14	4,514,000	7,604,000	?	
Dec.17,'14	Not plated	Not plated	Not plated	2,956,000
Jan.22,'15	6,100,000	2,206,000	12,667,000	9,967,000
Feb.19,'15	2,625,000	2,533,000	-10,000	900,000
Mar.22,'15	1,175,000	1,473,000	-10,000	5,506,000
Apr.13,'15	2,667,000	3,000,000	3,988,000	7,033,000

Results: From the tabulated data it might be inferred that the retention of moisture, as influenced by the special device used in these flasks, did not perceptibly delay the decrease in numbers of the four organisms tested. The figures, in fact, are quite comparable with those of the previous experiment where the soil was permitted to dry out gradually. The failure to secure a contrast between the results of imposing these different conditions may be partly attributed to the use in Experiment 3 of the rubber stoppers which so retarded evaporation as to make donditions during the first ten months too nearly like those of this experiment. Had evaporation in the former case been allowed to occur more rapidly, as would have been the case with cotton plugs, the results of the two experiments might have shown a contrast of some value.

It should be noted that while the soil in almost



all cases contained an amount of moisture sufficient to permit of processes of metabolism, the accumulation of toxic bi-products even in twelve months had not been such as to cause rapid diminution in numbers. In this connection the large adsorptive or absorptive surface offered by a fine garden soil may give it advantages over a solution where the long preservation of organisms in the moist condition is desired.

3.

(b). Three experiments designated here as trials 1, 2 and 3 respectively, were carried on, with the same general object. The methods used were varied slightly in the last two trials, but as the purpose of all three was practically identical, they are regarded here as sub-divisions of a single experiment, number 1.

Experiment 1.

Object of experiment: To determine whether an organism may receive protection from the solution in which it so suspended before being subjected to desiccation in sand.

Trial 1.

Plan: For this work were used cultures of Ps. radicicola grown five days at room temperature on nitrogen-free ash agar. For suspension the following solutions were employed:

1. Physiological salt solution.
2. " " " + .1% agar.
3. " " " + .1% gelatin.
4. " " " + .1% albumin.
5. " " " + .1% gum arabic.
6. " " " + .1% soluble starch.

With the exception of the albumin solution these were all prepared by dissolving 1 gram of the dry substance in a small amount of salt solution, and then making it up to a volume of 1000 c.c. They were found to be practically neutral to phenolphthalein. On account of the difficulty of dissolving powdered egg albumin it was found necessary to use raw egg white, a quantity being taken which by computation contained 1 gram of albumin. As albuminous solutions may be heated to 100° without coagulation, if slightly alkaline, this solution, before sterilization was made -10° F. S. by addition of N/NaOH. After sterilization (which, with all six was accomplished by the Tyndall method, 30 minutes heating in flowing steam on four successive days) the N/NaOH was neutralized with N/HCl, leaving the albumin solution like the other five, practically neutral.

Suspension of the bacterial growth from four agar slopes was made in 250 c.c. of each of the above solutions. For the purpose of securing initial counts, 1 c.c. of each suspension was diluted and plated on nitrogen-free ash agar. Twelve flasks of quartz sand were then inoculated from each of the six solutions, 5 c.c. to a flask. The sand had been prepared after the method described by Rahn[37]. It was heated

with diluted hydrochloric acid, washed several times, first with tap water, then with distilled water, heated on a water-bath till almost air-dry and then heated at least 30 minutes over a free flame. Fifty grams of the dry sand was placed in 100 c.c. Erlenmeyers which were plugged with cotton. Sterilization was accomplished by a heating of 45 minutes in the autoclave, under 15 pounds pressure.

The inoculated flasks were kept in a dark, well ventilated place at a temperature of 22 - 25°C. At intervals the number of organisms per gram of sand was determined by the plate method, samples being taken from two flasks representing each suspension solution. The procedure of plating was identical with that described in Experiment (b) 3, except that one medium, nitrogen-free ash agar, was used for all plates and these were kept ten days, at a temperature of 22° - 25°C. before counting.

Data given in Table 6.

Results: As is evident from the table, the counts are irregular and not such as to form a basis for any positive conclusions. This is, in part, due to the fact that the fluctuations in numbers, from time to time, were so extreme that it was difficult to determine what dilutions should be used, to obtain plates from which accurate counts might be made. One great mistake in this trial was the addition to the sand of a quantity of moisture which was sufficient to permit of multiplication of the organisms for three weeks after inoculation of the flasks. (In trials 2 and 3, addition of less moisture lessened the period of

TABLE 6.

Longevity of Ps. radicicola, dried in sand after suspension in different solutions.

Date	Salt Sol.	Agar Sol.	Gel.Sol.	Alb.Sol.	Gum A.Sol.	Starch Sol.
Jan.2	60,000	60,000	60,000	60,000	60,000	60,000
Jan.7	27,400	428,700	626,400	-10,000	30,000	60,500
Jan.15	1,711,000	3,651,000	3,974,000	?	63,160	2,143,000
Jan.27	674,800	328,600	1,335,600	3,677,000	60,000	468,100
Feb.13	-1,000	-1,000	-10,000	30,000	-1,000	-1,000
Feb.24	-50	-50	-50	200	50	50

multiplication). The bacteria were not actually subjected to desiccation until after January 27, by which time the difference in numbers of organisms developed on the five different substances used as food materials was such that a fair comparison of the influence of these substances, in their water-retaining capacity, during the process of drying, was not possible. Although it is true that after a desiccation extending over almost four weeks (from the last of January till February 24), there were greater numbers of living organisms in the flasks to which the albumin solution had been added, it is possible that this would not have occurred had not the organisms in those flasks reached enormous numbers, just previous to the period of drying, because of the superior nutritive qualities of this substance.

Trial 2.

Plan: The cultures used were the same as in Trial 1. The list of solutions, as revised for this trial, was as follows:

1. Physiological salt solution.
2. " " " + .1% agar.
3. " " " + .1% gelatine.
4. " " " + .1% gum arabic.
5. Nutrient broth.
6. Milk.
7. Soil solution (extracted from garden soil by method of Van Suchtelen).

The bacterial growth from one agar slope was suspended in 12 c.c. of each of the above solutions, and 1 c.c. diluted and plated quantitatively on nitrogen-free ash agar. From each of the seven suspensions 2 c.c. was added to each of five flasks of quartz sand, of the same quality and prepared exactly as in the preceding trial.

As in Trial 1, these flasks were kept in a dark place at 22° - 25°C. Quantitative determinations, made at intervals, are based on plates from but a single sample of each set instead of duplicate samples as in Trial 1.

Data given in Table 7.

Results: As the counts are based on plates made from but one sample of each set the opportunity for error is materially increased. It cannot, therefore, be claimed that these figures show accurate comparisons. However, allowing due margin for error, it is quite evident that between March 26 and April 17, (during which time the sand was so dry as to make multiplication of organisms impossible) the rate of decrease in numbers of organisms taken from broth, milk, and soil solution was noticeably less than that of organisms from the other solutions. This implies a certain protection gained from the presence of nitrogenous or albuminous constituents, in the milk or broth. To what substance or substances in the soil solution such protection should be credited cannot definitely be stated. The "slime", mentioned by Van Suchtelen may be of influence in this connection.

TABLE 7.

Longevity of Ps.radicicola, dried in sand after suspension in different solutions.

Date	Salt Sol.	Agar Sol.	Gel.Sol.	Gum A.Sol.	Broth	Milk	Soil Sol.
Mar. 18	1,100,000	1,500,000	1,440,000	1,613,000	1,024,000	1,176,800	1,460,000
Mar. 26	-10,000	-10,000	10,125,000	-10,000	19,967,000	185,000	40,000
Apr. 6	-25	25	50	-25	220,000	405,000	8,600
Apr.17	-25	-25	-25	-25	-25	-25	-25

Trial 3.

Plan: The cultures used were the same as in Trials 1 and 2. For suspension in addition to the solution tested in Trial 2, a .1% albumin solution was employed. The procedure was the same except that as a basis for quantitative determinations two samples were taken from each set instead of one. As the plates from several of the flasks, May 3, showed no colonies whatever, even in the lowest dilutions which represented 1/25 g., it was thought advisable in making the next determinations, May 13, to take 1 g. samples from these flasks and mix them directly with the melted medium in the Petri dish instead of plating 1 c.c. of a dilute suspension as previously done. It is quite evident that direct mixture of the sand with the plating medium tends to give higher counts than are secured by plating the washings of the sand, even in very low dilutions, for in the latter case a large number of organisms undoubtedly remain attached to the sand particles instead of being washed off into the suspension. This difference in technic may account for the apparent increase in numbers, in certain cases, as shown by the last plating.

Data given in Table 8.

Results: These figures offer little except a general confirmation of the results of Trial 2. As the sand was perfectly dry after April 26, it may be understood that the counts on May 3 and 13 represent the numbers surviving seven and seventeen days desiccation, respectively.

TABLE 8.

Longevity of Ps.radicicola, dried in sand after suspension in different solutions.

Date	Salt Sol.	Agar.Sol.	Gel.Sol.	Gum A.Sol.	Alb.Sol.	Broth	Milk	Soil Sol.
Apr.16	1,648,000	2,144,000	1,901,000	3,234,000	360,000	1,477,000	4,026,000	1,266,000
May 3	-25	25	-25	-25	56	428,625	515	391
May 13	—	—	116	—	2	432,000	106	3,080

Attention must be called to this fact, however, viz., that the lack of figures to show comparison in increase of bacteria in the different solutions, between April 16 and April 26, makes it impossible to overlook entirely the function of these different solutions in their nutritive capacity. Plates were made April 26 but the use of nitrogen-free agar made up with maltose instead of saccharose, proved an unfortunate choice for no colonies whatever, developed, although, as seen by the two subsequent platings, living organisms were then present in abundance. However, the favorable influence of soil solution, whether it may be as a food material for soil organisms or a protection during desiccation, cannot be disputed and this observation alone suggests a subject upon which further investigations might profitably be made.

4.

(c)

Experiment 1.

Object of experiment: To compare the longevity of Ps. radicicola, dried in quartz sand and in garden soil.

Plan: As in the foregoing experiment of section 3 (b), the organism was grown five days at room temperature on nitrogen-free ash agar. The bacterial growth from one agar slant was transferred to 12 c.c. of physiological salt solution and the mixture shaken thoroughly. One c.c. of the suspension was diluted and plated quantitatively. To two flasks each of garden soil and quartz sand were added 2 c.c. of the bacterial suspension. The garden soil had

been sifted and air-dried. The quartz sand had been prepared after Rahn's method, described previously. Fifty gram portions of each were placed in 100 c.c. Erlenmeyers, plugged with cotton and sterilized by heating in the autoclave 45 minutes under 15 pounds pressure.

The inoculated flasks were shaken to distribute the organisms throughout the sand or soil and then kept in a dark, well ventilated place at a temperature of 22° - 25°C. The number of living organisms per gram of sand and soil was determined, at intervals, by plating quantitatively from two samples of each.

Table 9.

Difference in longevity of Ps. radicicola dried in quartz sand and in garden soil.

Date	Sand	Garden Soil
April 16,	1,648,000	1,648,000
May 3,	-25	42,133
May 13,	-	33,025

Results: It is evident from the data above tabulated that a larger number of organisms survive a limited period of desiccation in garden soil than in quartz sand. This may be partly explained by the difference in grain-size and hygroscopic moisture of the two. A given weight of coarse quartz sand, consisting of large particles, has a surface much less than that of the same quantity of finely

divided garden soil, and therefore retains a much smaller amount of moisture in the hygroscopic form. If the grain-size were the only distinction between sand and garden soil it might properly be concluded that the longevity of organisms in such materials is directly proportional to the percentage of hygroscopic water, retained.su Such a conclusion is not permissible, however, for the garden soil differs from the sand not only in texture, but amount of organic constituents. The amount of such material in any sand is small and in this case, where the sand was treated with acid, it may be regarded as less than any appreciable quantity. The experiments in section 3 (b) indicated that soil solution contained substances which offered to the bacteria some protection against desiccation. This soil solution was extracted from just such a soil as was used in the experiment now under discussion. The possible function of these substances in the garden soil cannot, therefore, be wholly disregarded.

To prove that the amount of hygroscopic moisture is the only factor it would be necessary to compare the longevity of an organism in sands freed of all organic constituents and varying only in grain-size.

The value of organic constituents in the soil might be demonstrated by comparative tests made on sand and soil of equal grain-size but differing in content of organic materials.

(c)

Experiment 2.

Object of experiment: To compare the changes in numbers and kinds of organisms when soil solution is dried in different types of soils.

Plan: Soil solution exttracted from a rich garden loam was used for this experiment. The soils, obtained from the Soil Physics Department of this College, were of five different types:

Muck
Sand
Sandy loam
Clay
Clay loam.

Fifty gram portions of these soils (in the air-dry condition) were placed in 100 c.c. Erlenmeyers, plugged with cotton and were then sterilized by heating them in the autoclave 45 minutes under 15 pounds pressure. For greater exactness the total quantity of soil solution was agitated and then divided into five 250 c.c. portions; from each of these 1 c.c. was plated on ordinary agar in dilutions of 1:10,000, 1:100,000 and 1:1,000,000. Ten flasks of each type of soil were then inoculated with soil solution, all the solution used for any one type of soil being taken from a single flask. Although it was desired to have the inoculum approximately equal in all cases, a quantity of liquid which barely moistened the muck and clay loam was found to more than saturate the coarser soils. So, to make the physical conditions more nearly alike, 15 c.c. of solution were used for each flask of clay, clay loam, and muck, but only 10 c.c. for the flasks of sand and sandy loam.

The inoculated flasks were kept on a shelf in the laboratory at a temperature of 20° - 25°C., exposed to very dim diffused light and subject to the influence of normal variations in the humidity of the room atmosphere. At intervals of about four weeks quantitative determinations were made, samples being taken from two flasks of each soil. After the first plating samples were taken from one flask opened at the previous plating and from one new flask each time, the object being to secure more representative counts. The technic employed was the same as used in Experiment (b) 3, Section 1, plates being made with ordinary agar and kept one week at a temperature of 22° - 25°C. before counting. It should be explained here that ordinary agar was chosen for this purpose because comparative tests with plating soil solution on Lipman and Brown's synthetic agar and ordinary nutrient agar showed that the latter medium not only favored the development of a greater number of colonies but that these colonies attained larger size, within a shorter time and showed more marked differentiations than the colonies developed on Lipman and Brown's medium. As the use of any single medium offers limitation and the employment of a large number of media in such an experiment was not feasible, it was thought best to draw the comparisons on numbers and varieties of organisms which would develop on plain agar, under the conditions first described.

Moisture determinations, in duplicate, were made at the time of each quantitative plating, the bacterial counts being then computed on the oven-dry basis. Small

variations in percentage of moisture, occurring after the soils attained the air-dry condition (which with sand and sandy loam was by March 3, and with the other three soils between then and March 29), are probably the result of fluctuations in the humidity of the room air. As in the case of clay it was impossible to secure a thoroughly mixed sample, due to its drying into a sort of hard, baked condition, a slight irregularity in the moisture determinations could not be avoided.

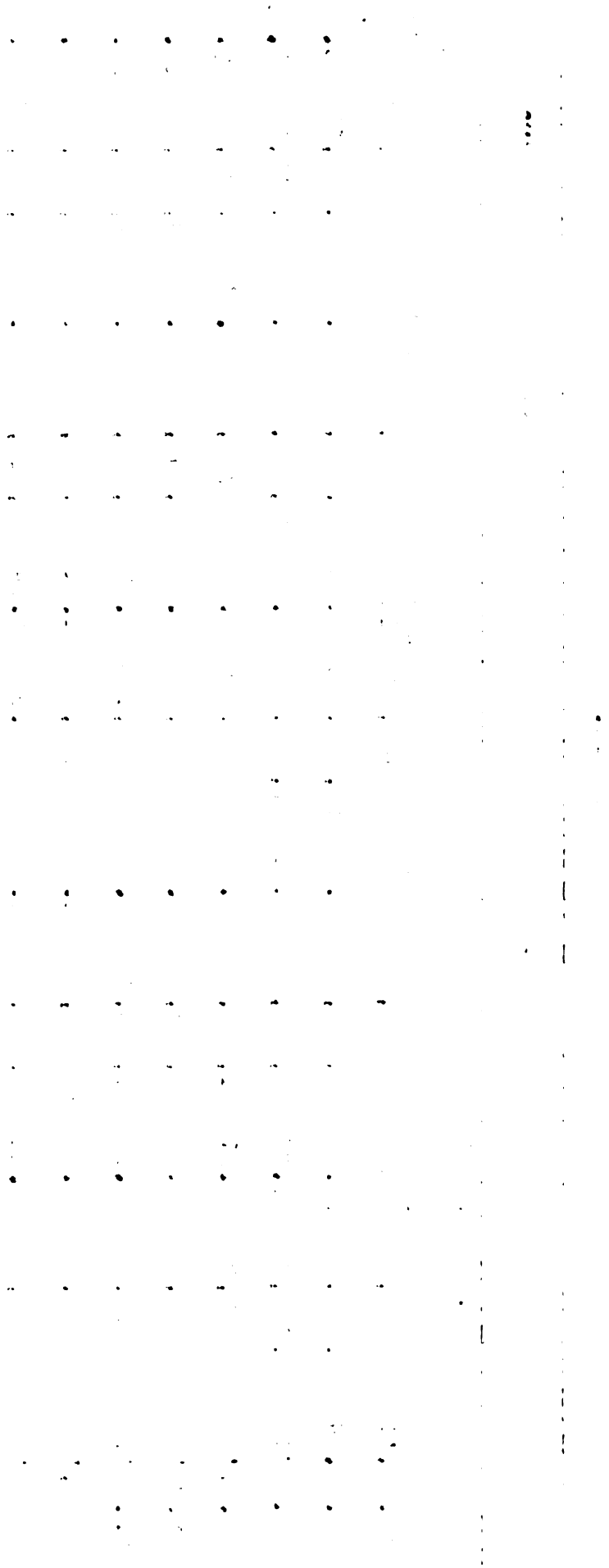
In table 10 are shown the changes in moisture content and bacterial count.

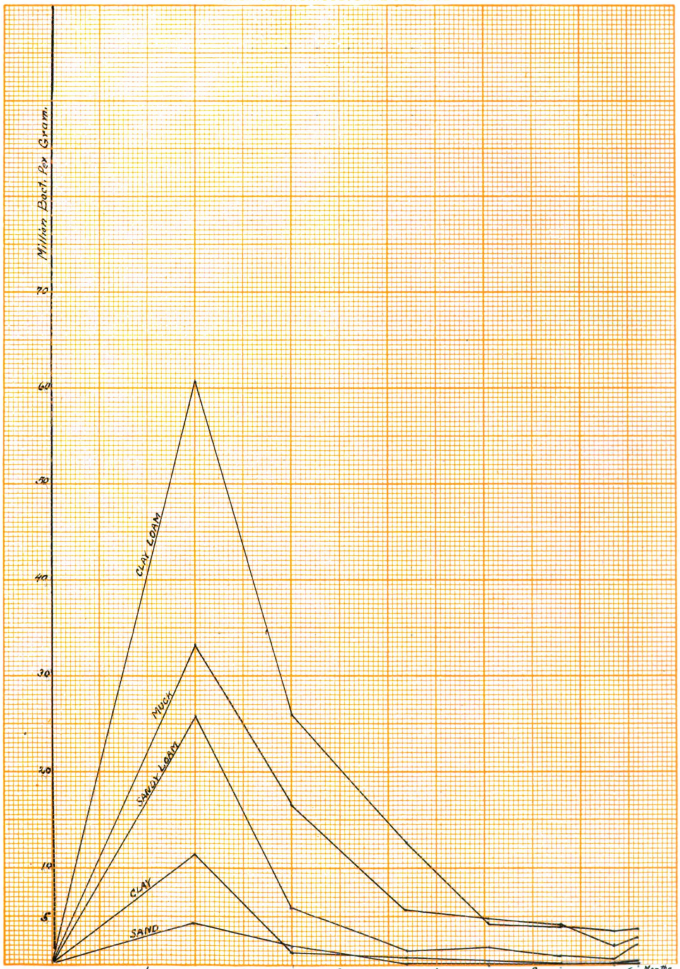
With a view to determining the predominant types of organisms placed in the soils, isolations were made from a few of the most common types of colonies occurring on the plates of the original soil solution. The characteristics of these organisms are given in tabulated form. It must not be assumed, however, from the fact that so few organisms were isolated, that the flora of the soil solution was limited to these species. The high dilutions, necessary for obtaining accurate quantitative plates of course failed to show up the organisms which were present in smaller numbers.

From the quantitative plates made after the soils reached the air-dry state, between March 3 and May 7, isolations were made of the most numberous types. As the muck plates were frequently overgrown with a downy white mold, but few pure cultures could be obtained from that source. In many cases several transfers from plates of a single soil

TABLE 10.

Date	Sand		Sandy loam		Clay		Clay loam		Muck	
	Bact.per g.	H_2O		H_2O		H_2O		H_2O		H_2O
Nov.17,'14	285,200	10cc in 50 g	170,000	10cc in 50 g	462,900	15cc in 50 g	225,000	15cc in 50 g	453,900	15cc in 50 g
Dec.29,14	4,318,000	14.54	26,170,000	14.38	11,500,000	28.96	60,840,000	31.81	33,689,000	26.13
Jan.28'15	1,912,000	6.25	5,806,000	2.81	1,492,000	19.17	26,006,000	16.96	16,613,000	24.85
Mar.3,'15	197,000	.19	1,555,000	.84	914,000	3.59	12,798,000	9.83	5,782,000	19.51
Mar.29'15	51,900	.36	1,967,000	.78	552,000	.93	4,659,000	2.93	4,924,000	16.33
Apr.21'15	18,900	.16	1,066,000	.84	447,100	1.57	4,135,000	3.31	4,217,000	16.32
May 7,'15	32,500	.27	983,000	1.08	278,800	1.81	3,845,000	3.65	2,220,000	16.25
May 14'15	37,000	.22	2,245,000	1.10	378,000	1.74	3,914,000	3.63	2,703,000	15.91





proved on cultivation to be the same organism. Where it was conclusively shown that any organism was identical with one previously isolated from the soil the record of its characteristics is omitted. The numbers, therefore, do not run consecutively in all cases.

Descriptions of the cultural and morphological characteristics of the organisms isolated are given in tabulated form. In general the terms used are thos which have been adopted by the American Society of Bacteriologists.

As to the method of obtaining the data tabulated in these descriptions some explanation is needed. The isolation of the bacteria was accomplished by use of ordinary nutrient agar. Transfers from individual colonies on this medium were then made to agar slants. After 24 - 48 hours growth on agar they were inoculated into the various other culture media and kept in darkness at 22° - 25° C. The cultures were usually described on the seventh day, later observations in some cases being necessary with litmus milk and gelatin stab cultures. Whenever the litmus milk was so discolored as to make the reaction uncertain, test of acidity was made with fresh litmus paper. Such biochemical features as indol and ammonia production and nitrate reduction were determined by making tests with the proper reagents on 7 day cultures in Dunham's peptone solution. Morphological features, except spore-formation, are based on observation of young cultures. The dimensions are taken from smears stained 2 - 3 minutes with aqueous alcoholic fuchsin. Motility was determined by careful examination of

Organisms from soil solution.

Number of organism	1	2	3	4
Morphology				
Form (Rod	+	+	+	+
(Spherical				
Diameter in microns	.6	.7	.6	.6
		.8	.8	.9
Length	.9	1.1	1	.9
	1.3	1.5	1.6	1.2
Grouping				
Endospores	-	+	-	-
Motility	+	+	+	+
Relation to oxygen				
Aerobic				
Facultative	+	+	+	+
Bouillon				
Turbid	+	+	+	+
Ring	-	-	-	-
Pellicle	+	+	+	+
Sediment	+	+	+	+
Agar streak				
Form of growth				
Spreading			+	+
Filiform	+	+		
Arborescent				
Beaded				
Topography				
Smooth	+	+	+	+
Contoured				
Rugose				
Verrucose				
Optical characters				
Opaque				
Translucent	+	+	+	+
Irridescent				
Litmus milk				
Curd	+	+	+	+
Acid	+	-	-	-
Alkaline	-	+	+	+
Discolored	+	+	+	+
Pep. begins	48h	7d	7d	7d
Pep. complete	-	-	-	-
Slimy consistency	-	-	-	-
Gelatine				
Needle form				
Surface growth	+	+	+	+
Filiform	+			
Beaded				
Papillate				
Arborescent				
Liquefaction	-	+	+	+
Crateriform			+	
Napiform		+		
Infundibuliform				+
Stratiform				
Liquefact. complete				

Organisms from soil solution. (Cont'd)

Number of organism	1	2	3	4
Fermentation				
Gas in Lactose	+	-	-	-
Chromogenesis				
Yellow				
Orange				
Pink				
White				
Fluorescent			+	+
Brown				
Grey	+	+		
Dunham's solution				
Indol produced	-	+	+	+
Ammonia	-	+	+	+
Nitrates reduced	+	+	+	+

Organisms from Sandy Loam Soil.

Number of organism	1	2	3	4	5	6
Morphology						
Form (Rod	+		+	+	+	+
(Spherical		+				
Diameter in microns	.6	1.2	.9	.4	.6	.3
		1.4	1.1			
Length	1.		2.5	.8	1.3	.7
	1.3			.9	1.4	.8
Grouping	Strep.	dip.	Strep.			Strep.
Endospores	-	-	+	-	-	-
Motility	-	-	+	+	+	+
Relation to oxygen						
Aerobic			+			
Facultative	+	+		+	+	+
Bouillon						
Turbid	-	+	-	+	+	+
Ring		+		+	+	
Pellicle	+		+	+		+
Sediment			+	+		
Agar streak						
Form of growth						
Spreading			+			
Filiform	+	+		+	+	+
Arborescent			+			
Beaded						
Topography						
Smooth		+	+		+	+
Contoured	+			+		
Rugose						
Verrucose						
Optical characters						
Opaque	+	+	+	+		+
Translucent					+	
Irridescent		+				
Litmus milk						
Curd	-	-	-	-	+	-
Acid	-	-	+	+	-	-
Alkaline	+	-	-	-	-	+
Discolored	-	-	-	-	-	+
Pep. begins	-	-	-	?	11d	-
Pep. complete	-	-	-	+30d	-	-
Slimy consistency	-	-	-	-	-	-
Gelatine						
Needle form						
Surface growth	+	+	+	+	+	+
Filiform		+	+	+	+	+
Beaded		+	+			
Papillate	+	-				
Arborescent						
Liquefaction	-		+	-	-	-
Crateriform						
Napiform						
Infundibuliform						
Stratiform						
Liquefact. complete						

Organisms from Sandy Loam Soil. (cont'd)

Number of organism	1	2	3	4	5	6
Gas in Lactose	-	-	-	-	-	-
Chromogenesis						
Yellow				+		
Orange						
Pink						
White	+	+			+	
Fluorescent						
Brown						
Grey			+			
Dunham's solution						
Indol produced	-	-	-	-	-	-
Ammonia	+	-	-	+	+	-
Nitrates reduced	+	-	-	+	+	+

Organisms from Sandy Loam Soil.

Number of organism	7	8	9	10	11
Morphology					
Form (Rod	+	+		+	+
(Spherical			+		
Diameter in microns	.6	.6	.9	.4	.2
			1.1		.3
Length	.9	.9		.7	.6
		1.		.9	.8
Grouping			Sarc.	Strep.	
Endospores	-	-	-	-	-
Motility	-	-	-	-	+
Relation to oxygen					
Aerobic	+	+	+		
Facultative				+	+
Bouillon					
Turbid	+	+	-	+	+
Ring	+		-	+	
Pellicle		+	-		+
Sediment	+	+	+	+	+
Agar streak					
Form of growth					
Spreading					
Filiform	+	+	+	+	+
Arborescent					
Beaded					
Topography					
Smooth	+			+	+
Contoured		+	+		
Rugose					
Verrucose					
Optical characters					
Opaque	+	+	+	+	+
Translucent					
Irridescent					
Litmus milk					
Curd	-	-	-	-	-
Acid	-	-	+	-	-
Alkaline	+	-	-	+	+
Discolored	-	-	-	-	-
Pep. begins	-	-	-	-	-
Pep. complete	-	-	-	-	-
Slimy consistency	-	-	-	-	-
Gelatine					
Needle form					
Surface growth	+	+	+	+	+
Filiform	+	+	+	+	+
Beaded		+			
Papillate					
Arborescent					
Liquefaction	-	-	-	-	-
Crateriform					
Napiform					
Infundibuliform					
Stratiform					
Liquefact. complete					

Organisms from Sandy Loam Soil (cont'd).

Number of organism	7	8	9	10	11
Gas in Lactose	-	-	-	-	-
Chromogenesis					
Yellow		(+			
Orange		(+			
Pink	+				
White				+	+
Fluorescent					
Brown			+		
Grey					
Dunham's solution					
Indol produced	-	-	-	-	-
Ammonia	-	+	+	+	+
Nitrates reduced	+	+	-	-	+

Organisms from Sandy Loam Soil				
Number of organism	13	14	15	16
Morphology				
Form (Rod	+	+	+	+
(Spherical				
Diameter in microns	.3	.3	1	.8
		.4	1.6	1.1
Length	.6	.5	1.6	1.2
	.9	.6	4	3
Grouping			Stre.	Stre.
Endospores	-	-	+	+
Motility	+	-	+	+
Relation to oxygen				
Aerobic				+
Facultative	+	+	+	
Bouillon				
Turbid	+	+	+	-
Ring	-	+	+	-
Pellicle	+	-	-	+
Sediment	+	+	-	-
Agar streak				
Form of growth				
Spreading				+
Filiform	+	+	+	
Arborescent				
Beaded				
Topography				
Smooth		+		+
Contoured	+		+	
Rugose				
Verrucose				
Optical characters				
Opaque	+		+	+
Translucent		+		
Irridescent			+	
Litmus milk				
Curd	-	-		-
Acid	-	-	-	-
Alkaline	+	+	-	+
Discolored	-	-	-	+
Pep. begins	-	-	-	24h
Pep. complete			-	48h
Slimy consistency	-	-	-	-
Gelatine				
Needle form				
Surface growth	+	+	+	+
Filiform	+	+	+	
Beaded				
Papillate				
Arborescent				
Liquefaction	-	-	+	+
Crateriform			+	
Napiform				
Infundibuliform				+
Stratiform				
Liquefact. complete				

Organisms from Sandy Loam Soil (cont'd).

Number of organism	13	14	15	16
Gas in Lactose	-	-	-	-
Chromogenesis				
Yellow				
Orange				
Pink				
White	+	+	+	+
Fluorescent				
Brown				
Grey		+	+	+
Dunham's solution				
Indol produced	-	-	-	-
Ammonia	+	+	+	-
Nitrates reduced	+	-	+	+

Organisms from Clay Loam Soil

Number of orgamism	2	3	4	5	6	7
Morphology						
Form (Rod	+	+	+		+	+
(Spherical				+		
Diameter in microns	.3	.3	.4	.8	.3	.4
		.4		1.		
Length	.9	2.	.8		.7	.7
	1.2	3.	1.1		1.	
Grouping				Sarc.		
Endospores	-	-	-	-	-	-
Motility	+	-	+	-	+	-
Relation to oxygen						
Aerobic						
Facultative	+	+	+	+	+	+
Bouillon						
Turbid	+	+	+	+	+	+
Ring				+		+
Pellicle	+	+	+		+	-
Sediment	+	+	+	+	+	+
Agar streak						
Form of growth						
Spreading				+	+	+
Filiform	+	+	+			
Arborescent						
Beaded					+	
Topography						
Smooth	+	+	+	+	+	+
Contoured						
Rugose						
Verrucose						
Optical characters						
Opaque				+	+	+
Translucent	+	+	+			
Irridescent						
Litmus milk						
Curd	-	-	+	-	-	-
Acid	-	+	+	+	-	-
Alkaline	+	-		-	+	+
Discolored	-	-	+	+	-	+
Pep. begins	+30d	+30d	-	+30d	-11d	11d
Pep. complete	+30d	-	-	+30d	-	-
Slimy consistency	-	-	-	-	-	+
Gelatine						
Needle form						
Surface growth	+	+	+	+	+	+
Filiform	+	+	+	+	+	+
Beaded						
Papillate						
Arborescent		+				
Liquefaction	-		-	+	-	-
Crateriform				+		
Napiform						
Infundubuliform						
Stratiform		+				
Liquefaction complete						

Organisms from Clay Loam Soil (cont'd).

Number of organism	2	3	4	5	6	7
Gas in Lactose	-	-	-	-	-	
Chromogenesis						
Yellow				+		
Orange		+				
Pink						
White	+					+
Fluorescent			+			
Brown						
Grey					+	
Dunham's solution						
Indol produced	-	-	-	-	-	-
Ammonia	-	-	+	-	+	+
Nitrates reduced	-	+	+	+	+	+

Organisms from Clay Loam Soil.

Number of organism	8	9	10	11	13	14
Morphology						
Form (Rod	+	+	+	+	+	+
(Spherical						
Diameter in microns	.4	.7	.3	.3	.4	.8
			.4			1.1
Length	.7	1.8	.7	1.	.7	1.5
		2.5	.9	1.2	.8	4
Grouping		Strep.			Dip.	Strep.
Endospores	-	-?	-	-	-	+
Motility	-	+	-	+	+	+
Relation to oxygen						
Aerobic						
Facultative	+		+	+	+	+
Bouillon						
Turbid	+	+	+	+	+	+
Ring	+			-	-	+
Pellicle	-		-	+	+	-
Sediment	+	+	+	+	+	+
Agar streak						
Form of growth						
Spreading	+	+				
Filiform			+	+	+	+
Arborescent						
Beaded						
Topography						
Smooth	+	+	+	+	+	+
Contoured						
Rugose						
Verrucose						
Optical characters						
Opaque	+		+			+
Translucent		+		+	+	
Irridescent						
Litmus milk						
Curd	-	-	-	-	-	-
Acid	-		-		-	-
Alkaline	+	+	-	+	-	-
Discolored	-		-		-	-
Pep. begins	3d		-		-	-
Pep. complete			-		-	-
Slimy consistency	-	-	-	-	-	-
Gelatine						
Needle form						
Surface growth	+	+	+	+	+	+
Filigorm	+	+	+	+	+	+
Beaded						
Papillate						
Arborescent						
Liquefaction	-	-	-	-	-	+
Crateriform						+
Napiform						
Infundubuliform						
Stratiform						
Liquefaction complete						

Organisms from Clay Loam Soil (cont'd).

Number of organism	8	9	10	11	13	14
Gas in Lactose	-	-	-	-	-	-
Chromogenesis						
Yellow			+			
Orange						
Pink						
White	+	+		+	+	+
Fluorescent						
Brown						
Grey						
Dunham's solution						
Indol produced	-	-	-	-	-	-
Ammonia	+	+	-	-	-	+
Nitrates reduced	+	+	-	+	-	+

Organisms from Clay Soil.

Number of organism	1	2	3	4	5	6
Morphology						
Form (Rod	+	+	+	+	+	+
(Spherical						
Diameter in microns	.6	.5	.6	.6	.5	.5
						.4
Length	1.3	1.	1.5	1.	1.	.9
	1.5	1.3		1.3		1.
Grouping	Dip.	Strep.		Strep.		
Endospores	-	-	-	-	-	-
Motility	+	+	+	-	+	-
Relation to oxygen						
(Aerobic			+			
(Facultative	+	+		+	+	+
Bouillon						
Turbid	+	+	-	-	+	+
Ring		+			+	+
Pellicle	+		+	+	+	+
Sediment						
Agar streak						
Form of growth						
Spreading						+
Filiform	+	+	+	+	+	
Arborescent						
Beaded						
Topography						
Smooth	+	+	+		+	+
Contoured				+		
Rugose						
Verrucose						
Optical characters						
Opaque		+		+		
Translucent	+		+		+	+
Irridescent						
Litmus milk						
Curd	-	-	-	-	-	-
Acid	-	-	-	-	-	-
Alkaline	+	+	+	+	+	+
Discolored	-	-	-	-	-	+
Pep. begins	-	+30d	-	-	-	6d
Pep. complete		+30	-	-	-	9d
Slimy consistency	-	-	-	-	-	-
Gelatine						
Needle form						
Surface growth	+	+	+	+	+	+
Filiform	+	+	+		+	+
Beaded						
Papillate				+		
Arborescent	-	+	-	-	-	-
Liquefaction		+				
Crateriform						
Napiform						
Infundibuliform						
Stratifrom						
Liquefact. complete						

Organisms from Clay Soil (cont'd).

Number of organism	1	2	3	4	5	6
Gas in Lactose	-	-	-	-	-	-
Chromogenesis						
Yellow						
Orange						
Pink						
White	+		+	+		+
Fluorescent						
Brown				+		
Grey		+				
Dunham's solution						
Indol produced	-	-	-	-	-	-
Ammonia	+	+	+	-	-	+
Nitrates reduced	+	+	+	+	-	+

Organisms from Clay Soil.

Number of organism	7	8	9	10	11	12
Morphology						
Form (Rod	+	+		+	+	+
(Spherical			+			
Diameter in microns	.5	.5	.6	.5	.3	.6
	.7	.6	.8	.7	.4	1.2
Length	1.	1.		1.	.7	1.
		1.3			.9	4.
Grouping			Staph.			
Endospores	-	+	-	-	-	+
Motility	-	+	-	-	-	+
Relation to oxygen						
Aerobic						+
Facultative	+	+	+	+	+	
Bouillon						
Turbid	+	-		+	+	+
Ring	+			+	+	
Pellicle	+	+		+	-	+
Sediment	+	-		+	-	-
Agar streak						
Form of growth						
Spreading	+		+	+		+
Filiform		+			+	
Arborescent						+
Beaded						
Topography						
Smooth	+		+	+	+	
Contoured						+
Rugose		+				
Verrucose						
Optical characters						
Opaque		+			+	+
Translucent	+		+	+		
Irridescent						
Litmus milk						
Curd	-	-	-	-	-	-
Acid	-		-	-	-	-
Alkaline	+	+	-	+	-	+
Discolored	-	+	-	-	-	+
Pep. begins	11d	-	-	11d	-	48h
Pep. complete	-	-	-		-	+4d
Slimy consistency	-	-	-	-	-	-
Gelatine						
Needle form						
Surface growth	+	+	+	+	+	+
Filiform	+	+	+	+	+	
Beaded						
Papillate	+			+		
Arborescent						+
Liquefaction	-	+	-	-	-	+
Crateriform		+				
Napiform						
Infundibuliform						
Stratiform						+
Liquefact. complete						

Organisms from Clay Soil (cont'd).

Number of organism	7	8	9	10	11	12
Gas in Lactose	-	-	-	-	-	-
Chromogenesis						
Yellow					+	
Orange						
Pink						
White				+		+
Fluorescent						
Brown		+				
Grey	+		+			
Dunham's solution						
Indol produced	-	-	-	-	-	-
Ammonia	+	-	-	+	+	-
Nitrates reduced	-	-	+	-	+	+

Organisms from Clay Soil.

Number of organism	13	15	16	17	18	19
Morphology						
Form (Rod	+		+	+	+	+
(Spherical		+				
Diameter in microns	.6	.8	.6	.6	.7	.3
	.7	1.	.7		1.1	.4
Length	2.		2.	1.	1.	.5
	4.		4.	1.2	3.	.7
Grouping	Strep.	Staph.	Strep.			
Endospores	+	-	+	-	+	-
Motility	+	-	+	+	+	-
Relation to oxygen						
Aerobic	+	+	+		+	
Facultative				+		
Bouillon						
Turbid	-	+	+	+	+	+
Ring	-	+	+	-	-	+
Pellicle	+	-	-	+	+	-
Sediment	-			+	+	-
Agar streak						
Form of growth						
Spreading	+		+	+	+	
Filiform		+				+
Arborescent						
Beaded						
Topography						
Smooth		+		+	+	+
Contoured			+			
Rugose	+					
Verrucose						
Optical characters						
Opaque	+	+	+	+		+
Translucent					+	
Irridescent						
Litmus milk						
Curd	-	-	-	-	-	-
Acid	-	-	-	-	-	--
Alkaline	+	-	+	+	+	+
Discolored	+	-	+	-	+	-
Pep. begins	48h	-	3d	8d	48h	-
Pep. complete	4d		7d	-	4d	-
Slimy consistency	-	-	-	-	-	-
Gelatine						
Needle form						
Surface growth	+	+	+	+	+	+
Filiform		+	+	+		
Beaded						
Papillate						
Arborescent						
Liquefaction	+	-	+	-	+	+
Crateriform			+			+
Napiform						
Infundibuliform	+					
Stratiform					+	
Liquefact. complete						

Organisms from Clay Soil (cont'd).

Number of organism	13	15	16	17	18	19
Gas in Lactose	-	-	-	-	-	-
Chromogenesis						
Yellow						
Orange		+				
Pink						
White			+	+		
Fluorescent						
Brown	+					
Grey					+	+
Dunham's solution						
Indol produced	-	-	-	-	-	-
Ammonia	+	-	-	-	+	+
Nitrates reduced	+	+	+	-	-	+

Organisms from Sand Soil.

Number of organism	1	3	4	5	6	8
Morphology						
Form (Rod	+	+	+	+	+	+
(Spherical						
Diameter in microns	.6	.5	.8	.4	.4	.4
			.9			.5
Length	1.3	1.	1.9	.6	.8	1.
	1.5		2.4	.7	.9	
Grouping			Dip.			-
Endospores	-	-	+	-	-	-
Motility	+	+	+	-	-	-
Relation to oxygen						
Aerobic			+			
Facultative	+	+		+	+	+
Bouillon						
Turbid	+	+	-	+	+	+
Ring				+	+	+
Pellicle	+	+	+	-	-	+
Sediment					-	-
Agar streak						
Form of growth						
Spreading						+
Filiform	+	+	+	+	+	
Arborescent						
Beaded						
Topography						
Smooth	+	+		+	+	
Contoured						+
Rugose			+			
Verrucose						
Optical characters						
Opaque		+	+	+	+	
Translucent	+					+
Irridescent						
Litmus milk						
Curd	-	-	-	-	-	-
Acid	-	-	-	-	-	-
Alkaline	+	-	+	+	+	+
Discolored	-	+	-	-	-	+
Pep. begins	-	?	?	?	?	6d
Pep. complete	-	11d	11d	+30d	+30d	9d
Slimy consistency						
Gelatine						
Needle form						
Surface growth	+	+	+	+	+	+
Filiform	+	+		+	+	+
Beaded						
Papillate						
Arborescent						
Liquefaction	?	+	+	+	+	-
Crateriform		+		+	+	
Napiform						
Infundabuliform			+			
Stratiform						
Liquefaction complete			+11d			

Organisms from Sand Soil (cont'd).

Number of organism	1	3	4	5	6	8
Gas in Lactose	-	-	-	-	-	-
Chromogenesis						
Yellow			+			
Orange		+				
Pink						
White	+			+		+
Fluorescent						
Brown			+			
Grey						
Durham's solution						
Indol produced	-	-	-	-	-	-
Ammonia	+	-	+	+	+	+
Nitrates reduced	+	-	+	+	-	-

Organisms from Sand Soil.

Number of organism	9	10	11	12	13	14
Morphology						
Form (Rod	+	+	+	+	+	+
(Spherical						
Diameter in microns	.6	.8	.8	.7	.6	.8
	1.2	1.	.9		.7	1.
Length	1.	2.	2.2	.9	2.	2.
	4.	5.	3.		3.	5.
Grouping						
Endospores	+	+	+	-	-	+
Motility	+	+	+	-	-	+
Relation to oxygen						
Aerobic	+	+	+			+
Facultative				+	+	
Bouillon						
Turbid	-	-	+	+	+	-
Ring				+	-	
Pellicle	+	+	+	-	-	+
Sediment	-	-	-	-	-	-
Agar streak						
Form of growth						
Spreading		+	+	+	+	
Filiform						+
Arborescent	+					
Beaded						
Topography						
Smooth						
Contoured	+			+		
Rugose		+	+		+	+
Verrucose						
Optical characters						
Opaque	+	+	+	+		+
Translucent					+	
Irridescent						
Litmus milk						
Curd	-	-	-	-	-	+
Acid	-	-	-	-	-	-
Alkaline	+	+	+	+	+	+
Discolored	+	+	+		+	+
Pepl begins	48h	24h	4 d	-	48h	72h
Pep. complete	+4d	48h	7d		7d	-
Slimy consistency	-	-	-	-	-	-
Gelatine						
Needle form						
Surface growth	+	+	+	+	-	+
Filiform			+	+	+	
Beaded						
Papillate						+
Arborescent	+					
Liquefaction	+	+	+	-		+
Crateriform						
Napiform						
Infundibuliform	+	+				
Stratiform			+			+
Liquefact. complete						

Organisms from Sand Soil (cont'd).

Number of organism	9	10	11	12	13	14
Gas in Lactose	-	-	-	-	-	-
Chromogenesis						
Yellow						
Orange						
Pink						
White			+	+	+	
Fluorescent						
Brown		+				+
Grey	+					
Dunham's solution						
Indol produced	-	-	-	-	-	-
Ammonia	+	-	+	-	+	+
Nitrates reduced	+	+	+	-	+	+

Organisms from Sand Soil.

Number of organism	15	16	17	18	19	20	21
Morphology							
Form (Rod	+	+	+	+		+	+
(Spherical							
Diameter in microns	.6	1.2	.6	.8	.6	.3	.8
	1.2	2.		1.5	1.1	.5	
Length	1.	2.	1.3	1.5	1.	1.2	1.
	4.	6.		4.	2.5	3.	1.1
Grouping		Strep.	Strep.	Strep.	Strep.	Strep.	
Endospores	+			+	+	-	-
Motility	+	+	+	+	+	-	+
Relation to oxygen							
Aerobic	+				+		
Facultative		+	+	+		+	+
Bouillon							
Turbid	-	+	+	+	+	+	+
Ring		+	-	+	-	-	-
Pellicle	+		+	-	+	-	-
Sediment	+	+	+	+	+	-	+
Agar streak							
Form of growth							
Spreading	+		+	+	+		+
Filiform		+				+	
Arborescent	+						
Beaded							
Topography							
Smooth			+		+	+	+
Contoured	+	+		+			
Rugose		+					
Verrucose							
Optical characters							
Opaque	+	+		+	+		
Translucent			+			+	+
Irridescent							
Litmus milk							
Curd	+		-	-	-	-	-
Acid	-		-	-	+	+	-
Alkaline	+		+	-	-	-	+
Discolored	+		-	-	-	-	-
Pep. begins	48h		-	-	-	-	-
Pep. complete	-		-	-	-	-	-
Slimy consistency	-						
Gelatine							
Needle form							
Surface growth	+	+	+	+	+	-	+
Filiform		+	+	+	+	+	+
Beaded							
Papillate			+				
Arborescent	+						
Liquefaction	+	+	-	+	+	-	-
Crateriform		+		+	+		
Napiform							
Infundubuliform							
Stratiform	+						
Liquefaction complete							

Organisms from Sand Soil (cont'd).

Number of organism	15	16	17	18	19	20	21
Gas in Lactose	-	-	-	-	-	-	-
Chromogenesis							
Yellow							
Orange							
Pink							
White	+		+	+	+		
Fluorescent							+
Brown							
Grey	+		+		+	+	
Dunham's solution							
Indol produced	-	-	-	-	-	-	-
Ammonia	+	+	+	-	+	-	-
Nitrates reduced	+	-	-	-	+	+	+

Organisms from Muck Soil.

Number of organism	1	2	3	4	5	6
Morphology						
Form (Rod	+	+		+	+	+
(Spherical			+			
Diameter in microns	.3	.3	.5 .7	.3	.4	.4
Length	.7 .9	1. 1.2		.8 1.	1. 1.2	.6 .7
Grouping			Sta.		Stre.	
Endospores	-	-	-	-	-	-
Motility	-	-	-	+	+	-
Relation to oxygen						
Aerobic						
Facultative	+	+	+	+	+	+
Bouillon						
Turbid	+	+	+	+	+	+
Ring	-	-	-	-	-	-
Pellicle	-	-	-	+	-	-
Sediment	+	+	+	+	+	+
Agar streak						
Form of growth						
Spreading					+	+
Filiform	+	+	+	+		
Arborescent						
Beaded	+					
Topography						
Smooth		+			+	+
Contoured	+		+	+		
Rugose						
Verrucose						
Optical characters						
Opaque				+		
Translucent	+	+	+		+	+
Irridescent						
Litmus milk						
Curd	+	-	-	-	+	-
Acid	-	-	+	-	+	-
Alkaline	+	+	-	+	-	+
Discolored	+	-	-	-	-	-
Pep. begins	20d	20d	-	-	-	48h
Pep. complete	+30d	-	-	-	-	10d
Slimy consistency	-	-	-	-	-	+
Gelatine						
Needle form						
Surface growth	+	-	+	+	-	+
Filiform	+	+		+	+	
Beaded	+		+			
Papillate						
Arborescent						
Liquefaction	+	-	-	-	-	+
Crateriform	+					
Napiform						
Infundibuliform						
Stratiform						+
Liquefaction complete						14d

Organisms from Muck Soil.(Cont'd)

Number of organism	1	2	3	4	5	6
Gas in Lactose	-	-	-	-	-	-
Chromogenesis						
Yellow			+			
Orange						+
Pink						
White		+				
Fluorescent						
Brown	+			+		
Grey					+	
Dunham's solution						
Indol produced	-	-	-	-	-	-
Ammonia	-	-	+	-	+	+
Nitrates reduced	+	-	+	-	+	+

Organisms from Muck Soil.

Number of organism	7	8	9	10	11
Morphology					
Form (Rod	+	+	+	+	
(Spherical					
Diameter in microns	.5	.3	.7	.3	.4
		.4	.8	.4	
Length	.7	.7	1.	1.1	.7
		.9	1.1	1.3	.8
Grouping					
Endospores	-	-	-	-	-
Motility	-	-	+	+	+
Relation to oxygen					
Aerobic					
Facultative	+	+	+	+	+
Bouillon					
Turbid	+	+	+	-	+
Ring	+	+	+	-	+
Pellicle		-	-	+	-
Sediment	+	-	-	-	-
Agar streak					
Form of growth					
Spreading	+		+		
Filiform		+		+	+
Arborescent					
Beaded					
Topography					
Smooth	+	+	+	+	+
Contoured					
Rugose					
Verrucose					
Optical characters					
Opaque	+	+			+
Translucent			+	+	
Irridescent					
Litmus milk					
Curd	-	-	-	-	-
Acid	-	-	-	-	-
Alkaline	+	-	+	+	+
Discolored	-	-	-	-	-
Pep. begins	48h	-	-	-	-
Pep. complete	-	-	-	-	-
Slimy consistency	-	-	-	-	-
Gelatine					
Needle form					
Surface growth	+	+	+	+	+
Filiform	+	+	+	+	+
Beaded			+		
Papillate					
Arborescent					
Liquefaction	-	-	-	-	-
Crateriform					
Napiform					
Infundubuliform					
Stratiform					
Liquefaction complete					

Organisms from Muck Soil (cont'd).

Number of organism	7	8	9	10	11
Gas in Lactose	-	-	-	-	-
Chromogenesis					
Yellow		+			
Orange					
Pink					
White	+			+	+
Fluorescent			+		
Brown					
Grey				+	+
Dunham's solution					
Indol produced	-	-	-	-	-
Ammonia	-	-	-	-	-
Nitrates reduced	+	+	+	+	-

a hanging drop from a 24 - 48 hour agar slant culture. Presence of spores was determined microscopically, by examination of stained and hanging drop preparations.

The following tabulation shows on which dates the different organisms were isolated:

Date.	Muck	Sand	Sandy loam	Clay	Clay loam
Mar. 3	1-6	1-7	1-6	1-5	1-6
" 29	---	8-11	---	6-8	8-8
Apr. 21	7-9	12-15	7-13	9-16	9-12
May 7	10-11	16-21	14-16	17-19	13-16

Results: As seen from Table 10, the loam soils and muck show a higher count, six months from the time of inoculation than do the clay and sand. During the first six weeks all five soils contained an amount of moisture sufficient for bacterial growth and during the last two months only, were the soils in the air-dry state. The amount of activity in the intermediate period between the optimum and minimum supply of moisture shows a gradual decrease, the rate varying in the different soils.

While there was not a great difference in the initial counts, the opportunity for bacterial growth, in the five types of soil was by no means the same. This is clearly evidenced by the contrast between their counts during the first period when the moisture content was yet sufficient to permit of multiplication. Since the sand

was saturated with the amount of soil solution used as an inoculum, it at first presented conditions more favorable to anaerobic than aerobic species. As this amount of moisture diminished and the oxygen supply increased, opportunity for growth of aerobic types was given, but the extent of this favorable period was limited not only by the small amount of organic food material but also by the extremely rapid evaporation of moisture. Conditions in the clay were, at first, comparable with those in the sand, it being practically water-logged. With the gradual reduction in moisture, and increase in aeration, growth of aerobic (and facultative) bacteria proceeded. Its having a smaller grain-size than the sand produced two noticeable effects, viz., a limited oxygen supply, inhibitory to the extensive multiplication of aerobic species, and a prolonged retention of moisture which favored the longevity, if not the activity, of non-spore-bearing bacteria. As in the sand a low content of organic nutrients acted as a natural limit to the growth of saprophytic species. In the clay loam, sandy loam, and muck, multiplication was possible from the start, for the amount of solution used for inoculation was just sufficient to moisten the soils without saturating them. Their higher content or organic substance also gave them an advantage in respect to nutrition.

However, in these soils also, differences in grain-size, thickness of moisture-film and oxygen supply proved to be factors of more influence than the mere abundance of organic food substances. The muck, for instance, although

containing the highest percent of such organic materials proved to be a less favorable medium for bacterial growth than did the clay loam for its smaller grain-size resulted in a thinner moisture-film around the individual particles and less aeration. This same quality, however, resulted in a materially slower rateof evaporation and this condition, as in clay, was favorable to the continued existance of non-sporebearing bacteria which, if they could not multiply, were at least not subjected to rapid and destructive desiccation. The grain-size of the clay loam appeared to be that which was most advantagious, with respect to aeration, thickness of moisture film and retnetion of hygroscopic water. Its content of decomposable substance while not so great as that of the muck, was more than sufficient for microbial development. The sandy loam with a lower amount of organic materials somewhat larger grain-size and consequently smaller hygroscopicity did not show as large numbers of living organisms at any time, as did the clay loam, although its oxygen supply in consequence of these same conditions must have been somewhat greater.

We, therefore, perceive that the optimum condition for microbial activity in soil is a proper adjustment of these fore-mentioned factores. With regard to longevity, fewer factors are concerned, the data so far obtained indicating that it is a function of both grain-size (and therefore hygroscopic moisture) and content of organic substances.

The influence of soil type was made evident not only in the numerical counts, but also in the varieties of organisms persisting in the different soils throughout the

two months that they were in the air-dry state.

As the condition of the sand had been such as to favor the development of organisms with high oxygen requirements, plates of high dilution always showed a predominance of those types. Such of these as were spore-bearers became a larger and larger proportion of the total number, as the period of desiccation extended and nonspore-bearing species died out. Among the spore-bearers most frequently found were B. mycoides and aerobes of similar morphological and cultural characters. Of the nonspore-formers an organism described as No. 1 sand, showed the greatest longevity, it being found in larger numbers than any other single species.

Physical conditions in the clay had somewhat inhibited the extensive multiplication of strongly aerobic types, but permitted the development of facultative bacteria. (Since anaerobic organisms could not be secured by the method of plating used, no mention of them is necessary) As nonspore-bearing types declined, the plates showed more evidence of spore-bearing, strictly aerobic varieties, similar to those met with in the sand. The fact that such colonies were not found till the diminishing numbers necessitated the use of lower dilutions suggests their development from spores which had merely remained latent in the clay, without passing through a process of multiplication and subsequent destruction like the majority of the facultative nonspore-bearing species. The nonspore-former showing greatest endurance of desiccation was a type identical with that persisting in the sand. It is described

as organism No. 1 clay.

During the period of extensive multiplication the plates from sandy loam, clay loam and muck showedquite similar types, although the sandy loam had slightly greater numbers of the strongly aerobic, spore-forming species. As the numbers diminished spore-bearing types became more frequent on plates from both sandy loam and clay loam, but were not evident on the plates from muck. It is to be inferred that the multiplication of those in the finest soil had not progressed to such an extent as to make their colonies numerous in high dilutions, their numbers apparently being in proportion to the grain-size and amount of aeration. The most persistent non-spore-bearer was of the type already referred to, as found in clay and sand. In addition to this certain chromogenic cocci and one variety of slime former were frequent on plates from all three of these soils throughout the time of desiccation. This slime-former, which was especially numerous on plates from muck, is described as No. 6 muck.

Attention should be called to the rather peculiar circumstance that not one of the organisms isolated during the last two months corresponds to any one of the four organisms which predominated in the original soil solution used for inoculation of the five soils. (See table of organisms from soil solution). The extinction of these species may have been due either to the unfavorable influence of association with other organisms during the period of active multiplication or to their lack of endurance when supplied with less than the optimum amount of moisture.

Conclusions.

1. The survival of nonspore-bearing bacteria in air-dry soil is due, in part, to the retention by the soil, of moisture in the hygroscopic form. This, however, is not the only factor, for the longevity of bacteria in a soil is not directly proportional to its grain-size and hygroscopic moisture.

2. Bacteria, at least those tested, resist desiccation longer in a rich clay loam than in sand, all other conditions being equal.

3. If bacteria are suspended in the solution extracted from a rich clay loam, before being subjected to desiccation in sand, they live longer than if subjected to desiccation after suspension in physiological salt solution.

4. The solution extracted from a rish clay loam contains substances which have a protective influence upon bacteria subjected to desiccation.

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