EFFECT OF TEMPERATURE ON GERMINATION AND GROWTH OF THE COMMON POTATO-SCAB ORGANISM

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INTRODUCTION

The causal organism of the common potato scab which has been known to phytopathologists since 1892 as *Oospora scabies* Thaxter was recently pronounced by Lutman and Cunningham[1] as identical with *Actinomyces chromogenus* Gasperini, which was described in 1891. The writer's studies were conducted upon several strongly pathogenic strains isolated from diseased specimens received from Maine, Vermont, and Wisconsin.[2]

All these strains fruit abundantly on the so-called Thaxter's potato agar. The gray film which almost invariably occurs on the scabby spots of naturally or artificially infected tubers when first removed from the soil is made up of the same elements which constitute the fruiting stage in artificial cultures. These elements, called "gonidia," are short, cylindrical segments of aerial filaments and when mature—i. e., when the aerial growth turns from white to dark gray—were employed in making the germination studies here described. They are 1.5 to 2μ long and 0.8 to 1μ broad, with truncate ends. These bodies, after having been sown in agar and shortly before germination, become somewhat broader and rounder, sometimes oval or nearly spherical. Germ tubes may be produced at either or both ends.

EXPERIMENTAL METHODS EMPLOYED

In making the germination tests the ordinary agar hanging-block used in studying the growth of bacteria was employed. A straight transfer needle was rubbed against the surface growth of cultures bearing mature gonidia and then gently drawn across the surface of solidified agar in Petri dishes. The agar blocks for germination studies were then removed from along this inoculated streak and mounted in moist cells

[1] Lutman, B. F., and Cunningham, G. C. Potato scab. Vt. Agr. Exp. Sta. Bul. 184, 64 p., 7 fig., 12 pl. 1914.

[2] The following method was used to obtain pure cultures from these and numerous other specimens: Both the operator's hands and the diseased tuber are thoroughly washed. Then the latter is rinsed in hydrogen peroxid and dried with sterilized absorbent paper. Next the corky covering of a scabby spot is lifted off by inserting the point of a flamed scalpel under one side of it. The layer of parenchyma underneath is greenish yellow in color, owing to the action of the parasite. The discolored area thus exposed is then gently scraped with a flamed knife and a small quantity (about 1 c. c.) of the pulp transferred to tubes containing 2 or 3 c. c. of sterilized, distilled water. One or more 2 mm. loops of this dilution are transferred to tubes containing 10 c. c. of melted beef agar and the plates poured in the usual way.

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in the usual way. Beef-extract agar, without salt,[1] was found to be the most satisfactory medium for the purpose.

Immediately after the hanging-block cultures were made, the slides bearing them were placed in incubators running at the requisite temperatures. They were not removed therefrom except for a short time at the close of each hour for examination with the microscope. To avoid inaccuracies, due to possible variations in temperature in different parts of the incubator chambers, care was taken to see that the temperatures recorded were those in the immediate vicinity of the preparations studied.

THERMAL EFFECT ON GERMINATION

The maximum temperature for growth is apparently a little below 41° C., although occasionally slight evidence of the beginning of germination of gonidia was observed at this point. Germination was most rapid between 35° and 40.5°, and little or no differences were noted in various trials between these limits. At the temperatures mentioned the first evidence of growth was observed at the end of three hours, and below this the time gradually increases: 5 hours are necessary at 30°, 8 hours at 25°, 11 hours at 20°, 18 hours at 15°, and 2 days at 10° C. (fig. 1). The largest percentage of germination is usually secured at from 30° to 37° C. Unevenness in germination is evident at 25°, and from this point down it becomes more and more apparent until it is especially pronounced at 10° C. Plate XV, figures 1 and 2, illustrates the characteristic appearance of the germinating gonidia at 35° C.

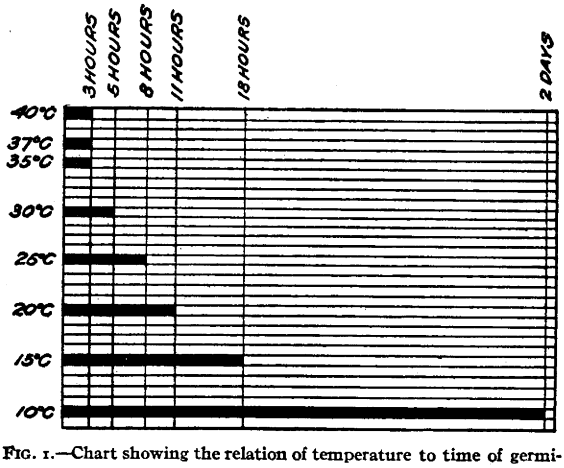


FIG. 1.—Chart showing the relation of temperature to time of germination.

No attempt was made to determine the exact minimum temperature for germination, but some previous unpublished studies of the writer indicate that it lies somewhere near 5° C. Twenty test-tube cultures, ten in beef broth and ten on potato cylinders, immediately after inoculation with material containing gonidia were placed in a refrigerator where the temperature varied from 5° to 7° C. Only a little growth was noted

[1] A modification of the formula given by F. D. Chester. (Chester, F. D. Manual of Determinative Bacteriology. p. 28. New York, 1901.)

in a few tubes at the end of one month. A few of the remaining cultures grew when taken to the laboratory, but the rest were dead.

Exposure to cold weather, several degrees below zero centigrade, does not always kill the parasite. During February and March, 1913, many test-tube cultures were exposed immediately after inoculation to freezing at outdoor temperatures and then again taken to the laboratory. The exposure in no case was longer than one week. In no instance were the organisms killed in tubes containing cooked potato cylinders, but in some cases with beef-broth cultures an exposure of five days was fatal when on some nights the thermometer registered as low as $-29°$ C.

RAPIDITY AND VIGOR OF GROWTH

Temperatures between 35° and 40° C. are most conducive to rapid germination. They are decidedly less favorable for the further development of the organism, except that at 35° the growth for the first day was more rapid than at any other temperature tested. No colonies visible to the unaided eye appear in cultures at 39.5°, and growth at this temperature practically ceases within one week. On the other hand, growth is very much retarded and slow below 20° C. Table I shows the comparative rates of germination and growth in cultures at various temperatures and at different intervals within one week.

TABLE I.—*Comparative rates of germination and growth of the common potato-scab organism at various temperatures and at different intervals*

Temperature.	Growth.						
	3 hours.	5 hours.	8 hours.	11 hours.	18 hours.	2 days.	1 week.
°C.							
40	Germination begins.	Threads 2 to 3μ	Very slight progress.	Very slight progress.	Very slight progress.	Threads 5 to 10μ.	Threads 15 to 20μ.
37	.....Do....	Threads 5 to 8μ.	Threads 8 to 14μ.	Threads 20 to 22μ.	A network of curled threads.	Small colonies formed.	Feeble colonies of curled threads.
35	.....Do....	Threads 5 to 14μ.	Threads 15 to 20μ.	Threads up to 70μ.	A complete network.	Colonies formed.	More or less complete growth along the line of inoculation.
30	.....	Germination begins.	Threads 5 to 8μ.	Threads 25 to 30μ.	A network..	.....do.....	Do.
25	.....	.....	Germination begins.	Threads 11 to 22μ.	.....do.....	.....do.....	Do.
20	.....	.....	.....	Germination begins.	Threads up to 50μ.	Formation of colonies.	Do.
15	.....	.....	.....	.....	Germination begins.	A network..	Formation of colonies.
10	.....	.....	.....	.....	.....	Germination begins.	Threads 27 to 30μ.

Observations upon cultures for longer periods, two to four weeks, indicated that the optimum temperatures for maximum growth are from 25° to 30°, with practically no difference between. The total growth produced was less above and below these points. While it was still proceeding normally but at a slower rate at the lower temperatures, at 35° it was not only less but appeared to have reached its end.

The discoloration of the medium which is very characteristic of cultures of this parasite was faint or absent at and above 35° and quite intense at and below 30° C.

INVOLUTION FORMS

High temperature is considered one of the factors influencing the production of degeneration forms of bacteria,[1] but this is apparently not the case with the potato-scab organism. While the individual filaments, which at lower temperatures are long and more or less curved, appear very short and curled at 37° C., and especially so at 39° and 40°, the writer does not consider them strictly involution forms. The gonidia, also, can not be considered as involution forms, since morphologically the same bodies occur normally upon scab spots on potato tubers and apparently serve as fruiting organs.

However, such abnormal growths may be produced by certain kinds of culture media. The writer has observed some very interesting involution forms which constantly appear at all temperatures when the scab organism is grown upon a synthetic agar that is much used in this laboratory for the cultivation of fungi.[2]

On this medium germination and growth proceeds normally at first, but after two days, if incubated at 35° to 37°, which, as has already been pointed out, are within the range of most favorable temperatures for germination and early growth, the threads become distorted and swollen at various places, both at the tips and in the middle. Sometimes even the gonidia themselves become abnormally enlarged at or before germination. At the end of a month the entire growth will consist of swollen, club-shaped, oval, or spherical segments of various sizes (Pl. XV, fig. 3 and 4). Not infrequently these abnormalities reach 4μ in diameter. The consistency of the growth thus produced is soft and somewhat slimy instead of being tough and hard, as is usually the case. By leaving out one of the ingredients of the medium at a time it was

[1] Migula, Walter. System der Bakterien . . . Bd. 1, p. 52–53. Jena, 1897.

[2] This synthetic agar was prepared according to the formula given by Darwin and Acton (Darwin, Francis, and Acton, E. H. Practical Physiology of Plants. ed. 3, p. 68. Cambridge, 1909) and consists of—

	Gm.		*Gm.*
Dextrose	50	Magnesium sulphate	2.5
Peptone	20	Potassium monophosphate	2.5
Ammonium nitrate	10	Calcium chlorid	0.1
Potassium nitrate	5	Distilled water	1,000

found that no such involution forms were produced when the potassium monophosphate alone was excluded, but they invariably appeared if it was present.[1]

SUMMARY

(1) Temperatures from 35° to 40° C. are most favorable for the germination of the gonidia of the potato-scab organism. They are unfavorable for long-continued growth, although at 35° a stimulating effect was produced at first.

(2) The maximum temperature for growth is about 40.5°, the optimum 25° to 30°, and the minimum about 5° C.

(3) Involution forms are produced, but not as the result of temperature conditions. They appeared abundantly when 0.25 per cent of potassium monophosphate was included in a synthetic culture medium.

[1] Münter observed similar involution forms in pure cultures of certain soil-inhabiting Actinomyces, but on a somewhat different synthetic medium than that used by the writer. Among the species studied by Münter *Actinomyces chromogenes* is mentioned. (Münter, F. Über Actinomyceten des Bodens. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 36, No. 15/18, p. 380–381. 1913.)

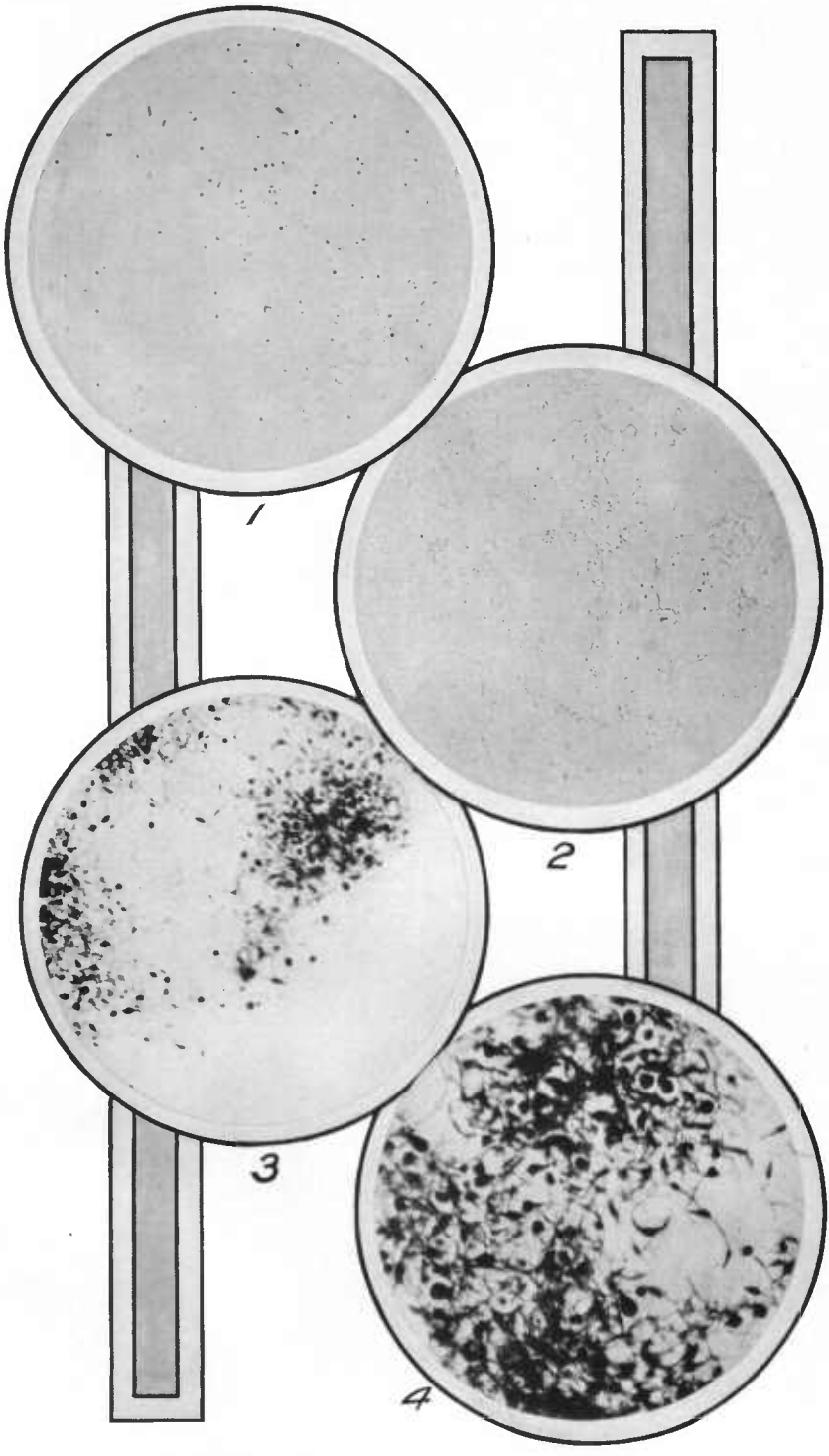
PLATE XV

Fig. 1.—Germinating gonidia of the potato-scab organism, agar hanging-block, 3 hours' incubation at 35° C. ×375.

Fig. 2.—Germinating gonidia of the potato-scab organism, agar hanging-block, 5 hours' incubation at 35° C. ×375.

Fig. 3.—Involution forms of the potato-scab organism on synthetic agar from a 1-month-old culture, stained with carbol fuchsin. ×425.

Fig. 4.—Involution forms of the potato-scab organism on synthetic agar from a 1-month-old culture, stained with carbol fuchsin. ×750.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