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Some Protein Analogies of the Mycelium of *Fusarium Lycopersici*

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Some Protein Analogies of the Mycelium of *Fusarium Lycopersici*

IRL T. SCOTT

ABSTRACT.—The experimental data reported in this paper show that when the living mycelial mats of the fungus, *Fusarium lycopersici*, are added to dilute buffered and unbuffered single-salt solutions at different reactions and the change in hydrogen-ion concentration is followed by means of electrometric or colorimetric methods the reaction is changed in every case to a final equilibrium point at or near a Sorensen value of 5.4. Dead mycelium does not give as consistent results as the living but suggests that it possesses an equilibrium point different from that for the living mycelium. Spore germination studies indicate that the toxicity of certain deleterious ions like the cations, mercuric, cupric, and basic dye—methylene blue—is greater in solutions more alkaline than p_H 5.4; while the anions, cyanide and acid dye-eosin—show greatest toxicity in solutions more acid than p_H 5.4. The simplest explanation of the observed phenomena that might be offered is that the living tissue of *F. lycopersici* behaves in a manner analogous to an amphoteric protein colloid, like gelatine, with an isoelectric point in the neighborhood of p_H 5.4.

INTRODUCTION

Proteins are formed by the condensation of a number of amino-acids, a conception due largely to the work of Emil Fischer. Amino-acids are characterized by the presence of one or more amino groups (NH_2) and generally one carboxyl group (-COOH). The former would confer basic properties on the protein; the latter, acidic properties. On the basis of their constituent amino-acids proteins are regarded as amphoteric electrolytes, reacting as acids in the presence of bases, and as bases in the presence of acids. It was pointed out by Hardy[9] that when a protein is placed under the influence of an electric field it slowly migrates toward the anode or toward the cathode, or not at all, depending upon the reaction of the dispersing medium. In acid solutions the protein behaves as a base and therefore bears a positive charge and migrates toward the cathode. In alkaline solutions the protein behaves as an acid and therefore migrates toward the anode due to its negative charge. That reaction of the dispersing medium, however, at which there is migration to neither cathode nor anode is called the "isoelectric point", and the protein is then said to be "isoelectric", no potential difference existing between the protein particles and the suspending medium.

At the isoelectric point there will be an equal number of positively charged ions and negatively charged ions, and the undissociated frac-

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tion is at a maximum. Michaelis[22] gives the following algebraic interpretation to show the condition at the isoelectric point: Let K_a and K_b be the acidic and basic dissociation constants, respectively; C_a the concentration of anions; C_k the concentration of cations; C_h and C_{oh} the concentration of the undissociated fraction. Then at equilibrium we would have the following equations:

$$\frac{C_a \cdot C_h}{x} = K_a \quad \text{(I), and} \quad \frac{C_k \cdot C_{oh}}{x} = K_b \quad \text{(II).}$$

But at the isoelectric point $C_a = C_k$, then dividing (I) by (II) gives

$$\frac{K_a}{K_b} = \frac{C_h}{C_{oh}} .$$

This relationship shows that when $K_a = K_b$ the isoelectric point would be at true neutrality (p_H 7.0). However, ampholytes are generally asymmetric as regards their acidic and basic properties so that K_a is either greater or less than K_b.

How are the physical and chemical properties of a protein affected at the isoelectric point? Hardy[9], Proctor[31], Proctor and Wilson[32], Michaelis[22], Sorensen[44], Pauli[25], Loeb[20], and others have shown that the physical and chemical properties of the proteins studied by them are at a minimum at the isoelectric point. Among the properties studied are swelling, viscosity, osmotic pressure, conductivity, combining power with anions and cations, etc.

The isoelectric point may be determined in several ways: (a) by study of the direction of movement of particles suspended in an electric field using as a dispersing medium solutions of different hydrogen ion concentration; (b) by observing swelling, viscosity, osmotic pressure, or electrical conductivity at different Sorensen units (see Loeb, et al); and (c) by observing the change in hydrogen ion concentration when an ampholyte is added to an acid or base at certain reactions. It is an elaboration of this last method that was used for the determination of the isoelectric point of plant tissue in the experimental work reported in this paper. To further elucidate this method the following is quoted from Michaelis[21]: "If a soluble ampholyte is added to a solution of definite hydrogen ion concentration, it behaves like any acid when the hydrogen ion concentration is less than that at the isoelectric point, and as a base if the hydrogen ion concentration is greater than that value. In other words, the ampholyte, like any acid, increases the hydrogen ion concentration in the first case quoted; in the second, like any base, it reduces the hydrogen ion concentration. If, however, the original hydrogen ion con-

centration is just equal to that at the isoelectric point the added ampholyte produces no change in its value."

In a series of investigations carried on in these laboratories by Robbins[11], Scott[41], Robbins[34], Robbins and Scott[36], Naylor[24], and Robbins[35], it has been shown that plant tissue responds to hydrogen ion concentration in many respects like a pure protein with a definite isoelectric point. The isoelectric point of *Fusarium lycopersici* was found to be near p_H 5.5. The object of the investigations reported in this paper was to determine how far this analogy is justified by observing whether the mycelium of the fungus, *Fusarium lycopersici*, affects the reaction of dilute buffer mixtures and unbuffered single salt solutions like a protein with an isoelectric point of p_H 5.5, and whether assuming the isoelectric point for this organism to be p_H 5.5 cations would be found to be more toxic in solutions alkaline to this point and anions more toxic in solutions acid to this point. The latter might be expected if cations combine with the protoplasm in larger quantities in solutions alkaline to the isoelectric point and anions combine in larger quantities in solutions acid to the isoelectric point.

REVIEW OF THE LITERATURE

Numerous observations can be found in the literature showing that bacteria, fungi, and seed plants, or their tissues, make the reaction of alkaline solutions more acid and that of acid solutions more alkaline.

Barratt[3], as early as 1905, determined the effect of living paramaecia upon solutions of dilute acids and alkalies. He observed that the living protoplasm of these protozoa decreased the acidity of dilute acids (HCl and H_2SO_4) and the alkalinity of dilute alkalies (NaOH and KOH) to which traces of NaCl, and Na_2SO_4 or KCl had been added. The effect was less marked with the alkalies than with the acids. Although no explanation was offered it is quite evident that there was an unequal uptake of ions.

Breazeale and Le Clerc[4] found that when they grew seedlings in culture solutions containing KCl, K_2SO_4, HCl or H_2SO_4 a selective action was evident whereby the potassium ion was absorbed by the roots and the chlorine and sulphate ions for the most part left in the solution. This produced greater acidity in the solutions which proved to be injurious to the plants. The addition of bases tended to keep the solutions alkaline thereby giving a favorable reaction for plant growth. The absorption of the ions from the solution was determined by chemical analyses at the end of the experiment.

Johnson[16] records that changes in reaction of culture solutions occurred when the roots of corn were allowed to penetrate the solution. He thinks that the amount of acidity and alkalinity developed in solu-

tions of neutral salts is too great to be accounted for by excretion from the roots, and therefore must be due to absorption by the roots of cations or anions. In order that the "electric stress" be maintained by the external solution he suggests an exchange of like ions between the plant and solution. Thus greater acidity could be accounted for by the uptake of cations for which an equivalent amount of hydrogen ion had been exchanged. Likewise there would be an exchange of hydroxyl ions for other anions. He further states that unequal absorption may be due to dead rather than living cells and presents experimental evidence showing that living beets absorbed practically equal amounts of calcium ion and chloride ion from a solution of $CaCl_2$, but that dead beet absorbed a greater amount of calcium ion (cation) than chlorine ion (anion).

Stiles and Jörgensen[49] report that potato tuber tissue reduces the acidity of dilute solutions of HCl, and the alkalinity of dilute solutions of NaOH.

Hoagland[10] studying the effect of hydrogen and hydroxyl ion concentration on the growth of barley seedlings noted that there was a strong tendency on the part of the plant to change the reaction of various potassium phosphate solutions toward neutrality. As a result of chemical analysis of the solutions he states that selective absorption of various phosphoric acid anions probably occurs accompanied by a removal of positive ions.

Arrehenius[1] reported that plant roots change the reaction of solutions, the direction of the change depending upon the plant. Rye shifted the reaction of solutions of p_H 3.0 and 9.0 to 5.8; peas shifted the reactions of p_H 3.0 and 8.0 to 4.5; and corn shifted the reactions of p_H 3.0 and 7.5 to 6.5.

Jones and Shive[19] found that when wheat seedlings grown in "Shive's Best" solution for 5 weeks were transferred to nutrient solutions the reaction became less acid after 24 and 52 hours. The initial reactions were in most cases p_H 4.0 and 4.8. At the end of the experiment the reactions were from p_H 5.3 to 6.1. Solutions with reactions near neutral remained practically unchanged. Later, Jones[18] found that the roots of wheat and soybeans in contact with one of Shive's solutions with initial p_H values of 4.3, 4.5, and 4.9, respectively, decreased the hydrogen ion concentration of the solutions.

Arrhenius[2] in a more recent paper, reported that rice shifted the reaction of nutrient solutions to p_H 6.2.

The work of Rudolfs[38] who studied the effect of seeds upon the hydrogen ion concentration of solutions of a number of single salts with different concentrations is quite interesting in connection with the experimental work presented in this paper. He determined the effect of a variety of seeds such as corn, wheat, watermelon, peas, buckwheat,

lupine, rape, and alfalfa upon solutions of $MgSO_4$, $NaNO_3$, $Ca(NO_3)_2$, NaCl, KCl, and K_2CO_3. The results show that there is a marked tendency for the seeds of a particular species to change the reaction of the solutions to a definite equilibrium point after immersion for a comparatively brief period of time. At the weaker concentrations, however, there was little or no change in reaction by the seeds, the behavior being comparable to that obtained in distilled water. He states that the only factor which could account for the rapid change in reaction in the solutions used "is that directly related to ion absorption by the seeds, the hydrogen ion concentration increasing as the cations are removed from solution by absorption at a more rapid rate than the anions."

Tarr and Noble[50] found that when Fulcaster wheat seedlings were grown in an unbuffered series of nutrient solutions the reaction of solutions with initial p_H values of 3.77 to 5.17 became less acid, while solutions with initial p_H values of 5.79 became slightly more acid, all final reactions occurring in a range between p_H 5.1 and 5.5. With more strongly buffered solutions there was but little shifting of reaction during the growth period used.

In rather extensive studies on water absorption by living potato tuber tissue, Robbins[33] has shown that disks of potato tuber show a minimum of water absorption in the vicinity of p_H 6.0 when immersed in dilute buffer mixtures at different hydrogen ion concentrations. He further notes that the tissue changed the reaction of solutions more acid than p_H 5.5 to less acid; those more alkaline than p_H 5-6.1 remained unchanged or became more acid. He suggests that the results obtained show an analogy between potato tuber tissue and a colloidal ampholyte with an isoelectric point in the neighborhood of p_H 6.0.

Hoagland[11] reports studies of ionic absorption by barley, peas and cucumbers in various salt solutions and finds that "there is a very general tendency for the plant to change the reaction toward the neutral point, whether the initial reaction be acid or alkaline" as far as solutions containing nitrate are concerned. The behavior of the seedlings immersed in single salts show this tendency to be quite consistent in nitrates, but differs with other salts.

Robbins and Scott[36] have recently reported results of experiments in which the change of reaction of dilute buffer solutions containing potato tuber tissue and root-tips of soybeans was followed electrometrically. They found disks of potato tuber tissue shifted the reaction of solutions initially p_H 6.5 or greater toward an equilibrium point in the vicinity of p_H 6.3, while the reaction of solutions initially p_H 6.14 or less became more alkaline. In a like manner, soybean root-tips caused solutions with reactions of p_H 6.68 or greater to become more acid, and solutions of p_H 6.2 or less to become more alkaline.

Rudolfs[39, 40] has recorded further experiments of selective absorption by seeds immersed in various solutions including single salts and certain inorganic and organic acids. Curves constructed for a single species show a shifting of reaction towards a constant equilibrium point with time comparable to those shown in this paper for the fungous mycelium of *Fusarium lycopersici*.

Several workers have cited data showing that fungi may shift the reaction of solutions from acid and alkaline reactions toward more or less fixed equilibrium points. Johnson[17] studied the growth of seven soil molds in relation to the hydrogen ion concentration and salt concentration. All the species changed the reaction of the nutrient solutions in which grown, acid reactions being made more alkaline, and strongly alkaline ones more acid. His graphs show a marked tendency on the part of most of the molds to shift the reaction toward a common equilibrium point located in a limited range of hydrogen ion concentration on the alkaline side of neutrality. He offers no explanation for the change in reaction but suggests that acid production from carbohydrates and the use of basic ions causing more acidity or of acid radicals causing more alkalinity could be contributing factors.

Sherwood[42] found *Fusarium lycopersici* to increase the acidity of nutrient solutions when the initial reactions of the solutions were between p_H 3.6 and 8.4. In other words, the reaction tended to shift from values alkaline to p_H 3.6 toward this point.

Scott[41] reported that, in general, the development of mycelial growth of *Fusarium lycopersici* in a rather strongly buffered nutrient solution shifted the reaction toward greater alkalinity when the initial p_H was less than 5.6 and toward greater acidity when greater than 6.0. The common point toward which the change tended was in a range between p_H 5.6 to 6.0. It was suggested that the behavior was somewhat analogous to that of a colloidal ampholyte with an isoelectric point in a range between p_H 5.6 and 6.1.

Robbins and Scott[36] also determined the effect of living mycelial mats of three species of fungi on the reaction of dilute buffer mixtures in the same manner as noted above for potato tuber tissue and soybean root-tips. On the basis of a protein analogy their results are interpreted to indicate that *Gibberella saubinetii* has an isoelectric point near p_H 6.2; the mycelium of *Fusarium lycopersici*, near p_H 5.5; and the mycelium of *F. oxysporum*, near p_H 4.9.

Sideris[43] presents evidence showing that the particular hydrogen ion concentration toward which *Fusarium cromyophthoron* shifted the reaction depended upon the composition of the nutrient medium especially as regards the carbon source and found the final reaction to vary when different substances like dextrose, amygdalin, peptone, potato starch,

etc., were used. He calls this critical point the "isometabolic point" which is different but specific for certain substances.

In conclusion, it might be added, that several workers with bacteria have observed these organisms to exert an effect on nutrient solutions to the extent of shifting the reaction from certain initial values toward a more or less common point. Itano[14] found that when *Bacillus subtilis* was grown at reactions between p_H 4.18 and 9.43 an alteration of the reaction occurred resulting in a final value at an average p_H of 8.076. Acid reactions were made distinctly alkaline, and strongly alkaline reactions were slightly changed toward increased acidity. He suggests that the alteration observed is an "automatic adjustment" due to the presence of a so-called "protective substance" familiar in enzymatic work. Fred and Loomis[8] showed that *Bacillus radicicola* grew throughout a wide range of hydrogen ion concentration (p_H 3.9-11.1), but that the reaction approaches the neutral point during growth. Wyeth[55] reported that *Bacillus coli* grown in 2% peptone with initial reactions ranging from p_H 4.29 to 9.37 gave a final reaction in the range p_H 5.92-8.55. Waksman and Joffe[51] state that Actinomycetes tend to change the reaction of the medium in which grown toward an optimum.

While the results recorded above are, in general, analogous to the effect of a protein like gelatine on the reaction of solutions, only certain of them can be ascribed to such an effect. Even though organisms or their tissues may affect the reaction of solutions in which they are placed or are growing in a way analogous to the effect of a protein there are many other factors which may be involved particularly in cases where the time is of any considerable duration. The metabolism of the organism involving the production of carbon dioxide, the formation of organic acids, the utilization of organic acids, proteolysis with the production of ammonia or other basic nitrogenous materials, the excretion of basic or acidic substances other than those mentioned may affect the reaction of the solution and frequently do when microorganisms are grown. Differences in permeability and the unequal absorption of ions due to the utilization of one in the synthetic processes of the plant may also be factors.

In order to eliminate the majority of the factors mentioned above the experiments performed with the mycelium of *Fusarium lycopersici* which are described later were limited to relatively short periods of time in which the formation of carbon dioxide, formation of organic acids and their utilization, proteolysis, production of ammonia or other basic substances were not contributing factors in the relatively simple salt solutions used.

Experimental Work

I. THE DETERMINATION OF THE ISOELECTRIC POINT OF LIVING MYCELIUM

The plant tissue employed was mycelium of a strain of *Fusarium lycopersici* used previously by Scott[41], Robbins[34], and Robbins and Scott[36]. The fungus was grown in all cases on a mineral nutrient solution of the following composition:

Potassium nitrate (KNO_3)	3 gms.
Magnesium sulfate ($MgSO_4.7H_2O$)	0.75 gms.
Monobasic potassium phosphate (KH_2PO_4)	3.64 gms.
Dibasic potassium phosphate (K_2HPO_4)	6.96 gms.
Ferric chloride ($FeCl_3$)	1 cc. of a 0.1% solution
Dextrose	10 gms.
Distilled water to make	1000 c.c.

After sterilization this solution has a reaction of about p_H 6.7.

Eighty c.c. of the nutrient solution were placed in 250-c.c. Erlenmeyer flasks, inoculated, and incubated at 28°C. By the end of four days mats of mycelium completely covered the surface of the culture solution. These mats increase in thickness until about the twenty-fifth day when autolysis begins. The ages of the mats used in the experiments varied from 4 to 21 days, all being of the same age in any single experiment as indicated in the experimental data.

Preceding each experiment a sufficient number of mycelial mats were carefully removed from the culture flasks and thoroughly washed in 0.2 M cane sugar solution or in redistilled water. The results obtained were the same in either case. After washing the mats were pressed between sheets of paper toweling or filter paper to remove the surplus water from the surface. The mats, being of equal diameter, were stacked one upon the other and sectors cut out from the stack for use in the experiment.

Various weights of fungous tissue were placed in given quantities of buffer solutions, and in solutions of unbuffered single salts, adjusted to different hydrogen ion concentrations by means of suitable acids and alkalies. The effect of the tissue upon the reaction of the solution was determined either electrometrically or colorimetrically. The results show that the behavior of the fungous tissue is analogous to that of a colloidal ampholyte in that the reaction of the suspending solution is changed to an equilibrium point which is remarkably constant and therefore comparable to the isoelectric point.

The electrometric procedure was as follows: The apparatus used consisted of a modified Hildebrand type of bubbling hydrogen electrode,

a Leeds Northrup type K potentiometer, type R galvanometer, and a saturated KCl-calomel electrode. A KCl bridge consisting of a glass siphon with a wooden plug inserted in the end making contact with the solution in the hydrogen electrode vessel reduced the diffusion of the KCl to a minimum but gave no difficulty in making the hydrogen ion determinations. The light used in the experiments was furnished by a 40-watt bulb. The solution was brought to equilibrium in the hydrogen electrode vessel before adding the mycelium. A given quantity of mycelium was then added and the change in reaction followed with the electrode until equilibrium was reached.

In earlier experiments the quantity of pressed mycelium added was weighed, but later no weights were taken as sufficient amounts were used to establish complete equilibrium in the solution.

The colorimetric procedure was as follows: A small quantity of the desired solution was placed in a 150-c.c. Pyrex beaker. To this increments of washed and pressed mycelium were added. After varying intervals of time 1 c.c. samples of the suspending solution were removed and the hydrogen ion concentration determined. For the hydrogen ion determinations the double wedge comparator made by Stirlen & Wallace was used. The standards were checked frequently with standard buffer solutions (phthalate and acetate), the reaction of which had been carefully checked with the hydrogen electrode. Determinations were also frequently checked by means of Gillespie's double-tube standards. The experiments in which the indicator method was employed were carried out in ordinary daylight in the laboratory. Experiments were run in duplicate or triplicate. The most striking differences between the electrometric and colorimetric methods were that in the former method carbon dioxide was excluded by the constant bubbling of hydrogen through the solution and anaerobic conditions existed. The equilibrium points reached in the experiments show that these differences were evidently without effect.

1. Experiment With Reactions Acid to the Isoelectric Point.

Buffered Solutions

EXPERIMENT 1.—Sodium phosphate. A 50 c.c. solution of 0.002M monobasic sodium phosphate adjusted to an initial reaction of p_H 4.03 with 0.002 M phosphoric acid was used. To this was added mycelium from 7-day-old cultures weighing 5.08 grams. The change in reaction was followed with the hydrogen electrode at a temperature of 22°C. Equilibrium was established after 65 minutes at p_H 5.50.

EXPERIMENT 2.—Sodium phosphate. Fifty c.c. of a 0.001 M solution of monobasic sodium phosphate having an initial reaction of p_H 4.55 were used. To this were added 5.08 grams of mycelium from 7-day-old cul-

tures. The change in reaction was followed with the hydrogen electrode at a temperature of 22°C. Equilibrium was established after 11 minutes at p_H 5.41.

EXPERIMENT 3.—Potassium phosphate. Ten c.c. of a 0.001 M solution of monobasic potassium phosphate adjusted to an initial reaction of p_H of 4.70 were used. To this were added 1.45 grams of mycelium from 16-day-old cultures. The change in reaction was determined electrometrically at a temperature of 22°C. Equilibrium was reached at p_H 5.0 after 59 minutes.

EXPERIMENT 4.—Sodium acetate--acetic acid. Ten c.c. of a 0.01 M sodium acetate—acetic acid buffer solution with an initial reaction of p_H 4.36 were used. Small increments of mycelium from 13-day-old cultures were added from time to time. Reaction changes were determined electrometrically at a temperature of 22°C. Equilibrium was reached at p_H 5.38 after 80 minutes.

EXPERIMENT 5.—Sodium acetate—acetic acid. Four beakers, each containing 25 c.c. of a 0.001 M sodium acetate—acetic acid buffer solution with an initial reaction of p_H of 4.6 were prepared. About 3 grams of mycelium from 21-day-old cultures were added to each of three of the solutions while the fourth was retained as a check. After equilibrium had been reached at p_H 5.35-5.4 in the first three solutions the excess liquid was poured from each into clean beakers and then 3 grams of fresh mycelium added to each including the check. The changes in reaction were determined colorimetrically at a temperature of 25°C. The equilibrium point in four cases is practically p_H 5.4, reached after from 30 to 45 minutes.

EXPERIMENT 6.—Potassium acid phthalate. Ten c.c. of a 0.01 M solution of potassium hydrogen phthalate with an initial reaction of p_H 3.95 were used. Mycelium from 8-day-old cultures weighing 1.5 grams was added. The reactions were determined electrometrically at a temperature of 23°C. The results are recorded in Table 1. The equilibrium point of p_H 5.41 was reached after 16 minutes.

EXPERIMENT 7.—Potassium acid phthalate. This experiment was a repetition of Experiment Six, using the electrometric method. An equilibrium point of p_H 5.47 was reached in 11 minutes.

EXPERIMENT 8.—Potassium acid phthalate. To 12 c.c. of a 0.001 M solution of potassium acid phthalate with an initial reaction of p_H 4.11 were added 1.31 grams of mycelium from 18-day-old cultures. Hydrogen ion determinations were made electrometrically at a temperature of 22.5°C. Equilibrium was reached after 80 minutes at p_H 5.46.

EXPERIMENT 9.—Potassium acid phthalate. Ten c.c of a 0.001 M solution of potassium acid phthalate with an initial p_H of 4.18 were used.

Increments of mycelium from 14-day-old cultures were added. Determination of reaction changes was made electrometrically at a temperature of 21°C. Equilibrium was established at pH 5.66 after 66 minutes.

TABLE 1.—Effect of mycelium of *Fusarium lycopersici* on the reaction of dilute single-salt buffer solutions.

Experiment 6		Experiment 14	
Min.	pH	Min.	pH
0	3.95	0	7.20
1	4.92	4	6.40
2	5.02	8	6.25
3	5.10	12	6.00
5	5.22	14	5.90
8	5.32	19	5.75
11	5.36	23	5.65
16	5.38	29	5.55
20	5.40	34	5.52
24	5.41	39	5.52
30	5.41	44	5.52

EXPERIMENT 10.—Potassium acid phthalate. Four beakers, each containing 25 c.c. of a 0.001 M potassium phthalate buffer solution adjusted with 0.001 potassium hydroxide to an initial reaction of pH 4.5 were prepared. About 3 grams of mycelium from 20-day-old cultures were added to each of three solutions while the fourth was retained as a check. After equilibrium had been reached at pH 5.30-5.36 in the first three solutions the excess liquid was poured from each into clean beakers and then 3 grams of fresh mycelium added to each including the check. Changes in reaction were determined colorimetrically at a temperature of 24°C. Equilibrium was established in all four cases at about pH 5.3-5.4 after 30 to 40 minutes. No further change in reaction resulted in the first three solutions upon the addition of fresh mycelium except 0.05 of a Sorensen unit in the first solution, and 0.01 in the third.

Unbuffered Single Salt Solutions

EXPERIMENT 11.—Potassium chloride. Twenty c.c of 0.001 M potassium chloride were adjusted to an initial reaction of pH 4.6 using 0.001 M hydrochloric acid. Increments of mycelium from 9-day-old cultures were added to duplicate solutions at 23°C. and the change in reaction determined colorimetrically. The final reaction reached in both solutions was pH 5.6 after from 27 to 40 minutes.

EXPERIMENT 12.—Calcium chloride. Twenty c.c. of a 0.001 M calcium chloride solution were adjusted to an initial reaction of pH of 4.5

using 0.001 M hydrochloric acid. Increments of mycelium from 9-day-old cultures were added to duplicate solutions at 22°C. and the change in reaction determined colorimetrically. The reactions at equilibrium in the two solutions were p_H 5.7 and 5.6, respectively, after 35 minutes.

EXPERIMENT 13.—Copper sulfate. Twenty c.c. of 0.001 N copper sulfate solution were adjusted to an initial reaction of p_H 4.1 with 0.001 N sulphuric acid. Increments of mycelium from 19-day-old cultures were added to duplicate solutions at 21°C. and the reaction determined colorimetrically. The final reactions were p_H 5.30 and 5.40 respectively, after 30 to 70 minutes.

2. Experiments With Reactions Alkaline to the Isoelectric Point.

Buffered Solutions

EXPERIMENT 14.—Sodium phosphate. Ten c.c. of a 0.001 M solution of sodium phosphate buffer with a reaction of p_H 7.20 were used. To this were added 1.63 grams of mycelium from 4-day-old cultures and the changes in reaction determined electrometrically at 23.5°C. The results are recorded in Table 1. Equilibrium was reached after 34 minutes at p_H 5.52.

EXPERIMENT 15.—Sodium phosphate. A solution identical to that in Experiment 14 was used, except that the reaction was adjusted to an initial reaction of p_H 6.66. Changes in reaction were determined electrometrically after adding 0.635 grams of mycelium from 5-day-old cultures at a temperature of 24°C. Equilibrium was reached after 39 minutes at p_H 5.46.

EXPERIMENT 16.—Sodium phosphate. A solution identical to that used in Experiments 14 and 15 was employed, except that the reaction was adjusted to p_H 8.50. Small increments of mycelium from 7-day-old cultures were added until no further change in reaction occurred. Reaction changes were determined electrometrically at a temperature of 23°C. The addition of fresh mycelium after 62 minutes did not change the final reaction reached, p_H 5.52.

EXPERIMENT 17.—Potassium phosphate. Twenty c.c. of 0.001 M potassium phosphate buffer solution with a reaction of p_H 6.1 were placed in each of two 150 c.c. beakers to which increments of mycelium from 16-day-old cultures were then added at a temperature of 22°C. Changes in reaction were determined colorimetrically. After about 100 minutes equilibrium was reached at p_H 5.45, and 5.40, respectively.

EXPERIMENT 18.—Sodium acetate—acetic acid. Duplicate solutions of 0.001 M sodium acetate buffer of 20 c.c. each with a reaction of p_H 7.3 were prepared. To these were added increments of mycelium from 12-day-old cultures and the change in reaction determined colorimetrically

at a temperature of 23°C. After 136 minutes both solutions reached equilibrium at p_H 5.4 after which the addition of fresh increments of mycelium produced no further change.

EXPERIMENT 19.—Potassium phthalate. Two solutions, consisting of 20 c.c. each of 0.001 M potassium phthalate adjusted to an initial reaction of p_H 6.5 with 0.001 M potassium hydroxide were prepared. To each solution increments of mycelium from 21-day-old cultures were added and the changes in reaction determined colorimetrically at a temperature of 24°C. Equilibrium was established after about 140 minutes at a reaction of p_H 5.5.

Unbuffered Single Salt Solutions

EXPERIMENT 20.—Potassium chloride. Twenty c.c. of 0.01 M potassium chloride were placed into each of two 150 c.c. Pyrex beakers. The initial reaction of the solutions was p_H 6.7. To these solutions increments of mycelium from 9-day-old cultures were added at a temperature of 22°C. until equilibrium was established after about 60 minutes at p_H 5.45-5.50, the changes in reaction being determined colorimetrically.

EXPERIMENT 21.—Potassium chloride. Twenty c.c. of 0.001 M potassium chloride with an initial reaction of p_H 6.9 were used. To this solution increments of mycelium from 16-day-old cultures were added until equilibrium was reached at p_H 5.58 after which no further change was produced by the addition of fresh mycelium. The reactions were determined colorimetrically at 22°C.

EXPERIMENT 22.—Potassium chloride. This experiment was identical to Experiment 21, excepting that 25 c.c. of solution were used to which increments of mycelium from 9-day-old cultures were added at a temperature of 23°C. Duplicate solutions were prepared. Determinations of hydrogen ion concentration were made colorimetrically. Equilibrium was reached at p_H 5.40 after 90 minutes.

EXPERIMENT 23.—Calcium chloride. Twenty c.c. of 0.001 M calcium chloride with a reaction of p_H 6.85 were placed into each of two 150-c.c. Pyrex beakers. To the two solutions were added increments of mycelium from 9-day-old cultures at 22°C. until equilibrium was reached. Reaction changes were determined colorimetrically. The equilibrium reaction was reached after about 135 minutes at p_H 5.3 and 5.4, respectively.

EXPERIMENT 24.—Magnesium chloride. Using the same procedure as in the preceding experiment duplicate solutions containing 20 c.c. of 0.001 M magnesium chloride with a reaction of p_H 6.1 were prepared. To these were added increments of mycelium from 10-day-old cultures. Changes in reaction were determined colorimetrically at 21°C. Equilibrium was reached after about 100 minutes at p_H 5.30-5.40.

EXPERIMENT 25.—Copper sulfate. Duplicate solutions of 20 c.c. each of 0.001 N copper sulfate with a reaction of p_H 5.9 were prepared,

to which were then added increments of mycelium from 19-day-old cultures. Changes in reactions were determined colorimetrically at a temperature of 21°C. Equilibrium was reached after 90 to 120 minutes at p_H 5.4 to 5.5.

3. Experiments with the Reactions at or near Isoelectric Point

EXPERIMENT 26.—Sodium phosphate. Fifty c.c. of 0.001 M sodium phosphate buffer solution adjusted to an initial reaction of p_H 5.43 were used. To this solution 4.04 grams of mycelium from 9-day-old cultures were added and the changes in reaction determined electrometrically at 22.5°C. Practically no change in reaction occurrred in this solution equilibrium resulting after a change of only 0.05 of a Sörensen unit.

TABLE 2.—Effect of mycelium of *Fusarium lycopersici* on the reaction of dilute single-salt unbuffered solutions (KCl).

Experiment 27			
I		II	
Min.	p_h	Min.	p_h
0	5.30	0	5.30
10	5.40	10	5.45
20	5.40	22	5.45
38	5.40	39	5.45
*40	.....	*40	.....
60	5.40	60	5.42
70	5.40	70	5.40

* Indicates the addition of fresh mycelium.

EXPERIMENT 27.—Potassium chloride. Twenty c.c. of 0.001 M potassium chloride adjusted to an initial reaction of p_H 5.3 with 0.001 M hydrochloric acid were prepared in duplicate and placed in 150 c.c. Pyrex beakers. Increments of mycelium from 9-day-old cultures where then added and the changes in reaction determined colorimetrically at 22°C. The results are given in Table 2. No appreciable change in reaction occurred in these solutions.

EXPERIMENT 28.—Calcium chloride. Twenty c.c. of 0.001 M calcium chloride adjusted to a reaction of p_H 5.25 with 0.001 M hydrochloric acid were placed in each of two 150 c.c. Pyrex beakers. To these solutions were added increments of mycelium from 9-day-old cultures. Colorimetric methods were used to determine the changes in reaction at a temperature of 22.5°C. The changes in reaction in the two flasks were from the initial at p_H 5.25 to a final of p_H 5.5, and 5.6, respectively.

EXPERIMENT 29.—Mercuric chloride. Duplicate solutions of 20 c.c. each of 0.001 M mercuric chloride adjusted to an initial reaction of p_H 5.3, using 0.001 M hydrochloric acid, were prepared. To each were added increments of mycelium from 10-day-old cultures. Reaction changes were determined colorimetrically at 21.5°C. The reaction of the solutions changed to p_H 5.5 and 5.4, respectively, as equilibrium was established.

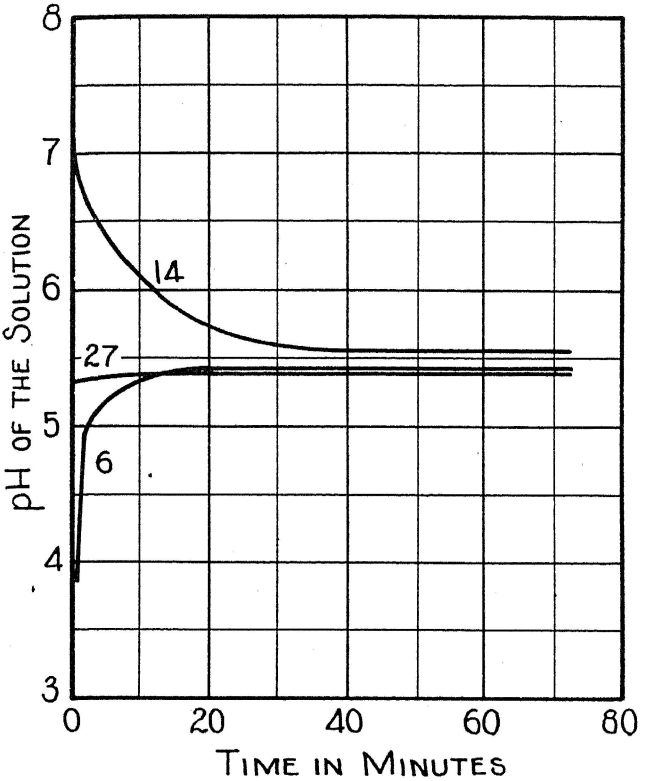

FIG. 1.—Typical curves showing the effect of living mycelium of *Fusarium lycopersici* on the reaction of solutions with different hydrogen-ion concentrations. Experiments Six, Fourteen, and Twenty-seven.

General Summary of the Experiments with Living Mycelium

In Table 3 are summarized the results of Experiments 1 to 29, inclusive. In Tables 1 and 2 are recorded the results of three typical experiments, i. e., experiments 6, 14, and 27, respectively. Curves are shown in Fig. 1 for these three experiments. It may be observed that with the buffered and unbuffered solutions the acidity in solutions acid to about p_H 5.4 is decreased, while in solutions more alkaline than p_H 5.4, the alkalinity is decreased, the equilibrium point in all cases being at or near p_H 5.4. In all solutions with an initial reaction at or near the equi-

TABLE 3.—Summary of the experiments showing the effect of the living mycelium of *Fusarium lycopersici* on the reaction of buffered and unbuffered single-salt solutions at different hydrogen-ion concentrations. Experiments 1 to 29, inclusive.

Expt. No.	Solution Used	Concn.	Age of mat	Wt. of mat	Initial pH	Final pH	H-ion method used
			da.	*gms.*			
1	Na-Phosphate	0.002 M	7	5.08	4.03	5.50	Electrode
2	Na-Phosphate	0.001 M	7	5.08	4.55	5.41	Electrode
3	K-Phosphate	0.001 M	16	1.45	4.70	5.26	Electrode
4	Na-Acetate	0.01 M	13	----	4.36	5.38	Electrode
5-I	Na-Acetate	0.001 M	21	3.00	4.60	5.35	Indicator
5-II	Na-Acetate	0.001 M	21	3.00	4.60	5.40	Indicator
5-III	Na-Acetate	0.001 M	21	3.00	4.60	5.40	Indicator
5-IV	Na-Acetate	0.001 M	21	5.50	4.60	5.45	Indicator
6	K-Phthalate	0.01 M	8	1.50	3.95	5.41	Electrode
7	K-Phthalate	0.01 M	8	1.50	3.95	5.47	Electrode
8	K-Phthalate	0.001 M	18	1.31	4.11	5.46	Electrode
9	K-Phthalate	0.001 M	14	----	4.18	5.66	Electrode
10-I	K-Phthalate	0.001 M	20	3.00	4.50	5.30	Indicator
10-II	K-Phthalate	0.001 M	20	3.00	4.50	5.30	Indicator
10-III	K-Phthalate	0.001 M	20	3.00	4.50	5.36	Indicator
10-IV	K-Phthalate	0.001 M	20	3.00	4.50	5.34	Indicator
11-I	K-Chloride	0.001 M	9	----	4.60	5.60	Indicator
11-II	K-Chloride	0.001 M	9	----	4.60	5.60	Indicator
12-I	Ca-Chloride	0.001 M	9	----	4.50	5.70	Indicator
12-II	Ca-Chloride	0.001 M	9	----	4.50	5.60	Indicator
13-I	Cu''-Sulfate	0.001 N	19	----	4.10	5.30	Indicator
13-II	Cu''-Sulfate	0.001 N	19	----	4.10	5.40	Indicator
14	Na-Phosphate	0.001 M	4	1.63	7.20	5.52	Electrode
15	Na-Phosphate	0.001 M	5	.635	6.66	5.46	Electrode
16	K-Phosphate	0.001 M	7	----	8.50	5.52	Electrode
17-I	K-Phosphate	0.001 M	16	----	6.10	5.45	Indicator
17-II	K-Phosphate	0.001 M	16	----	6.10	5.40	Indicator
18-I	Na-Acetate	0.001 M	12	----	7.30	5.50	Indicator
18-II	Na-Acetate	0.001 M	12	----	7.30	5.40	Indicator
19-I	K-Phthalate	0.001 M	21	----	6.50	5.50	Indicator
19-II	K-Phthalate	0.001 M	21	----	6.50	5.55	Indicator
20-I	K-Chloride	0.01 M	9	----	6.70	5.50	Indicator
20-II	K-Chloride	0.01 M	9	----	6.70	5.45	Indicator
21	K-Chloride	0.001 M	16	----	6.90	5.58	Indicator
22-I	K-Chloride	0.001 M	9	--	6.90	5.40	Indicator
22-II	K-Chloride	0.001 M	9	----	6.90	5.40	Indicator
23-I	Ca-Chloride	0.001 M	9	----	6.85	5.30	Indicator
23-II	Ca-Chloride	0.001 M	9	----	6.85	5.40	Indicator
24-I	Mg-Chloride	0.001 M	10	----	6.10	5.30	Indicator
24-II	Mg-Chloride	0.001 M	10	----	6.10	5.40	Indicator
25-I	Cu''-Sulfate	0.001 N	19	----	5.90	5.40	Indicator
25-II	Cu''-Sulfate	0.001 N	19	----	5.90	5.50	Indicator
26	Na-Phosphate	0.001 M	9	4.04	5.43	5.48	Indicator
27-I	K-Chloride	0.001 M	9	----	5.30	5.40	Indicator
27-II	K-Chloride	0.001 M	9	----	5.30	5.40	Indicator
28-I	Ca-Chloride	0.001 M	9	----	5.25	5.60	Indicator
28-II	Ca-Chloride	0.001 M	9	----	5.25	5.55	Indicator
29-I	Hg''-Chloride	0.001 M	10	----	5.30	5.50	Indicator
29-II	Hg''-Chloride	0.001 M	10	----	5.30	5.40	Indicator

Note: Where the weight of the mat has been omitted increments of mycelium were added and therefore no weights taken.

librium point the reaction is not appreciably altered. This is what would be expected if we assume the living plant tissue to behave as an amphoteric protein with an isoelectric point at about p_H 5.4. The results do not vary with the different salts used, with the concentration of the salts, with the temperature ranges employed, with the method of determining the hydrogen ion concentration, with kind of light used, with the age of the mycelium, nor with the solutions used in washing the mats. It might be added that larger increments of mycelium are required to bring about equilibrium when the reactions of the solutions are initially farther removed from p_H 5.4, when buffer salts are used, and when the solutions are more concentrated. This would indicate that the reaction between the plant tissue and the suspending solutions was more or less quantitative as would be the case if chemical combination occurs. It was observed that when solutions in which equilibrium had been reached were allowed to stand for as long as 15 or 20 hours no appreciable change of reaction from that at the equilibrium point occurred, but when allowed to stand for longer periods of time a change in reaction was brought about. These changes were undoubtedly promoted by growth of the mycelium which exerted an influence due to metabolic activities.

Since the living mycelium in the preceding experiments shows remarkable constancy in its chemical behavior the question arises as to whether the dead mycelium would behave in a like manner when added to acid and alkaline solutions, and exhibit a definite equilibrium point comparable to the isoelectric point of an ampholyte. It is interesting to note in this connection that Michaelis and Davidsohn[23] showed that denatured albumin exhibited an isoelectric point at a lower H-ion concentration than that of the genuine albumin. Endler[6] found that death of the cell changed the isoelectric point of elodea to a more alkaline reaction. Johnson[16] reports that dead roots of beets absorbed greater amounts of cation from a solution of calcium chloride than living roots, the latter absorbing practically equal amounts of cation and anion. However, Robbins[33] found, as far as his methods permitted, that dead potato tuber tissue showed the same minimum for water absorption as the living tissue. Therefore the following experiments were undertaken for the purpose of ascertaining the behavior of the dead mycelium of *Fusarium lycopersici*.

II. THE DETERMINATION OF THE ISOELECTRIC POINT OF DEAD MYCELIUM

The general procedure of the experiments with dead mycelium was the same as with the living except that after washing and drying the mycelial mats by pressing between sheets of paper toweling or filter paper the mats were killed by using one of the following methods: (a)

treating for various lengths of time with 50 per cent ethyl alcohol, (b) placing in water at a temperature of 80-90°C. for varying periods of time, and (c) treating with toxic concentrations of bichloride of mercury. In all cases the mats became flaccid, sticky and considerably shriveled as a result of the killing as contrasted to the more or less turgid, elastic and smooth condition of the living ones. Definite weights of the dead mycelium were not taken in all cases but increments were added until equilibrium was reached. All mats were thoroughly rinsed in redistilled water before placing them in the salt solutions.

1. Experiments with Reactions Acid to the Isoelectric Point

EXPERIMENT 30.—Potassium phosphate. Ten c.c. of 0.001 M potassium phosphate buffer solution with an initial reaction of p_H 4.9 were prepared. To this solution 3.16 grams of mycelium from 15-day-old cultures killed by immersion for 30 minutes in 50 per cent ethyl alcohol were added. Changes in reaction were determined electrometrically at a temperature of 23°C. Equilibrium was established in about 45 minutes at p_H 5.90.

EXPERIMENT 31.—Sodium acetate—acetic acid. Twenty-five c.c. of 0.001 M sodium acetate buffer solution with a reaction of p_H 4.55 were prepared in duplicate in 150 c.c. Pyrex beakers. Increments of mycelium from 16-day-old cultures were added after killing by immersion in water at 80-90°C. for 20 minutes. Hydrogen ion determinations were made colorimetrically at a temperature of 22°C. Equilibrium was reached at p_H 5.90 and 5.85, respectively.

EXPERIMENT 32.—Sodium acetate—acetic acid. Duplicate solutions were prepared as in the preceding experiment except that the reaction was adjusted to p_H 4.6. Increments of mycelium from 19-day-old cultures killed by immersion for one hour in 50 per cent ethyl alcohol were added to the solutions. Reaction changes were determined colorimetrically at 24°C. Equilibrium was reached at p_H 4.8 and 4.85, respectively, although fresh quantities of mycelium were added.

EXPERIMENT 33.—Sodium acetate—acetic acid. Ten c.c. of 0.01 M sodium acetate buffer solution with a reaction of p_H 4.37 were prepared. Increments of mycelium from 13-day-old cultures killed by immersion for 40 minutes in 50 per cent ethyl alcohol were added. Hydrogen ion determinations were made electrometrically at 22°C. Equilibrium was at p_H 7.59 after 78 minutes.

EXPERIMENT 34.—Potassium phthalate. Duplicate solutions of 20 c.c. each of 0.001 M potassium phthalate buffer at a reaction of p_H 4.5 were prepared. Increments of mycelium from 17-day-old cultures were used after killing by immersion in water at 80-90°C. for 20 minutes. Reaction changes were determined colorimetrically at 23°C. Equilibrium was reached after 65 minutes at p_H 5.65 and 5.70, respectively.

EXPERIMENT 35.—Potassium phthalate. This experiment was identical with Experiment 34 except that the initial reaction of the solution was p_H 4.45, and that mycelium from 19-day-old cultures killed by immersion in 50 per cent ethyl alcohol for one hour was used. Equilibrium was reached at p_H 5.65 after 76 minutes.

EXPERIMENT 36.—Potassium phthalate. This experiment was carried out in order to determine whether immersion for different periods of time in 50 per cent ethyl alcohol would have any effect upon the behavior of the dead mycelium. Consequently, mycelium from 15-day-old cultures was killed by immersion in 50 per cent ethyl alcohol for 30 minutes, 24 hours, and 46 hours, respectively. Increments of mycelium were added in each case to 10 c.c. of 0.001 M potassium phthalate buffer solution with a reaction of about 4.2, and the changes in reaction determined electrometrically at 22°C. With a killing period of 30 minutes equilibrium was established in about 90 minutes at p_H 5.79; with a killing period of 24 hours, at p_H 5.58 in about 90 minutes; and with a killing period of 46 hours, at p_H 5.265 after 66 minutes.

EXPERIMENT 37.—Potassium phthalate. Increments of mycelium from 16-day-old cultures killed by 30 minutes immersion in 50 per cent ethyl alcohol were added to 10 c.c. of 0.001 M potassium phthalate buffer with a reaction of p_H 5.58. Changes in reaction were determined electrometrically at a temperature of 23°C. Equilibrium was reached at p_H 5.74.

EXPERIMENT 38.—Potassium phthalate. Increments of mycelium from 18-day-old cultures killed by immersion for 20 minutes in 50 per cent ethyl alcohol were added to 12 c.c. of 0.001 M potassium phthalate buffer solution with a reaction of p_H 4.22. Changes in reaction were determined electrometrically at 23°C. Equilibrium was established at p_H 6.0.

2. Experiments With Reactions Alkaline to the Isoelectric Point.

EXPERIMENT 39.—Sodium phosphate. Mycelium from 12-day-old cultures was killed by immersion in 50 per cent ethyl alcohol for 30 minutes, 2 hours, 17 hours, and 24 hours, respectively. Quantities of mycelium weighing from 4.5 to 5.8 grams from each treatment were respectively added to each of four Pyrex beakers containing 20 c.c. of 0.001 M sodium phosphate buffer solution with a reaction of p_H 7.7. A check of living mycelium from the 12-day-old cultures was also run. Determinations of the changes in reaction were made colorimetrically at 23°C. Equilibrium was established in about 2 hours at the values indicated in Part I of Table 4. In order to check the foregoing results, the experiment was repeated, but in this case increments of mycelium were added, and the buffer solution used was taken at an initial reaction of p_H 7.1. Duplicates were pre-

pared of the 2-hour killing period in order to check up more closely the apparently anomalous behavior of this mycelium in the first experiment. A check of living mycelium was run as before. The results of this second trial are recorded in Part II of Table 4. The lower Sorensen values found in the first part of the experiment are increased in the second trial thus showing that the first results are probably due to the given quantity of mycelium being insufficient to bring about equilibrium.

TABLE 4.—Effect of the dead mycelium of *Fusarium lycopersici* on the reaction of a sodium phosphate buffer solution after immersion for various periods of time in 50 per cent ethyl alcohol. Experiment 39.

Part 1

Period of immersion in alcohol	Reaction of solution in Sorensen units after					
	0 min.	20 min.	60 min.	80 min.	120 min.	900 min.
30 minutes	7.7	7.0	7.0	6.9	5.9	5.9
2 hours	7.7	7.35	7.0	6.9	5.3	5.3
17 hours	7.7	7.4	7.1	6.8	6.1	6.0
24 hours	7.7	7.25	7.0	6.9	6.2	6.2
Check (living)	7.7	6.8	6.1	5.7	5.5	5.4

Part 2

Period of immersion in alcohol	Reaction of solution in Sorensen units after					
	0 min.	20 min.	60 min.	80 min.	120 min.	900 min.
30 minutes	7.1	7.0	6.8	6.2	6.2	6.2
2 hours	7.1	7.0	6.7	6.4	6.3	6.4
2 hours	7.1	7.1	6.75	6.4	6.4	6.45
17 hours	7.1	7.1	6.9	6.1	6.1	6.1
24 hours	7.1	7.1	6.8	6.2	6.2	6.2
Check (living)	7.1	6.5	6.2	5.6	5.4	5.4

EXPERIMENT 40.—Potassium phosphate. Increments of mycelium from 11-day-old cultures killed by immersion in 50 per cent ethyl alcohol for 30 minutes were added to 11 c.c of a 0.001 M potassium phosphate buffer solution with a reaction of p_H 7.92. Reactions were determined electrometrically. The reaction remained constant after reaching a value of p_H 6.10.

EXPERIMENT 41.—Sodium acetate—acetic acid. Increments of mycelium from 12-day-old cultures killed by immersion for 1 hour in 50 per cent ethyl alcohol were added to 20 c.c. of 0.001 M sodium acetate buffer solution in duplicate. Changes in reaction were determined colorimetrically at 23°C. The reaction was changed from an initial value of p_H 7.3 to p_H 6.15 and 6.10, respectively.

EXPERIMENT 42.—Sodium acetate—acetic acid. Increments of mycelium from 16-day-old cultures killed by immersion for 1 hour in 50

per cent ethyl alcohol were added to duplicate solutions of 25 c.c. each of 0.001 M sodium acetate buffer solution with a reaction of p_H 6.6. Reaction changes were determined colorimetrically at 22°C. Equilibrium was reached at p_H 5.9 and 5.85, respectively.

EXPERIMENT 43.—Potassium phthalate. Mycelium from 21-day old cultures was killed by immersion for one hour in 50 per cent ethyl alcohol. Four grams of the dead tissue were added to 20 c.c. of 0.001 M potassium phthalate buffer solution at p_H 6.5. Changes in reaction were determined colorimetrically at 23°C. No further change in reaction was obtained at the equilibrium point p_H 6.20-6.25 by the addition of fresh quantities of dead tissue.

EXPERIMENT 44.—Potassium phthalate. Increments of mycelium from 16-day-old cultures killed by immersion for 30 minutes in 50 per cent ethyl alcohol were added to 10 c.c. of 0.001 M potassium phthalate buffer solution with a reaction of p_H 6.77 and changes in reaction followed with the hydrogen electrode at a temperature of 23°C. Equilibrium was established at p_H 6.0.

EXPERIMENT 45.—Sodium phosphate. Mycelium from 17-day-old cultures was killed by immersing in a 1:1000 solution of bichloride of mercury for 30 minutes. Increments of thoroughly washed mycelium were added to duplicate solutions containing 20 c.c. each of 0.001 M sodium phosphate buffer solution. Changes in reaction were determined colorimetrically at a temperature of 23°C. The initial reaction of the solutions was changed from p_H 6.85 to p_H5.4 and 5.5, respectively.

3. General Summary of the Experiments With Dead Mycelium.

In Table 5 is given a general summary of the results from the experiments with dead mycelium. The isoelectric point of the dead tissue does not appear to be identical with that of living tissue but is nearer a value of p_H 5.8. There were greater variations in the behavior of the killed mycelium than with the living. This is even true when tissue from cultures of the same age and those killed by the same methods were used. Only two methods of killing were employed with some variation in the duration of the treatments, excepting the single experiment with bichloride of mercury. A treatment as long as 46 hours in 50 per cent alcohol, as recorded in Experiment 36, shows considerable variation from the treatments for shorter periods. The effect of dead mycelium in the sodium acetate buffer mixtures is not the same in all cases, as shown in Experiment 33 where the reaction was changed from an initial of p_H 4.37 to a final reaction of p_H 7.59, and in Experiment 32 where but little change in reaction was obtained with an initial p_H of 4.6 although increments of the dead tissue were added. Larger quantities of the dead mycelium

than of the living were required to bring about equivalent changes in reaction.

TABLE 5.—Summary of experiments showing the effect of the dead mycelium of *Fusarium lycopersici* on various buffered single-salt solutions with different H-ion concentrations. Experiments 30 to 45, inclusive.

Expt. No.	Solution Used	Concn.	Age of mat	Initial pH	Final ph	H-ion method used	Method of killing mats
			da.				
30	K-Phosphate	0.001 M	15	4.90	5.90	Electrode	30 min. 50% alc.
31-I	Na-Acetate	0.001 M	16	4.55	5.90	Indicator	H_2O 80-90°C. 20^m
31-II	Na-Acetate	0.001 M	16	4.55	5.85	Indicator	H_2O 80-90°C. 20^m
32-I	Na-Acetate	0.001 M	19	4.60	4.80	Indicator	1 hr. 50% alc.
32-II	Na-Acetate	0.001 M	19	4.60	4.85	Indicator	1 hr. 50% alc.
33	Na-Acetate	0.01 M	13	4.37	7.59	Electrode	40 min. 50% alc.
34-I	K-Phthalate	0.001 M	17	4.50	5.65	Indicator	H_2O 80-85°C. 20^m
34-II	K-Phthalate	0.001 M	17	4.50	5.70	Indicator	H_2O 80-85°C. 20^m
35-I	K-Phthalate	0.001 M	19	4.45	5.65	Indicator	1 hr. 50% alc.
35-II	K-Phthalate	0.001 M	19	4.45	5.65	Indicator	1 hr. 50% alc.
36	K-Phthalate	0.001 M	15	4.20	5.79	Electrode	30 min. 50% alc.
36	K-Phthalate	0.001 M	15	4.20	5.58	Electrode	24 hrs. 50% alc.
36	K-Phthalate	0.001 M	15	4.20	5.26	Electrode	46 hrs. 50% alc
37	K-Phthalate	0.001 M	16	5.58	5.74	Electrode	30 min. 50% alc.
38	K-Phthalate	0.001 M	18	4.22	6.00	Electrode	20 min. 50% alc.
39-1	Na-Phosphate	0.001 M	12	7.70	5.90	Indicator	30 min. 50% alc.
39-1	Na-Phosphate	0.001 M	12	7.70	5.30	Indicator	2 hrs. 50% alc.
39-1	Na-Phosphate	0.001 M	12	7.70	6.00	Indicator	17 hrs. 50 %alc.
39-1	Na-Phosphate	0.001 M	12	7.70	6.20	Indicator	24 hrs. 50% alc.
39-2	Na-Phosphate	0.001 M	12	7.10	6.20	Indicator	30 min. 50% alc.
39-2	Na-Phosphate	0.001 M	12	7.10	6.40	Indicator	2 hrs. 50% alc.
39-2	Na-Phosphate	0.001 M	12	7.10	6.45	Indicator	2 hrs. 50% alc.
39-2	Na-Phosphate	0.001 M	12	7.10	6.10	Indicator	17 hrs. 50% alc.
39-2	Na-Phosphate	0.001 M	12	7.10	6.20	Indicator	24 hrs. 50% alc.
40	K-Phosphate	0.001 M	11	7.92	6.10	Electrode	30 min. 50% alc.
41-I	Na-Acetate	0.001 M	12	7.30	6.15	Indicator	1 hr. 50% alc.
41-II	Na-Acetate	0.001 M	12	7.30	6.10	Indicator	1 hr. 50% alc.
42-I	Na-Acetate	0.001 M	16	6.60	5.90	Indicator	1 hr. 50% alc.
42-II	Na-Acetate	0.001 M	16	6.60	5.85	Indicator	1 hr. 50% alc.
43	K-Phthalate	0.001 M	21	6.50	6.20	Indicator	1 hr. 50% alc.
44	K-Phthalate	0.001 M	16	6.77	6.00	Electrode	30 min. 50% alc.
45-I	Na-Phosphate	0.001 M	17	6.85	5.40	Indicator	30^m 1:1000 $HgCl_2$
45-II	Na-Phosphate	0.001 M	17	6.85	5.50	Indictaor	30^m 1:1000 $HgCl_2$

III. THE EFFECT OF REACTION ON THE TOXICITY OF CERTAIN COMPOUNDS AS DETERMINED BY SPORE GERMINATION STUDIES AND ITS CORRELATION WITH THE ISOELECTRIC POINT OF THE LIVING TISSUE

If we assume that the living tissue of *Fusarium lycopersici* behaves like an ampholyte with an isoelectric point at about p_H 5.4, then on the acid side of this point the amphoteric tissue would act as a base and combine with cations. On the basis of such an assumption cations should be more toxic on the alkaline side, and anions more toxic on the acid side of p_H 5.4. In an effort to justify such conclusions the following spore germination studies were undertaken in which the percentage of conidial germination of *F. lycopersici* was noted in solutions at different hydrogen ion concentrations containing a toxic cation or anion at a concentration near its toxic limit. Among the toxic cations used were mercuric, cupric, and methylene blue (a basic dye); among the toxic anions used were cyanide and eosin (an acid dye). Previous workers have shown that spore germination is greatly influenced by the hydrogen ion concentration of solutions alone. Numerous checks were run in the following experiments using only the standard solution of potassium phosphate at different reactions and without the toxic cation or anion in order to obtain data with which that obtained in experiments with toxic ions might be compared. The recent work of Webb[52, 53] presents detailed experiments in which the toxic effect of hydrogen and hydroxyl ions were studied. He also gives a review of the literature pertaining to spore germination. While many earlier workers have studied the effect of toxic agents upon spore germination none have taken the hydrogen ion concentration into consideration. However, numerous studies have been made of the effect of acidity and alkalinity on the toxicity of various substances to bacteria and protozoa. Quite complete reviews are given of this latter work in the papers of Stearn and Stearn [46,47,48] and of Robbins[35].

EXPERIMENT 46—Before determining the effect of toxic substances upon spore germination two experiments were conducted to determine the effect of the hydrogen ion concentration upon spore germination in dilute potassium phosphate buffer mixtures.

In the first procedure van Tiegham hanging-drop cells were used following the method recommended by Duggar[5]. Two glass rings were sealed to glass slides by means of beeswax. Five drops of 0.001 M potassium phosphate adjusted to different hydrogen ion concentrations were placed in the bottom of each pair of cells. A drop of the same buffer mixture was placed on a cover slip, inoculated with spores by means of a platinum needle and sealed to its corresponding ring with petrolatum.

The cultures were incubated at 28°C., the optimum temperature for growth of *Fusarium lycopersici*. Counts were made after 3, 6, and 22 hours. Curves are plotted in Fig. 2 showing the average percentage of germination after 3, 6, and 22 hours, respectively. Maxima of spore germination occur between p_H 4.0 and 4.5, and 6.0 and 8.0, with a marked minimum between P_H 5.0 and 5.5.

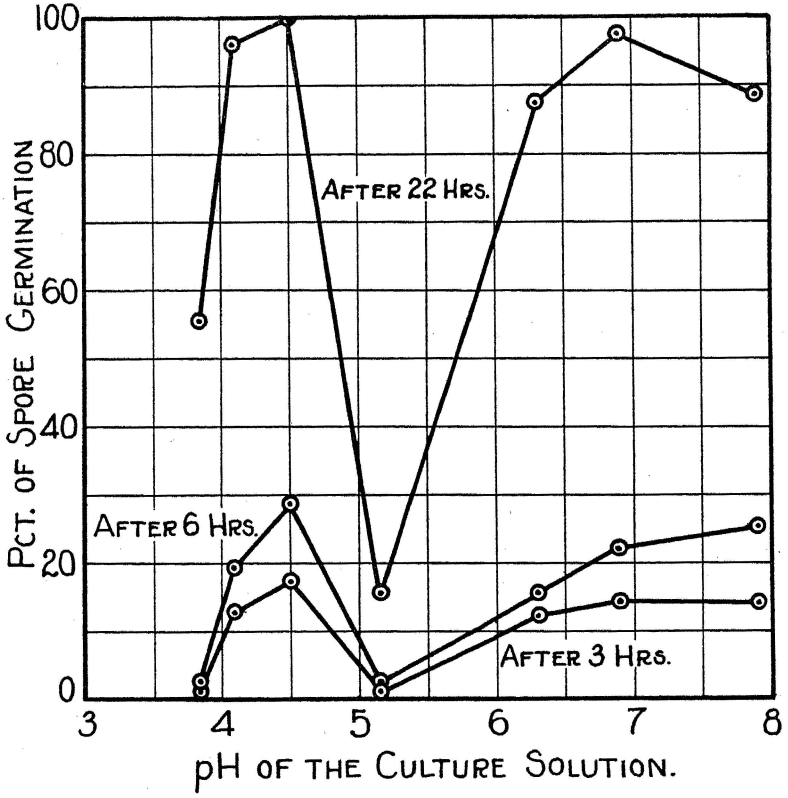

FIG. 2.—Curves showing the per cent of germination of spores of *Fusarium lycopersici* in 0.001 M potassium phosphate buffer mixtures at different H-ion concentrations. Experiment 46 (first procedure).

No determination of the final reaction of the hanging drop was made. The cover glass is likely to be a source of alkali, however, and thus change the reaction of the small volume of solution used. A change in reaction may also occur where the spore mass is large in proportion to the volume of the hanging drop due to the shifting of the reaction toward an equilibrium point as found for mycelial mats in the preceding experiments. Webb[52, 53] found some change in reaction in hanging drop cul-

tures in his spore germination studies. Another factor of importance in the hanging drop method is the possible crowding of the spores in the drop with a resulting decrease in germination.

It was therefore decided to employ another method of studying spore germination and thus overcome some of the objections to the use of hanging drop cells.

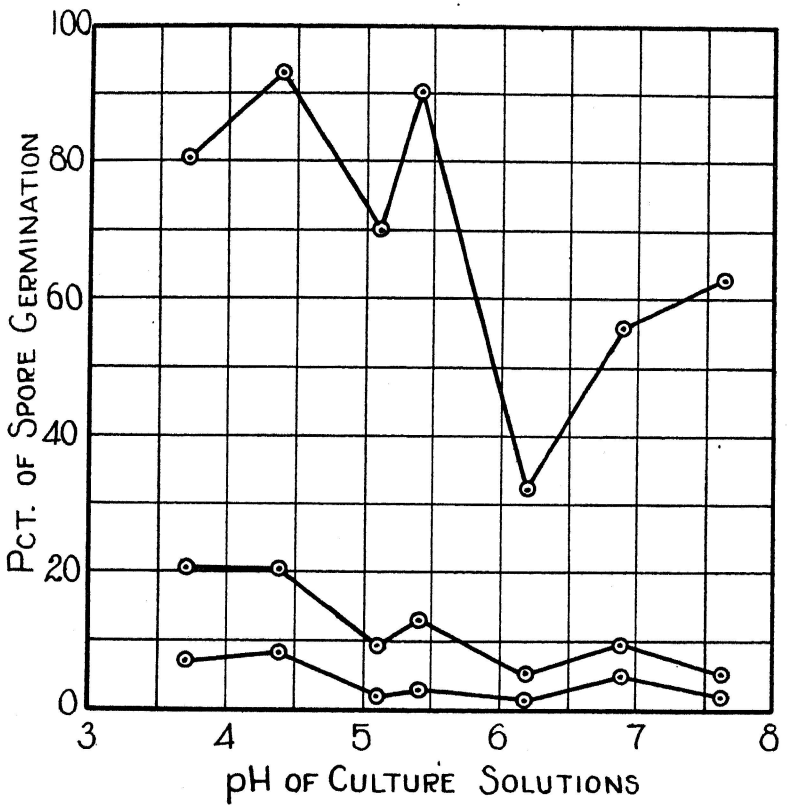

FIG. 3.—Curves showing the per cent of germination of spores of *Fusarium lycopersici* in 0.05 M potassium phosphate buffer mixtures at different H-ion concentrations. Experiment 46 (second procedure).

In the second procedure sterile Syracuse watch crystals were used as culture vessels. Duplicate cultures containing 3 c.c. each of 0.05 M potassium phosphate buffer mixture at different hydrogen ion concentrations were prepared and inoculated with spores using a platinum needle. Each watch crystal was rimmed with petrolatum and covered with another watch crystal thus preventing evaporation of the solution. All solutions were incubated at 28°C. Counts were made after 3, 6, and 19

hours using low power, the spores in five fields being counted each time. Both macro- and micro-conidia of *Fusarium lycopersici* are easily seen under low power.

Attempts were made to obtain as uniform seeding of the cultures as possible. Thorough distribution of the spores throughout the solution was made by vigorously stirring with a platinum needle. Approximately 100 to 150 spores could be seen in a low power field. All counts were made from fields showing such distribution. The greater volume of solution used here in proportion to the spore mass insured a constant value of hydrogen ion concentration as far as any effect of the fungous tissue is concerned, throughout the duration of the experiment. All reactions were determined colorimetrically. No change in reaction occurred in the solutions. While the glass of watch crystals is likely to be somewhat soluble it was evident that this factor did not affect the reaction of the solutions in experiments where they were used. Most of the conidia of *F. lycopersici* settle to the bottom of the culture dish. This results in decreased oxygen supply but all counts were made from the submerged spores so that the results are comparable.

Curves are plotted in Fig. 3 showing the average percentage of spore germination at the different hydrogen ion concentrations after the given time intervals. The maxima of spore germination occurred in 0.05 M potassium phosphate buffer mixtures between p_H 4.0 and 4.5, and at 5.5, respectively.

EXPERIMENT 47.—Quantities of 0.02 N hydrochloric acid varying from 18 to 30 c.c. were added to 10 c.c. of 0.01 potassium cyanide. The volume was then brought to 100 c.c. by adding distilled water. This gave a series of solutions each containing 0.001 M concentration of the cyanide, a toxic anion, at different hydrogen ion concentrations from p_H 4.1 to 8.0. Hanging drop cells were prepared as in the first procedure in Experiment 46 with duplicate solutions at each reaction. All cultures were inoculated with spores by means of a platinum needle and incubated at 28°C. Counts were made after intervals of 3, 10, and 19 hours. Curves are plotted in Fig. 4 showing the average percentage of spore germination of each pair of cultures at the different reactions after the given time intervals. It seems evident from the results obtained that spore germination is markedly inhibited on the acid side of p_H 5.5. The percentage of germination increases slowly from this point to a value of p_H 7.0 from which point germination rapidly increases to a maximum at p_H 8.0.

EXPERIMENT 48.—To given amounts of 0.05 M potassium phosphate buffer mixtures adjusted to different reactions sufficient mercuric

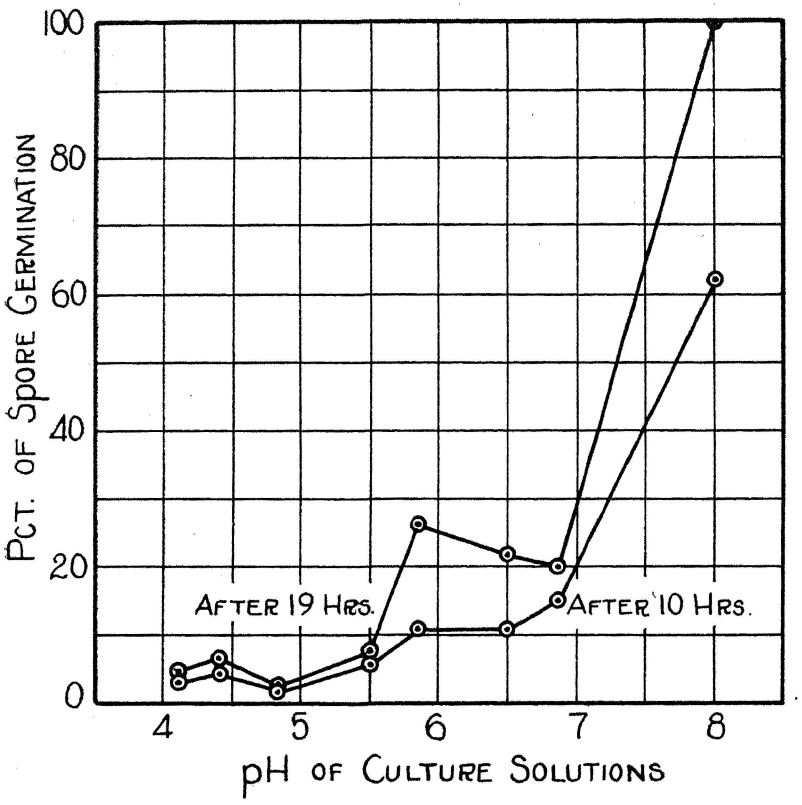

FIG. 4.—Curves showing the per cent of germination of spores of *Fusarium lycopersici* in 0.001 M potassium cyanide adjusted to different H-ion concentrations with 0.02 M HCl. Experiment 47.

chloride was added to give a concentration of 1:400,000. Duplicate solutions at each reaction were prepared by placing 3 c.c. of each in watch crystals as in the second procedure of Experiment 46. The solutions were then inoculated with spores and incubated at 28°C. Counts were made after intervals of 6 and 24 hours. In Fig. 5 are plotted curves showing the average percentage of spore germination after the given time intervals at the different hydrogen ion concentrations. After 24 hours but slight germination had occurred at reactions more alkaline than P_H 6.0. It seems evident that the toxic cation (mercuric) was an inhibiting factor. The inhibitory effect of the cation was less marked at reactions more acid then P_H 6.0.

EXPERIMENT 49.—A sufficient quantity of copper sulphate was added to 0.05 potassium phosphate buffer mixtures to give a concentration

of 0.001 N copper sulphate. A series of solutions with different reactions were then prepared by placing 3 c.c. of each solution in a watch crystal. All solutions were prepared in duplicate. After inoculation with spores the cultures were incubated at 28°C. Counts were made after intervals of 6, 17, and 24 hours. Curves are plotted in Fig. 6 showing the average percentage of spore germination at the different reactions after the given time intervals. A marked maximum occurs at p_H 3.7, falling rapidly to a minimum between p_H 4.3 and 5.0. A slight maximum occurs at p_H 5.8 above which value the per cent of germination is quite small. It is evident that spore germination is markedly inhibited on the alkaline side of p_H 4.5. At p_H 7.0 the copper begins to precipitate as hydroxide as indicated by slight turbidity in the dilute solutions used.

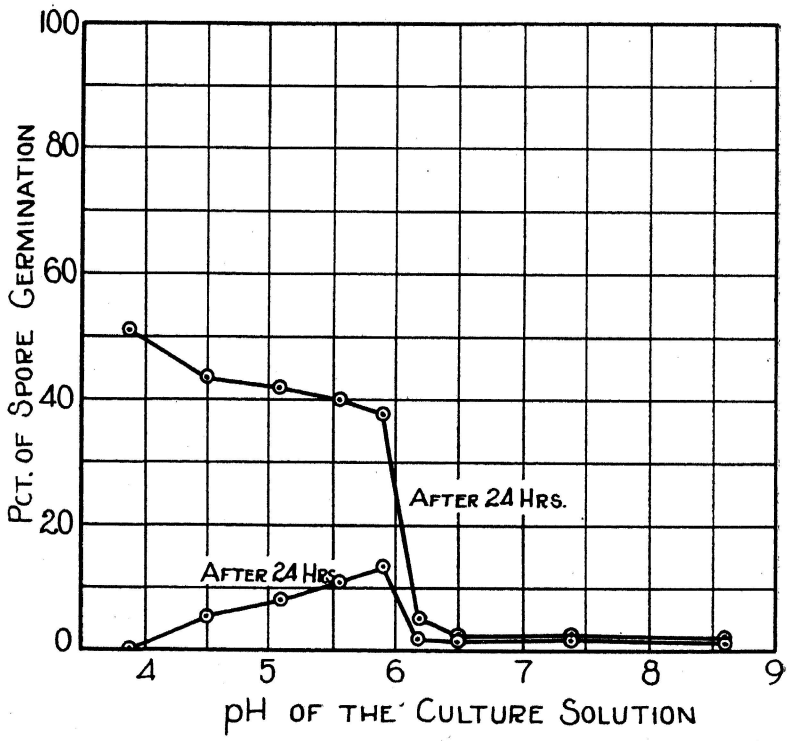

FIG. 5.—Curves showing the per cent of germination of spores of *Fusarium lycopersici* in 1:400,000 mercuric chloride in 0.05 M potassium phosphate buffer solutions at different H-ion concentrations. Experiment 48.

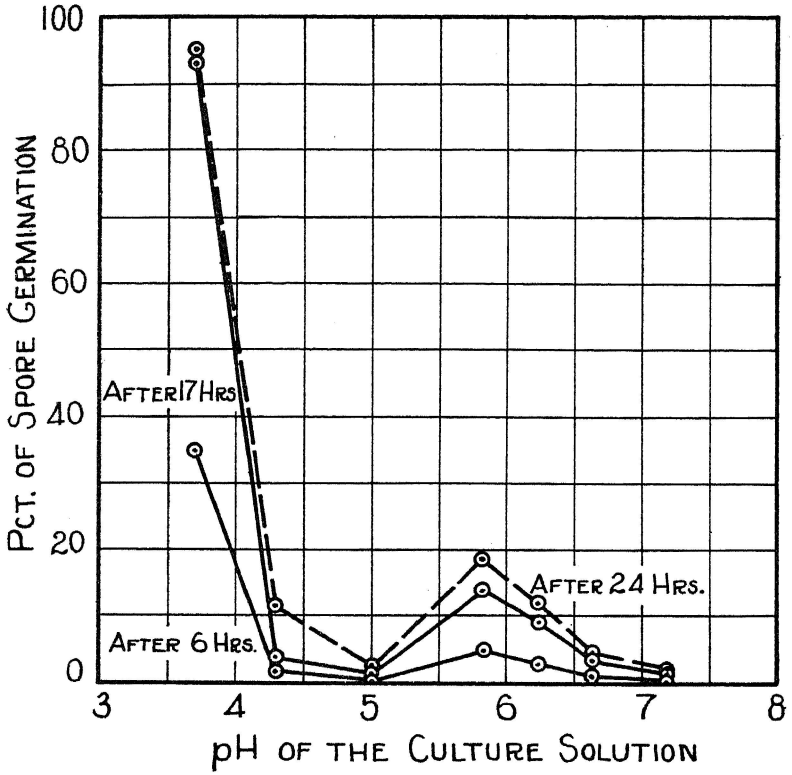

FIG. 6.—Curves showing the per cent of germination of spores of *Fusarium lycopersici* in 0.001 N copper sulfate in 0.05 M potassium phosphate buffer solutions at different H-ion concentrations. Experiment 49.

EXPERIMENT 50.—This experiment was identical to Experiment 49 excepting that the concentration of the copper sulphate was reduced to $\frac{N}{1800}$ and the concentration of the potassium phosphate buffer mixture increased to 0.1 M. Reactions with p_H values as great as 7.3 and 7.9 were used. However, copper hydroxide was precipitated at these reactions as indicated by the increasing turbidity of the solutions with increase in alkalinity. A single series of solutions of the 0.1 M potassium phosphate buffer were used as a check. In Fig. 7 are plotted curves showing the average percentage of spore germination in the copper sulphate solutions at different reactions after 8 and 22 hours, and in the phosphate buffer solutions alone after 22 hours. Spore germination is at a maximum in

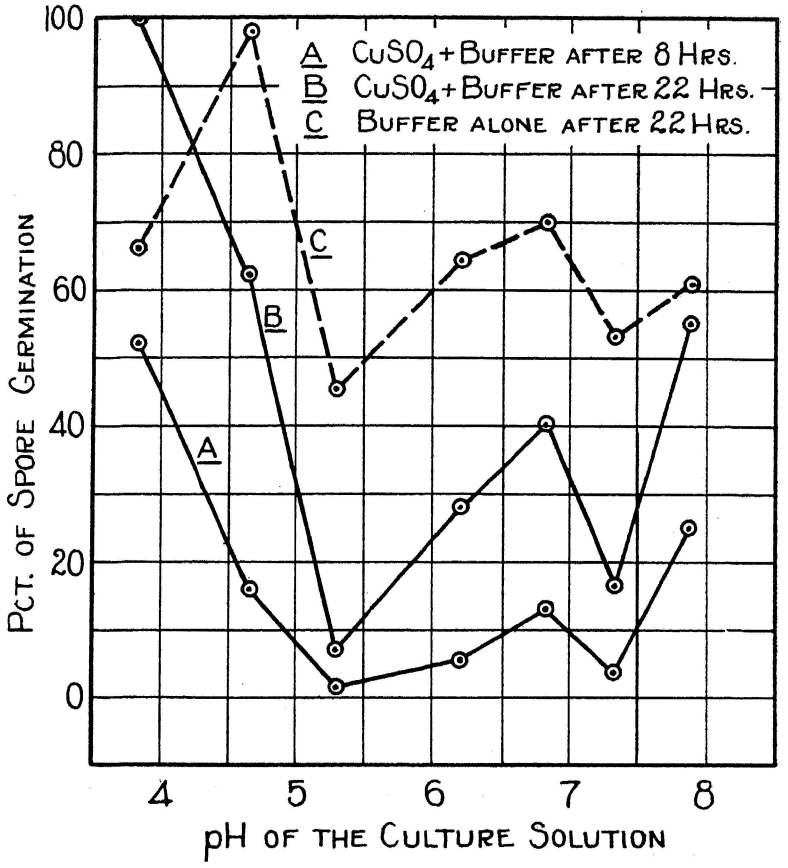

FIG. 7.—Curves showing the per cent of germination of spores of *Fusarium lycopersici* in N/1800 copper sulfate in 0.1 M potassium phosphate buffer mixtures at different H-ion concentrations. Experiment 50.

the copper sulphate solutions at p_H 3.9 uniformly decreasing to a minimum at p_H 5.3. A second, but less marked, maximum appears at p_H 6.8, followed by a second minimum at p_H 7.3. In solutions at p_H 7.9 the percentage of germination again increases, probably due to the precipitation of the copper as hydroxide. It is evident from these experiments that copper sulphate exerts a toxic effect on the alkaline side of p_H 5.0 at the concentrations used.

EXPERIMENT 51.—An acid dye, eosin, was chosen for the toxic substance in this experiment. The toxic radical is the anion in this case, therefore it would be expected to exert an inhibitory influence on spore germination on the acid side of the isoelectric point of the living fungous

tissue, about p_H 5.4. Duplicate solutions of 3 c.c. each in watch crystals containing aqueous eosin with a concentration of 1:10,000 in 0.1 M potassium phosphate buffer mixtures at different hydrogen ion concentrations were prepared. A single series of the 0.1 M phosphate buffer mixtures alone was run as a check. Inoculation and incubation were as in previous experiments. Curves showing the percentage of spore germi-

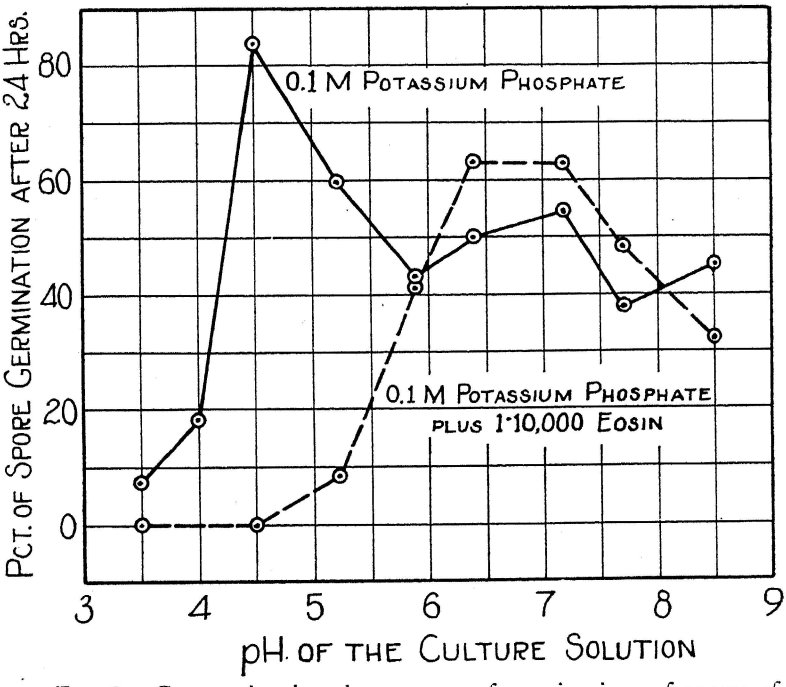

FIG. 8.—Curves showing the per cent of germinations of spores of *Fusarium lycopersici* in 1:10,000 eosin (an acid dye) in 0.1 M potassium phosphate buffer solutions at different H-ion concentrations, and in 0.1 M potassium phosphate buffer solutions alone. Experiment 51.

nation after 24 hours are plotted in Fig. 8. Spore germination was completely inhibited by the eosin at p_H values of 3.5, 4.0, and 4.5. Germination was somewhat less in the 0.1 M potassium phosphate alone at p_H 4.0 than in previous experiments. At p_H 5.0 spore germination increases rapidly in the presence of eosin reaching a maximum between p_H 6.4 and 7.2, after which there is some decrease. Microscopic examination reveals marked penetration of the dye into the spores at the more acid reactions becoming less intense as the reaction approaches p_H 5.2. At Sorensen units higher than this point little staining of the spores is evident.

EXPERIMENT 52.—A basic dye, methylene blue, was chosen for this experiment. The toxic radical in this case is the cation and therefore would be expected to exert an inhibitory influence on spore germination on the alkaline side of the isoelectric point, p_H 5.4. Triplicate solutions of 3 c.c. each in watch crystals were prepared consisting of 0.1 M potassium phosphate buffer mixtures at different hydrogen ion concentrations plus a 1:10,000 concentration of methylene blue. Duplicate series of the 0.1 M potassium phosphate buffer mixtures alone were also prepared. The solutions were inoculated with spores and incubated at 28°C. Counts were made after 8 and 24 hours, respectively. In Fig. 9 curves are plotted showing the average percentage of spore germination at the end of 8 and 24 hours at the different reactions. Spore germination is markedly inhibited at p_H values greater than p_H5.5 by the dye, while it approaches that in the buffer solutions alone in cultures more acid than p_H 5.4. The basic dye radical thus appears to exert a toxic influence on the alkaline side of the isoelectric point of the plant tissue.

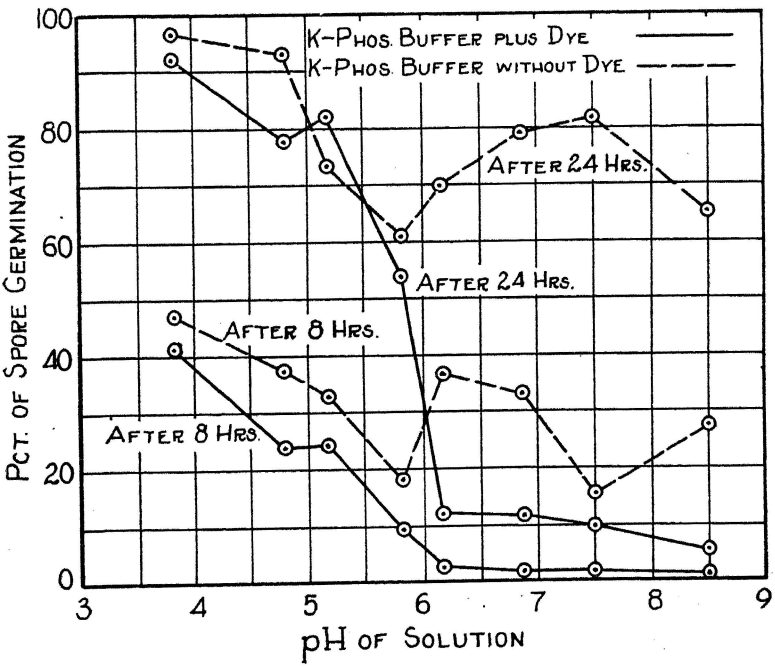

FIG. 9.—Curves showing the per cent of germination of spores of *Fusarium lycopersici* in 0.1 M potassium phosphate buffer mixtures plus 1:10,000 methylene blue (a basic dye) at different H-ion concentrations, and in the 0.1 M phosphate mixtures alone. Experiment 52.

DISCUSSION

The results of the experiments with the living mycelium of *Fusarium lycopersici* show that when this tissue is placed in dilute solutions of buffered or unbuffered single salts at different hydrogen ion concentrations the initial reaction is changed to a value at or near p_H 5.4. This is interpreted to mean that in solutions more acid than p_H 5.4 anions are taken up from the solutions more rapidly than cations resulting in a change in reaction until uptake of anion ceases or until anion and cation are taken up at equal rates, which appears to be at a reaction of about p_H 5.4. In solutions more alkaline than p_H 5.4, cations are taken up more readily than anions resulting in a shifting of the reaction until a value of p_H 5.4 is reached. At reactions at or near p_H 5.4 neither anion nor cation appears to be absorbed, or both are absorbed at equal rates, resulting in no change in reaction. This behavior is analogous to that of an amphoteric colloid and would seem to indicate that this plant tissue is amphoteric in character and has an isoelectric point at or near p_H 5.4.

For normal tissue, age does not appear to exert any effect upon the final results obtained with the living tissue. It must be remembered that while mycelial mats from cultures of different ages were used yet the tissue in the mat varies in age. Development of the fungus in the culture flasks was circular, centering around the point where the inoculum started growth, therefore the edges of the mats were composed largely of tissue somewhat younger than that in the center of the mat. It was for this reason that sectors of tissue were cut out for the absorption studies. Old cultures which showed any evidences of autolysis were found to give anomalous results and were discarded.

As far as experiments with the fungous tissue used are concerned the method of determining the hydrogen ion concentration did not seem to have any influence upon the final results. As noted by Robbins and Scott[36] the production of carbon dioxide by plant tissue is a factor which is likely to upset experiments in which the reaction changes are determined by the indicator method. With living plant tissue like potato tuber tissue and root-tips this seems to be of importance and could be overcome by using the electrometric method altogether thus eliminating the carbon dioxide from the solution by means of the vigorously bubbling hydrogen gas. The results with *Fusarium lycopersici* are identical regardless of the method of determining the reaction.

However, an experiment was performed to determine whether the production of an appreciable amount of carbon dioxide in the solution would result in a displacement of the equilibrium point. This was done by placing a quantity of a washed 10-day-old mat of mycelium in a 0.001

M potassium phosphate buffer solution with an initial reaction of p_H 6.7. After about 40 minutes the reaction determined by the indicator method, had changed to p_H 5.45, at which point it remained constant. The solution containing the mycelium was then charged with carbon dioxide by blowing the breath somewhat vigorously into the solution through glass tubing, mixing and stirring the mycelium and solution at the same time with a glass rod. Upon immediately withdrawing 2 c.c. of solution and determining the reaction it was found that it had reached a value of p_H 4.8. After allowing the solution and mycelium to stand for 20 minutes another determination was made, when it was found that the reaction had returned to practically the same point, p_H 5.4, at which point equilibrium was maintained. A similar experiment with a solution in which the initial reaction was p_H 4.2 was conducted. Similar results were again obtained, the reaction returning to the equilibrium point after being shifted by the bubbling of carbon dioxide through the solution. It is evident from the results obtained by the use of both methods of hydrogen ion determination and from the foregoing experiments that carbon dioxide production is not a factor in the experiments with the living mycelium of the organism used when sufficient quantities of mycelium are present to insure establishment of the equilibrium point.

If the equilibrium point found for the living mycelium is the isoelectric point of the plant tissue of the organism studied the results show that at acid reactions the anions, phosphate, acetate, phthalate, chloride and sulphate are absorbed, and at alkaline reactions the cations, sodium, potassium, calcium, magnesium, cupric, and mercuric. It would seem that the cupric and mercuric anions at the concentrations used would be toxic to the plant tissue and result in death of the protoplasm during the duration of the experiment and that results similar to those with dead tissue be obtained. However, this apparently was not the case. Nevertheless, it must be noted that only one set of experiments with the mercuric ion was performed and here the initial acidity was only slightly greater than that at the isoelectric point. Under such circumstances the cation was probable but slightly absorbed and therefore, exerted no influence upon the small absorption of anion necessary to change the reaction from p_H 5.3 to 5.4 and 5.5 in the two solutions used.

The dead mycelium of *Fusarium lycopersici* does not behave like the living tissue. In general, there is a tendency for equilibrium to be established in the neighborhood of p_H 5.8 when the dead tissue is placed in buffered and unbuffered single salt solutions with different reactions. There are many discrepancies in its behavior, however, as particularly evident in the experiments with acetate (Expts. 32 and 33) and in some cases with sodium phosphate (Expts. 39 and 40). The equilibrium point

varies throughout a zone reaching from p_H 4.80 to 7.59. However, the majority of the experiments show a final value in a range from p_H 5.6 to 6.2. The average p_H value at equilibrium in 33 trials was at 5.88. Two methods of killing the mycelium were employed, both of which have serious objections. Alcohol and heat are known to coagulate proteins. This effect probably has an influence upon the activities of the proteins as ampholytes. It must therefore be concluded that the dead mycelium of this organism does not behave like a stable ampholyte with a definite isoelectric point comparable to that obtained in the experiments with living tissue of this fungus. The anomalous behavior may be attributed to a difference between living and dead protoplasm, or to an effect depending upon the method of killing.

Robbins[33] found dead potato tuber tissue to show the same minimum for water absorption as the living tissue. However, the tissue was not subjected to the rigorous killing treatments as the fungous mycelium in the experiments presented here. It is possible that the soaking in strong alcoholic solutions or in hot water may destroy the cell walls to some extent and allow some of the cell constituents to diffuse out into the killing solution. Whether we are dealing with the same material in dead tissue as in living as far as the experiments reported here are concerned will have to be proven by further experiments. While the results with dead tissue are not altogether consistent, it may be possible that the tissue killed under exactly the same conditions would show a definite isoelectric point though different from that for living tissue. As Michaelis and Davidsohn[23] have shown in the case of albumin, an alteration of the properties of the protein would cause a shifting of the isoelectric point.

The spore germination studies furnish further evidence that the assumption of a definite isoelectric point for the tissue of *Fusarium lycopersici* is tenable. These studies were undertaken to show that when certain toxic cations (cyanide, and acid dye, eosin), and anions (mercuric, cupric, and basic dye, methylene blue) were chosen at concentrations near their toxic limits the isoelectric point of the species of fungi in question exerts a profound influence upon the intensity of the toxic action depending upon the reaction of the suspending medium. In other words, the toxic action of a deleterious cation or anion is largely due to its ability to combine with the proteins of the plant tissue and the protein is capable of combining with toxic cations only when it exists as an anion, and with toxic anions only when it exists as a base. This could occur when the surrounding medium was more alkaline, or more acid, respectively, than the isoelectric point. With greater concentrations of the toxic salts than those used spore germination is inhibited at all reactions.

The toxicity of the mercuric ion and the basic dye, methylene blue, does not seem to exert a marked influence upon spore germination until

a hydrogen ion concentration of about p_H 6.0 is reached, while the critical point for all other ions used is between p_H 5.0 and 5.5. It is interesting to note that Joachimoglu[15] found that mercuric chloride at a concentration of 1:600,000 exerted greatest antiseptic power at p_H 5.0 to 6.6, while from p_H 7.8 to 10.1 its antiseptic power was slight. It would be expected that the amount of free base would increase in alkaline solutions and therefore show the greatest toxic influence in such solutions.

Spore germination in the non-toxic potassium phosphate buffer mixtures alone shows a minimum between p_H 5.0 and 6.0 in most cases. This agrees quite closely with the minimum point for growth previously found by Scott[41] for this organism in nutrient solutions. The double-maximum curves obtained are quite similar to those shown by Webb[52,53] for spore germination in a number of species of fungi, and with the results obtained by Hopkins[12] for conidial germination of *Gibberella saubinetti*. On the basis of the ampholyte hypothesis a minimum of spore germination would be expected at or near the isoelectric point of the plant tissue, provided that the spores have the same amphoteric properties as the fungous mycelium. Water absorption would be at a minimum at the isoelectric point and, as is well known, water absorption is necessary for the promotion of growth activities such as spore germination.

The phenomena observed appear to be due largely to simple chemical combination between constituents of the plant tissue and ions in the external solution, the direction of the reaction depending upon the hydrogen ion concentration of the surrounding solution. Such a conception offers the simplest explanation for the results presented in this paper. While it is not probable that only one amphoteric protein is present in the plant tissue, yet the ampholytic content for a given organism, or at least for a given tissue system of an organism, should be fairly specific when developed under the same conditions. The suggested isoelectric point for such tissue may be the resultant due to the combined influence of its several constituent ampholytes. With the living mycelium of *Fusarium lycopersici* under the experimental conditions given the isoelectric point appears to be very near p_H 5.4.

As suggested in the recent paper by Robbins and Scott[36], the determination of the isoelectric point of closely related fungi offers the possible use of a new method as an aid in identification. The limiting reactions of various bacteria grown in standard media have been found to be remarkably consistent and to be an aid in differentiating closely related groups and species.

To what extent is the foregoing protein analogy substantiated by other investigators? As early as 1912, Endler[6], studying the effect of salts on absorption of methylene blue by cells of elodea, concluded that the

protoplasm of this plant behaved as an amphoteric colloid with an isoelectric point between p_H 3.8 and 4.1. Rohde[37] explained the retention of basic and acid dyes by plant tissue as being due to an effect of the acidity or alkalinity upon the properties of the cell colloids, and presents a curve showing the intensity of absorption of acid and basic dyes as affected by reaction which resembles very much the curves shown in this paper (Fig. 1) for the reaction changes by fungous mycelium. Falk and Shaugnessy[7] obtained results which they believed to indicate that bacteria may act like an ampholyte with two isoelectric points.

Robbins[33] was one of the first workers to present extensive investigations based upon the idea that plant tissue might act like an amphoteric colloid with a definite isoelectric point and pointed out the significance of such an assumption. As heretofore cited, he found that living potato tuber tissue showed a minimum of water absorption at a hydrogen ion concentration near p_H 6.0 when placed in dilute buffer mixtures at different reactions, and that disks of potato tuber tissue retained acid dyes, like eosin, more strongly when the stained disks were washed with buffer solutions more acid than p_H 6.0 than they did when washed with buffer solutions more alkaline than this point. The reverse was found to be true for basic dyes. On the basis of such observations he suggests that the behavior of potato tuber tissue is analogous to that of a pure protein with an isoelectric point in the vicinity of p_H 6.0.

Stearn and Stearn[45, 46, 47, 48] demonstrated that bacteria act like amphoteric proteins in their behavior toward acid and basic dyes and explain their results on such an assumption.

Pearsall and Priestley[30] as well as Weber[54] have attempted to account for the formation of meristem upon the conception that the plant cell acts as an ampholyte. Irwin[13] suggests that the increasing penetration of the dye cresyl blue into living cells of *Nitella* with increasing alkalinity can be attributed to "an increase in active protein (or other amphoteric electrolyte) in the cell which can combine with the dye."

In further studies Robbins[34] presents experimental evidence that suggests an isoelectric point for the mycelium of *Rhizopus nigricans* near p_H 5.0. He found a minimum of growth at p_H 5.2 on potato dextrose agar adjusted to different hydrogen ion concentrations. The dry matter produced by the fungus when grown in potato dextrose broth at different reactions was at a minimum near p_H 5.0. When the mycelium of *Fusarium lycopersici* was stained with certain acid and basic dyes the former were retained quite strongly when the stained sections were washed with dilute buffer solutions more acid than p_H 5.5, but quite weakly when washed with solutions more alkaline than 5.5. The reverse was true with basic dyes.

Pearsall and Ewing[27] report that potato tuber tissue and carrot root

tissue showed less outward diffusion of chlorine ions in solutions more alkaline than p_H 4.3-4.4, but greater outward diffusion when the solution was more acid. They note, however, that solutions more acid than p_H 4.3 killed the potato tissue. A relationship between the isoelectric points of the principal proteins of these plants and the behavior of the tissue seems to exist. These authors give further summaries of their work in a later paper[29], and further[28] report rather extensive determinations of isoelectric points of various plant proteins. Pearsall[26] reviews the recent work on hydrogen ion concentration and growth and calls attention to the significance of the isoelectric point conception of plant tissue.

Robbins and Scott[36], as heretofore cited, studied the effect of potato tuber tissue, soybean root-tips, and the fungous mycelium of *Gibberella saubinetii*, *Fusarium lycopersici* and *Fusarium oxysporum* on dilute buffer solutions using electrometric methods. They found that the behavior of the plant tissue in each case was analogous to that of colloidal ampholytes showing definite isoelectric points which in the case of potato tuber tissue appeared to be near p_H 6.3. for soybean root-tips about p_H 6.3, for the fungous mycelium of *Gibberella saubinetii* at about p_H 6.2, for the fungous mycelium of *Fusarium lycopersici* at p_H 5.4, and for the mycelium of *Fusarium oxysporum* between p_H 4.5 to 5.0. When the change in hydrogen ion concentration was followed by means of the hydrogen electrode the plant tissue was found to cause the reaction of those solutions more acid than the isoelectric point to become less acid reaching equilibrium at the isoelectric point. Solutions alkaline to the isoelectric point were rendered acid reaching equilibrium at the isoelectric point. Plant tissue in solutions at or near the isoelectric point produced little or no change in the reaction of the external solution. There were, however, some discrepancies in the case of *F. oxysporum*.

Naylor[24], in these laboratories, found that the retention of acid and basic dyes by sections of root-tip tissue bears a definite relation to the reaction of buffer solutions in which the sections were washed. The retention of acid and basic dyes by the tissue when subjected to the different reactions gives evidence for the existence of an isoelectric point. However, he finds that different parts of the cell such as the cytoplasm, nucleus, nucleolus, and chromosomes show staining reactions that would indicate different isoelectric points for the cell part.

It is evident that the conception of plant tissue as an amphoteric compound with a definite isoelectric point is an important and significant hypothesis.

GENERAL SUMMARY

1. It is suggested that the behavior of the living mycelium of *Fusarium lycopersici* is analogous to that of an amphoteric colloid with isoelectric point at or near p_H 5.4.

2. When the living mycelium of *Fusarium lycopersici* is placed in both buffered and unbuffered single salt solutions at different hydrogen ion concentrations a change in reaction of the solution occurs which reaches equilibrium at or near p_H 5.4 under the experimental conditions reported in this paper.

3. This is interpreted to mean that on the acid side of p_H 5.4 the tissue acts as a cation and combines with anion resulting in a shifting of the reaction towards greater alkalinity. On the alkaline side of p_H 5.4 the tissue acts as an anion and combines with cation resulting in a shifting of the reaction towards greater acidity. At p_H 5.4 the tissue acts as if it were electrically neutral and combines equally with cation and anion, or with neither, resulting in no change of reaction.

4. Dead tissue of the organism studied does not show a definite equilibrium point in absorption of single salts as did the living mycelium. There is a possibility that an isoelectric point for dead tissue may exist at or near p_H 5.88.

5. The germination of spores of *Fusarium lycopersici* in potassium phosphate buffer solutions at different reactions shows a double maximum near p_H 4.5 and 7.0, respectively, with a minimum between p_H 5.0 and 5.5, in the vicinity of the suggested isoelectric point for the fungous mycelium.

6. The germination of spores of *F. lycopersici* is greatly inhibited in solutions acid to p_H 5.4 when toxic anions like cyanide and the acid dye eosin are used with concentrations near their toxic limits. The reverse is true of the toxic cations mercuric, cupric, and the basic dye methylene blue.

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