STUDIES ON BACTERIAL NUTRITION.

IV. Effect of Plant Tissue upon Growth of Pneumococcus and Streptococcus.

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Study of the growth requirements of *Bacillus influenzæ* (1–4) indicated that for the cultivation of this organism at least two substances must be present in the culture medium. Both of these substances are present in red blood corpuscles. One, a vitamine-like substance, can be extracted not only from red blood corpuscles but from yeast and vegetable cells as well, and is relatively thermolabile. This substance has been called the V factor. The other substance is also present in red blood corpuscles and in plant tissue, but it is thermostable and is effective in amounts so small as to suggest the possibility that it functions as a catalyst. It has been called the X factor. These observations on the growth of *Bacillus influenzæ* in the absence of blood have been extended (5). The organism can be readily grown in plain broth in which small pieces of sterile, unheated yellow or white turnip, carrot, beet, parnsip, or sweet potato have been placed. Knowledge of the growth-stimulating property of unheated plant tissue for organisms as sensitive to cultural conditions as the bacteria of the hemophilic group encouraged an extension of the study to other groups of bacteria. The present paper presents the results of a study of the effect of plant tissue upon the growth of certain Gram-positive cocci.

Material.

Media.—The plant tissues used in this study were white potato, sweet potato, carrot, white turnip, yellow turnip, parsnip, beet, and banana. Care was observed in selecting fresh young specimens. With the same technique described in a previous communication (4),

sterile pieces of these vegetables were placed in phosphate broth of pH 7.8. When air is not excluded from media prepared in this way oxidation of the plant tissue results in brown discoloration of the vegetable and of the supernatant broth. To prevent this the media were sealed with sterile vaseline. The media could then be stored in the ice box for many weeks without discoloration.

Organisms.—Three strains of pneumococci, three of hemolytic streptococci, and five of non-hemolytic streptococci were employed in these experiments.

Growth of Pneumococcus in Media Containing Sterile Plant Tissue.

The rate of growth of pneumococcus in broth is dependent upon several factors, the most important of which are (*a*) the growth activity of the bacteria used for seeding (6), (*b*) the size of the inocula, and (*c*) factors pertaining to the media such as its pH (7), its content in nutritive substances, etc. Depending upon the balance between these factors, growth as determined by the number of viable organisms present at different intervals during the life of the culture is divisible into four periods: (*a*) period of lag, during which the number of viable organisms in the culture decreases, remains constant, or slowly increases; (*b*) logarithmic period, in which the culture maintains its maximum rate of growth; (*c*) stationary period, in which the maximum rate of growth is not maintained, the organisms present, though viable, reproducing more slowly or not at all; and (*d*) period of decline, in which the number of viable organisms decreases rapidly, the pneumococcus culture eventually becoming sterile (6).

Effect of the Addition of Sterile Unheated Potato on the Growth of Pneumococcus.

With the above facts in mind, an experiment was planned to show what effect the addition of sterile unheated plant tissue to fluid media would have on the growth of pneumococci. The period of lag was first studied. Plain broth containing sterile unheated potato was used as the test medium. As a control, broth to which 1 per cent of dextrose had been added, was employed. Dextrose is readily fermented by pneumococcus, and is known to accelerate the growth of this organism.

Experiment 1.—Into a 300 cc. flask containing 150 cc. of phosphate meat infusion broth (pH 7.6) were placed four cylindrical pieces of unheated, sterile potato, each about 1.5 cm. long and 0.5 cm. in diameter. The flask was then incubated at 37°C. to insure sterility. A control flask, containing 150 cc. of broth of the same lot, to which 1 per cent dextrose had been added, was prepared. With the media at 37°C. each flask was seeded with 0.1 cc. of a 6 hour broth culture of Pneumococcus Type I. The culture used for seeding was actively growing, as indicated by the presence of long chains in the stained film. At frequent intervals over a period of 9 hours, fractions of both the dextrose broth and the potato broth cultures were removed and estimations made of the number of viable organisms present in each. In making these estimates the following method was employed. Several accurate dilutions of the culture were made. 0.5 cc. from each of two or more of these dilutions was added to a tube of 1 per cent dextrose agar and plates were poured. The necessary calculations for the different dilutions having been made, the average

TABLE I.

Acceleration of Growth of Pneumococcus in Broth Containing Sterile Unheated Potato.

Time after seeding.	Log of No. of colonies per 0.5 cc. of culture.	
	Plain broth + potato.	Plain broth + 1 per cent dextrose.
hrs.		
0	3.7	3.8
1	4.0	4.1
2	4.2	4.0
3	4.9	3.8
4	5.1	
5	5.8	4.0
6	6.3	4.0*
7	7.1	4.0*
9	7.9	4.0*

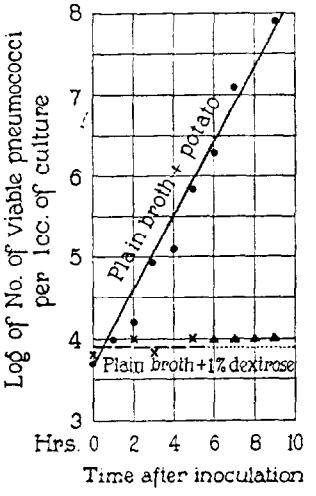
* No colonies in the dilutions plated. Therefore the exact determination was not made. The log is less than 4.

of the number of colonies present in the plates after 48 hours at 37°C. was used for the colony count per 0.5 cc. of culture for that hour. The results of these estimations as expressed in the logarithms of the number of colonies present at the time of each examination, are given in Table I. By using these logarithms as ordinates and the hours of examination as abscissæ, Text-fig. 1 was constructed.

From Table I and Text-fig. 1 it is evident that the organisms in broth containing sterile unheated potato grew steadily and at a constant rate. There occurred no period of lag. In contrast with this,

organisms seeded into broth containing an abundance of an easily fermentable substance, dextrose, but no plant tissue, failed to attain this maximum rate of growth for a period of at least 9 hours.

A second experiment was now performed in order to extend these observations and to determine the effect of the presence of unheated plant tissue upon the rate of growth during the entire cycle of a pneumococcus broth culture and also the effect upon changes in reaction which usually occur in the medium during growth. It is



TEXT-FIG. 1. Influence of sterile unheated plant tissue on the acceleration of growth of pneumococcus. ▲ indicates less than 4.

known that during growth of pneumococci in broth an increase in the pH occurs. When the medium is enriched by the addition of readily fermentable substances, as dextrose or certain other sugars, the acid production by the growing pneumococcus is still more marked (8).

Experiment 2.—The medium was prepared as in Experiment 1. Two 300 cc. flasks, one containing 150 cc. of 1 per cent dextrose broth, the other 150 cc. of plain broth to which had been added about 5 gm. of sterile unheated potato, were seeded with small identical amounts of a growing broth culture of Pneumococcus Type I. At intervals, as noted in Table II, samples were removed from each of the flasks. Estimations of the number of viable organisms were made, as in Experiment 1. Determinations of pH, by means of the colorimetric method, were made at the same time. The results of these observations are shown in Table II, and are graphically represented in Text-fig. 2.

From Table II and Text-fig. 2 it is seen that, under the conditions of the experiment, no period of lag occurred in the broth culture containing unheated potato. The control culture in dextrose broth, a medium quite favorable for prompt and abundant growth of pneumococcus when the inoculum is sufficiently large, showed a period of lag of 18 hours, during which the viable organisms in the culture apparently decreased in number. However, when once the culture

TABLE II.

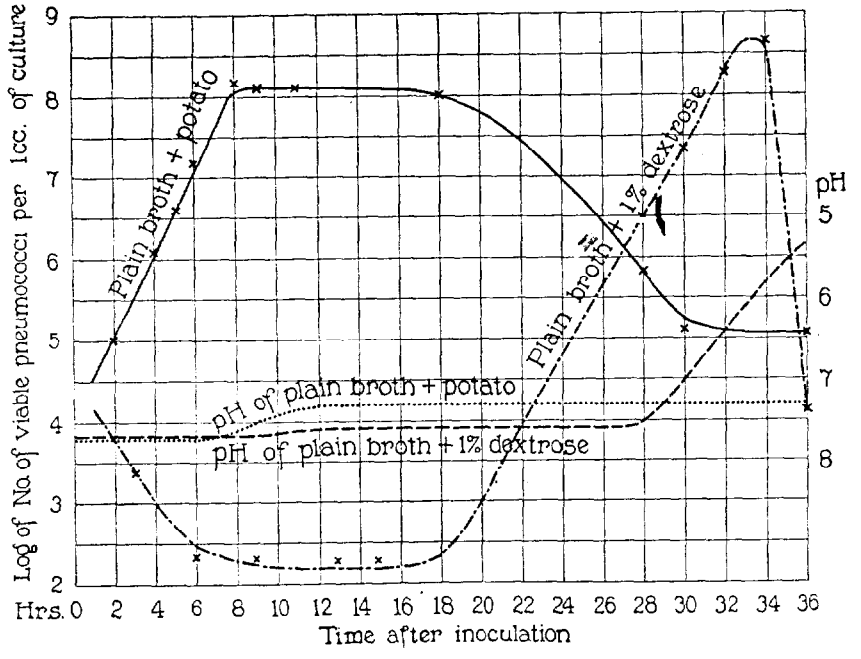
Comparison of Growth of Pneumococcus in Potato Broth and Dextrose Broth.

Time after seeding.	Broth containing unheated potato.		Broth containing 1 per cent dextrose.	
	Log of No. of colonies per 0.5 cc. of culture.	pH of culture.	Log of No. of colonies per 0.5 cc. of culture.	pH of culture.
hrs.				
1	4.5	7.7	4.2	7.7
2	5.0			
3	5.5		3.3	
4	6.1			
5	6.7	7.7		7.7
6	7.3		2.3	
8	8.2	7.5		7.65
9	8.1		2.3	
11	8.1			
13	8.1	7.3	2+*	7.6
15	8.1		2+*	
18	8.0		2.3	
28	5.8	7.3	6.5	7.6
30	5.1	7.3	7.4	7.1
32			8.4	6.3
34			8.7	5.3
36	5.1	7.3	4.1	

* Actual number of colonies not determined.

entered upon the logarithmic phase of growth, the generation time was approximately the same in the dextrose broth culture as in the potato broth culture; that is to say, cell division, when once initiated, proceeded at about the same rate in the two cultures. It is noteworthy that the stationary phase of the pneumococcus culture in potato broth extended over a period of 10 hours, while that of the culture in dextrose broth was of extremely short duration.

Other observations have shown that in the potato broth culture there is a prolongation of the period of decline in the growth curve; that is, the period in which cell death is proceeding at a more rapid rate than multiplication. It is especially prolonged when compared with a culture in dextrose broth. Moreover, when young actively growing potato broth cultures are removed from the incubator and are placed on ice, the bacteria may remain viable for very long periods



TEXT-FIG. 2. Influence of sterile unheated plant tissue on the growth curve of pneumococcus.

of time, even 8 months. These facts indicate that not only is the initiation of growth accelerated in the potato broth, but death of the bacteria occurs more slowly in this medium.

Effect of the Addition of Other Sterile Unheated Plant Tissues on the Growth of Pneumococcus.

The following experiment illustrates the effect on growth of pneumococcus when vegetable tissue, other than potato, is added to plain broth.

Experiment 3.—Sterile pieces of parsnip, sweet potato, carrot, yellow turnip, white turnip, and banana, each weighing about 0.5 gm., were placed in tubes containing 10 cc. of plain broth and these were sealed with vaseline. After incubating several days to insure sterility, the seals were removed and 2 cc. of the broth were taken from each tube for colorimetric pH determinations. Each tube was then seeded with 0.05 cc. of an actively growing broth culture of Pneumoccocus Type II. A tube containing 8 cc. of broth of the same lot, but no vegetable tissue, was seeded with the same amount of the same culture. The tubes were placed in the incubator at 37°C. Observations on the degree of clouding present in the media were made after 5 hours, and again after 17 hours incubation. The results are given in Table III.

TABLE III.

Acceleration of Growth of Pneumococcus in Broth Containing Sterile Unheated Vegetable Tissue.

Media.			Inoculum.	Growth of pneumococcus.*	
Broth.	Plant tissue.	pH at time of seeding.		5 hrs.	17 hrs.
cc.			*cc.*		
8	None.	7.8	0.05	−	++
8	Parsnip.	6.3	0.05	+	++++
8	Banana.	6.4	0.05	+++	++++
8	Sweet potato.	6.7	0.05	++	++++
8	Carrot.	6.9	0.05	++	++++
8	Yellow turnip.	7.1	0.05	++	++++
8	White "	7.2	0.05	±	++++
8	Potato.	7.4	0.05	+++	++++

* − indicates no macroscopic growth; ±, faint haze confined to vicinity of plant tissue; +, faint clouding throughout media; ++, moderate clouding throughout media; +++, marked clouding throughout media; ++++, maximum growth attained.

From Table III it is evident that other plant tissues have the same growth-stimulating effect upon pneumococcus that potato possesses. It is also evident that this stimulation operates in spite of the fact that the initial reaction of the media may be unfavorable for growth of this organism.

The Limiting pH for the Growth of Pneumococcus in Potato Broth.—It has been shown that the optimum hydrogen ion concentration for growth of pneumococcus is pH 7.8 and that growth in plain broth

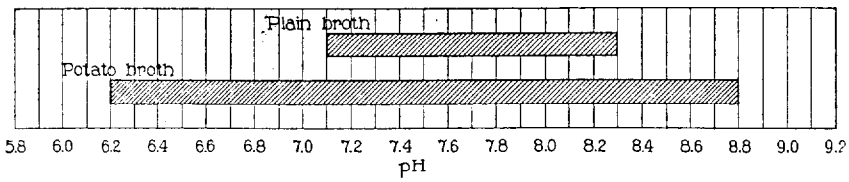
can be initiated only if the hydrogen ion concentration is between pH 7 and 8.3 (7). The observations made in Experiment 3 indicate that if there is present in the medium a bit of unheated vegetable tissue growth of pneumococcus can be initiated even though the hydrogen ion concentration is only pH 6.3. An attempt was made, therefore, to determine the limiting hydrogen ion concentrations for the initiation of growth of pneumococcus in broth containing sterile unheated potato.

TABLE IV.

Growth of Pneumococcus at Different Hydrogen Ion Concentrations in Broth Containing Sterile Unheated Plant Tissue.

Inoculum.	pH of media.	Growth of pneumococcus in plain broth after 18 hrs.*	Growth of pneumococcus in plain broth + potato after 18 hrs.*
cc.			
0.05	5.8	−	−
0.05	6.2	−	+
0.05	6.8	−	+
0.05	7.1	+	+
0.05	8.3	+	+
0.05	8.6	−	+
0.05	8.8	−	+
0.05	9.0	−	−

* − indicates no macroscopic growth; +, growth.



TEXT-FIG. 3. Influence of sterile unheated plant tissue on the range of hydrogen ion concentration for initiation of growth of pneumococcus.

Experiment 4.—Tubes containing 10 cc. of potato broth were prepared in the usual way and incubated to insure sterility. In a number of tubes the hydrogen ion concentration was adjusted, by the addition of suitable amounts of sterile N and 0.1 N HCl or NaOH, so that a series of tubes was obtained in which the reaction ranged between pH 5.8 and 9. In the same way there was prepared a series of tubes of plain broth, without potato, in which the hydrogen ion concentration ranged between the same points. All these tubes were then seeded, each with

0.05 cc. of a growing culture of Pneumococcus Type I, and incubated at 37°C.[1] Growth of pneumococcus occurred as is recorded in Table IV, and as represented diagrammatically in Text-fig. 3.

The results in Table IV indicate that the presence of sterile unheated potato in broth greatly increases the range of pH in which the growth of pneumococcus can be initiated. This increased range extends between pH 6.2 and 8.8.

Effect of the Addition of Sterile Unheated Potato on the Growth of Hemolytic and Non-Hemolytic Streptococci.

In order to determine whether unheated plant tissue might exert a growth-stimulating effect on other cocci, strains of hemolytic and of non-hemolytic streptococci were grown in plain broth alone and in the same broth to which unheated plant tissue had been added. Differences in growth activity of the same organisms in the two types of media were noted.

TABLE V.

Acceleration of Growth of Hemolytic and Non-Hemolytic Streptococci in Broth Containing Sterile Unheated Plant Tissue.

Organisms.	Growth in 10 cc. of plain broth.*	Growth in 10 cc. of potato broth.*
Results after 14 hrs. incubation.		
1. Hemolytic Streptococcus 3/22	−	+++
2. " " 23/20	−	+++
3. " " 84/18	−	+++
Results after 18 hrs. incubation.		
4. Non-hemolytic Streptococcus A141	−	++
5. " " A135	−	+
6. " " B39	+	+++
7. " " 38d	±	+++
8. " " A149	±	++

* − indicates no macroscopic growth; ±, faint haze; +, bacterial whirl; ++, marked growth; +++, maximum growth.

[1] A set of uninoculated tubes containing potato broth at reactions between pH 7.2 and 4.6 was also incubated to determine whether plant tissue alone, at 37°C., might cause a change in the pH. After 3 days at 37°C. the pH determinations on the broth in these tubes showed that no changes had occurred.

Experiment 5.—The media were incubated for 3 days to insure sterility. 24 hour blood broth cultures of three strains of hemolytic streptococci and five strains of non-hemolytic streptococci were used for seeding. In each instance the tip of a platinum wire was inserted the distance of 1 cm. into the supernatant fluid of the blood broth culture and then carried into the test medium. The latter was then placed in the incubator at 37°C. The degree of clouding of the medium was noted at the hours given in Table V.

From the results shown in Table V it is evident that sterile unheated plant tissue has a marked growth-stimulating effect upon hemolytic and non-hemolytic streptococci. Heavy clouding of the potato broth cultures of the three strains of *Streptococcus hæmolyticus* and, to a less degree, of two of the five strains of non-hemolyzing streptococci occurred before there was macroscopic evidence of growth of these organisms in the plain broth without potato. In the remaining cultures of non-hemolytic streptococci at the end of 18 hours growth was much more abundant in the potato broth tubes than in the tubes of plain broth.

SUMMARY.

In previous papers it has been shown that unheated plant tissue, in the form of potato, contains the two factors necessary for the growth of organisms of the hemoglobinophilic group. Further studies (5) confirmed these findings and showed that yellow and white turnip, carrot, beet, parsnip, and sweet potato can replace blood in the cultivation of *Bacillus influenzæ.*

In the present paper it has been shown that vegetable tissues also greatly facilitate and stimulate the growth of other organisms entirely unrelated to *Bacillus influenzæ.* Three varieties of Gram-positive cocci have been used in the present study, pneumococcus, *Streptococcus hæmolyticus,* and *Streptococcus viridans.* With pneumococcus it has been previously shown that prompt and luxuriant growth will occur in broth containing unheated potato even though the seeding be so small that no growth whatever will occur with the same seeding in plain broth (5). In the present study it has been shown that even in dextrose broth this minimal inoculation is followed by a prolonged period of lag, whereas in potato broth this same inoculum serves to initiate immediate and rapid growth. When pneumococci are grown in potato broth not only is the period of lag abolished, but the sta-

tionary period of growth is extended and cell death is delayed. Moreover, in plant tissue medium the zone of hydrogen ion concentration within which growth of pneumococcus can be initiated is considerably extended beyond the acid and alkaline limits of the optimal range in ordinary bouillon.

It has been found also that the presence of unheated plant tissue in the media likewise stimulates growth of hemolytic and non-hemolytic streptococci.

In this investigation no attempt has been made to determine the exact nature of the substances in plant tissue upon which these properties depend. That they are not of the nature of readily fermentable carbohydrates, however, is made evident by the fact that no increased production of acid occurs in the pneumococcus culture when potato is present.

CONCLUSIONS.

1. The initiation of active growth in broth culture of pneumococcus, *Streptococcus hæmolyticus*, and *Streptococcus viridans* is accelerated by the presence of unheated plant tissue.

2. Cultures of pneumococcus in broth containing unheated plant tissue show a prolongation of the stationary phase of growth.

3. Death of the individual organisms in pneumococcus broth cultures containing unheated plant tissue does not proceed so rapidly as in cultures without plant tissue.

4. The zone of hydrogen ion concentration within which growth of pneumococcus in ordinary broth can be initiated is considerably extended, both on the acid and on the alkaline side, by the addition of unheated vegetable tissue to the media.

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