FIELD AND CULTURAL STUDIES WITH PHYTOMONAS BETICOLA

THESIS

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by

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INTRODUCTION.

It is commonly recognized that the sugar beet industry, especially in many western states, is in precarious condition. The small margin of profit on the manufactured sugar, the distance from large markets, and the vicissitudes of water supply together with many other conditions, such as diminishing labor supply, have served not only to hamper development of the industry, but even to threaten its existence. But still more important than these economic and environmental factors are the disease factors which in recent years have caused the closing of many factories and which, if not controlled, will drive the beet industry from large areas in the west.

The epidemic diseases, leaf spot and curly top, stand first in importance in causing losses to the sugar beet industry, but second only to them is the group of diseases known collectively as the root diseases of the beet. During the last few years the epidemic diseases of the beet in certain areas are limiting factors in crop production, but each year the root diseases levy a definite toll upon the crop, and many fields are rendered unprofitable. These root diseases are numerous, and some have been but little studied. Their effects may be to cause complete destruction of the roots by rotting, such as is found in the Phoma and Rhizoctonia root rot, or there may be produced malformations which upset the normal metabolism, as occurs in tuberculosis of the beet.

The continued existence of the sugar beet industry is a vital necessity to successful agriculture in many western states. It is, therefore, of utmost importance that intensive study be made of all the im-

portant sugar beet diseases in order that proper methods of control for each of the disease factors may be discovered so that the losses, which year after year are jeopardizing the industry, may be reduced to a minimum.

This paper presents the results of experiments carried on during 1926 and 1927 upon the root disease which has been called sugar beet tuberculosis. The investigations here presented deal with the nature and etiology of the disease, the effect of the disease upon the metabolism of the sugar beet, the anatomy of the gall which is characteristic of the disease, and includes studies upon the reaction and affinities of the causal organism as well as observations and experiments to throw light upon the saprophytic existence of the organism in the soil.

HISTORY AND OCCURRENCE.

The recognition of tuberculosis of sugar beets as a distinct disease is comparatively a recent thing.

In the course of his classic work on crown-gall of plants, Dr. Erwin F. Smith and associates (14) discovered the tuberculosis of beets and called attention to it as a distinct disease. Working with material obtained from Colorado, Dr. Smith isolated and described the pathogen, a yellow bacillus, and he proved that this organism produced an entirely different type of overgrowth from that which is always found in the crown-gall disease. Because of the anatomical similarity of the overgrowths found in the newly discovered beet disease to tubercles found in tuberculosis of animals, Dr. Smith called the sugar beet disease tuberculosis, whereas the crown-gall caused by Phytomonas tumefaciens (Smith and Townsend) S. A. B. he considered plant cancer.

However, in spite of Dr. Smith's work, the sugar beet tuberculosis has continued to be confused with the true crown-gall, which also

occurs on beets. Both diseases are indiscriminately termed crown-gall by field workers and growers.

With a single exception of a publication by Townsend (16) entitled "Field Studies of the Crown-gall of Sugar Beets" in which the effects of the disease upon the sugar content and the purity as well as upon the actual per cent of sugar in the gall are given, there seems to have been no further contribution to the literature dealing with this disease. Townsend in his account gives no indication of the nature of the disease on the particular specimens analyzed, but many of the photographs given are very typical of tuberculosis as it occurs in beets in Colorado.

Such other references to the disease have been found, Sackett (12) and Peklo (9). The first author gives an abstract of Dr. Smith's work, while the latter worker worked with both the crown-gall and tuberculosis organism sent to him by Smith.

GEOGRAPHICAL DISTRIBUTION.

The disease is known only from the United States, and the reports of its occurrences are limited. As has been said, the first material was received by Dr. Smith from Colorado at the time he was working on the crown-gall problem. The material used by Dr. Townsend came from Girard, Kansas. The disease was next collected in September 1926 by G. H. Coons and Dewey Stewart from a field at Lamar, Colorado, in which some hail injury had occurred. They reported at least ten per cent of the beets affected in certain portions of the field. Since that time various collections have been made at Rocky Ford, Colorado, and in other fields in the Arkansas Valley, so that it can safely be said that the distribution of the disease is fairly general in this area. Mr. Asa C. Maxson of the Great

Western Sugar Company has found the disease at Lowell, Wyoming.**

Because of the common confusion of the tuberculosis of sugar beets with crown-gall and perhaps with overgrowths of other causes, it is impossible to approximate the field distribution of the disease. Dr. G. H. Coons states that he has not seen any gall resembling the tuberculosis gall in Michigan material, although true crown-gall occurs frequently.* Observations made by the writer in 1926 and 1927 of beets grown at Michigan State College showed a few cases of crown-gall, but no tuberculosis. Inspection of nine carloads of Michigan beets as well as inspection at the factory in Lansing, Michigan, of a large pile of beets revealed only one galled beet which proved to be crown gall. During the 1926 beet harvest at Lansing, Michigan, Mr. Lill of the U. S. Dept. of Agriculture sent in two crown-gall beets.

It seems then that the distribution of tuberculosis is limited to the western beet area, and it seems safe to predict that additional observations will undoubtedly reveal that the disease is more extensively distributed in the western territory than the few collections made so far would indicate.

ECONOMIC IMPORTANCE.

The occurrence of galled beets has been noted for many years, and these beets have always attracted attention at harvest time. Townsend (16) states that the crown-gall of beets has increased rapidly in recent years and that it is still on the increase. From the meager data at present available as to the distribution of the tuberculosis of sugar beet as distinct from crown-gall, it is obvious that no determination of actual loss caused by this disease can be made. The nearest approach that can

* Verbal communication.

** Letter.

be had to a determination of the potentialities for loss from the disease organism must be based upon the studies which have been made of the actual conditions in certain fields in Colorado. Estimations of prevalence in various fields at Rocky Ford and Lamar, Colorado, have shown a 3 to 4 per cen t infection, while one field was known to have run as high as 10 per cent. It is doubtful if the percentage of galled beets in the general factory run approaches 1 per cent, but the high percentage found in certain fields indicates that under certain conditions the disease may become of increasing importance.

Losses from the tuberculosis of beets may be considered under three heads:

(1) Rotting: Although tuberculosis does not itself cause rotting of the affected sugar beet, the loss which occurs from secondary invaders, chiefly saprophytic fungi, may be serious. The ruptured epidermis and the more or less disintegrated nature of the gall tissue afford easy avenues of entrance for fungi, and considerable decay of affected beets is to be expected in storage piles from these secondary invaders.

(2) Waste from topping: The tuberculosis of the beet as will be obvious from the photographs causes loss because of the wasteful topping which is given to diseased beets. Since the crowns contain such a high percentage of salts which prevent the sugar from crystallizing in the mill, it is essential that all of the crown be removed just below the scars of the lowest whorl of leaves. The tubercles are often on the crown and extending down onto the main part of the root. (Fig. 1). This means a very large portion of the beet is likely to be cut off by the topper, thus losing considerable tonnage for the grower, especially if the field is running 5 to 10 per cent diseased.

(3) The effect of quality in roots: The tuberculosis of the beet, however, produces its losses very largely because of the profound upset of

the sugar beet metabolism which it causes. Townsend has already for at least two types of sugar beet gall called attention to the injurious effects that some overgrowths produce upon the quality of the roots. He shows that the galls themselves are low in purity and are detrimental in the milling process.

In order to obtain further information on this point, the writer while stationed at the U. S. Dept. of Agriculture Field Station at Rocky Ford, Colorado, made analyses of galled beets and compared these with the analyses of the nearest nearby normal beets of approximately the same size. The samples were taken from a field of commercial beets of a high sugar strain which was being sampled at weekly intervals for the purpose of determination of the course of sugar production during the season. Whenever a naturally occurring gall was found, a nearby beet (within a radius of 4 feet) of approximately the same size was chosen for a comparative test. The beets were analyzed by the usual methods of handling individual beets and before wilting had taken place to any marked degree.

TABLE I.

Comparative analyses of normal beets and of beets affected with the tuberculosis disease. Analyses made when beets were approximately two-thirds grown.

Condition of Beets	Date 1927	Weight in Pounds	Sugar in Juice	Coefficient of Purity**	Size of Galls
1. Galled Beet	9 - 15	2 1/4	11.2	65.5	Small
*Normal "		2 1/4	12.8	77.7	"
2. Galled "	9 - 15	3 1/4	10.8		"
Normal "		3 3/4	12.0		"
3. Galled "	9 - 21	6	11.4		"
Normal "		4 1/2	13.0		"

Condition of Beets	Date 1927	Weight in Pounds	Sugar in Juice	Coefficient of Purity**	Size of Galls
4. Galled Beet	9 - 25	2 1/4	10.2	68.0	Small
Normal "		2 1/2	13.6	78.4	"
5. Galled "	9 - 25	2 3/4	11.2		"
Normal "		3	11.1		"
6. Galled "	9 - 25	3	11.0		"
Normal "		4	12.6		"
7. Galled "	9 - 25	2 1/2	11.0		"
Normal "		3 1/4	11.2		"
8. Galled "	9 - 15	2	10.8	63.0	Medium
Normal "		2 1/4	14.8	78.9	"
9. Galled "	9 - 15	3	10.4	64.5	"
Normal "		2 1/2	13.0	78.3	"
10. Galled "	9 - 30	2 1/2	10.4	66.2	"
Normal "		3	11.3	78.3	"
11. Galled "	9 - 25	3 1/4	11.2		"
Normal "		3 1/4	11.8		"
12. Galled "	9 - 15	4 1/4	9.4		Large
Normal "		3 3/4	13.0		"
13. Galled "	9 - 21	4	10.0	65.2	"
Normal "					
14. Galled "	9 - 21	2 1/2	9.2		"
Normal "		2 1/2	12.4		"
15. Galled "	9 - 30	2	11.6	72.0	"
Normal "		4 1/4	11.6	78.0	"
16. Galled "	9 - 25	4 1/2	9.8	64.0	"
Normal "		3 3/4	12.3	79.3	"
17. Galled "	9 - 25	4	10.3		"
Normal "		4 1/2	13.6		"
18. Galled "	9 - 25	6	9.2		"
Normal "		4 1/2	10.8		"
19. Galled "	9 - 25	2 1/2	10.6		"
Normal "		2 1/2	13.2		"
20. Galled "	9 - 15	6	10.6	61.2	"
Normal "		4 1/2	13.0	81.9	"

* The normal beet used as a check was adjacent to the galled one, except where a considerable difference in size made it desirable to choose some other beet within a radius of approximately four feet to serve as a check.
** Computed from solids in beet juice as determined by refractometer

The sugar content and coefficient of purity are the factors which determine the value of beets for sugar-making purposes. A considerable percentage of the total weight of the beets consists of soluble solids, of which sugar forms the largest portion. The galled beets show a very much lower coefficient of purity due to the larger amounts of impurities in the form of soluble solids other than sugar. The greater the proportion of other soluble solids present the lower the purity, hence the more difficult it is to extract the sugar. These diseased beets with their low sugar and purity percentage give syrup from which recovery of crystalizable sugar is poor, and the handling of such beets in the factory is unprofitable.

An additional experiment to the above was performed to determine the odds that the tuberculosis disease is significant in the reduction of the sugar content.

A number of artificially inoculated beets from three different varieties of plots were used. These beets were inoculated at intervals so that on August 29, 1927, when the sugar determinations were made, there were three stages of the galls present i.e. small, medium, and large. The checks were, in the majority of cases, the two adjacent beets; however, if this was not practical, the checks were taken within a four foot radius. Attempts were made to choose the normal and diseased beets as nearly the same size as possible, as shown in photographs. (Fig. 2).

In computing the data obtained in this experiment, the galled beet value is the actual beet sugar percentage, while the check value is the average of the two adjacent normal beets. The following tables give the odds calculated by Student's Method as to the significance of the differences, grouping the data according to gall size, small, medium, and large.

TABLE II.

Sugar percentages of diseased sugar beets contrasted with percentages found in normal beets. Tested at Rocky Ford, Colorado, summer 1927.

Variety	Sugar per cent in juice: Galled Beets	Normal Beets	Difference	Size of Gall	Odds
	12.5	14.60	-2.10	Small	
50165	11.3	12.53	-1.23	"	
166	11.5	12.03	- .52	"	
	11.0	12.69	-1.69	"	
					77 to 1
4088-23	10.0	10.84	- .84	Medium	
T	9.5	10.00	- .50	"	
	10.0	10.53	- .21	"	
	9.6	10.83	-1.23	"	
	11.1	11.80	- .70	"	
					9999 to 1
F. F.	9.8	12.82	-3.02	Large	
24	10.2	12.64	-2.44	"	
	8.8	11.51	-2.71	"	
	10.0	12.23	-2.33	"	
	9.5	11.90	-2.40	"	
					9999 to 1

The analyses given in the tables I and II show such consistency in the sugar and purity percentages found in beets affected with tuberculosis when compared with normal beets, and these differences as seen in the tables are so significant even in the case of beets affected with small galls, that the writer believes that the early statement of Townsend (16) is confirmed.

Thus with these three sources of loss the disease may become of very considerable economic concern to both the beet grower and the manufacturer.

SYMPTOMS.

The overgrowths of this disease are generally confined to the crown, but they are also at times observed down almost to the tip of the root. (Fig. 3).

There are as Smith (14) has pointed out two distinct types of overgrowths occurring on the sugar beet roots, and these have been designated as "tumors and tuberculosis" Townsend (16). It is almost impossible to differentiate the two types of galls apart unless certain well marked characteristics of the older galls, which make it possible to identify the two types of overgrowth are observed. In the tuberculosis of beet the young tubercle is a smooth, yellowish white proliferation, which shows no sign of outer cell disintegration. At this point it is almost impossible to determine the two above types of galls apart without taking into consideration internal structure, which will be described later. As the galls mature the older overgrowths are characterized by their roughness and badly fissured surfaces. The diseased root generally shows a tendency to be turnip shape, that is the beet does not develop with the normal long tapering root, but becomes short and very broad at the crown. Also a marked profusion of leaves may accompany this type of growth. The leaves come out in groups over the entire crown, and often extend down onto the root proper. Along with the flattening out of the crown, there generally arises swellings which extend as ridges from the middle of the root up to the crown. These ridges generally terminate in a large rough overgrowth. (Fig. 1). The primary tubercular focus which is the cause of this ridge development is generally deep within the tissue of the root with secondary tubercles at the apex of the ridges. These aspects are in marked contrast to the condition in other beet root galls, which generally are lateral fleshy out growths upon the affected roots. Over the surfaces of the old galls, secondary

tubercles can be noticed, and these nodular growths together with the deep fissure formation give the proliferation a very rough appearance. Near the end of the growing season the tubercles show a more or less marked decay of the outer layers of cells. The large fissures and cracks are found to penetrate as deep as one inch into the tissue of the root. These openings offer excellent avenues for entrance of secondary fungous invaders, and these under ordinary conditions lead to additional decay of the crown.

The internal appearance of this disease is very distinct from that of crown-gall. In this disease localized brownish watery spots with a general distribution can be noticed, hence the name tuberculosis. The galls also show hyperplasia and hypertrophy tissue which extends out in all directions. Upon microscopic examination of the spots, large masses of bacteria are found to be present. The spots vary from 2 mm. to 10 mm. in diameter, and extend up and down the gall 1 mm. up to 3 to 4 cm. These bacterial pockets have a viscid consistency, and under moist conditions are sometimes so very mucilaginous that the bacterial slime when touched may be drawn out in sticky threads. (Fig. 4). The diameter of the galls varies from about 1 cm. in young infections to as large as 1 dm. in old beets.

On Petioles: The only galls observed on leaf petioles were results of artifical inoculations. The proliferations are at first small, and appear to come from under the epidermis at the point inoculated. As the gall becomes older, the epidermis begins to crack and small fissures are formed. The overgrowth then takes much the same rough appearance as the galls on the roots. The galls when young are green, but as the epidermis is ruptured the outer cells disintegrate, becoming brown and subsequently dry.

Since crown-gall and tuberculosis of the sugar beet have so generally been confused, the following table is given. (See also the

photograph plates Figs. 1 and 5.)

TABLE III.

Comparison of Tuberculosis and Crown-Gall of Sugar Beets.

Cause	
Tuberculosis.	Crown-Gall.
A bacterial disease caused by Phytomonas beticola (Smith Brown and Townsend) S.A.B.	A bacterial disease caused by Phytomonas tumefaciens (Smith and Townsend) S.A.B.
Symptoms on the Root.	
1. Rough overgrowth produced.	1. Smooth or at most wrinkled overgrowth produced.
2. Rough appearance due to ruptured epidermis, and large fissures over surface of over growth.	2. Contour uneven in old galls, but epidermis intact without fissure formation.
3. Ridges extending from middle of root to crown terminating in the galled out growths.	3. No ridges present; gall generally separated from root by stipe.
4. Turnip shaped beet roots generally results from this disease, generally with a large amount of newly formed leaves coming out over entire crown.	4. The shape of root not changed except at place of tumor formation; no extra growth of leaves.
5. Secondary fungous invasion of the beet commonly found.	5. No secondary fungous growth generally associated with this type of overgrowth.

6. Small newly developed galls yellowish white, even contour, epidermis intact, no fissures.

6. Small young galls white, even contour, epidermis intact, no fissures.

Internal Symptoms of Roots.

1. Numerous yellowish watery spots present within galls. (Bacterial pockets) Surrounding tissue white.

2. Strands not very noticeable, outer cells of overgrowth decayed, fissures extending into tissue.

1. No bacterial pockets present; beet tissue white.

2. Strands of tissue extending in all directions within gall; outer cells not decayed.

Environmental conditions necessary for the Development of the Disease:

From field observations made in 1926 and 1927 in Colorado, it appears that the incidence of the tuberculosis disease providing wounds which occurs in young growing beets is strongly influenced by temperature and water factors. The observed correlations with the above factors has received positive confirmation from greenhouse tests in which temperature and humidity factors have been found to play a role in the success of inoculations. Injury to plants by hail usually results in abundant infections, as the hail is generally accompanied by heavy showers which give the organism exceptionally favorable conditions for penetration and establishment within the host tissue.

Various fieldmen for the western sugar beet companies have observed that after a hail storm certain fields were found to run very high in tubercle formation. It was also noticed by Coons, in the field in which this type of gall was first brought to his attention, that a great

amount of hail injury was evident. A letter from Asa C. Maxson at Longmont, Colorado, stated that "Several times during the past 18 years I have found this type of growth very common in fields badly injured by hail." Observations made in the field by the writer indicated that an injury to a beet root was necessary for entry of the organism into the host as is shown by the following table.

TABLE IV.

Results of Field Experiment on wounded and sound sugar and garden beet roots exposed to P. Beticola and P. tumefaciens.

Method of Inoculation	No. of plants showing symptoms.			
	5 days	10 days	15 days	20 days
12 plants - check	0	0	0	0
P. beticola - No. 1 25 plants - Injured	0	10	12	24
10 plants - check	0	0	0	0
P. beticola - No. 3 25 plants - Injured	0	5	14	25
P. beticola - No. 3 25 plants not injured	0	0	0	0
No inoculum 25 plants - not injured	0	0	0	0
Beet organism - No. 2				
25 plants - Injured	0	0	0	0
25 G. Beets - Injured	0	3	4	9
P. tumefaciens - No. 146 25 plants - Injured	0	0	0	15
P. tumefaciens - No. 146 25 plants - not injured	0	0	0	0

This experiment shows that infection in the field took place from seven days up to twenty days after inoculations were made, and that the

Phytomonas beticola cultures No. 1 and No. 3 are very virulent for sugar beets, but the No. 2 strain is only pathogenic on garden beets. Phytomonas tumefaciens (Smith and Townsend) S. A. B., M. S. C. No. 146, was also virulent to sugar beets and produced typical crown-gall.

Mechanical injuries, such as made by a cultivator just before irrigation water is applied to a field of beets, generally results in 50 to 60 per cent of the injured beets becoming infected. Mr. A. W. Skuderna of the American Beet Sugar Company has reported a field of beets which following rolling showed practically 100 per cent tuberculosis infection.*

Attempts at Rocky Ford, Colorado, to isolate the organism from irrigation water was unsuccessful, but it is evident that irrigation water can serve to carry organisms in the soil to the wounds arising from hail, rolling, or other cultural practices, and thus materially influencing infection. On the other hand, beets injured under dry soil conditions in the field have shown practically no invasion by the organism. The growing season for beets in the western states affords favorable soil conditions for the organism. The variation in weather conditions, such as temperature outside of the amount of hail, will show very little effect on the epidemic nature of the disease.

Under greenhouse conditions in Michigan, however, temperature has a definite effect upon disease production. Under cool conditions when the beet root is in a very slow growing condition, the disease may not be produced, even with direct inoculation. There seems to be a distinct ratio between the rate of root growth and disease incidence. Under the above circumstances then, it seems to indicate that temperature, root injury, and soil water are important factors influencing the severity of outbreaks of the disease.

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*Verbal communication.

PATHOGENICITY.

The causal organism was isolated by pouring plates in the usual manner, using a small amount of the yellow portion taken from a firm gall. In 48 hours, when the gall was of the tuberculosis type, yellow organisms such as described by Dr. Smith appeared in great abundance on the plates. Potato dextrose agar was found to be a good medium for isolation, and for continued growth of the organism.

In making inoculations ten rapidly growing beets with roots that showed the anomalous secondary growth were used. The method of inoculation was the puncturing of the beet crown with a needle whose point had been dipped in a suspension of the bacteria to be tested. Ten beets of the same type as above were used as checks. They were injured by puncturing the same as the others except the needles were not dipped into the suspension.

After twenty-six days all ten beets showed the symptoms of the disease, while no check beet developed overgrowths. The galls of the diseased beets were cut open, and numerous bacterial pockets were observed in each one. Platings from these pockets produced a great abundance of yellow colonies. A second series of inoculations of the reisolated organism into ten beets gave typical tubercles again. The check plants again showed negative results. There can be no question of the etiological relation of the yellow organism isolated to the tuberculosis of the beet.

With the pathogenicity of the yellow organisms established on sugar beets, the question of cross inoculation arose. The first cross inoculation tried was on ordinary garden beets (Beta vulgaris L.) Inoculations both from agar slants and broth cultures were made on ten roots; infection on these showed a 100% positive. Symptoms on these beets (Fig. 6) showed up in 19 days under greenhouse conditions. The galls showed unusual-

ly large fissures, which were formed from an excess amount of splitting, and foreign soil invaders were plentiful. Crown-gall organism, P. tumefaciens, was inoculated as a check; they only showed an 83 per cent infection. The first gall was noticed 32 days after inoculation, with an average of 34 days for all.

In the course of the work a number of organisms have been isolated and used in infection experiments. These organisms have also been used in serological investigations, as will be discussed later; these isolations have shown certain characteristics. For convenience these organisms have been called "strains". The following is the list of isolations used with a summary of their outstanding peculiarities.

Strain No. 1. Isolated from sugar beet received from Rocky Ford, Colorado, October 16, 1926. Good grower. Strong virulence until Sept. 1927, when strain died out.

Strain No. 2. Isolated by Miss M. C. Carpenter of Michigan State College from a sugar beet sent from Lamar, Colorado, Oct. 13, 1926. Good grower. Strong virulence on garden beets, but not virulent to sugar beets.

Strain No. 3. Isolated from soil obtained at Lamar, Colorado, by use of agglutination method later described. Good grower. Strong virulence to both sugar and garden beets.

Strain No. 4. Reisolated from garden beet, Rocky Ford, Colorado, which had been inoculated with strain No. 2. Same type of virulence as No. 2.

Strain No. 5. Reisolated from beet inoculated with Strain No. 1. Good grower. Strong virulence for sugar and garden beets, also to Swiss Chard. "R" form seems to be present.

Strain No. 6. Reisolated from sugar beet artificially inoculated with No. 5. Reactions same as No. 5. "S" forms probably present.

Strain No. 7. Same source as No. 1 but did not die out.

P. tumefaciens, M. S. C. No. 146. A culture obtained from Dr. E. F. Smith. Isolated from hop. Has been grown for some years at Michigan State College, and was used in comparison with the tuberculosis pathogen.

HOST CONSIDERATION.

The cultivated sugar beet (Beta vulgaris L.), as represented by two varieties grown at Rocky Ford, Colorado, namely Pioneer and Flat Foliage, was found very susceptible to the tuberculosis disease discussed in this paper. No varietal differences as to resistance or susceptibility were found in sixteen varieties of commercial beets which were tested by artificial inoculation. The disease has also been produced on garden beets (B. vulgaris L.) and Swiss chard (B. vulgaris L.). When inoculations were made into tomatoes (Lycopersicon esculentum), no typical gall formation resulted. However, a small, smooth, yellowish spot on the stem at the point of injection (Fig. 9) was noticed 28 days after inoculation. Pure cultures of the organism were obtained from this spot at that time. What probably is the case here is that the tomato can harbor the organism without reacting to gall formation. Tobacco (Nicotiana sp.), raspberry (Rubus sp.), apple (Malus sp.), and geranium (Geranium sp.) were all inoculated but showed no symptoms of gall formation. In contrast to these results, all the above plants with the exception of tobacco developed overgrowths when inoculated with the crown-gall organism in a series of tests paralleling the above mentioned inoculations. No wild host has been found, although various weeds, many of them in the same family as the beet, were

examined in fields where beets were developing the disease naturally.

So far as information goes at present, the tuberculosis disease is confined to the members of the genus Beta, a group of plants commonly thought to be a closely related species, if not members of the same species.

TAXONOMY.

The organism was first described by Dr. Smith in 1910 under the name Bacterium beticolum n. sp. Smith, Brown, and Townsend.* According to the revision of Migula's and Smith's classifications adopted by the Committee of the Society of American Bacteriologists in 1920 (10), the terminology would be Pseudomonas beticola. In a later report (11) of another committee of the same society the genus name Phytomonas was made to include organisms of this character, whence the name Phytomonas beticola which is used in this paper.**

DESCRIPTION OF THE ORGANISM.

The following description of the organism is based upon strain No. 1, isolated by the writer from Colorado beets. In a large part the tests outlined follow the work reported by Dr. Smith; however, where significant variation from Dr. Smith's results have been found and additional information has been obtained, the statements are starred.

Morphological Characters: The organism in the host is a small rod with rounded ends occurring singly, but at times in pairs or clumps; in different media, however, it varies greatly. The measurements of

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* The word 'cola' being a Latin noun meaning inhabitant, cannot be changed to 'colum' as used by Dr. Smith and associates in naming the organism. They assumed erroneously that this came from an adjective--colus, a, um. The species name when a noun cannot be changed to correspond with the gender of the genus, this only taking place when the species name is an adjective. Verbal explanation by Dr. E. A. Bessey.

** The species name in Bergey's Manual is written 'betacola' instead of 'beticola'.

single rods vary from 0.5 to 0.9μ by 1.0 to 1.9μ, *the average size being 0.6 by 1.4μ.

The organism is motile by the means of polar flagella, which vary in numbers from one up to four. *The flagella are very tenuous and range from 2.2 to 3.7μ in length, the length being much larger on the large cells. The best results in staining flagella were obtained by using a mordant of 1 part 2% Osmic acid and 2 parts 10% tannic acid followed by standard carbol-fuchsin stain. (Fig. 7). No endospores were observed in any cultures examined, which included 24 hour old cultures to one six months old.

Capsules were demonstrated by the method given in Giltner's Manual, when the organism was grown in Uschinsky's solution. However, the organism when grown upon nutrient broth or agar and potato dextrose agar shows no capsule formation.

Zoogloeae occur when Uschinsky's solution was offered, also psuedozoogloeae was produced in 48 hour old potato dextrose agar slant cultures. Microscopic examination of such material shows the cells embedded in a gelatinous mass.

Behavior toward Stains:

*The organism is gram positive, and stains readily with all the ordinary basic anilin stains such as gentian-violet, carbol-fuchsin, methyl-green, safranin, and methylene-blue.

Cultural characteristics:

Potato dextrose and beef bouillon proved to be very favorable for prolonged growth.

Gelatine Stabs:

There is a slight granular growth along the entire line of the

puncture. The best growth is at the top where it is villous or papillate, the length of the villi or papillae decreasing downward. After 18 to 21 days at 20° C. it shows a fair degree of liquefaction, but this is not complete until sometime between the 30th and 40th day.

Gelatine Plates: Colonies of the organism when planted on these plates showed up within 36 hours, being whitish at first then becoming yellow. On the surface of the media the colonies appear round, glistening with a smooth flat surface, but in the substratum they appear elliptical in shape. Slight liquefaction begins in five days at a temperature of 20° C. In thickly sown plates, liquefaction proceeded rapidly, becoming completely so in eighteen to twenty-five days. In thinly sown plates the colonies average size was 1.2 cm., and small saucer-shape liquefactions were formed.

Agar plates: *On potato dextrose agar plates Ph. 7.2 kept at 26° C. colonies appeared in 24 to 30 hours. They are at first white, round, smooth, and after 48 hours they start to turn yellow. The margin is entire without any conspicuous zonation; the yellow color is diffused throughout. The individual colony is somewhat sticky to the touch of a needle and becomes viscid in old cultures; it is glistening, somewhat raised or convex, and internally appears amorphous. Submerged colonies are elliptical in shape and showed small growth.

*Potato sucrose agar: Same as Potato dextrose, except colonies become a more deeply yellow.

Plain nutrient agar plate: Same as potato dextrose, only the growth is not nearly so abundant.

Plain nutrient agar slant: A streak culture will show moderate normal filiform growth for the first five days then a change to filiform-fimbriate type takes place. (Fig. 8).

Loeffler's blood serum: Growth restricted, whitish yellow, dull, filiform, convexed slightly; medium was not liquified.

Maltose Agar: *Very scanty filiform growth, but the organism secretes a very bright yellow-brown pigment producing more color than with other sugars.

Lima bean agar: *Growth very feeble to nil.

Prune juice agar: *Scanty growth.

Nutrient broth: In beef-peptone bouillon Ph 7.2, there is a moderate clouding in 24 hours, and heavy in 48 hours. A thin pellicle sometimes forms over the surface which readily breaks up and falls in thin flakes. *But the usual case is moderate clouding settling to the bottom with a formation of a scant amount of yellow precipitate.

Uschinsky's solution: A moderate to copious growth is produced in this medium. Growth is best at the surface in the form of clouding, zoogloeae, and a slight pellicle. After a few days the entire liquid is very viscid, and stains show large thin walled capsules present. Color is first white becoming yellow in old cultures.

Cohn's solution: *Growth very scanty.

Nitrate Reduction: Nitrates are promptly reduced. Tests were made on cultures in peptonized broth with use of Sulphanitic acid (sol.I) and a-naphthylamin (sol.II).

Indol Production: Indol is produced in small but definite amounts in 1% peptone, 0.5% disodium phosphate, 0.1% magnesium sulphate in distilled water. Also good amounts were formed in 2% peptone water. B. coli was used as a check and showed much stronger reaction.

Coon's synthetic: *Growth first white, not as abundant as on potato dextrose; later turning yellow.

Carrot plugs (Raw): *It grows readily with a yellow color covering the entire slab, but not rotting the tissues. The non-rotting action indicates the absences of a pectic enzyme.

Steamed potato cylinders: It grows at a moderate rate, flat, spreading, smooth, dull yellow, and somewhat glistening. *The potato changes very slightly to a dry grayish brown color in 5 days. Diastasic action is very marked. Tests were made by use of this method for diastasic action on starch. At four and six days, tests were made by pouring Lugol's iodine solution over the plates. Light areas appeared around the colonies to a very marked degree. P. tumefaciens M. S. C. No. 146 streaks used as a check showed less diastasic reaction.

Hydrogen sulphide: *Hydrogen sulphide is not produced. Tests were made by hanging pieces of lead acetate paper in mouths of tubes of bouillon, potato, and milk cultures.

GAS PRODUCTION.

A basal solution was made by adding two per cent of peptone to water. Six solutions were made from this, each containing one per cent respectively of one of the following carbon compounds: glycerin, sucrose, mannite, dextrose, maltose, lactose. Five fermentation tubes were filled with each of these solutions and sterilized by heating for twenty-five minutes on three successive days. Three tubes of each set were inoculated and two were left as a control. The inoculated tubes each received a one-millimeter loop of culture material from a twenty-four hour old bouillon culture. Thirty-six hours after inoculation, a cloudiness appeared in the open arm of each of the inoculated tubes. This turbidity increased with age, extending up into the closed arm in the dextrose, sucrose, and maltose cultures, but not in the tubes containing glycerin and

lactose. There was a well-defined line between the growth in the bulb and the clear liquid in the closed arm in the latter two tubes. This points toward the facultative nature of the organism, except where certain sugars are offered. No gas was produced in the closed arm in any of the substances listed during a twenty-five day period.

ACID PRODUCTION.

*Various experiments indicate that this organism does not produce gas in any of the ordinary nutrient media. In the presence of certain sugars, however, the acid reaction was very marked, as shown in the following tables.

TABLE V.

Behavior of P. beticola strains and P. tumefaciens on various sugars with an indicator of Bromo-Cresol-Purple.

	Sucrose and gas		Mannose		Dextrose		Lactose		Glycerin		Levulose		Maltose		Galuctose		Plain	
	Acid	Gas	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
B. beticola No. 5	+	-	+	-	+	-	-	-	-	-	+	-	+	-	+	-	-	-
P. tumefaciens No. 146	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. betiola No. 3	S	-	-	-	-	-	-	-	-	-	+	-	S	-	+	-	-	-
Check	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

TABLE VI.

Behavior of P. beticola on various sugars when the Quinhydrone Method was applied.

Type of Carbohydrate Plus Nutrient Broth	Original Ph	Ph at end of 24 hrs.	Ph at end of 5 days	Ph at end of 10 days
Plain Broth	7.13	7.46	7.90 -	8.00 -
Dextrose 1%	6.91	6.76	5.54	5.32
Sucrose	6.98	6.75	5.41	5.07
Lactose	7.31	7.45	7.53	7.62
Mannose	7.24	6.98	6.61	5.98
Glycerine	7.02	7.12	7.24	7.30
Distilled - H_2O - Sucrose	6.90	6.90		6.75
Dextrose - Check	6.91			6.91
Plain Broth - Check	7.13			7.12

THERMAL DEATH POINT.

In preliminary tests it was found that the thermal death point lies between 49° and 50° C. The following tests were made to determine the death point more exactly. Seven sets of fourteen tubes each of peptonized beef broth were inoculated with P. beticola from six day old slants of potato dextrose cultures. The tubes were placed in water at a constant temperature of 49° C, 50° C, 51° C, 52° C, 53° C, 54° C, and 55° C, respectively. The tubes were removed at the end of ten minutes and were kept at 25° C for two weeks. At the end of six days growth had taken place in all the tubes of 49° C and eleven tubes of 50° C. Growth had also appeared in two of the fourteen tubes that had been kept at 51° C. No growth appeared at the end of two weeks in tubes that had been kept for

ten minutes at 52°, 53°, 54°, and 55°C., except one tube at 53°C.

The experiments at 50°, 51°, and 52°C. were repeated several times with culture isolation strains No. 7, No. 1, No. 5, and No. 3, and in most all cases there would be one to three tubes showing growth at 51°C, but in no other series was growth observed in 53° or 54°C. *The thermal death point, therefore, is between 50°C and 51° C. Smith in 1911 placed the thermal death point of P. beticola at 51°C.

Resistance to drying: Small drops of four day old bouillon cultures were placed on sterile cover glasses in a covered sterile petri dish and left at room temperature which ranged from 18°C to 23°C. At intervals of twenty-four hours one of the cover glasses was transferred to a tube of sterile bouillon. These tubes were used for each test. Growth occurred up to the eighteenth day, after which the tubes remained sterile.

This experiment was repeated a number of times with bouillon cultures from four to ten days old. As a rule there was no growth after the eighteenth day; in one case, however, growth occurred on the twenty-first, and another case on the sixteenth day.

*Optimum temperature. The optimum temperature for growth appears to lie between 25° and 28°C., preferably 26°C. Growth at 26°C on potato dextrose and peptonized beef bouillon was considerably better than at 32°C. Growth at 20°C. and 37°C. was about the same but was considerably less than at 32°C. and much less than at 26°C.

Maximum temperature: Four day old bouillon cultures of No. 5, No. 6, and No. 7 isolations of the organism were used to inoculate slants of potato dextrose medium. Six tubes were used for each culture; three tubes of each lot were placed in an incubator at 39°C; and three were kept at 26°C. All cultures, both in the incubator and at 26°C. made good growths; however, those exposed to the higher temperature were of the color

of the medium, while those in the lower temperature had developed the normal yellow color after thirty-six hours. On the tenth day two of those at 39°C. were placed at 26°C., and in twenty-four hours they took on the normal yellow color. At 42°C. no growth occurred. *The maximum* temperature for the organism for growth can be placed between 39°C. and 42°C.

Effect of sunlight: Moderately thick sowings of 24 hour broth cultures were made on potato dextrose poured plates. One-half of each plate was covered with black paper, and the plates were set on a white enamel surface so that reflection would have effect also.

These plates were exposed to October sun for 2 1/2, 5, 10, 15, and 20 minutes, duplicates being used for each time period. They were then incubated at 26°C. No difference in number of colonies could be detected for any of the periods in the exposed as against the covered half. The results were rather unexpected since bacterial pathogens are often described as sensitive to sunlight, certainly within the range employed, 5 to 20 minutes being sufficient to kill or very much retard colony development in a large number of forms. The experiment was repeated in July at Rocky Ford, Colorado, with exposures the same as above, except additional exposure of 30, 40, and 45 minutes were included. The sun was exceptionally bright, but there was hardly more than 55 per cent reduction in the number of colonies on plates exposed and those not exposed. The organism does not appear to be so very sensitive to sunlight, unless exceptionally bright and over considerable length of time.

Loss of virulence: Park (6) stated that, "bacteria differ --- as to ease and rapidity with which they grow in any nutrient substance and the amount of poison they produce. Both of these properties not only

vary greatly in different members of the same species, but each variety of bacteria may to a large extent be increased or diminished in virulence."

In October 1926 three cultures were isolated from sugar beets. All the cultures were grown continually on potato dextrose medium. It was observed that a culture designated as No. 1 was the most vigorously growing strain in hand. The inoculation work in the greenhouse at Michigan State College showed its pathogenicity was very marked. Also the first field inoculations at Rocky Ford, Colorado, in July, 1927 showed the marked virulence of the organism. Late in the middle of August after a month without new transfers the strain seemed to lose its vigor on culture media, and the beet injury was also far less marked when it was inoculated into beet roots. Only 43 per cent of the plants inoculated produced galls, while formerly it was not uncommon to get 100 per cent infection with rapidly growing cultures. The original culture finally died out, but reisolations from beets with this organism proved to be a rapid grower, producing the same color on potato dextrose. It was designated as No. 5 which has a high virulence. From these results it seems possible that the rate of growth of the pathogen may be one of the controlling factors in the disease production of the organism.

AGGLUTINATION TEST.

As a method of Identification of a specific phytopathogenic bacteria.

The writer's researches are concerned with the production of specific agglutinins of P. beticola, for use in diagnosis and for studies on the relationships to other plant pathogenes.

Production of Antiserum: The methods followed were in general similar to those given by Zinsser (17). However, in order to get a high titre, modifications had to be made. It was discovered, as the following data will show, that a dead organism produced a very low titre. It was

necessary to use living cultures to get a sufficiently high titre.

The organisms used for antigens were the various strains, listed on page 17, of P. beticola, P. tumefaciens, and a culture of E. carotovora. A 48 hour culture from potato dextrose agar (Ph 7.2) was washed from the slant with normal saline, and after filtering through sterile cotton and being shaken with beads, it was adjusted to standard density No. 2 by use of McFarland nephelometer.

In the first attempts the standardized suspensions of P. beticola were heated to 56°C. for one hour before the intravenous injections. The titres obtained with this material were not very high, namely 1 to 950 and 1 to 1200. In another series of rabbits the antigen was injected without the preliminary heating. After 5 injections of 1 cc. of the antigen given intravenously at 2 to 4 day intervals, serum of satisfactory titre was obtained, 1 to 2200 and up to 1 to 3600.

The following are the titres obtained by the latter method of rabbit serum immunized against the indicated antigens.

Rabbit 407	Titre	1 - 1200	Anti - P. beticola No. 1
Rabbit 239	"	1 - 2200	Anti - P. beticola No. 5
Rabbit 101	"	1 - 2750	Anti - P. tumefaciens " 146
Rabbit 397	"	1 - 3050	Anti - P. tumefaciens " 146
Rabbit 294	"	1 - 3250	Anti P. beticola No. 5
Rabbit 295	"	1 - 3600	Anti - P. beticola No. 2
Rabbit 395	"	1 - 650 (intraperitoneal)	Anti E. Carotovora.

Having obtained the high titre serum , various plant and soil bacteria were subjected to cross agglutination tests. In the tests the macroscopic method of determination was used. Some difficulty was experienced with P. tumefaciens in that it had a tendency to form small clumps,

which formed spontaneously in the .85 per cent salt solution; however, with a 0.5 per cent salt solution this was obviated.

The results of the various tests are shown in the following tables:

TABLE VII.

Test of Phytomonas beticola Strain No. 5 antiserum against various antigens.

Animal	Antigen	Dilutions						
		1/10	1/20	1/50	1/100	1/200	1/500	Check
239	P. beticola No.5	+ +	+ +	+ +	+ +	+ +	+ +	-
	P. beticola No.4	+	±	-	-	-	-	-
	P. beticola No.2	-	-	-	-	-	-	-
	B. noctuarum	-	-	-	-	-	-	-
	E. atroseptica (1)	-	-	-	-	-	-	-
	E. atroseptica (2)	-	-	-	-	-	-	-
	P. vignae	+	±	-	-	-	-	-
	B. sphingidis	-	-	-	-	-	-	-
	E. carotovora	±	-	-	-	-	-	-
	*P. tumefaciens	+ +	+ +	+ +	+ +	+ +	+ +	+ +

*Saline solution .85% caused floculation.

+ + Strong flucculation.
+ Medium "
± Doubtful "
- No "

TABLE VIII.

Test of Phytomonas tumefaciens No. 146 antiserum against various antigens.

Animal	Antigen	Dilutions						
		1/10	1/20	1/50	1/100	1/200	1/500	Check
397	P. beticola No. 5	+	-	-	-	-	-	-
	P. beticola No. 4	-	-	-	-	-	-	-
	P. beticola No. 2	±	-	-	-	-	-	-
	B. noctuarum	-	-	-	-	-	-	-
	E. atroseptica (1)	-	-	-	-	-	-	-
	E. atroseptica (2)	-	-	-	-	-	-	-
	P. vignae	+	±	-	-	-	-	-
	B. sphingidis	-	-	-	-	-	-	-
	E. carotovora	-	-	-	-	-	-	-
	P. tumefaciens (In .05 saline)	+ +	+ +	+ +	+ +	+ +	+ +	- -

TABLE IX.

Test of Erwinia carotovora antiserum against various antigens.

Animal	Antigen	Dilutions						
		1/10	1/20	1/50	1/100	1/200	1/500	Check
396	P. beticola No. 5	±	-	-	-	-	-	-
	P. vignae	-	-	-	-	-	-	-
	P. tumefaciens	+	+	±	-	-	-	-
	E. carotovora	+ +	+ +	+ +	+ +	+ +	+ +	-

TABLE X.

Test of Phytomonas beticola Strain No. 2 antiserum against various antigens.

Animal	Antigen	Dilutions						
		1/10	1/20	1/50	1/100	1/200	1/500	Check
406	P. beticola No. 5	-	-	-	-	-	-	-
	P. beticola No. 4	+ +	+ +	+ +	+	±	-	-
	P. tumefaciens	-	-	-	-	-	-	-
	P. beticola No. 2	+ +	+ +	+ +	+ +	+ +	+ +	-

TABLE XI.

Test of Phytomonas beticola Strain No. 5 antiserum against various antigens.

Animal	Antigen	Dilutions						
		1/10	1/20	1/50	1/100	1/200	1/500	Check
294	P. beticola No. 5	+ +	+ +	+ +	+ +	+ +	+ +	-
	P. beticola No. 7	+ +	+ +	+ +	+ +	+ +	+ +	-
	P. beticola No. 2	-	-	-	-	-	-	-
	E. atroseptica (1)	-	-	-	-	-	-	-
	E. atroseptica (2)	-	-	-	-	-	-	-
	E. amylovora	-	-	-	-	-	-	-
	E. carctovora	+	-	-	-	-	-	-
	Ps. flourescens	-	-	-	-	-	-	-
	P. vignae	±	-	-	-	-	-	-
	P. hyacinthi	-	-	-	-	-	-	-
	P. tumefaciens No. 146	+	±	-	-	-	-	-

The tabulation of the first antiserum experiment (Table VII) indicated that the antiserum of P. beticola No. 5 shows no very strong reaction with any antigen except the homologous antigen. However, a yellow organism reisolated from a garden beet at Rocky Ford, which was observed to have gall formation, showed a slight reaction. It is suspected that this particular organism is also a soil organism of that vicinity. E. carotovora showed a very slight tendency toward a reaction, but could only be detected with microscopic methods. P. vignae showed a slight reaction with serum from rabbit 239, but from 294 no reaction was noticed. This organism, much the same as P. tumefaciens, has a tendency to flocculate in saline .85% solution, which may have helped to cause the conflicting results in the two.

The antiserum of P. tumefaciens proved to be very specific for only its own antigen, except in the case of a 1-10 dilution reaction with P. vignae and P. beticola No. 5. The first reaction was very marked and a group relationship between these two organisms seemed close. The agglutination with P. beticola No. 5 was very slight, and no strong group agglutination is apparent.

The antiserum E. carotovora from rabbit 396, which was the result of intraperitoneal injections, showed a very low titre. However, it was specific for its own antigen except when P. tumefaciens was used. Here a very noticeable reaction was found. Link (5) has also observed the same phenomena. The antiserum of P. tumefaciens showed no affinity, however, for E. carotovora. This seems to indicate that a specific protein is present in E. carotovora for the production of antibodies which will react with P. tumefaciens, but the production is not vica versa.

The antiserum of P. beticola strain No. 2 was specific to its own antigen except when the No. 4 strain which was reisolated from the red

garden beet at Rocky Ford was used. No agglutination whatsoever was noticed with P. beticola No. 5 or P. tumefaciens. These two are evidently a soil organism from the Rocky Ford district which shows pathogenicity for beets with a very low resistance.

The antiserum of P. beticola No. 5 from rabbit 294 showed a slight tendency toward agglutination with 1 - 10 dilution of P. tumefaciens and E. carotovora. No other reactions could be noticed. The above P. beticola No. 5 antigen showed a tendency for roughness (R), while a culture designated as No. 6 was obtained which seems to show smooth (S) appearance. These two showed no difference in the agglutination reaction of the above antiserum.

The work has shown that when titres are obtained which run above 1 - 1200, they are sufficient for diagnostic purposes, as given under the identification experiment. The antisera of P. beticola and P. tumefaciens are specific respectively for their own antigens to a very marked degree. It can safely be said that in relatively high dilutions (1 - 500) the identification of specific plant pathogenes studied can be made.

OVERWINTERING OF THE ORGANISM.

In common agricultural practice, beets are topped in the field, and it is obvious with a disease of the type of tuberculosis that debris containing the organism is thus scattered in the field. To determine if the organisms lived over in the tops, two beets showing gall formation were placed on Nov. 3, 1926 in sandy loam at a depth of six inches. One of these beets was examined March 3, 1927 and the other April 29, 1927; both were somewhat disintegrated. (Fig. 10). Isolations made from the interior of the decaying galls gave a great variety of organisms, but in dilutions of 1 - 10,000 a very great preponderance of yellow organisms

were found, which by cultural tests and inoculations were shown to be P. beticola. The experiment was run under Michigan conditions, and the air temperature during this time reached as low as 10 F below zero. It is evident from these isolations that bacteria in the galls can withstand severe conditions, as the soil around the beets was frozen from the last of November to March.

Three tubercles from a beet that had been kept in the ice box from October 16, 1926 to January 15, 1927 were ground in a meat grinder. This material was mixed thoroughly with sterilized potted soil from the greenhouse, having a PH reaction of 6.80. Ten pots were used and the following quantities of the ground material were distributed to the various pots of the series: 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 30.0, 50.0, 100.0, and 200.0 grams. Young sugar and garden beets showing about 7 sets of leaves and whose root bundles had started to differentiate into the collateral type were transplanted to the pots. After the plants were firmly established, the dirt was carefully removed from one side of the root. The crown and root was injured by scratching with a sterile needle, care being taken to injure the roots by a longitudinal scratch so as to expose the lower portions of the beet to the material to be tested. Over a period of twenty-seven days the pots were watered uniformly with sterile water; after this time the plants received ordinary care. The results of the tubercle formation are given in Tables 12 and 13.

TABLE XII.

Infections secured in a 40-day period with varying amounts of ground tubercle material. Young sugar beets exposed to infection after being wounded at crown and along the roots.

Pot	Quantity	Symptoms showed up					
		28 days	29 days	30 days	31 days	32 days	40 days
1	0.5 gms.	0	0	0	0	0	0
2	1.0 "	0	0	0	0	0	0
3	2.0 "	0	0	0	0	0	0
4	5.0 "	0	0	0	+	+	0
5	10.0 "	0	0	0	0	+	+
6	20.0 "	0	0	+	0	+	+
7	30.0 "	±	+	0	+	0	+
8	50.0 "	0	0	0	+	+	+
9	100.0 "	0	0	+	0	+	+
10	200.0 "	0	0	±	+	+	+

0 No symptoms.
+ New symptoms each.
± Doubtful.

TABLE XIII.

Infections secured in a 40-day period with varying amounts of ground tubercle material. Young garden beets exposed the same as preceding table.

Sample	Dilutions	Symptoms showed up.					
		18 days	19 days	20 days	21 days	22 days	23 days
1	0.25 gms.	0	0	0	0	0	0
2	0.5 "	0	0	0	0	0	0
3	1.0 "	0	0	0	0	0	0
4	2.0 "	0	0	+	0	0	0
5	5.0 "	0	0	+	+	0	0
6	10.0 "	+	0	0	0	+	0
7	20.0 "	0	+	0	0	+	+
8	30.0 "	0	0	+	+	+	0
9	50.0 "	0	+	+	0	+	0
10	100.0 "	+	+	+	0	0	+

The persistence of the organism capable of producing an infection when harbored in a gall over a long period of time is clearly shown by the above tables. It is evident that overwintering is particularly likely to take place when infected crowns are left in the field, especially if covered by soil so as to prevent excessive desiccation.

In another experiment 48-hour old broth cultures of P. beticola were poured onto two flats of sandy loam soil. These two flats were left outside from November 29, 1926 until January 20, 1927, after which sugar beet seeds were planted in one of them. When the seedlings were at the second leaf stage, the hypocotyls of a portion of the plants were injured with a sterile needle, others were left uninjured. In the other flat older

sugar beet plants which had attained a root size of one half inch in diameter were transplanted, and a portion of these were stabbed with a sterile needle, some being left uninjured. The two series were allowed to run forty-two days after injuring the plants. In the first flat, with seedlings, no symptoms of the disease were noticed. In the second flat, with large roots, two beets showed remarkable tubercle formation. It is shown by this experiment that some of the organisms had lived over in the soil.

The above series of experiments indicate that the bacteria causing tuberculosis are capable of living overwinter in both beet debris and soil. This datum, coupled with the finding of the organism in the field soil one year after affected beets were observed, indicates that the organism may be of a saprophytic nature as well as parasitic.

EXISTENCE OF P. BETICOLA IN THE SOIL AS FREE LIVING FORMS.

In determining the existence of the tuberculosis organism in the soil, two different methods have been employed: first, the selection from yellow organisms obtained by platings by the use of P. beticola antiserum; second, a method as employed by Patel (7) with use of his Crystal Violet Bile medium for selection of P. tumefaciens in the soil.

It has been shown by Goldsworthy (2) that it is possible to select specific organisms from soil by the use of an antiserum. After positive results were obtained in agglutination test from laboratory experiments, it was thought possible that Goldworthy's method for determining organisms from the soil would be helpful as an aid in the study of western soil where the disease is observed.

Greenhouse test: In order to find out if P. beticola could be reisolated from soil and to find out how serviceable the agglutinating

serum would be in this connection, the following preliminary work was done. An antiserum of P. beticola with titre 1 - 1200 was used.* A composite broth mixture of P. beticola No. 1 and several other undetermined yellow organisms which were isolated from soil at Rocky Ford, Colorado, were poured on soil in flower pots in which young sugar beet plants were growing. The beets were then punctured with a sterile needle and kept in a very moist condition with sterile water; they later developed galls. Fourteen days after the suspension was poured on the soil, cultures were made in serial dilutions as shown in Table 14. The numerous yellow organisms which developed on the plates were isolated and grown on potato dextrose agar, and then tested with the P. beticola antiserum in the usual manner. The results are given in column 3 of the table.

TABLE XIV.

Results of the selected number of yellow organisms on the dilution plates which showed agglutination reaction with P. beticola antiserum.

Soil Dilutions Used	Yellow Colonies Selected	No. of Colonies Agglutinated at 1 - 1000 titre	Per cent
1/1,000	14	4	28
1/10,000	9	3	33 1/3
1/100,000	1	0	0
1/1,000,000	2	0	0
1/2,000,000	1	0	0
1/5,000,000	3	1	33 1/3

*See page 29.

With the organisms showing an agglutination reaction with the antiserum, inoculations were made into rapidly growing sugar beets. One month after inoculation, the following results were obtained: Out of the eight organisms which were agglutinated, seven produced typical tubercles on inoculated beets, thus proving that P. beticola by the above technique can easily be reisolated from a composite of soil organisms.

With the above evidence, field samples of soil were collected from several fields at Rocky Ford and Lamar, Colorado. Some samples were from beet fields where the disease, if present, was certainly not serious. Other samples were from the field at Lamar, Colorado, which the previous year had shown 10 per cent diseased in certain portions, and still other samples were obtained in which so far as could be ascertained from the records of the American Beet Sugar Company, beets had never been grown. Water from two irrigation canals was also tested.

The first selection was twenty samples taken at random July 2, 1927 from the Lamar field. Serial dilutions of 1 - 10,000 to 1 - 2,000,000 were made of each sample in duplicate on potato dextrose agar (PH 7.56). From these plates, organisms which showed the most typical pigment formation and growth characteristics were picked out and cultured. The organisms were then tested by use of an agglutinating serum. Six organisms from the forty plates showed a strong agglutination reaction with antiserum at a dilution 1 to 1,000. The pathogenicity of each of the six organisms was then tested by inoculating into rapidly growing beets in a field with appropriate checks left. Five of the organisms thus selected produced galls on sugar beets, whereas all of the organisms proved pathogenic to garden beets. It is evident that P. beticola had persisted over winter in the soil under the dry conditions prevalent in the Colorado district.

Other soil samples taken from the various Rocky Ford, Colorado, fields have only yielded one other definitely identified culture of P. beticola, although numerous platings have been made. Both Catlin ditch and Rocky Ford ditch irrigation water was tested by the above method, but in all cases the results proved negative. Soil that as far as is known may be termed virgin showed no tubercle producing organisms present; however, the amount of material worked with was insufficient to prove that the organism is not present in that type of soil.

TABLE XV.

Results of the Agglutination test with P. beticola antiserum and organisms isolated from the twenty samples obtained at Lamar, Colo.

Dilutions	Plates		Antiserum Dilutions					Remarks
	No. of Colonies Average	No. yellow Colonies Average	No. yellows showing Agg. 1:5	1:50	1:100	1:500	1:1000	
1/1,000	Too numerous	Too numerous						None selected
1/1,000	"	"	"	"				" "
1/10,000	96	27	2	1	0	0	0	
1/10,000	103	16	6	4	2	2	2	Pathogenic
1/50,000	47	22	2	1	1	1	1	Path. on Garden Beet
1/50,000	30	11	1	1	0	0	0	
1/100,000	12	7	1	1	1	1	1	Pathogenic
1/100,000	17	3	1	?	?	?	?	"
1/1,000,000	4	1	0	0	0	0	0	
1/1,000,000	8	0	0	0	0	0	0	
1/2,000,000	9	2	1	1	1	1	?	Pathogenic
1/2,000,000	5	0	0	0	0	0	0	

The Patel's Crystal Violet Bile medium was next tested as to its behavior when P. beticola was grown on it. The medium was produced by Patel especially for the differentiation of P. tumefaciens from other organisms in the soil. However, the reaction of P. beticola, that of a large halo, is a very marked difference from either P. tumefaciens or any other organism tested with the single exception of Ps. pyocyanea which in a few cases showed slight halo formation. With the above demarcations showing up and a tendency for P. beticola and Ps. pyocyanea to show somewhat the same reaction, a modification of Patel's medium was made. This gave P. beticola distinctive reaction from all other organisms tested, as shown in Table 16.

The modification of Patel's agar is as follows:

*4 grams Sodium Taurocholate.

*20 grams Sucrose.

20 grams Agar.

*7 grams Peptone.

2 cc. Crystal violet 1/1,000 aq. solution.

1000 cc. of water.

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*Amounts were changed to make possible selectiveness.

TABLE XVI.

Behavior of various soil organisms when grown on a modification of Patel's Crystal Violet Bile Medium.

Organisms	Growth Started	60 hours Growth in diameter	72 hours Growth in diameter	84 hours Growth in diameter	Description
P. tumefaciens	48 hrs.	2.3 mm.	3.2 mm.	7.1 mm.	Colony glossy.
P. beticola No. 5	44 hrs.	2.4 mm.	3.5 mm.	7.0 mm.	Halo produced 4.25 cm. across.
P. beticola No. 2	72 hrs.			2.1 mm.	Takes up dye.
P. beticola No. 4	None				No growth.
B. prodigiosus	48 hrs.	2.04 cm.	2.52 cm.		Red color.
B. violaceus	60 hrs.		1.7 mm.	2.0 mm.	Deep purple.
Ps. radiciccola	72 hrs.			1.2 mm.	Light purple.
P. pyocyanea	24 hrs.	1.92 cm.	2.20 cm.	2.29 cm.	Col. spreading.
B. flourescens	60 hrs.		3.4 mm.	3.6 mm.	Light purple.
B. mesentericus	None				
B. subtilis	None				
B. mycoides	None				
Asp. niger	None				
Pen. italicum	60 hrs.		3.3 mm.	7.5 mm.	Very fuzzy.
M. varians	None				

A test was performed on field materials sent to Michigan from Rocky Ford, Colorado, with the use of the modified medium. The technique was followed as given by Patel (7) for isolation of P. tumefaciens from the soil. From the ten samples on hand only one P. beticola colony was obtained, and this from a field that had been in one year clover following a beet crop.

The above experiments along with the overwintering results gives some evidence that P. beticola can persist for eighteen months in Colorado soils under ordinary climatic conditions. This evidence coupled with the development of the disease here and there in the Arkansas Valley leads the writer to the contention that with this organism we have to do with a pathogen capable of existence for a considerable period in the soil.

INFLUENCE OF PH CONSENTRATION OF SOIL AND ARTIFICIAL MEDIA ON P. BETICOLA.

The effect of the hydrogen -ion concentration upon the growth of P. beticola is very marked. The optimum range is between 7.00 and 7.60; the complete range, however, where growth has taken place is approximately between 5,80 and 8.60 +. Below 5.80 very scanty growth resulted, while above 8.60 + growth was not found. A check on different soil samples at Rocky Ford and Lamar, Colorado, showed that the pH. range in that locality was between 7.42 and 8.10. This finding was observed to be very close to that which, as stated above, was found best in cultural media for growth of the organism.

After the determination of the PH values of western soil, local beet soil from Michigan was obtained and the PH of it was obtained. It was thought that the PH of a soil might be one of the deciding factors in keeping the organism within a definite area. Samples of the local beet soil showed a range of 5.83 to 6.03.

It can plainly be seen that the soil which is known to have the organism present is of a much more alkaline reaction than soil where the disease has not been observed. This may be one of the factors restricting the organisms to the west.

THE INFLUENCE OF FREEZING ON P. BETICOLA.

Temperature is considered one of the cardinal factors influencing life activities of micro-organisms. The majority of bacteria are unable to exercise normal metabolism at temperatures below 6°C. or above 45°C. according to Hilliard (3). In order to determine the resisting power of P. beticola, experiments with both freezing and thawing were conducted. Prudden (8) in 1887 found pure cultures of B. prodigiosus and B. proteus (common soil organisms) to be sterile after fifty-one days at temperatures ranging between -10° to 1°C. B. typhosus survived for at least one hundred and three days at a temperature between 14° and 30°F. Keith (1923) contrary to Prudden has emphasized the importance of solid freezing as compared with cold in relation to the death rate of bacteria. B. coli frozen solidly in water at -20°C. shows 99 per cent killed in five days, but when not actually frozen, a large per cent remained alive for months. When P. beticola was frozen in diluted soil solutions, the death rate increased with the dilution. If suspended in aqueous mixture of 5 to 40 per cent glycerine, a large percentage of the P. beticola remained alive for three months at temperatures ranging from a few degrees above zero to -12°F. below.

TABLE XVII.

Results showing resisting power of both Freezing and Thawing of P. beticola.

Distilled Water		Soil Solution	
Frozen Solid	Alternate Fz.	Frozen Solid	Alternate Fz.
Phytomonas beticola			
Count per cc	Count per cc	Count per cc	Count per cc
Before Freeze 50,428	Before Freeze 50,428	Before Freeze 50,428	Before Freeze 50,428
Frozen 1 hr. 15,600	*Fz. 3 times 920	Frozen 1 hr. 39,042	*Fz. 3 times 20,049
Frozen 12 hrs. 1,620	Fz. 5 times 116	Frozen 12 hrs. 16,422	Fz. 5 times 1,940
Frozen 24 hrs. 160	Fz. 6 times 124	Frozen 24 hrs. 10,327	Fz. 6 times 832
Frozen 3 days 156	Fz. 8 times 8	Frozen 3 days 949	Fz. 8 times 73
Frozen 5 days 93		Frozen 5 days 926	Fz. 10 times 4
Various degrees		Various degrees	

* 10 min. at -10°C.

TABLE XVIII.

Results of Resisting power to Freezing and Thawing of Senatia marcescens.

Distilled Water		Soil Solution	
Frozen Solid	Alternate Fz.	Frozen Solid	Alternate Fz.
Senatia marcescens (B. prodigiosus)			
Count per cc	Count per cc	Count per cc	Count per cc
Before Freeze 39,516	Before Freeze 39,516	Before Freeze 39,516	Before Freeze 39,156
Frozen 24 hrs. 6,417	*Fz. once 5,257	Frozen 24 hrs. 24,007	*Fz. once 1,673
Frozen 30 hrs. 7,679	Fz. 2 times 76	Frozen 30 hrs. 15,090	Fz. 2 times 1,579
Frozen 3 days 1,973	Fz. 3 times 15	Frozen 3 days 11,730	Fz. 3 times 973
Frozen 5 days 986	Fz. 4 times 17	Frozen 5 days 4,872	Fz. 4 times 74
Various degrees		Various degrees	

* 10 min. at -10°C.

It is apparent by the above data that P. beticola can endure a continued freeze, especially in a soil solution. This compared favorably with the common soil organism Serratia marcescens. The organism when in the soil below six inches in depth is not subject to a very great fluctuation during the winter; hence it is possible for them to overwinter as a soil organism.

The distilled water gave a much higher mortality than the soil solution. This is probably due to the lowering of crystallization and possible mechanical crushing of the bacteria during the freezing of the medium.

When in decaying vegetable parts, the beets may only be slightly covered with soil, but the solution within the disease tissue will be sufficiently concentrated to offer good living conditions. Thus it is further shown that P. beticola has the ability to withstand conditions necessary for successful overwintering of a soil organism.

THE ANATOMY OF THE OVERGROWTH.

Smith (15) before the Societe de Pathologie Vegetale et d'Entomologie Agricola de France stated that he believed that there were structures produced in plant diseases which are analogous to the tubercles found in diseased animals. He further stated that the P. savastanoi (E.F.S.) S.A.B., causing olive knot and P. Beticola, causing tuberculosis of sugar beet furnished proof of his statement. In addition to the two already mentioned, there is also to be included R. leguminosum (Frank) S. A. B. which causes the tubercles on plants of the Leguminosae. According to Smith's conception then, there are three distinctly different organisms causing plant tubercles. All three produce symptoms in plants which, if Smith's idea is accepted, are closely analogous in structure to those growths caused by organisms pathogenic to animals.

Macroscopic examination of a cross-section of the tuberculosis type of overgrowth in sugar beets reveal small, yellowish, watery, spots surrounded by much distorted tissue. Upon microscopic examination it is seen that these spots are bacterial pockets. The crown-gall type of proliferation presents no such aspect.

A study was made of the overgrowth to determine the correlation of the cellular behavior with the localization of organisms, and to compare the type of pathological growth of the beet with animal tubercles. The material selected for this study was fixed, and embedded in paraffin, sectioned 10 in thickness, and stained with Haidenhain's Iron Alum Haema-

toxylin.

The organism causing the neoplasm was found in a wide range of tissues including phloem, xylem, cambium, and parenchyma. The overgrowths appear to be the result of a stimulant produced by material secreted by the bacteria. It was evident that the cells of the plant are also mechanically pushed apart by bacteria passing from one place in the tissue to another, this being especially true in the cambium region of the host. The overgrowths show a tuberculoma-like type of neoplasm, in that the tissue involved tends to hold the parasite within localized areas. (Fig. 11). The organism causes no decay of the tissues with which they come in contact, but new host cells or enlarged old cells restrict the bacteria to small pockets. This condition is somewhat analogous to granulomatous tissue of animals.

Microscopic study of cross-sections of diseased material at various stages of development reveal the origin of the bacterial pockets. The organisms in early stages of infection, or as they are squeezing through the tissue, are intercellular. They multiply at certain advantageous points. Such points are usually in the cortex, where the newly differentiated parenchyma cells with their commonly occurring intercellular spaces allow the colony of organisms to increase in size. (Fig. 12). A similar growth may also be found between the cells of the cambium. As the bacteria increase in numbers at these points, the pressure from the growing mass crushes the bordering host cells, whose structures may also have been weakened by seepage of bacterial by-products, thus forming the disorganized cavities. When the bacteria are found located in the vicinity of the meristematic cells, hyperplasia is the most characteristic type of proliferation. When the bacterial cavities are surrounded by parenchyma, hypertrophy of

the cells is the most common type of response. However, the stimulating substance from the parasite has an effect over a zone at least ten to fifteen cells in thickness, and when this substance acts upon newly differentiated parenchyma cells, hyperplasia often takes place. With such increases in cell divisions, poly-nucleated cells are often found, and the rate of division is accelerated (Fig. 13). These fast dividing cells often form strand-like structures in the overgrowth which may either be purely hyperplastic or hypertrophic, or at times both growth types intermingled.

In a large number of sections the pockets were found within the cambium layer, and this is especially true in the case of petiole infection (Fig. 14). The cells of the cambium are in a state of rapid division, but with the stimulation produced by the organism these cells seem to divide even more rapidly; thus abundant hyperplastic growth is caused. The young rapidly dividing cells are very easily crushed, and hence we find a great number of both large and small bacterial pockets. The rapid internal cell production along with the enlargement of old cells, which are the results of the stimulating effect of the colonies of the organism, cause a great internal pressure to take place. Since no new outer cells are being formed, the tubercle splits, thus forming the deep fissures over the surface of the overgrowth.

The tubercles, besides having large fissures present, are also very rough and show extremely large amounts of disintegration. This was observed to be caused when the organisms are located in the old parenchyma tissue, or the stimulant reaches these cells. The effect is mostly hypertrophic in nature (Fig. 15). These cells take on various shapes--cylindrical, polyhedral, and spherical with large intercellular spaces. There is no evidence of an epidermal development in later stages of tubercle growth. As the proliferation develops, the epidermis is ruptured so

that the large hypertrophic cells with enlarged intercellular spaces are exposed, which allows soil organisms to enter. This causes the very rough and decayed surfaces spoken of earlier as a distinguished characteristic under the external symptoms.

The parenchyma cells next to the xylem are thick walled, and are spoken of by Artschwager (1) as the "sugar sheath". Cells of the "sugar sheath" attacked by P. beticola lose their turgidity, and suffer partial or complete collapse, producing primary bacterial pockets. The bacteria are able to break down the "sugar sheath" cells very easily, whereas the strength of the tracheae and the tracheids will withstand the pressure from the bacterial growth. However, the organisms at times do penetrate into these tubes after the pockets have been formed in the "sugar sheath", and travel along in them only to develop in large masses again, breaking out of the tubes and forming new pockets. This condition is also present in the bundles of the petiole which are of the open collateral type, with the cambium taking the place of the "sugar sheath" as the point of bacterial invasion (Fig. 16). Certain pockets over two inches in length have been observed in the beet root. These pockets extend in longitudinal direction, and are more or less associated with the vascular system.

The above considerations undoubtedly play the decisive role in determining the nature and localization of the galls upon the sugar beet. In the root tubercles the situation is as follows: A mediam horizontal section through a beet shows a number of annular zones which are more or less equidistant, except near the periphery, where they are very close together. (cf. Artschwager (1)). With the cambium located so near the periphery, and being the most susceptible tissue to the attack of the organism, it is evident that infection may take place following even a very shallow wound.

In the course of the studies of beet tuberculosis, the question arose as to why at certain stages of beet growth there are apparently different degrees of susceptibility. A structural difference is readily observed when the age of beets is taken into consideration, and there are probably physiological differences as well. In the hypocotyls of the young beet seedlings, there is no differentiated cambium present which has been seen in the tissue especially subject to attack by P. beticola. The cortical cells prevent the organism from becoming established. In certain artificial inoculation experiments, it has been noticed that the resistance in young seedlings has been broken down and an infection secured, only to have the cortex cells of the hypocotyl sloughed off, thus eliminating the infection.

In petiole infection the shallow wounds which are sufficient for beet infection are of no consequence. It will be recalled that petiolar galls have not been found in nature, but it was found necessary to puncture one of the bundles before disease symptoms could be produced. The histological composition of the vascular tissue, according to Artschwager (1), is that of a typical collateral bundle (Fig. 14b). The phloem forms a narrow zone composed chiefly of sieve tubes with their companion cells and some phloem parenchyma with only a thin layer of cambium. Although this cambium layer is favorable tissue for pocket formation, it is surrounded b y thick walled cortex cells which are unfavorable for bacterial extension and development. It would seem that the time explanation of the absence of petiolar tubercles in nature is to be found in the anatomical structure of the petioles.

As is evident from the entire discussion given of the pathological anatomy and as is shown by the photomicrographs, the tubercle formed in this disease fits the criteria used by animal pathologists for their concept

of a true tuberculosis, and the writer believes Dr. Smith's characterization of the galls as a similar structure is fully warranted.

SUMMARY.

Sugar beet tuberculosis, a disease characterized by overgrowths on the crown and root of beets, is one of the many root diseases of this plant.

The literature of this disease is limited to the original contribution of Smith, Brown, and Townsend, and a few brief notes.

The disease up to the present time has only been reported from Colorado, Kansas, and Wyoming, and seems to be limited in its distribution to the West.

The disease may eventually become of considerable economic importance, since diseased beets are subject to rot in storage piles and are likely to be wastefully topped. The diseased beets show consistently lower sugar percentage and purity than normal beets.

The disease is distinguishable from crown-gall in that the tubercles are more rough; they extend generally from ridges perpendicular to the root; cross-section shows yellow watery spots present; and frequently the roots assume turnip shape.

In the areas where P. beticola is in the soil, hail or mechanical injury of some sort has been found to produce heavy infection on rapidly growing roots. Irrigation water has been found to carry the organism to the wounds.

The pathogenicity of the organism has proven according to Koch's rules of proof. The bacterial pathogen was found to occur in large masses in the yellowish, watery spots in the tubercles.

Cross inoculations have only proved successful on garden beets and Swiss Chard, with tomatoes harboring the parasite for one month without the production of the typical symptoms. Inoculations on many other hosts gave negative results.

The classification as given by Bergey's Manual is followed; hence the name used in this paper Phytomonas beticola.

The description of the organism is given. In a large part the test outlined follows the work reported by Dr. Smith; however, some significant variations have been noted, along with additional information.

The production of specific agglutinins of P. beticola for use in diagnostic purposes and relationships of the organism to other plant pathogens are of practical use in this work.

The existence of tuberculosis organism in the soil was determined by two methods; first by use of P. beticola antiserum, second by use of Patel's Crystal Violet Bile medium (modified).

The organism causing the tubercles was found to be located in a wide range of tissue, namely phloem, xylem, cambium, and parenchyma, with the bacteria having a special preference for the cambium.

The overgrowths appear to be the result of a stimulation of the cells by material produced by the bacteria.

The overgrowths showed a tuberculoma-like type of neoplasm, in that the tissue involved tends to hold the bacteria within localized areas.

Hypertrophic and hyperplastic tissue was found to make up the greater part of the galls, hypertrophy being associated with parenchyma cells, and hyperplasia with the cambium cells.

The apparent resistance of the young seedlings to the disease seems attributable to the absence of the cambium layer at this stage. When infection was produced by artificial inoculation, the diseased parts are

usually eliminated from the plant at the time of the rupture and sloughing of the primary cortical layer.

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EXPLANATION OF FIGURES.

Fig. 1. Sugar beet tuberculosis caused by Phytomonas beticola. Beet from a hail-injured field Lamar, Colorado, natural infection. Photo by Dewey Stewart.

Fig. 2. Type of diseased and normal beets used in report on Table II. Disease produced by artificial inoculation.
(a) Medium size tubercle. (b) Large size tubercles.

Fig. 3. Natural infection of sugar beets following injury from field practices. Tubercles produced on main part of root.

Fig. 4. (a) Cross-section of crown tubercles showing central, brownish, water-soaked, bacterial areas surrounded by white flesh.
(b) Longitudinal section of beet showing bacterial pockets.

Fig. 5. (a) Sugar beet crown-gall produced by inoculation with Phytomonas tumefaciens. M. S. C. strain 146.
(b) Cross-section of the same gall.

Fig. 6. Tuberculosis on garden beet caused by Phytomonas beticola. Beet was artificially inoculated at Rocky Ford, Colorado.

Fig. 7. Phytomonas beticola stained to show flagella.

Fig. 8. Typical growth of Phytomonas beticola on nutrient agar slants. Growth characterized by brush-like projections.

Fig. 9. Tomatoes inoculated with (a) Phytomonas beticola, and (b) Phytomonas tumefaciens.

Fig. 10. Disintegrated condition of sugar beet left in soil overwinter.

Fig. 11. Cross-section of a sugar beet tubercle with typical bacterial cavities marked. Result of a pure culture inoculation using Phytomonas beticola No. 5.

Fig. 12. Tubercles on sugar beet showing pockets filled with rod shape bacteria. Ten micron sections of a twenty day gall X1000.

Fig. 13. Tubercles in young cortical parenchyma of a sugar beet petiole. The rapidly dividing polynucleated cell may be seen. From a pure culture inoculation.

Fig. 14. Section through a sugar beet petiole showing (a) the cambium of the bundle destroyed by the formation of a bacterial pocket, (b) a normal bundle of the same petiole.

Fig. 15. Cross-section of a tubercle showing large hypertrophic parenchyma cells exposed, and the large intercellular spaces. Epidermis has been ruptured and has fallen away.

Fig. 16. Longitudinal section showing bacterial pockets produced at intervals along the tracheae. From pure culture inoculations.

FIGURE 1.

FIGURE 2.

a.

5.

FIGURE 3.

FIGURE 4.

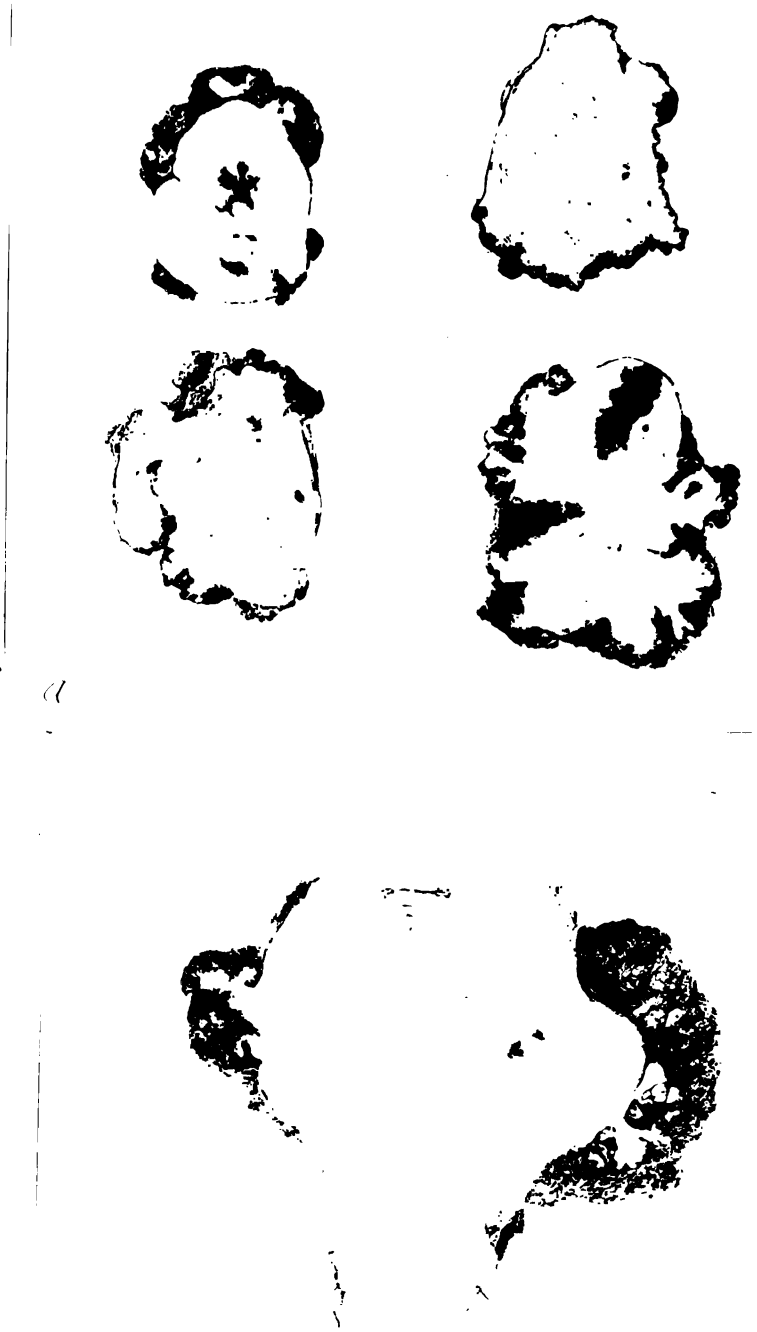


FIGURE 5.

a.

b.

FIGURE 6.

FIGURES 7 & 8.

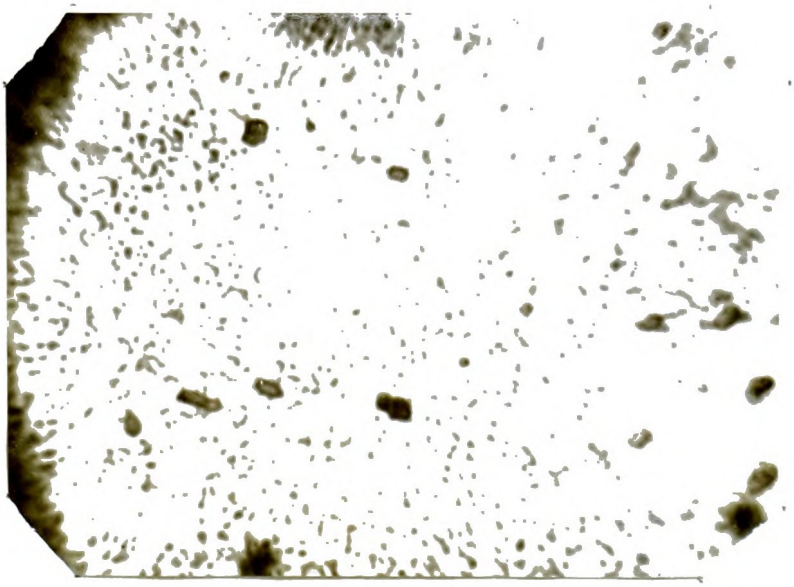
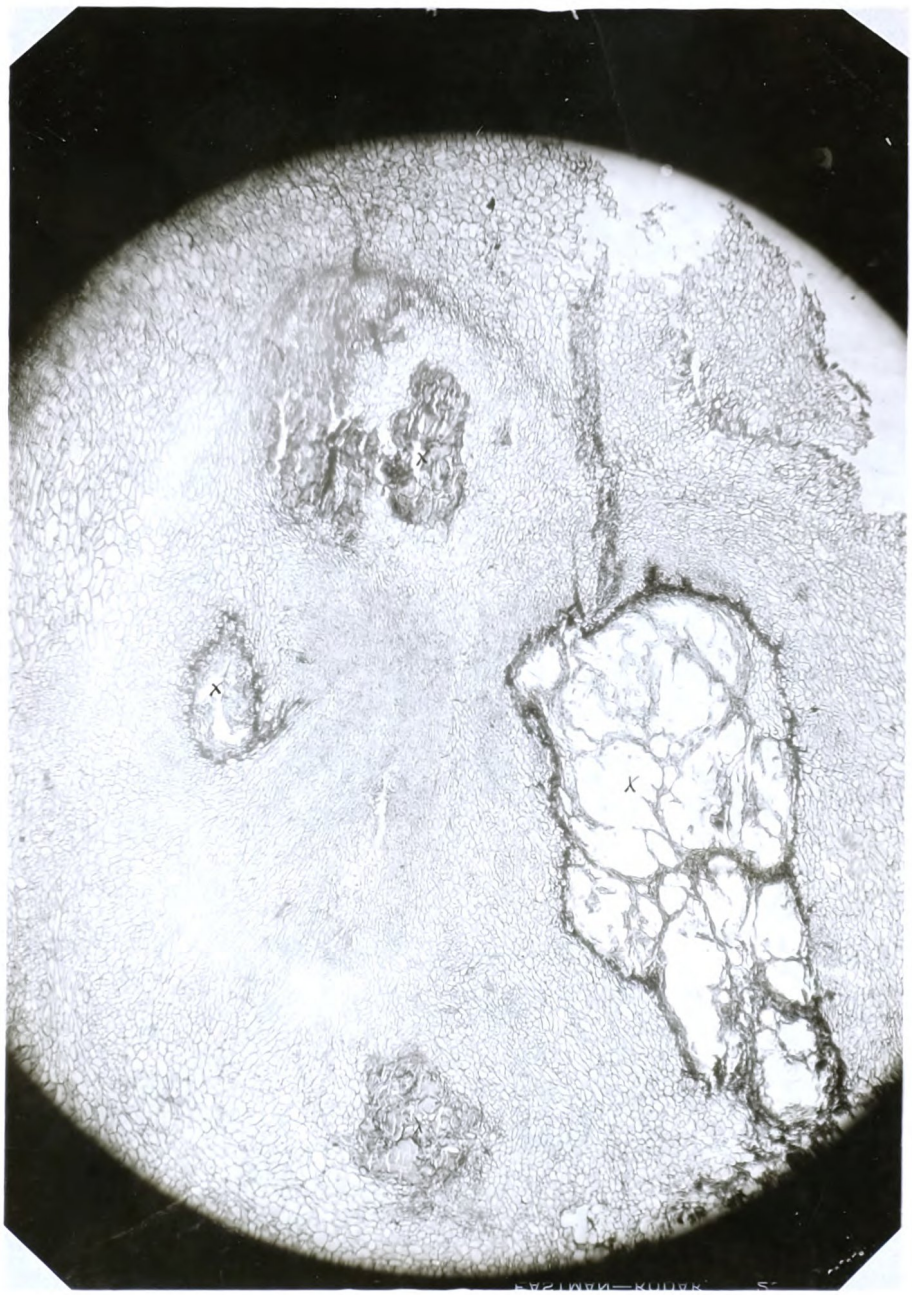


FIGURE 9.

FIGURE 10.

FIGURE 11.



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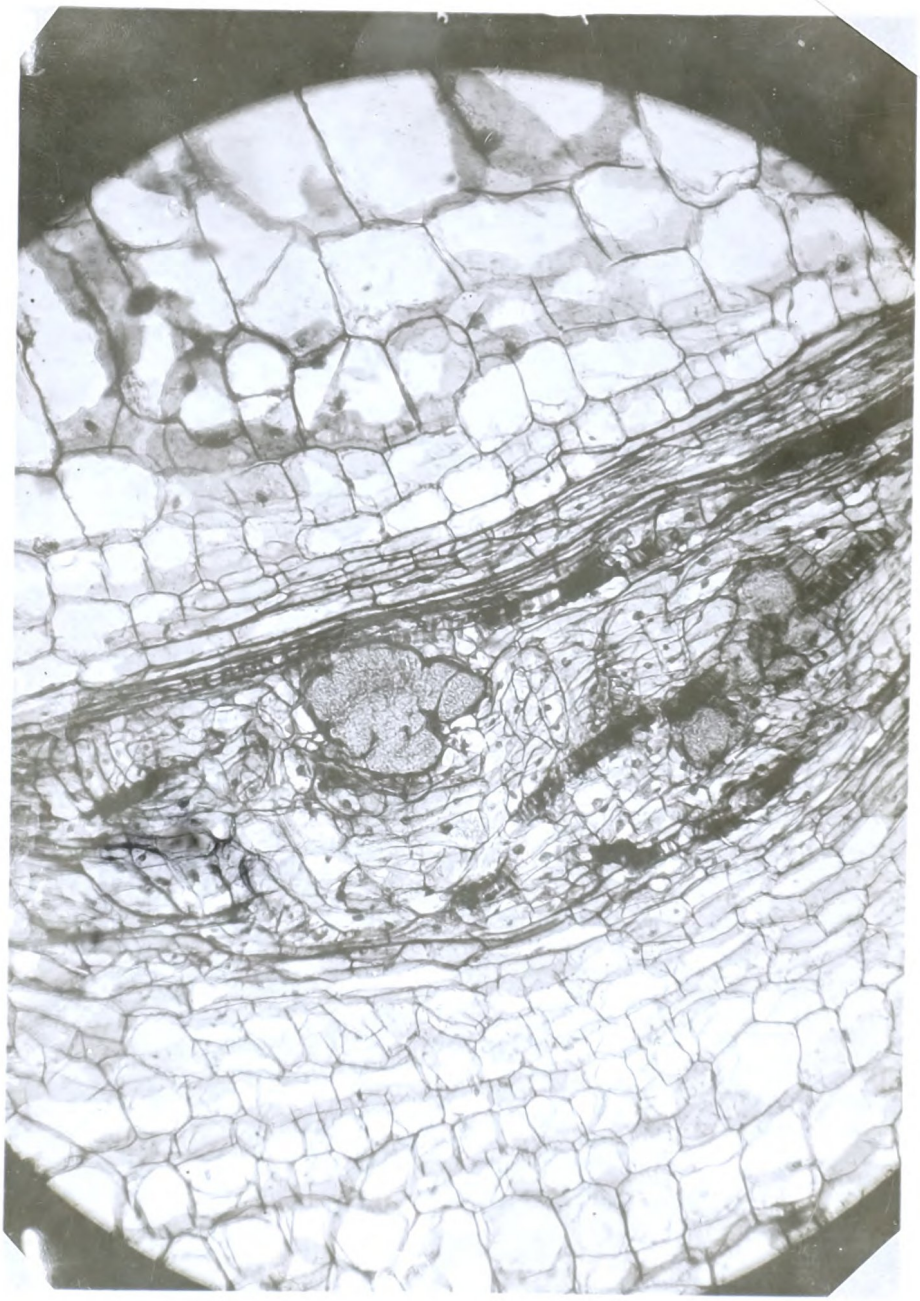

FIGURE 13.

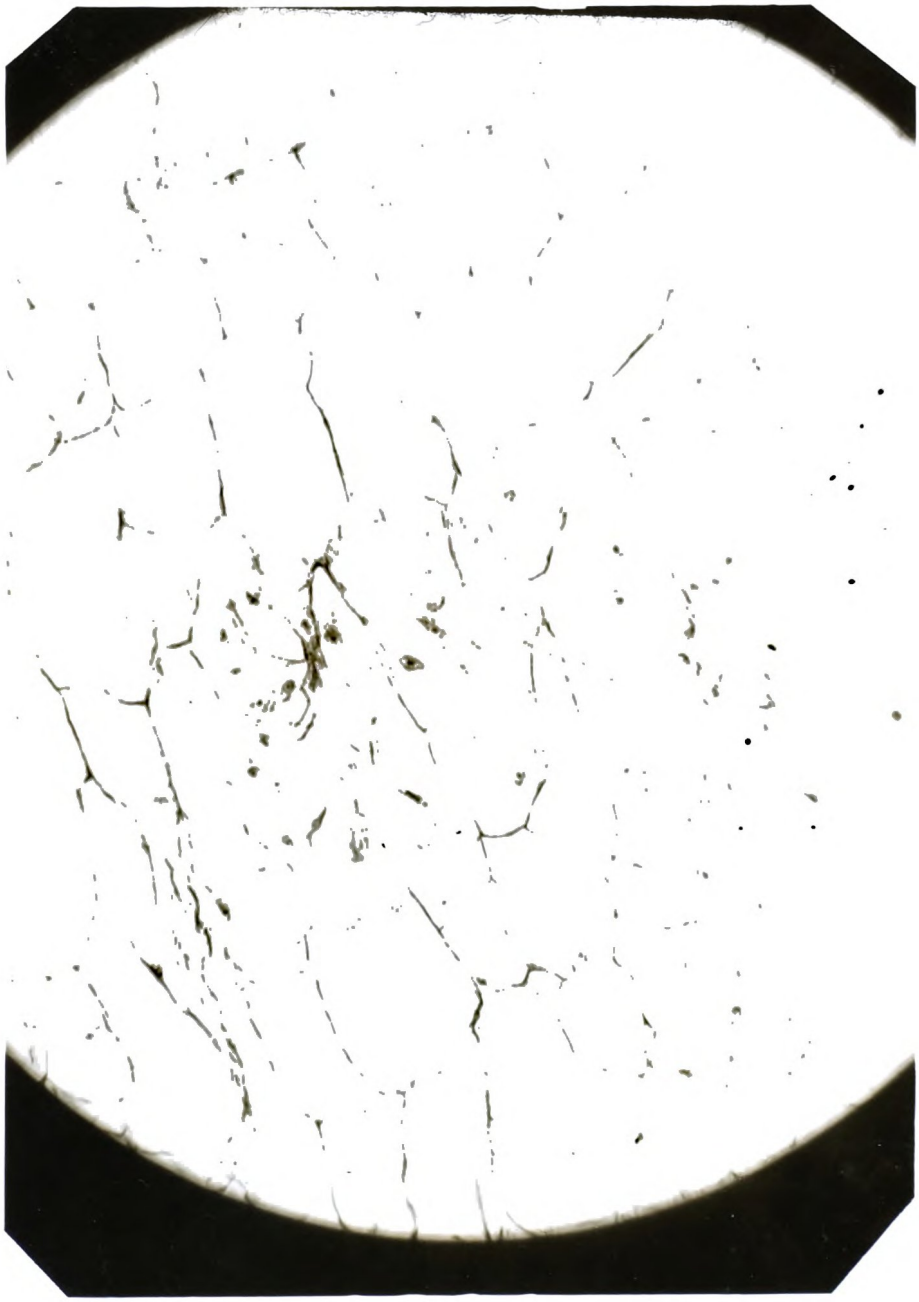

FIGURE 14.

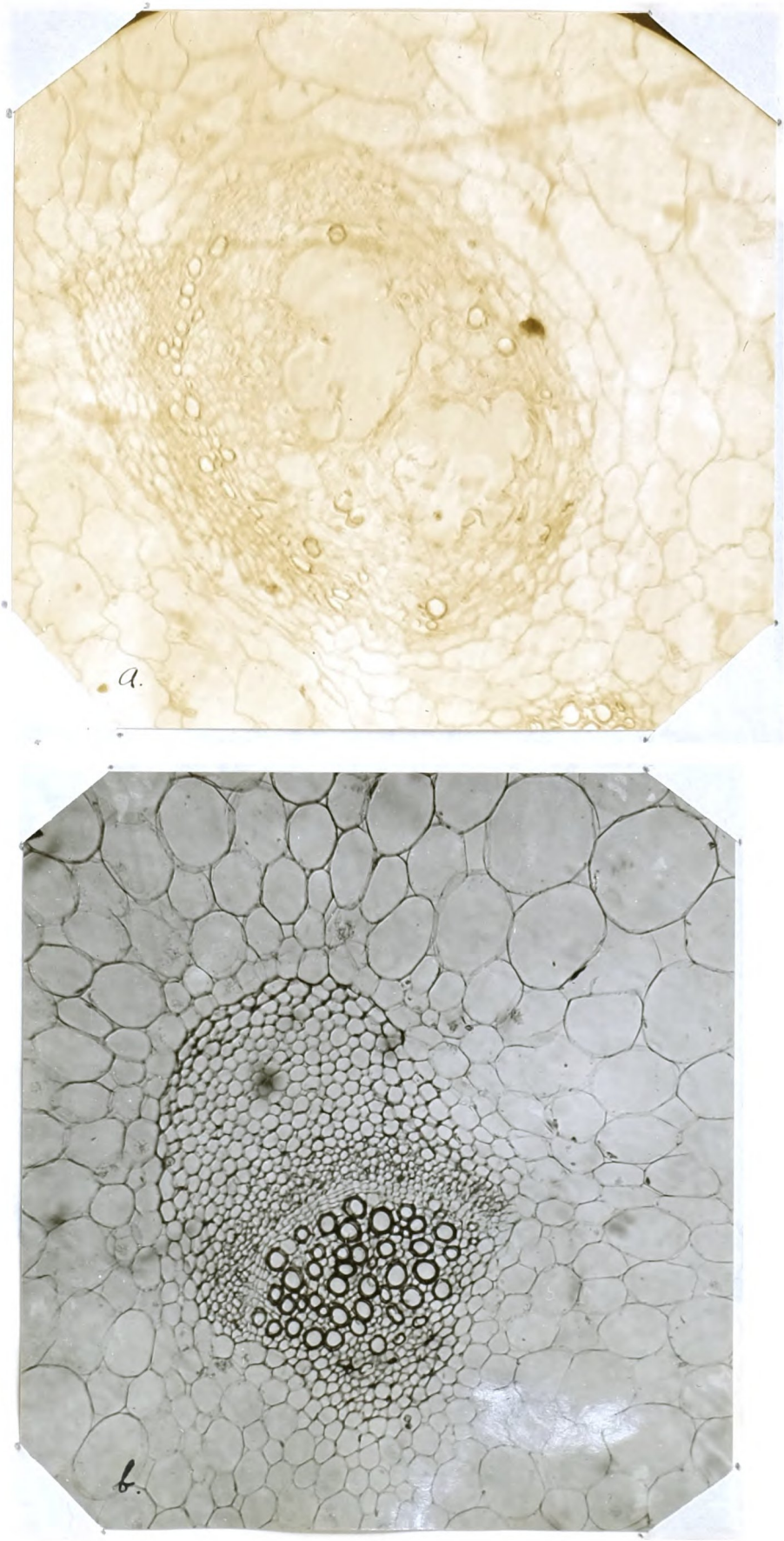

FIGURE 15.

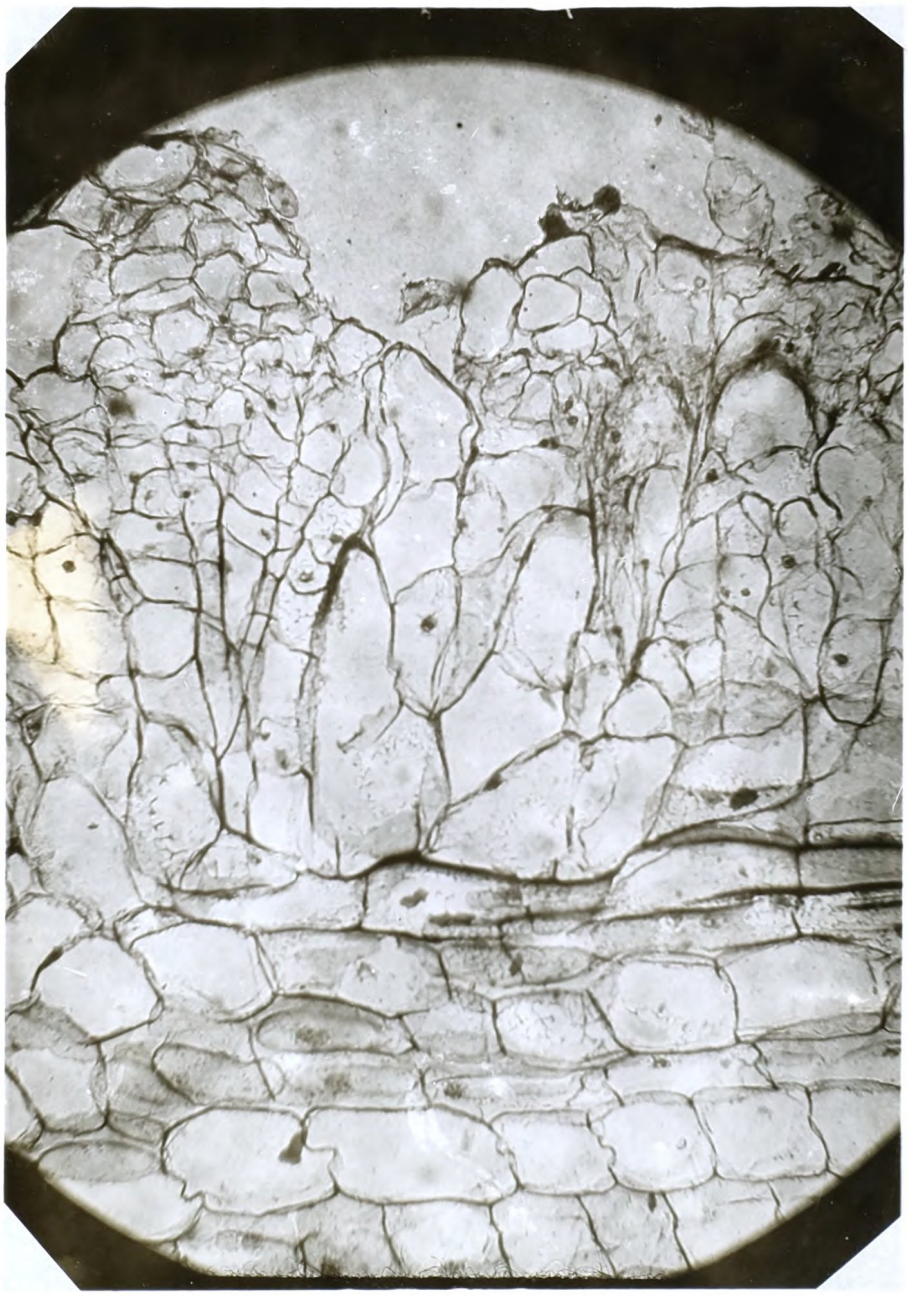

FIGURE 16.