# THESIS

## STUDIES ON BACTERIUM PULLORUM

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## INTRODUCTION

Although considerable work has been carried out on Bacterium pullorum, both culturally and serologically, still it was believed that work carried out on a large number of cultures obtained from different sources would either bring out any variations that had not been observed or it would be an excellent check and confirmation of previous work.

Dr. Stafseth of this Station has had considerable difficulty in diagnostic serological work and it was desired to determine definitely whether the organisms used for antigen were giving satisfactory results. It is quite generally known that strains of some organisms vary considerably in their agglutinating power. Very little work concerning this property of Bacterium pullorum has been carried out.

It was further hoped that cultural and fermentation studies might shed some light on the causes of variation which are particularly frequent with Bacterium pullorum.

## HISTORICAL RESUME.

The study of chicken diseases dates back many years, although, as in other diseases, the work was largely observational. It is of interest, however, to know of these early investigators and their work.

Hadley (1) gives a very interesting historical resumé of these early workers. As I do not have these early articles at hand, I take the liberty of quoting Hadley.

"Baronco, a celebrated Lombardy (Italy) physician, appears to have been among the first to seriously consider the nature and the cause of serious typhoid-like epidemics which had been noted by poultry raisers even before this time. Together with Miocchi and Brugna he examined with considerable care the extensive epidemics which occurred among poultry in Lombardy between the years 1789-1798 and which were commonly referred to by the poultrymen of the Turin district under the name 'calcinaccio' and by the Piemontese as 'causinera'. Somewhat later similar epidemics in Piemonte were studied by Brugnone and Toggia. In view of the lack of bacteriological knowledge and technique in these early years, it is, of course, clear that there are few data, save the description of the clinical features of the malady to serve as a basis of connection with cholera-like diseases of poultry as we know them at the present day; and it is therefore impossible definitely to say whether they represented true fowl cholera or some other disease. But in view of the circumstances

that when advances in bacteriological knowledge and technique finally did permit a certain degree of differentiation (about 1875) the non-cholera type of infection appeared to be prevailing poultry malady of the Italian provinces, it can perhaps justifiably be assumed that, even in earlier years, it was a well established poultry scourge in these sections of southern Europe whence much of our poultry has been derived.

"In the early days of the science, bacteriologists were laboring with the problem of anthrax and it is not surprising that Ercolani, who in 1861 made a study of many epidemics, described some of the then prevailing types of poultry infection under the name of epidemic anthrax (carbonchis). Others who contributed to the early knowledge of these diseases were Lemaistre (1869) Delafond, Reynal and Benion."

From 1877, considerable work on chicken diseases was carried on. All of this work centered around fowl typhoid and fowl cholera. No worker, apparently, isolated or studied the organism causing white diarrhea of chicks.

It was not until 1899 that the organism causing this disease was discovered. At this time, Rettger (2) reported a case of fatal septicemia in young chicks. It is interesting to know that many bacteriologists were working on this problem at this time and were unable to make any progress. Rettger

examined a number of chicks suffering from what he describes as a peculiar ailment. A bacteriological examination was made and an organism was isolated from the liver and the spleen. This organism was injected into normal young chicks with fatal results. The organism was reisolated.

In 1900, another epidemic was studied by Rettger (3) and again he was able to isolate the same organism. Inoculation experiments were again successful.

In the same year, Pernot (4) at the Oregon Agricultural College published a report on chicken diseases in which under the heading indigestion, he mentions a heavy loss of incubator chicks through lack of understanding of their requirements when young. He states "Some of the chicks dissected were found to have their craws filled with food and a fair quantity of unabsorbed yolk in their abdomens. All the conditions gave evidence that they had died of an acute indigestion. Particles of food were found in all parts of the intestinal tract." Without doubt, Pernot was describing white diarrhea of chicks, but he apparently knew nothing of its cause.

In 1908, Milks (5) working in Louisiana isolated an organism from the liver of a chick dying from the so-called white diarrhea. He gives a description of the organism that checks very closely with the one isolated by Rettger in 1899 and 1900. His inoculation experiments on young chicks were successful.

Rettger and Stoneburn (6) in 1909 in further studies give a complete description of the organism, which Rettger at this time named Bacterium pullorum. In commenting on the properties of the organism he states "Some of the strains do not produce gas in any of the sugar media, though acid production is quite apparent." I quote this statement because of its bearing on work presented in this paper. Their conclusions show that the mother hen is the original source of the disease and that a certain percetage of the chicks oninfected farms have the disease when hatched.

A little earlier, 1908, Morse (7) published a paper in which he stated that much of the so-called trouble in chickens was due to coccidiosis, identifying the parasite as Coccidium tenellum. In 1909, Hadley (8) claimed that the etiological factor in white diarrhea was Coccidium cuniculi which was found in the ceca. He believed the organism to be similar to that causing blackhead in turkeys.

In 1909, Rettger (9) investigated the significance of Coccidium tenellum. He states that he found Bacterium pullorum in cases where Coccidium tenellum was present and concludes that Bacterium pullorum is the etiological factor and not Coccidium tenellum. At this time, he again states that considerable variation was found in gas production by the strains. He states "The behavior of the organism on agar is quite constant. The same thing is true with reference to the development in gelatin, milk and potato. The different strains vary, however, in their gas producing power in dextrose bouillon. Some of the organisms which I have isolated have failed and still fail to produce gas in this

medium. On the other hand, two particular strains fermented the sugar with 5 percent and 25 percent respectively, in this medium. The more active ferments lost this property within a period of seven to eight months so that for a while, it was strictly anerogenic. At the present time, the fermenting power is gradually being restored. Work on this particular department is being carried on from this standpoint of variation, and some very interesting results have already been obtained. These will be reserved for a later publication."

"All of the strains of organisms studied produced acid in dextrose bouillon. Lactose is apparently not affected. Mannite is fermented by some strains but not by others."

Jones (10) in 1910 confirmed the work of Rettger and Stoneburn and further found that the disease might be induced through intravenous injection.

In 1911, Gage (11) again confirmed the work of Rettger and Hadley in their contention that the disease is transmitted to the chick from the hen through the egg.

In 1913, Jones (12) reports an epidemic where adult birds were infected by feeding infected incubated eggs. In the same year, in another paper (13) he again suggests the use of the agglutination test for detecting birds infected with white diarrhea. This test was first suggested for use by Jones in 1911 (14). He recommends that in using the test the antigen should be made up of several strains of the organism and that dilutions of 1-50, 1-100, 1-200 for routine should be used. An efficiency of one hundred percent is claimed for the test. The

latter claim is rather a little premature as the number of tests is not sufficient for such conclusions.

Rettger, Kirkpatrick and Stoneburn (15) in 1912 state that the period of greatest danger from bacillary white diarrhea of young chicks lies within the first 24 hours after hatching. They further state that hens may become carriers of the disease after they have reached maturity. They believe that the ovaries may be become infected by contact of the hens with infected hens and that the infection is likely acquired through the mouth. In 1914, a second report (16) considers the relationship between infection as chicks and the carrier state when mature. They state "Of 138 chicks which grew to maturity and lived until the termination of the experiment, 88 were infected with bouillon cultures of Bacterium pullorum when quite young, while the remaining 57 were not subjected to this treatment, but served throughout the investigation as checks. Of the 88 that were infected as chicks, 21 gave positive agglutination tests when about a year old and at the same time, they were killed all of the positive agglutination tested pullets showed unmistakable evidence of ovarian infection with Bacterium pullorum. In many instances, the ovaries were in advanced stages of infection. More than 25 percent that were artificially infected as chicks became permanent carriers." This also confirms Jones' macroscopic agglutination test and shows that all birds showing positive reactions had infected ovaries. Gage (17) in the same year also confirms the value of the agglutination test as a means of detecting carriers. He also agrees with Jones in the use of polyvalent antigens. He confirmed Jones' method of infecting the ovaries through intravenous injection.

In the following year (1915) Rettger, Kirkpatrick and Jones (18) presented an extensive check on the use of the agglutination test. They state that they examined 14,617 birds. Of this number, 1440 were reactors, or 9.85 percent. The number of hens tested was 13,831, of which 1417 were reactors, or 10.24 percent. The number of males tested was 786, of which 23, or 2.9 percent, were reactors. The following year some of these flocks were retested with the following results. Four flocks out of 13 failed to show any reactors and in the other 9 the percentage of infection varied from 0.6 to 25.7 percent, the number in each instance being decidedly less than before.

Smith and TenBroeck (19) in the same year published a paper showing differences between Bacterium pullorum and Bacterium sanguinarum. The following differences are given:

Action on maltose

Bacterium sanguinarum produces acid

Bacterium pullorum does not produce acid

Gas production on dextrose and mannite

Bacterium sanguinarum - No gas

Bacterium pullorum - Gas produced

Milk

Bacterium sanguinarum - alkaline

Bacterium pullorum - no alkaline

Rettger and Koser (20) in 1916 working independently from Smith and TenBroeck made a similar study in which they found a few additional differences. Morphologically, they state that

Bacterium sanquinarum is a shorter and thicker rod than Bacterium pullorum. They found that Bacterium sanquinarum produced appreciable acidity in maltose, dextrin and dulcite, while Bacterium pullorum had a tendency to alkali production in these sugars. They also found, which is partially confirmatory, that gas production by Bacterium pullorum is quite variable in mannite and galactose.

In immunizing experiments carried out at the same time they found that where birds were immunized with the fowl cholera organism, they were not protected against fatal doses of Bacterium pullorum, whereas when the birds were immunized with Bacterium pullorum they were immune to the fowl cholera organism. These experiments were carried out on a small scale and so do not justify any conclusions.

Ward and Gallagher (21) in 1917 suggest the use of an intradermal test for Bacterium pullorum in place of the agglutination test which they consider as cumbersome and impractical as compared with the above test. Although they do not claim one hundred percent efficiency for the test, they do urge its use in the field in comparison with the agglutination test.

One of the most complete studies of the fermenting properties of Bacterium pullorum was carried out by Goldberg (22) in 1917. He studied 5 strains of Bacterium pullorum and determined the fermentative properties on 20 sugars and alcohols, as shown in table I.

TABLE I

Gas Production by Various Strains of Bacterium pullorum on Various Carbohydrates.

| Strain No. | Dextrose | Lactose | Sucrose | Mannite | Dextrin | Inulin | Galactose |
|---|---|---|---|---|---|---|---|
| 1 | B | - | - | B | - | - | + |
| 2 | + | - | - | B | - | - | + |
| 3 | B | - | - | B | - | - | + |
| 4 | + | - | - | + | - | - | + |
| 5 | - | - | - | - | - | - | - |

| Strain No. | Levulose | Raffinose | Amygdalin | Arabinose | Adonite | Dulcite |
|---|---|---|---|---|---|---|
| 1 | B | - | - | + | - | - |
| 2 | B | - | - | + | - | - |
| 3 | B | - | - | + | - | - |
| 4 | B | - | - | + | - | - |
| 5 | - | - | - | + | - | - |

| Strain No. | Xylose | Starch | Isodulcite | Salacin | Mannose | Glycerin | Erythol |
|---|---|---|---|---|---|---|---|
| 1 | - | - | + | - | + | - | - |
| 2 | - | - | + | - | + | - | - |
| 3 | - | - | + | - | + | - | - |
| 4 | - | - | + | - | + | - | - |
| 5 | - | - | - | - | - | - | - |

Mulsow (23) in 1919 reported a study of various strains of Bacterium pullorum. Table II gives the results that he obtained. An examination of Tables I and II shows a marked irregularity in gas-forming characteristics. Both Goldberg ( Table I ) and Mulsow (Table II ) show a strain that is a non-gas-producer. Several organisms in Table II show a decided variation on arabinose, rhamnose, sorbite and xylose. Mulsow states that two strains of Bacterium pullorum produced acid and gas in maltose and a few strains produced slight acidity after several days.

Berry (24) found three strains of Bacterium pullorum that fermented lactose with gas. These three strains were otherwise normal.

Hadley (25) in 1917 confirmed Goldberg's work on fermentation. He also confirmed Rettger's fermentative studies and suggests that the gas-producing type of Bacterium pullorum be called Bacterium pullorum Alpha, and the non-gas-producing type be called Bacterium pullorum Beta. Hadley states that the Alpha type is pathogenic to young chicks only, while the Beta type is able to produce a generalized infection in adult fowls, but only to a slight degree, if at all, in young chicks.

In 1919, Hadley, Caldwell and Heath (26) again observed the two types of Bacterium pullorum and state that the anaerogenic (Beta) type is uncommon. They believe the difference between the two types depends upon envrionmental factors and is independent of the specific fermentable sugars.

TABLE II

Acid and Gas Production of Various Strains of *Bacterium pullorum* on Various Sugars.

| Strain | Dextrose | | Maltose | | Arabinose | | Rhamnose | | Sorbite | | Xylose | | Dulcite | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | G | A | G | A | G | A | G | A | G | A | G | A | G |
| 4 | + | + | + | - | + | + | + | + | + | + | + | + | - | - |
| 5 | + | + | + | - | + | + | + | + | + | + | + | + | - | - |
| 6 | + | + | - | - | + | - | + | + | + | - | + | - | - | - |
| 7 | + | + | - | - | + | + | + | + | + | - | + | - | - | - |
| 8 | + | - | - | - | + | - | + | - | + | - | + | - | - | - |
| 9 | + | + | - | - | + | + | + | + | + | - | + | + | - | - |
| 10 | + | + | + | - | + | - | + | + | + | - | + | - | - | - |
| 16 | + | + | - | - | + | + | + | - | + | - | + | - | - | - |
| 17 | + | + | + | - | + | + | + | + | + | - | + | + | - | - |
| 19 | + | + | + | - | + | + | + | - | + | + | + | + | - | - |
| 20 | + | + | + | + | + | + | + | + | + | + | + | + | - | - |
| 21 | + | + | - | - | + | + | + | + | + | - | + | - | - | - |
| 22 | + | + | + | - | + | - | + | + | + | + | + | + | - | - |
| 23 | + | + | - | - | + | - | + | + | + | - | + | - | - | - |
| 24 | + | + | - | - | + | + | + | + | + | + | + | + | - | - |
| 25 | + | + | - | - | + | - | + | + | + | - | + | - | - | - |
| 26 | + | + | - | - | + | - | + | + | + | - | + | + | - | - |
| 27 | + | + | - | - | + | + | + | + | + | - | + | + | - | - |
| 28 | + | + | - | - | + | + | + | + | + | + | + | - | - | - |
| 29 | + | + | + | - | + | + | + | + | + | - | + | - | - | - |
| 30 | + | + | - | - | + | + | + | + | + | - | + | - | - | - |
| 31 | + | + | - | - | + | + | + | + | + | + | + | + | - | - |
| 32 | + | + | + | - | + | + | + | + | + | + | + | + | - | - |

They report that the kind of broth medium used has an effect upon gas production. They found that when the anaerogenic types from chicks were grown in dextrose extract broth no gas was produced while gas was produced in dextrose infusion broth. The anaerogenic strains isolated from adults gave negative gas production on both kinds of media.

In regard to occurrence of the two types of organisms (Alpha and Beta) they ask "(1) Are both types present in the intestinal tract of fowls and does the Beta type produce generalized infection because of a difference in aggressivity; or (2) may the Alpha type give rise to the Beta type during the progress of ovarian or intestinal infection as a result of adaptation or as a result of a selection of anaerogenic mutants? Is Bacterium pullorum an instance of Bacterium gallinarum in the making?"

In a very extensive report (1918) Hadley, Elkins and Caldwell draw the following conclusions:

"The present methods for and results of the diagnosis of Bacterium pullorum infections by agglutination reactions are not satisfactory because they fail to differentiate (1) between ovarian and non-ovarian infections, (2) between culminated and current infections (3) between Bacterium pullorum and infections with the fowl cholera bacillus which is widely disseminated among adult stock; and (4) between infections caused by Bacterium pullorum Alpha and Bacterium pullorum Beta."

A new view on the transmission of the disease in the adult fowl is given by Rettger, Kirkpatrick and Card (28) in 1919. They state that progressive infection in a flock is not confined to

young chickens but may also be spread through the adult fowls, particularly the females. The introduction of the organism into the cloaca and oviduct of laying hens may readily lead to permanent ovarian infection. The rôle of the male has not been demonstrated but it undoubtedly plays a part as a passive carrier. Very few males, they state, show infection of the testicles although they did find a few cases.

## HISTORY OF THE CULTURES STUDIED.

### Cultures 4 and 5.

These two cultures were isolated May 5, 1922, from a Rhode Island cockerel sent in for examination from Lawrence, Michigan. The gross examination showed epicarditis, heavy injection of the intestinal vessels and also of the vessels of the testicles. The bird had shown signs of white diarrhea and leg weakness for a considerable period previous to death. The disease was a generalized septicemia as evidenced by the isolation of the organism from the liver, kidneys and the blood. The two cultures were picked from the same plate, two being fished because of a difference in the size of the colonies. Culture 4 was a small colony while Culture 5 produced a somewhat larger colony.

### Culture 6.

This culture was isolated April 22, 1922, from an adult bird (hen) from Dearborn, Michigan. The bird was autopsied in the field and was diagnosed without hesitation as white diarrhea. Only the liver was submitted for examination, from which the organism was isolated.

### Culture 7.

This culture was isolated from a baby chick from Big Rapids, Michigan, April 14, 1922. The liver was studded with small necrotic foci. The culture was isolated from the liver.

Cultures 8 and 9.

These two cultures were isolated August 1, 1922, from chicks shipped to the laboratory from Vicksburg, Michigan. No gross pathological description was taken. The chicks all showed positive agglutination tests. The two cultures represent two colonies showing slight cultural differences.

Culture 10.

This culture was obtained from Dr. Hadley of the University of Michigan in 1921. The culture was carried to Michigan by Dr. Hadley when he became connected with the University. The culture was one he originally isolated while at the Rhode Island Experiment Station. The culture was originally an aerogenic type, Bacterium pullorum Alpha.

Culture 11.

This culture was isolated May 8, 1922, from baby chicks shipped from St. Johns, Michigan. The livers of these chicks were ochre color and showed typical white diarrhea lesions.

Culture 12.

This culture was isolated from baby chicks from Hillsdale, Michigan, May 9, 1922. Typical ochre colored livers were found.

Culture 13.

This culture was isolated from baby chicks from Ann Arbor, Michigan, May 19, 1922. Typical ochre colored livers were found. The culture was isolated from the liver.

Culture 14.

This culture was isolated from white Leghorn baby chicks obtained from Plainville, Michigan, May 22, 1922. No pathological description was taken.

Culture 15.

This culture was obtained from baby chicks from Marion, Michigan, June 6, 1922. Typical lesions were present on the liver.

Culture 16.

This culture was isolated June 20, 1922, from baby chicks shipped from Benzonia, Michigan. Typical lesions were present on the liver.

Culture 17.

This organism, isolated June 11, 1922, was obtained from baby chicks shipped from Bately, Michigan. Typical lesions were present on the liver.

Culture 18.

This culture was isolated June 21, 1922, from baby chicks from Paw Paw, Michigan. Typical lesions were found on the liver.

Culture 19.

This culture was isolated June 21, 1922, from baby chicks from Grass Lake, Michigan. Typical lesions were present on the liver.

Culture 20.

This culture was isolated June 23, 1922, and was obtained from baby chicks from Allegan, Michigan. Typical lesions were present on the liver.

Culture 21.

This culture was isolated June 24, 1922, and was obtained from baby chicks from Emmet, Michigan. Typical lesions were present on the liver.

Culture 22.

This culture was isolated June 28, 1922, from baby chicks sent in from Oakley, Michigan. Typical lesions were present on the liver.

Culture 23.

This culture was isolated June 28, 1922, from baby chicks obtained from Lawrence, Michigan. Typical lesions were present on the liver.

Culture 24.

This culture, isolated July 1, 1922, was obtained from baby chicks from Royal Oak, Michigan. The liver showed only one slightly colored spot.

Culture 25.

This culture was isolated July 8, 1922, from baby chicks from Williamston, Michigan. A retained yolk the size of a pea was found. The adjoining tissue had a slight ochre color. No outstanding lesions were present.

Cultures 26 to 41.

These cultures were obtained from the Kansas Agricultural College in 1922. No history of the organisms was given. The names of the organisms were:

Culture 26 was marked 2205-40
Culture 27 " " 2142-29
Culture 28 " " PP7
Culture 29 " " 2248-30
Culture 30 " " PP1
Culture 31 " " 3848-39
Culture 32 " " PP3

Culture 33 was marked Chick

Culture 34 " " 2353-32

Culture 35 " " 2248-39

Culture 36 " " 2648-37

Culture 37 " " 190-3

Culture 38 " " 2317-40

Culture 39 " " 2533-Ad

Culture 40 " " PP5

Culture 41 " " Bacterium pullorum

Cultures 42 to 45

These cultures were obtained from Ohio State University in 1922. No history of these cultures is given. The cultures were labeled as follows:

Culture 42 was marked Bacterium pullorum

Culture 43 " " Rettger B16

Culture 44 " " Rettger W17

Culture 45 " " Rettger MR

Unfortunately strains 42, 43 and 45 were lost, in fact we were unable to grow strains 43 and 45 from the slants sent us.

Culture 46.

This culture was isolated July 20, 1922, from a hen belonging to Dr. Giltner, diagnosed by him as having white diarrhea. Only the ovaries were brought in to the laboratory. The organism was isolated from these.

Culture 47.to 53

These cultures were obtained from Perdue University in 1922. No history of the cultures was given. They were labeled as follows:

Culture 47 was marked Field Strain 5

Culture 48 " " Field Strain 7

Culture 49 " " Field Strain 4

Culture 50 " " Field Strain 6

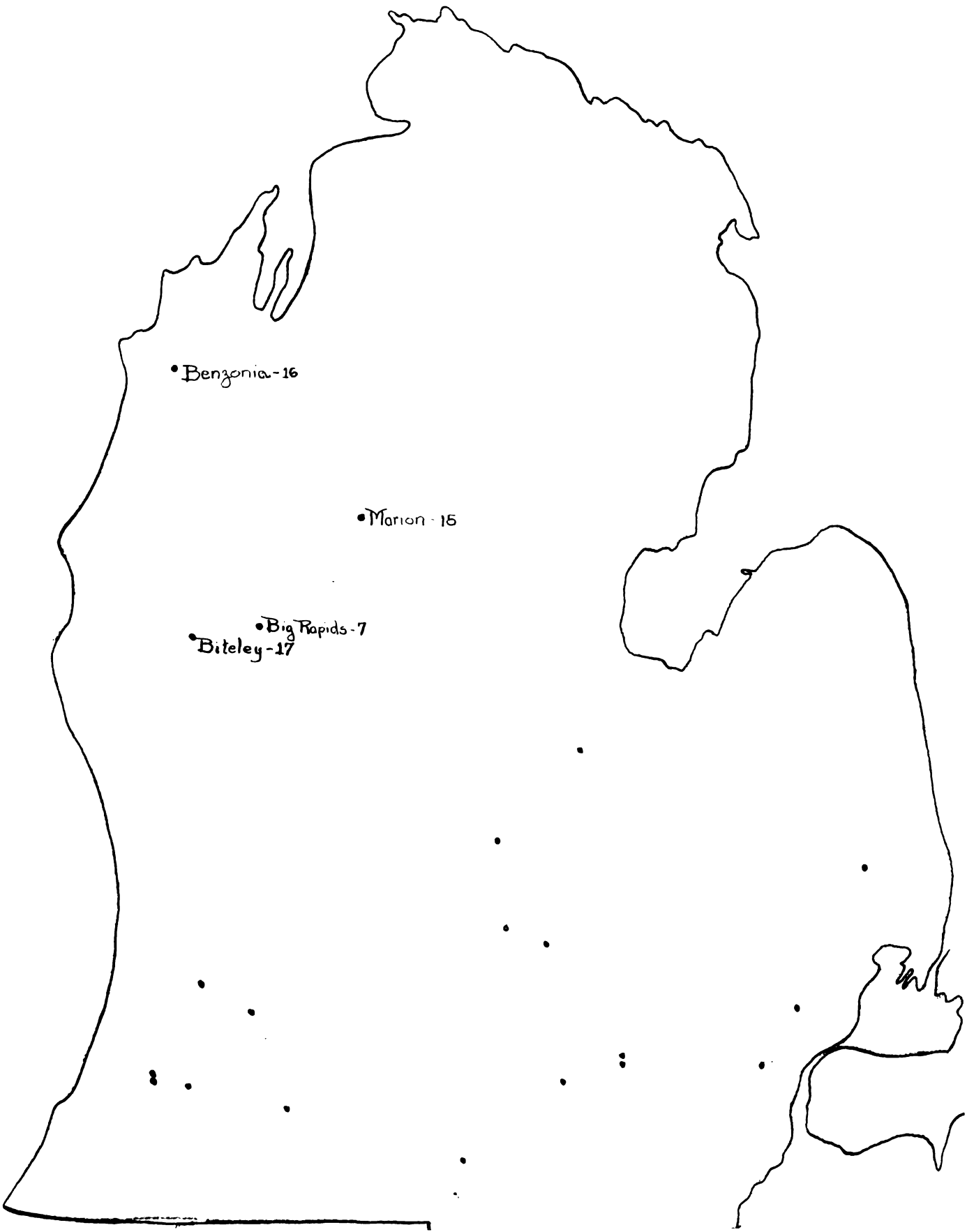
Culture 51 " " Field Strain 1

Culture 52 " " Field Strain 2

Culture 53 " " Field Strain 3

A map of the lower peninsula of Michigan is given in Figure I, showing the sources of the various cultures that were isolated in Michigan. This map shows that the cultures were obtained from a wide area representing practically the whole of the lower peninsula. Thus the cultures studied would represent many strains, as the disease would very likely be introduced from various sources, from outside states as well as the state of Michigan.

MAP IN THE LOWER PENINSULA OF MICHIGAN SHOWING FIELDS WHERE *BACTERIUM PULEBRUM* CULTURES WERE OBTAINED



## PREPARATION OF MEDIA USED

Considerable variation occurred in the preparation of the media used and this should be known as it may play some part in the reaction obtained as has been demonstrated by Rettger and others.

### Liver Agar.

This medium was selected for growing Bacterium pullorum as Dr. Stafseth had highly successful results using it for carrying stock cultures. At first the medium was made according to the direction of Stafseth and Huddleson. Briefly, this method consisted in cooking the liver with tap water, added at the rate of 500 c.c. per pound of finely ground lean liver. The cooking was done in an Arnold steamer for about 1 hour. The liquid was pressed out and filtered through glass wool. The liver agar medium was now prepared, using the following ingredients:

500 c.c. liver infusion,
500 c.c. tap water,
15 gm. agar,
10 gm. peptone,
5 gm. salt.

The mixture was steamed until the agar melted, after which it was cooled to 60° C. and 10 gm. of egg albumin added. The mixture was now cooked another hour to coagulate the egg albumin and precipitate any other albuminous substances that would be precipitated by heat. After heating, the hot mixture was filtered until clear through glass wool.

Due to the fact that the above method was quite difficult, modifications were introduced.* I might state here that the glass wool was used as it was believed (Torrey) that paper or cotton removed certain growth-producing substances. This has recently been proved by Hasley in a very striking manner with freshly isolated Bacterium abortus. However, the writer discarded the use of glass wool and substituted cotton and also dropped clarifying the medium with egg albumin. This made the preparation of the medium as simple as making ordinary agar. No change in the amount of growth of the Bacterium pullorum resulted, as was the case with Bacterium abortus.

Extract Broth.

The extract broth was made according to the method recommended by the American Public Health Association for water analysis. The broth was fermented with Bacillus coli to remove any fermentable agar present and then adjusted to a titre of pH 6.8. The last run of fermentation tests (1924) was made on extract broth with a titre of pH 7.

The sugars were used at the rate of 1 percent in the case of the cheaper sugars, as lactose, sucrose, mannite, dextrose, starch, inulin, maltose, glycerine and dextrin, and one tenth percent in the case of the more expensive sugars as adonite, dulcite, mannose, levulose, rhamnose, galactose, arabinose and raffinose.

* Huddleson, Hasley and Stafseth now have a procedure whereby the above process of preparation is relatively simple.

Infusion Broth.

This broth was prepared from ground lean beef in the usual manner. The resulting broth was fermented with Bacillus coli and the titre adjusted to a pH 6.8.

Preparation of the Sugar Broths.

All sugar broth used, with the exception of lactose, maltose and sucrose were prepared as usual and sterilized at 15 pounds pressure for 20 minutes. The three sugars mentioned, due to the fact that they are very sensitive to heat were prepared as follows:

The required amount of broth needed was measured into a suitable flask and sterilized by heat. A 20 percent solution of the sugars to be used was prepared, using sterile distilled water. This solution was now passed through a sterile Berkefeld filter to remove any organisms present. The sterile sugar filtrate was added, aseptically in the required amounts, to the sterile broth. Using the filling apparatus pictured in Figure II, the broth was tubed aseptically. (This apparatus was not original with the writer.) The broth was incubated to detect contamination and any tubes showing growth or even a suspicion of growth were discarded. However, it was necessary to discard but few tubes.

Figure II

Indicator for Acidity.

The indicator used to determine acidity was Andrade's indicator, which was added at the rate of 1 c.c. per 100 of broth.

## Microscopic Studies of Bacterium pullorum.

Transfers of all the cultures to be studied were made on liver agar slants. These were incubated at 37° C. for 24 hours, and then ordinary stains were made to study the morphology and staining properties and to make sure that all strains were pure cultures. Contrary to expectations, the microscope showed large heavy stained organisms inclined to form long chains, and occasional apiculated forms, instead of observing typical Bacterium pullorum cells. Few cells appeared normal. The strains appeared to be contaminated with an entirely different type of organism.

Several strains showing pronounced differences, namely, cultures 10, 23, and 24 were plated out on liver agar, in order to determine whether they were contaminated or whether they were passing through some stage of pleomorphism. Colonies which were fished from these plates were planted on liver agar slants. Stains were made and the following observations noted:

Culture 10 - Stain showed fairly large rods which were mostly in singles, but still a few chains were observed.

Culture 23 - The stain showed smaller rods than Culture 10 and very few chains. The apiculated forms observed before were still in evidence.

Culture 24 - This strain showed cells somewhat the same as Strain 23, except that many more of the apiculated forms were present, which in this case were always in pairs.

None of the colonies fished appeared normal (microscopically) for Bacterium pullorum. Apparently the forms observed were involution forms or marked pleomorphic types. The colon-typhoid group is characterized by their decided pleomorphism and it would appear that Bacterium pullorum was no exception.

In order to be able to make microscopical studies to determine whether all of the strains were the same it was necessary to find some medium upon which the organisms would appear normal. As previous observations were made from plain agar, this medium was selected.

All of the strains were invigorated on liver agar and then liver agar and plain agar slants were made. These were incubated at 37° for 24 hours when plain and Gram's stains were made. The results of these studies are tabulated in Table III.

A close study of Table III shows that a distinct difference exists in the appearance of the cells on plain agar and on liver agar. It will be noticed that on plain agar, the size and shape of the cells is similar to earlier observations, the cell being slightly longer than wide with slightly rounded ends and with a size of about 1.25 by 0.75 microns. The organisms appear generally as singles with an occasional pair. No chains were observed in any of the strains.

On liver agar, the cells are totally different, the cells being larger, staining more easily and more deeply and having a tendency to chain formation. Cells are seldom in singles as compared to the cells appearing on plain agar.

Table III.

A Comparison of the Morphological Appearance of

Bacterium Bullorum on 24 Hour Plain Agar and Liver Agar Slants.

| Strain | Plain Agar Slants. | Liver Agar Slants. |
|---|---|---|
| 4 | No chains-organisms single-size 1-2 x 0.5 | Chains frequent-mostly singles-size 2.4-3.6 x 0.75 microns |
| 5 | No chains, organisms single- 1.2-1.8 x 0.5 | Long chains frequent, A 72 micron chain observed-generally single pairs frequent with ends slightly pointed-1.2-3 x 0.75 |
| 6 | No chains-organisms single-short rods nearly coccoid size 0.75 - 1.8 x 0.5 | Long chains-singles and pairs Size 3.6-2 x 0.75 u. |
| 7 | No chains-cells single short rods-uneven staining- 0.75-1.25 x 0.25-0.5 | No chains-cells single, slightly larger-even staining, 1-1.5 x.5 |
| 8 | No chains-cells generally single, even staining-1.25-2.5 x 0.5 u. | chains frequent- pairs common even staining-1-1.5 x 0.5 u |
| 9 | No chains-cells single-even staining, short coccoid rods- 1-1.5 x 0.25 - 0.5 u. | Chain infrequent- pairs occasional-even staining-1-2.5 x 0.5 u. |
| 10 | No chains-coccoid to slender rods- 1-2.5 x 0.5 u. | Chains very frequent- pairs common- long rods- stain deeply 1.5 -3 x 0.5 u. |
| 11 | No chains-very short rods-nearly round-always single- 9.75 -1.25 x 0.5-0.75 u. | No chains-heavy thick rod - short- stains deeply 1.2 -2 x 0.75 u. |
| 12 | No chains-uneven staining-always single 1-2 x 0.5 u. | Chains present-2 types of cells in regard to staining- one deep and one light 1.5 -2.5 x 0.5 -0.75 u. |
| 13 | No chains-generally single-very short to moderate rods- 0.75 -1.25 x 0.5u. | Chains present-singles predominate-stains evenly 1.5 -2.5 x 0.5 u. |

Table III continued.

| Strain | Plain Agar Slants. | Liver Agar Slants. |
|---|---|---|
| 14 | No chains-generally single short rods-nearly coccoid in some cases-stain evenly-o.75 x 0.5 | Chains present-commonly in short chains of 2 and 3. Stain heavily and evenly 2.5 x 0.5-size uniform |
| 15 | No chains-generally single short rods-uniform size, even staining .75-1.5 x 0.5 | Chains present-pairs frequent-stains deeply & evenly 1.2-3 x 0.5 |
| 16 | No chains-generally single -very uneven staining 0.78-1.5 x 0.5 | Chains present-stains deeply & evenly-uniform size-pairs frequent- 1.5-2.5 x 0.5 |
| 17 | No chains-generally single uneven staining-short rods 1.25 - 0.75 | Pairs common-stains more evenly-slightly larger |
| 18 | No chains-singles-stain fairly even- 1-1.25 x 0.25 -0.7 | Few chains-pairs frequent-1.25-1.5 x 0.75 - stained deeply & evenly. |
| 19 | No chains-singles-stain fairly even altho variation is noticeable - 1 x 0.5 | Chains frequent- 2 & 3's common-even staining- 1.25-2 x 0.5-0.75 |
| 20 | No chains-singles-decided variation in staining-1-1.25 x 0.5 | 3's and 4's common-singles fairly so - 1.5-2 x 0.75 |
| 21 | No chains-singles-decided variation in staining-1-1.25 x .5 | Chains frequent-stain heavier but variable-generally single-1.25-1.5 x 0.5-0.75 |
| 22 | No chains-singles-stains well & fairly even-1 x 0.5 | Chains very frequent-singles few-stains heavily - 1.25-2 x 0.75 |
| 23 | About the same as #21-very lightly stained | Chains present - poorly stained |
| 24 | No chains-singles B.coli shaped-twice as long as wide 1-1.25 x 0.5 | No chains-pairs occasional rods slenderer & slightly longer. |
| 25 | No chains-singles B.coli shaped-1-1.25 x 0.5 | Pairs frequent-stains well-larger & longer rods - 1.25-2 x 0.5 |
| 26 | Same as #25-not stained as well | Short chains-pairs common-variable but heavy staining-size same as #25 |

Table III continued.

| Strain | Plain Agar Slants. | Liver Agar Slants. |
|---|---|---|
| 27 | No chains-singles-stain evenly-cells slightly more slender than #25- 1-1.25 x 0.5 | Chains frequent-stains heavily-pairs common- 1.5-2 x 0.5 |
| 28 | No chains-singles-stain well-nearly coccoid in some cases-1 x 0.5 | Few chains-stains very irregular-size of cells irregular-somewhat larger. |
| 29 | No chains-singles-stains evenly-generally twice as long as wide- 0.75-1 x 0.5 | Extremely long chains & very frequent-stain heavy- 1.25-1.75 x 0.75 |
| 30 | No chains-singles-stain evenly- 1-1.25 x 0.5 | No chains-stain deeply-& evenly-considerable débris-somewhat larger |
| 31 | No cells visible | No cells visible |
| 32 | No chains-singles-stain evenly- 0.75-1.25 x 0.75 | Few chains present-stains deeply considerable débris- somewhat larger |
| 33 | No chains-singles-stain lightly but evenly- 0.75-1.25 x 0.5 | Some chains-generally pairs or 3's and 4's-staining extremely uneven-stains lightly- 1.25-1.5 x 0.75 |
| 34 | No chains-singles-size same as #33 | Chains frequent-stain deeply & evenly altho some cells stain very poorly- 1.5-1.75 x 0.75 |
| 35 | No chains-singles-stain well & evenly - uniform size- 1-1.25 x 0.5 | Chain frequent-staining even within the cells but decided variation in staining of different cells. Cells long & cylindrical- 1.5-2.5 x 0.75 |
| 36 | No chains-singles-stain well & evenly-uniform size nearly coccoid- 0.75-1.25 x 0.5 | Chains frequent-some cells stain deeply-others no stain at all-cells long and slender. 1.5-2.5 x 0.75 |
| 37 | No chains-singles-stain well-uniform size, more slender than #36 | Chains but short-similar to 36 in staining and shape- 1.5-2.5 x 0.75 |
| 38 | No chains-singles-stain well but lightly-uniform size-very short rods 1 x 0.5 | Pronounced chains-most cells poorly stained (degenerated appearance)-staining and shape same as in #36 |

Table III continued.

| Strain | Plain Agar Slants. | Liver Agar Slants. |
|---|---|---|
| 39 | Very poorly stained-appears as #38 | Very poorly stained-appears as #38 |
| 40 | No chains-singles-some extremely long-about twice as long as wide, may be pairs altho no evidence shows- 1-2 x 0.5 | Chains-much débris or degenerate appearing cells-normal appearing cells are 1.5-2 x 0.75 |
| 41 | No chains-singles-stain well-slightly longer than #38- 1-2 x 0.5 | Chains few-much débris-cells slightly larger than on plain agar |
| 42 | No chains-singles-stain well-size uniform- 0.75-1.25 x 0.5 | No chains observed-cells stain deeply-long and slender- 1.5-2.5 x 0.75 |
| 46 | Very poorly stained-probably like #38 | Very poorly stained-seem to be long rods |
| 47 | No chains-very short rods-nearly coccoid-stain evenly 0.75-1 x 0.5 | No chains-pairs common-staining of cells irregular-banded or polar- 1.5-2 x 0.75 |
| 48 | No chains-short rods-nearly coccoid-stain evenly- 1-0.5 x 0.5 | No chains-pairs-staining somewhat irregular 1-2 x 0.75 |
| 49 | Too poorly stained | Chains-staining poor-appears to be long rods |
| 50 | No chains-cells short-poorly stained | Slide dirty-cells clumped-appear longer |
| 51 | Poor stain-cells coccoid | Chain and long rods |
| 52 | No chains-cells evenly stained-some very short 0.75-1 x 0.5 | No chain-cells slightly larger. |
| 53 | No chains-cells evenly stained-very short rods- 0.75-1 x 0.5 | No chain-cells longer and wider-slender appearing-some pairs. |

All strains do not behave the same on the same medium. Strains 10 and 11 and to a lesser extent Strain 47 show a marked coccoid shape. Strains 10 and 11 were particularly prominent and stand out from the rest. However, the strains vary considerably in this characteristic and it is very doubtful whether the shape is constant and even if it was it is too unreliable to try to base a separation of strains on.

On the liver agar, the production of chains varied considerably, but here again it is too inconstant to have any differential value.

This table shows that Bacterium pullorum is decidedly pleomorphic and hence too much stress should not be placed upon a microscopic examination in diagnosis.

The effect of the medium used on the Gram's Stain was also studied. These stains were made at the same time that the plain stains were prepared (Table III) The results of the Gram's stains are shown in Table IV.

The medium does not apparently influence the Gram's stain to any extent although the results in Table IV show slightly better results where plain agar was used. The stains were made on the same slide in every case to avoid any variations in technic. The results show that occasionally (Strains 5 and 12) a positive stain will appear. This is not a constant characteristic and might occur on any stain. Its occurrence is not constant. However, in making Gram's stains, plain agar should be used where possible as the results obtained on liver agar are too variable.

TABLE IV

The Effect of the Media on the Gram's Stain.

| Strain | Plain Agar Slant | Liver Agar Slant | Strain | Plain Agar Slant | Liver Agar Slant |
|---|---|---|---|---|---|
| 4 | - | -? | 27 | - | No good |
| 5 | - | + | 28 | - | - |
| 6 | - | - | 29 | - | - |
| 7 | No good | No good | 30 | - | - |
| 8 | - | - | 31 | - | - |
| 9 | - | - | 32 | - | - |
| 10 | - | - | 33 | - | - |
| 11 | - | - | 34 | - | - |
| 12 | -? | + | 35 | - | - |
| 13 | No good | - | 36 | - | - |
| 14 | - | - | 37 | - | - |
| 15 | - | - | 38 | No good | - |
| 16 | - | - | 39 | - | - |
| 17 | -? | -? | 40 | - | - |
| 18 | - | - | 41 | - | - |
| 19 | - | - | 42 | - | - |
| 20 | - | - | 46 | + | + |
| 21 | - | - | 47 | - | - |
| 22 | - | - | 48 | - | -? |
| 23 | - | No good | 49 | - | -? |
| 24 | - | No good | 50 | +? | long rods - short coccoids + |
| 25 | -? | -? | 51 | - | - |
| 26 | - | - | 52 | - | - |
| | | | 53 | - | - |

- indicates a negative Gram's stain, and + indicates a positive.

## CULTURAL STUDIES

### Biochemical Changes Produced in Dunham's Solution.

These tests were made in series of two years, using duplicate tubes in each year. Examinations, on the Dunham's Solution were made for hydrogen sulphide, ammonia, nitrites and indol. All tests were made after incubation of the broth at 37° for 7 days. The results obtained are given in the accompanying tables.

Hydrogen Sulphide Production. Previous work by others (Hadley) has shown Bacterium pullorum quite a constant producer of hydrogen sulphide. The determinations (Table V) fail to show any confirmation of such work, in fact, it is just the opposite. Hydrogen sulphide production appears to be extremely variable. It will be noticed that the last determinations showed very few positives (4 strains) while the 1922 determinations show 15 strains positive. The same strains in 1923 do not show necessarily the same as in 1922. Culture 10 showed negative in 1922 and slightly positive in 1923, and only cultures 7, 8 and 9 show positive in both series of tests. Considerable variation is shown but has no apparent significance.

Ammonia Production. The production of ammonia by all strains is apparently constant. (Table VI) All strains with the exception of one tube showed approximately the same amount of ammonia. This one tube showed an increased amount. Ammonia production is then a constant property of all Bacterium pullorum strains.

TABLE V

Formation of Hydrogen Sulphide in Dunham's Solution by Bacterium pullorum.

| Strain | Determinations 1922 | | 1923 | | Strain | Determinations 1922 | | 1923 | |
|---|---|---|---|---|---|---|---|---|---|
| 4 | - | - | - | - | 28 | - | - | - | - |
| 5 | - | +++ | - | - | 29 | - | - | - | - |
| 6 | - | - | - | - | 30 | - | - | - | - |
| 7 | +++ | ++ | ± | - | 31 | - | - | - | - |
| 8 | ++ | - | - | ± | 32 | - | - | - | - |
| 9 | +++ | +++ | ± | ± | 33 | - | - | - | - |
| 10 | - | - | ± | ± | 34 | - | - | - | - |
| 11 | ++ | + | - | - | 35 | - | - | - | - |
| 12 | - | - | - | - | 36 | - | - | - | - |
| 13 | +++ | +++ | - | - | 37 | - | - | - | - |
| 14 | + | ++ | - | - | 38 | - | - | - | - |
| 15 | - | ++ | - | - | 39 | - | - | - | - |
| 16 | + | ++ | - | - | 40 | - | - | - | - |
| 17 | ++ | ++ | - | - | 41 | - | - | - | - |
| 18 | - | - | - | - | 44 | - | - | - | - |
| 19 | - | - | - | - | 46 | - | - | - | - |
| 20 | + | - | - | - | 47 | + | + | - | - |
| 21 | - | ++ | - | - | 48 | - | - | - | - |
| 22 | + | - | - | - | 49 | - | - | - | - |
| 23 | ± | + | - | - | 50 | - | - | - | - |
| 24 | - | - | - | - | 51 | - | - | - | - |
| 25 | - | - | - | - | 52 | - | - | - | - |
| 26 | - | - | - | - | 53 | - | - | - | - |
| 27 | - | - | - | - | | | | | |

- indicates negative ± indicates questionable or very slight
+ indicates slight, ++ indicates fair, +++ indicates fairly strong.

TABLE VI

Formation of Ammonia in Dunham's Solution by Bact. pullorum.

| Strain | Determinations 1922 | | 1923 | | Strain | Determinations 1922 | | 1923 | |
|---|---|---|---|---|---|---|---|---|---|
| 4 | + | + | + | + | 28 | + | + | + | + |
| 5 | + | + | + | + | 29 | + | + | + | + |
| 6 | + | + | + | + | 30 | + | + | + | + |
| 7 | + | + | + | + | 31 | + | + | + | + |
| 8 | - | + | + | + | 32 | + | + | + | + |
| 9 | + | + | + | + | 33 | + | + | + | + |
| 10 | + | + | + | + | 34 | + | + | + | + |
| 11 | + | + | + | + | 35 | + | + | + | + |
| 12 | + | + | + | + | 36 | + | + | + | + |
| 13 | + | + | + | + | 37 | + | + | + | + |
| 14 | + | + | + | + | 38 | + | + | + | + |
| 15 | + | + | +++ | + | 39 | + | + | + | + |
| 16 | + | + | + | + | 40 | + | + | + | + |
| 17 | + | + | + | + | 41 | + | + | + | + |
| 18 | + | + | + | + | 46 | + | + | + | + |
| 19 | + | + | + | + | 47 | + | + | + | + |
| 20 | + | + | + | + | 48 | + | + | + | + |
| 21 | + | + | + | + | 49 | + | + | + | + |
| 22 | + | + | + | + | 50 | + | + | + | + |
| 23 | + | + | + | + | 51 | + | + | + | + |
| 24 | + | ± | + | + | 52 | + | + | + | + |
| 25 | + | + | + | + | 53 | + | 0 | + | + |
| 26 | + | + | + | + | | | | | |
| 27 | + | + | + | + | | | | | |

Nitrate Production. None of the strains studied were able to produce nitrites in Dunham's Solution. This checks with previous work.

Indol Production. Bacterium pullorum is not supposed to produce indol and the strains studied did not show any evidence of it in 1922, except strain 23 which showed positive in both tubes. In the 1923 determinations, strains 20 and 22 showed positive in one tube only and strains 14, 15 and 19 showed very slight in one tube (Table VII).

TABLE VII

Formation of Indol from Dunham's Solution by Bact. pullorum

| Strain | Determinations 1922 | | | 1923 | Strains | Determinations 1922 | | | 1923 |
|---|---|---|---|---|---|---|---|---|---|
| 4 | - | - | - | - | 28 | - | - | - | - |
| 5 | - | - | - | - | 29 | - | - | - | - |
| 6 | - | - | - | - | 30 | - | - | - | - |
| 7 | - | - | - | - | 31 | - | - | - | - |
| 8 | - | - | - | - | 32 | - | - | - | - |
| 9 | - | - | - | - | 33 | - | - | - | - |
| 10 | - | - | - | - | 34 | - | - | - | - |
| 11 | - | - | - | - | 35 | - | - | - | - |
| 12 | - | - | - | - | 36 | - | - | - | - |
| 13 | - | - | - | - | 37 | - | - | - | - |
| 14 | - | - | - | - | 38 | - | - | - | - |
| 15 | - | - | - | ± | 39 | - | - | - | - |
| 16 | - | - | - | ± | 40 | - | - | - | - |
| 17 | - | - | - | - | 41 | - | - | - | - |
| 18 | - | - | - | - | 44 | - | - | - | - |
| 19 | - | - | - | ± | 46 | - | - | - | - |
| 20 | - | - | - | + | 47 | - | - | - | - |
| 21 | - | - | - | - | 48 | - | - | - | - |
| 22 | - | - | - | + | 49 | - | - | - | - |
| 23 | - | - | - | - | 50 | - | - | - | - |
| 24 | + | + | - | - | 51 | - | - | - | - |
| 25 | + | - | - | - | 52 | - | - | - | - |
| 26 | - | - | - | - | 53 | - | - | - | - |
| 27 | - | - | - | - | | | | | |

Biochemical Changes Produced in Nitrate-Peptone Solution by Bacterium pullorum.

Only one set of determinations was made for the oxidation of nitrites in Nitrate-Peptone Solution. These determinations were made December 20, 1923. The results of these tests are given in Table VIII.

Hadley (29) states that Bacterium pullorum does not reduce nitrites, however, the results obtained show that this property of lack of property is variable. A number of tubes showed what might be designated as a slight nitrite test. This was confined, with one exception, to only one tube of the two tubes used of each organism. The results obtained should be duplicated before any conclusions can be drawn. No ammonia was shown in any of the tubes tested.

TABLE VIII

Biochemical Changes Produced in Nitrate-Peptone Solution by Bact. pullorum.

| Strain | $NH_3$ | | Nitrites | | Strain | Ammonia | | Nitrites | |
|---|---|---|---|---|---|---|---|---|---|
| 4 | - | - | - | - | 28 | - | - | - | - |
| 5 | - | - | - | ± | 29 | - | - | - | - |
| 6 | - | - | - | - | 30 | - | - | + | - |
| 7 | - | - | - | - | 31 | - | - | - | - |
| 8 | - | - | - | - | 32 | - | - | - | - |
| 9 | - | - | - | - | 33 | - | - | - | - |
| 10 | - | - | - | - | 34 | - | - | + | + |
| 11 | - | - | ± | - | 35 | - | - | - | - |
| 12 | - | - | + | - | 36 | - | - | - | - |
| 13 | - | - | ± | - | 37 | - | - | - | ± |
| 14 | - | - | - | - | 38 | - | - | - | - |
| 15 | - | - | - | - | 39 | - | - | - | - |
| 16 | - | - | - | - | 40 | - | - | - | - |
| 17 | - | - | - | - | 41 | - | - | + | - |
| 18 | - | - | - | - | 44 | - | - | ± | - |
| 19 | - | - | - | - | 46 | - | - | - | - |
| 20 | - | - | - | - | 47 | - | - | - | - |
| 21 | - | - | + | - | 48 | - | - | - | - |
| 22 | - | - | - | - | 49 | - | - | - | - |
| 23 | - | - | - | - | 50 | - | - | ± | - |
| 24 | - | - | - | - | 51 | - | - | - | - |
| 25 | - | - | - | - | 52 | - | - | - | - |
| 26 | - | - | + | - | 53 | - | - | ± | ± |
| 27 | - | - | - | - | | | | | |

Effect Produced on Milk by Bacterium pullorum

The milk used was adjusted to a titre of 1.5 percent normal acid, using phenolphthalein as an indicator. Two percent azolitmin was added and the medium was sterilized by autoclaving. Two series of tests were made, one in 1922 and the other in 1923. Duplicate tubes were used in both cases.

In all previous work reviewed, Bacterium pullorum was described as producing a slight acidity in litmus milk in a week or ten days. In no place was an alkaline reaction obtained. One of the points of differentiation between Bacterium pullorum and Bacterium sanguinarum is the reaction produced in litmus milk, Bacterium pullorum producing a slight acidity and Bacterium sanguinarum an alkaline condition.

In this work, the reaction of the milk (Table IX-1922) on the 12 day reading shows but very little variation, the only variation being the degree of acidity produced. Some of the tubes showed no change from normal, while the greater share showed a very slight acidity.

The tubes run in 1923 showed a much greater variation. However, it should ne remembered that the final readings in this case were made in 25 days instead of 12 days as in the case of the 1922 results. Due to the fact that some of the cultures showed decidedly alkaline and in 5 cultures, one of the tubes was contaminated, these cultures were re-inoculated into litmus milk and a second observation was made, as shown in the following table (Table X).

Showing Changes Produced in Milk by Bact.pullorum.

| Strain | 1922 12 day reading | | 1923 25 day reading | | Strain | 1922 12 day reading | | 1923 25 day reading | |
|---|---|---|---|---|---|---|---|---|---|
| 4 | ± | ± | ± | ± | 29 | ± | ± | - | cont. |
| 5 | ± | ± | ± | r.c. | 30 | ± | ± | ± | ± |
| 6 | ± | ± | - | - | 31 | ± | ± | ± | ± |
| 7 | ± | ± | ± | ± | 32 | ± | ± | - | - |
| 8 | ± | ± | ± | ± | 33 | ± | ± | ± | ± |
| 9 | ± | ± | ± | ± | 34 | ± | ± | ± | ± |
| 10 | ± | ± | alk. | alk. | 35 | ± | ± | - | - |
| 11 | ± | ± | ± | ± | 36 | - | - | - | cont. |
| 12 | ± | ± | ± | ± | 37 | - | - | ± | ± |
| 13 | ± | ± | ± | ± | 38 | - | - | - | - |
| 14 | ± | ± | ± | ± | 39 | - | - | ± | cont. |
| 15 | ± | ± | ± | ± | 40 | - | - | - | - |
| 16 | ± | ± | - | - | 41 | - | - | ± | ± |
| 17 | ± | ± | ± | cont. | 44 | - | - | ± | ± |
| 18 | ± | ± | ± | ± | 46 | - | - | ± | ± |
| 19 | ± | ± | ± | ± | 47 | - | - | ± | ± |
| 20 | ± | ± | ± | ± | 48 | - | - | ± | ± |
| 21 | ± | ± | ± | ± | 49 | - | - | - | - |
| 22 | ± | ± | ± | ± | 50 | - | - | ± | cont. |
| 23 | ± | ± | ± | cont. | 51 | - | - | ± | ± |
| 24 | - | - | ± | ± | 52 | - | - | ± | ± |
| 25 | - | - | ± | ± | 53 | - | - | alk. | alk. |
| 26 | ± | ± | ± | ± | | | | | |

- or + indicate slight acidity.
r.c. indicates rennet curd.
cont. indicates contamination.

Table V.

Reactions Produced in Litmus Milk by Bacterium pullorum.

| Strain | Reaction Produced. |
|---|---|
| 4 | No change in 14 days. Later - slight acidity. |
| 5 | No change in 14 days. Later - slight acidity. |
| 10 | Alkaline in 14 days |
| 17 | Slightly acid in 14 days |
| 23 | Slightly acid in 14 days |
| 27 | Alkaline in 14 days. Later - reduction and curd. |
| 39 | Alkaline in 14 days |
| 29 | Slightly alkaline in 14 days. Later - decidedly alkaline, with rennet curd. |
| 36 | Slightly alkaline in 14 days. |
| 50 | Neutral |
| 53 | Neutral |

These confirmatory tests show that strains 10 and 27 still persist in alkali production while strain 53 which showed decidedly alkaline has changed and shows neutral. Strains 29, 36 and 39 in which one tube was contaminated before and where the second tube was neutral or slightly acid (strain 39) now show slight alkalinity. In fact after 25 days incubation these tubes showing alkali production in 14 days, now show decided rennet curd.

The reaction of milk, then, is not always a reliable characteristic of Bacterium pullorum.

## Special Biochemical Tests.

Dextrose dipotassium phosphate broth was inoculated with all strains and incubated at 37° C. for 5 days. At this time all tubes were tested by the methyl red test for acidity and by the Voges-Proskauer reaction to determine the presence of acetyl-methyl carbinol, a substance produced by Bacterium aerogenes in this broth.

All tubes showed positive to methyl red, that is, all strains produced approximately the same amount of acid.

All of the tubes showed negative to the Voges-Proskauer reaction.

TABLE XI.

Gas Production on Various Sugars by

Bacterium Pullorum.

| Strain | Days | Dextrose 1922 | Dextrose 1924 | Levulose 1922 | Levulose 1924 | Mannose 1922 | Mannose 1924 | Galactose 1922 | Galactose 1924 | Mannite 1922 | Mannite 1924 | Arabinose 1924 | Rhamnose 1924 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 1 | 10 | B 5 | B | B | 5 | 5 | - | - | 10 | 10 | - | - | 5 | 5 | - | - | B | B | - | - | - | - | - | - |
| | 2 | 20 | 10 | 5 | 5 | 10 | 10 | - | - | 10 | 10 | B | - | 5 | 5 | - | - | 15 | 15 | B | B | B | - | - | - |
| | 3 | 20 | 10 | 10 | 10 | 10 | 10 | - | - | 10 | 10 | B | - | 5 | 5 | - | - | 15 | 15 | 5 | 5 | B | - | - | - |
| 5 | 1 | 10 | - | B | B | B | B | - | - | 10 | 10 | - | - | 5 | 5 | B | 5 | 10 | 10 | - | - | 5 | - | - | - |
| | 2 | 10 | 5 | 5 | 3 | B | B | B | B | 10 | 10 | B | - | 5 | 5 | 10 | 5 | 10 | 10 | - | 10 | 10 | B | - | - |
| | 3 | 10 | 10 | 10 | 10 | 5 | 5 | B | B | 10 | 10 | B | - | 5 | 5 | 10 | 5 | 10 | 10 | -* | 10 | 10 | B | -* | -* |
| 6 | 1 | B | B | B | B | - | - | - | - | 10 | 10 | - | * | 5 | 5 | - | - | 5 | 10 | - | - | - | - | - | - |
| | 2 | 5 | 10 | 5 | 5 | B | B | - | - | 10 | 10 | - | - | 5 | 5 | - | - | 5 | 10 | - | - | - | - | - | - |
| | 3 | 5 | 10 | 10 | 5 | 5 | 5 | B | B | 10 | 10 | - | - | 5 | 5 | - | - | 5 | 10 | - | B | - | - | - | - |
| 7 | 1 | 10 | 0 | B | B | - | - | - | - | 10 | 10 | - | - | 5 | 5 | - | B | 5 | 5 | - | - | - | - | - | - |
| | 2 | 20 | - | 5 | 5 | - | - | - | - | 10 | 10 | - | - | 5 | 5 | - | B | 5 | 5 | - | - | - | - | - | - |
| | 3 | 20 | 10 | 8 | 10 | B | B | B | -* | 10 | 10 | - | - | 5 | 5 | - | B | 5 | 5 | 5 | - | - | - | - | - |
| 8 | 1 | 20 | 15 | B | B | - | B | - | - | 5 | 5 | - | - | 5 | 5 | - | - | 5 | 20 | B | 5 | - | - | - | - |
| | 2 | 30 | 20 | 5 | 5 | 10 | 5 | - | - | 5 | 5 | - | - | 5 | 5 | B | - | 10 | 40 | 20 | 40 | - | - | - | - |
| | 3 | 30 | 20 | 10 | 10 | 20 | 10 | - | - | 5 | 5 | - | - | 5 | 5 | B | - | 10 | 40 | 30 | 50 | - | - | - | - |
| 9 | 1 | 20 | 30 | B | B | B | - | - | - | 10 | 10 | - | - | 5 | 5 | - | - | - | 30 | 5 | 5 | B | B | - | - |
| | 2 | 40 | 40 | 10 | 5 | B | B | - | - | 10 | 10 | - | - | 5 | 5 | - | B | 5 | 30 | 30 | 30 | B | B | - | - |
| | 3 | 40 | 40 | 30 | 10 | 5 | 10 | - | - | 10 | 10 | - | - | 5 | 5 | - | B | 5 | 40 | 30 | 30 | B | B | - | - |
| 10 | 1 | - | - | B | B | - | - | - | - | 10 | 10 | - | - | B | B | - | - | B | 5 | - | - | - | - | - | - |
| | 2 | - | - | 5 | 5 | - | B | - | - | 10 | 10 | - | - | 5 | 5 | - | B | 5 | 5 | - | - | B | B | - | - |
| | 3 | - | - | 10 | 10 | B | B | - | - | 10 | 10 | - | - | 5 | 5 | - | B | 5 | 5 | B | B | B | B | - | - |
| 11 | 1 | - | - | - | - | - | - | - | - | B | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | 2 | - | - | - | - | - | - | - | - | B | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | 3 | - | - | -* | -* | - | - | - | - | B | - | - | - | - | - | - | - | - | 0 | - | - | - | - | - | - |
| 12 | 1 | 20 | 10 | B | B | B | - | - | - | 10 | 10 | - | - | 5 | 5 | B | - | B | 10 | B | B | B | - | - | - |
| | 2 | 30 | 30 | 5 | 10 | 5 | B | - | - | 10 | 10 | - | B | 5 | 5 | B | - | 5 | 20 | 30 | 60 | B | B | - | - |
| | 3 | 30 | 30 | 10 | 15 | 5 | 5 | - | B | 10 | 10 | B | - | 5 | 5 | B | B | 5 | 20 | 40 | 60 | B | B | - | B |

TABLE XI (continued)

| Strain | Days | Dextrose 1922 | | Dextrose 1924 | | Levulose 1922 | | Levulose 1924 | | Mannose 1922 | | Mannose 1924 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13 | 1 | B | B | B | B | B | B | - | - | 5 | 5 | - | - |
| | 2 | 10 | 20 | 3 | B | 20 | 10 | - | - | 5 | 5 | - | - |
| | 3 | 10 | 20 | 5 | B | 20 | 10 | - | - | 5 | 5 | - | - |
| 14 | 1 | B | B | B | B | B | B | - | - | 5 | 5 | - | - |
| | 2 | 30 | 10 | 5 | 5 | 5 | 5 | - | - | 10 | 10 | - | B |
| | 3 | 30 | 10 | 5 | 5 | 5 | 5 | - | - | 10 | 10 | B | B |
| 15 | 1 | 10 | 10 | B | B | B | B | - | - | 5 | 5 | B | B |
| | 2 | 20 | 20 | 3 | 3 | 10 | 30 | B | - | 10 | 10 | B | B |
| | 3 | 20 | 20 | 10 | 5 | 10 | 30 | B | -* | 10 | 10 | B | B |
| 16 | 1 | 10 | 10 | B | B | B | B | - | - | 10 | 10 | - | - |
| | 2 | 30 | 30 | 10 | 5 | 10 | 10 | - | - | 10 | 10 | - | - |
| | 3 | 30 | 30 | 20 | 10 | 10 | 10 | -* | B | 10 | 10 | - | 8 - |
| 17 | 1 | 40 | 10 | B | B | B | B | - | - | 10 | 10 | - | - |
| | 2 | 40 | 20 | 10 | 25 | 10 | 30 | - | B | 10 | 10 | - | - |
| | 3 | 40 | 20 | 20 | 30 | 10 | 30 | - | B | 10 | 10 | - | - |
| 18 | 1 | 10 | 15 | B | B | 10 | 10 | - | - | 10 | 10 | B | 5 |
| | 2 | 20 | 30 | 15 | 15 | 20 | 20 | 5 | B | 10 | 10 | 10 | 5 |
| | 3 | 20 | 30 | 20 | 20 | 20 | 20 | 10 | 10 | 10 | 10 | 10 | 5 |
| 19 | 1 | 10 | B | B | - | - | 10 | - | - | 10 | 10 | - | - |
| | 2 | 30 | 30 | 10 | 5 | 10 | 20 | 5 | 15 | 10 | 10 | B | B |
| | 3 | 30 | 30 | 10 | 10 | 20 | 20 | 10 | 20 | 10 | 10 | B | B |
| 20 | 1 | 5 | 5 | B | B | B | B | - | - | 10 | 10 | - | - |
| | 2 | 15 | 20 | 10 | 5 | 10 | 20 | B | - | 10 | 10 | - | - |
| | 3 | 15 | 20 | 15 | 10 | 15 | 20 | B | - | 10 | 10 | - | - |
| 21 | 1 | 10 | 10 | B | B | 5 | 5 | - | - | 10 | 10 | - | - |
| | 2 | 40 | 30 | B | 15 | 15 | 15 | - | - | 10 | 10 | - | - |
| | 3 | 40 | 30 | 5 | 20 | 15 | 15 | B | - | 10 | 10 | - | - |
| 22 | 1 | 10 | 10 | B | B | 5 | 5 | - | - | 10 | 10 | - | - |
| | 2 | 30 | 30 | B | B | 10 | 10 | - | - | 10 | 10 | - | - |
| | 3 | 30 | 30 | B | B | 10 | 10 | - | - | 10 | 10 | - | - |

| Strain | Days | Galactose 1922 | | Galactose 1924 | | Mannite 1922 | | Mannite 1924 | | Arabinose 1924 | | Rhamnose 1924 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13 | 1 | B | B | B | - | B | B | - | - | B | B | - | - |
| | 2 | 5 | 5 | B | - | 10 | B | 40 | B | B | B | - | B |
| | 3 | 5 | 5 | B | -* | 10 | B | 40 | 5 | B | B | - | B |
| 14 | 1 | B | B | B | B | B | B | - | - | B | B | - | - |
| | 2 | 5 | 5 | B | B | 5 | 5 | 3 | 10 | B | B | - | - |
| | 3 | 5 | 5 | B | B | 5 | 5 | 10 | 20 | B | B | - | - |
| 15 | 1 | B | B | - | - | 10 | 5 | B | B | B | - | - | - |
| | 2 | 5 | 5 | B | B | 20 | 10 | 10 | 10 | B | B | - | - |
| | 3 | 5 | 5 | B | - | 20 | 10 | 20 | 20 | B | B | - | - |
| 16 | 1 | 5 | 5 | B | B | 5 | 5 | - | - | B | - | - | - |
| | 2 | 5 | 5 | B | B | 5 | 5 | 15 | 20 | B | B | - | - |
| | 3 | 10 | 10 | B | B | 5 | 5 | 20 | 30 | B | B | - | - |
| 17 | 1 | - | 5 | B | B | B | 10 | - | - | B | B | - | - |
| | 2 | - | 5 | B | B | 5 | 30 | B | - | B | 5 | - | - |
| | 3 | - | 5 | 5 | 5 | 5 | 30 | B | B | B | 5 | - | - |
| 18 | 1 | B | B | B | B | 40 | 40 | - | B | B | B | - | - |
| | 2 | 5 | 5 | B | 5 | 40 | 40 | 20 | 30 | B | B | - | - |
| | 3 | 10 | 10 | B | 5 | 50 | 50 | 30 | 30 | B | B | - | - |
| 19 | 1 | 5 | 5 | B | B | - | - | - | - | B | B | - | - |
| | 2 | 5 | 5 | B | B | 30 | 30 | 25 | 25 | 5 | B | - | B |
| | 3 | 10 | 10 | B | B | 40 | 40 | 30 | 30 | 5 | B | - | B |
| 20 | 1 | B | B | - | B | 20 | 10 | - | B | - | - | - | - |
| | 2 | B | B | - | B | 20 | 20 | 15 | 10 | B | B | - | - |
| | 3 | 5 | 5 | - | B | 20 | 20 | 15 | 10 | B | B | - | - |
| 21 | 1 | B | B | B | B | 10 | 10 | - | - | B | - | - | - |
| | 2 | B | B | B | B | 20 | 20 | B | B | B | B | - | - |
| | 3 | 5 | 5 | B | B | 20 | 20 | B | B | B | B | - | - |
| 22 | 1 | B | B | B | - | 10 | 5 | - | - | B | B | - | - |
| | 2 | B | B | B | - | 20 | 20 | - | 3 | B | B | - | - |
| | 3 | 5 | 5 | B | - | 20 | 20 | B | B | B | B | - | - |

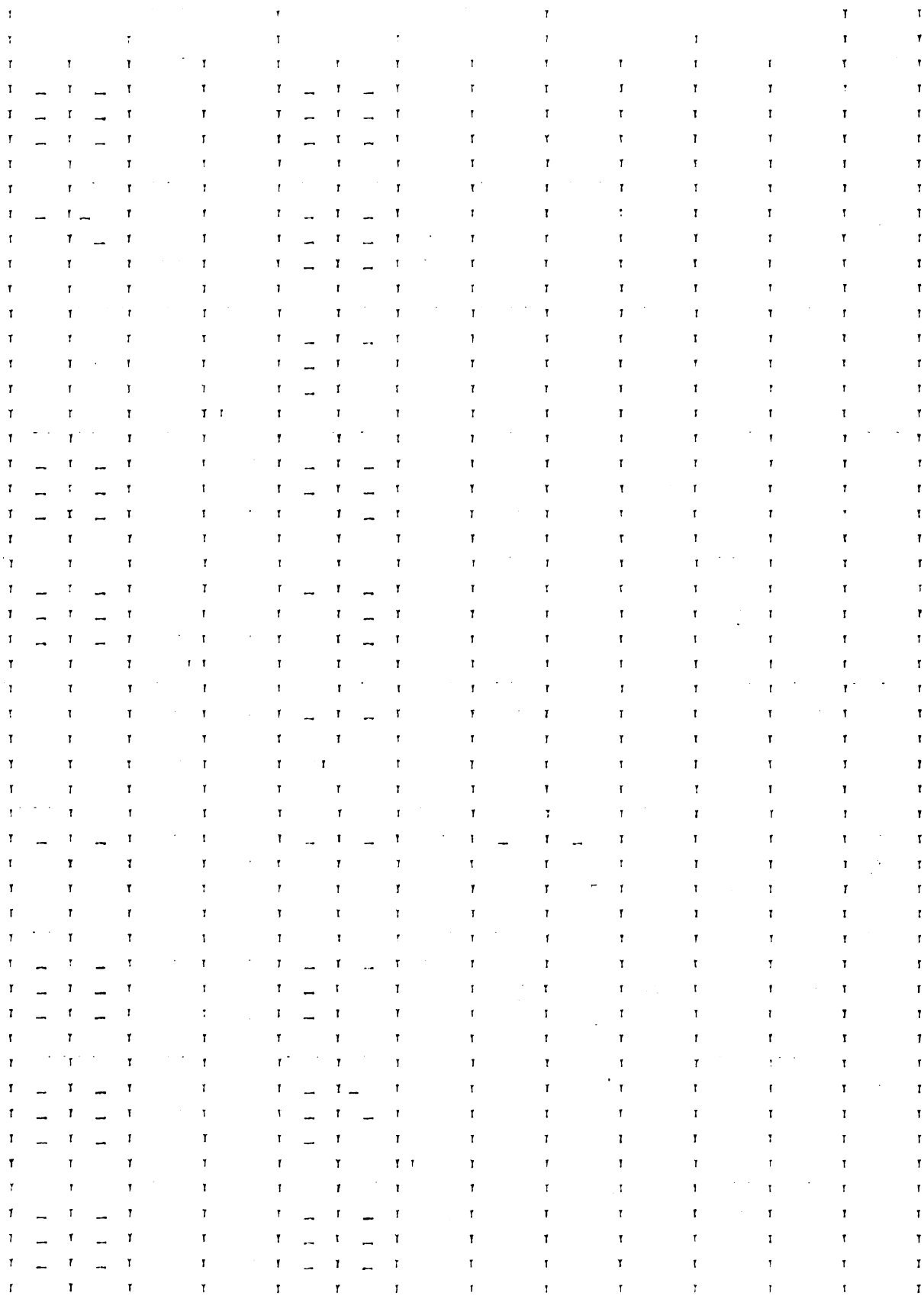


TABLE XI (continued)

| Strain | Days | Dextrose 1922 | | Dextrose 1924 | | Levulose 1922 | | Levulose 1924 | | Mannose 1922 | | Mannose 1924 | | Galactose 1922 | | Galactose 1924 | | mannite 1922 | | mannite 1924 | | Arabinose 1924 | | Rhamnose 1924 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 23 | 1 | 10 | 10 | B | B | 5 | 5 | - | - | 10 | 10 | - | - | B | B | B | B | 15 | 15 | 0 | - | - | B | - | - |
| | 2 | 30 | 30 | 10 | 5 | 20 | 20 | - | - | 10 | 10 | - | - | B | B | B | B | 50 | 40 | B | B | B | B | - | - |
| | 3 | 30 | 30 | 10 | 5 | 20 | 20 | - | - | 10 | 10 | - | - | 5 | 5 | B | B | 50 | 40 | 15 | 10 | B | B | - | - |
| 24 | 1 | 20 | 15 | - | - | 5 | 5 | - | - | 5 | 5 | B | - | B | B | B | B | B | 10 | - | - | - | - | - | - |
| | 2 | 20 | 20 | 10 | B | 10 | 10 | - | - | 10 | 10 | B | - | 5 | 5 | B | B | 10 | 20 | - | - | B | B | - | - |
| | 3 | 20 | 20 | 10 | B | 10 | 10 | B | B | 10 | 10 | B | - | 5 | 5 | B | B | 10 | 20 | B | 5 | B | B | - | - |
| 25 | 1 | 10 | B | B | B | 10 | 10 | - | - | 10 | 10 | B | - | 10 | 10 | B | B | B | 10 | - | - | B | B | - | - |
| | 2 | 40 | 30 | B | B | 20 | 20 | - | B | 10 | 10 | B | B | 10 | 10 | 5 | 5 | 10 | 30 | - | - | B | B | - | - |
| | 3 | 40 | 30 | 5 | 5 | 30 | 30 | - | B | 10 | 10 | B | B | 10 | 10 | 10 | 10 | 15 | 30 | B | B | B | B | - | - |
| 26 | 1 | 20 | 20 | B | B | B | BB | - | - | - | - | - | - | - | 15 | B | B | 20 | 20 | - | - | - | B | - | - |
| | 2 | 30 | 20 | 20 | 10 | B | B | - | - | B | B | - | - | B | 15 | 10 | B | 20 | 30 | B | B | B | B | - | - |
| | 3 | 30 | 20 | 20 | 10 | 5 | 5 | - | - | B | B | -* | - | 5 | 15 | 10 | 10 | 20 | 30 | 5 | 5 | B | B | - | - |
| 27 | 1 | 20 | B | B | B | B | - | - | - | - | - | - | - | - | - | B | - | 20 | 15 | - | - | B | B | - | - |
| | 2 | 20 | 10 | 10 | 10 | B | - | - | - | B | B | - | - | 10 | - | B | - | 20 | 20 | B | B | B | B | - | - |
| | 3 | 30 | 10 | 10 | 10 | 5 | - | - | - | B | B | - | - | 10 | B | B | - | 20 | 20 | 5 | B | B | B | - | - |
| 28 | 1 | 10 | 20 | B | B | B | 5 | - | - | - | - | - | - | - | - | B | B | 20 | 20 | - | - | B | B | - | - |
| | 2 | 10 | 20 | B | B | 10 | 5 | - | B | - | - | - | - | B | - | B | B | 20 | 20 | B | B | B | B | - | - |
| | 3 | 10 | 20 | 5 | B | 10 | 10 | - | B | - | - | - | - | 10 | B | B | B | 20 | 20 | 10 | 10 | B | B | - | - |
| 29 | 1 | 10 | B | B | B | B | - | - | - | - | 5 | - | - | - | - | - | - | 5 | 10 | - | - | - | - | - | - |
| | 2 | 10 | 10 | B | B | B | - | - | - | - | 10 | - | - | - | B | - | - | 5 | 5 | - | B | B | B | - | - |
| | 3 | 10 | 10 | B | 5 | B | - | - | - | -* | 10 | - | - | B | B | - | - | 5 | 5 | - | B | B | B | - | - |
| 30 | 1 | B | 20 | B | - | B | B | - | - | - | - | - | - | - | - | - | - | B | 20 | - | - | B | B | - | - |
| | 2 | 10 | 25 | 10 | 10 | B | B | B | - | - | - | - | - | - | - | B | B | 20 | 20 | B | B | B | B | - | - |
| | 3 | 10 | 25 | 10 | 10 | 5 | 5 | B | - | 5 | - | - | - | B | - | B | 5 | 20 | 20 | B | B | B | B | - | - |
| 31 | 1 | 10 | 10 | - | - | B | B | - | - | - | - | - | - | 10 | - | - | - | 10 | 10 | - | - | B | - | - | - |
| | 2 | 30 | 10 | B | B | B | B | - | - | - | - | - | - | 10 | 5 | B | B | 10 | 15 | - | - | 5 | - | - | - |
| | 3 | 30 | 10 | 5 | 5 | B | B | - | B | -* | - | - | - | 10 | 10 | 5 | 10 | 10 | 15 | 10 | 5 | 5 | B | - | - |
| 32 | 1 | 5 | 10 | B | B | B | B | - | - | - | - | - | - | - | - | - | B | 20 | 20 | - | - | B | B | - | - |
| | 2 | 10 | 10 | 5 | 5 | 5 | 5 | - | - | 15 | B | B | - | B | B | B | B | 20 | 20 | - | - | B | B | - | - |
| | 3 | 10 | 10 | 10 | 5 | 5 | 5 | - | - | 15 | B | B | - | 10 | 10 | B | B | 20 | 20 | B | B | B | B | - | - |

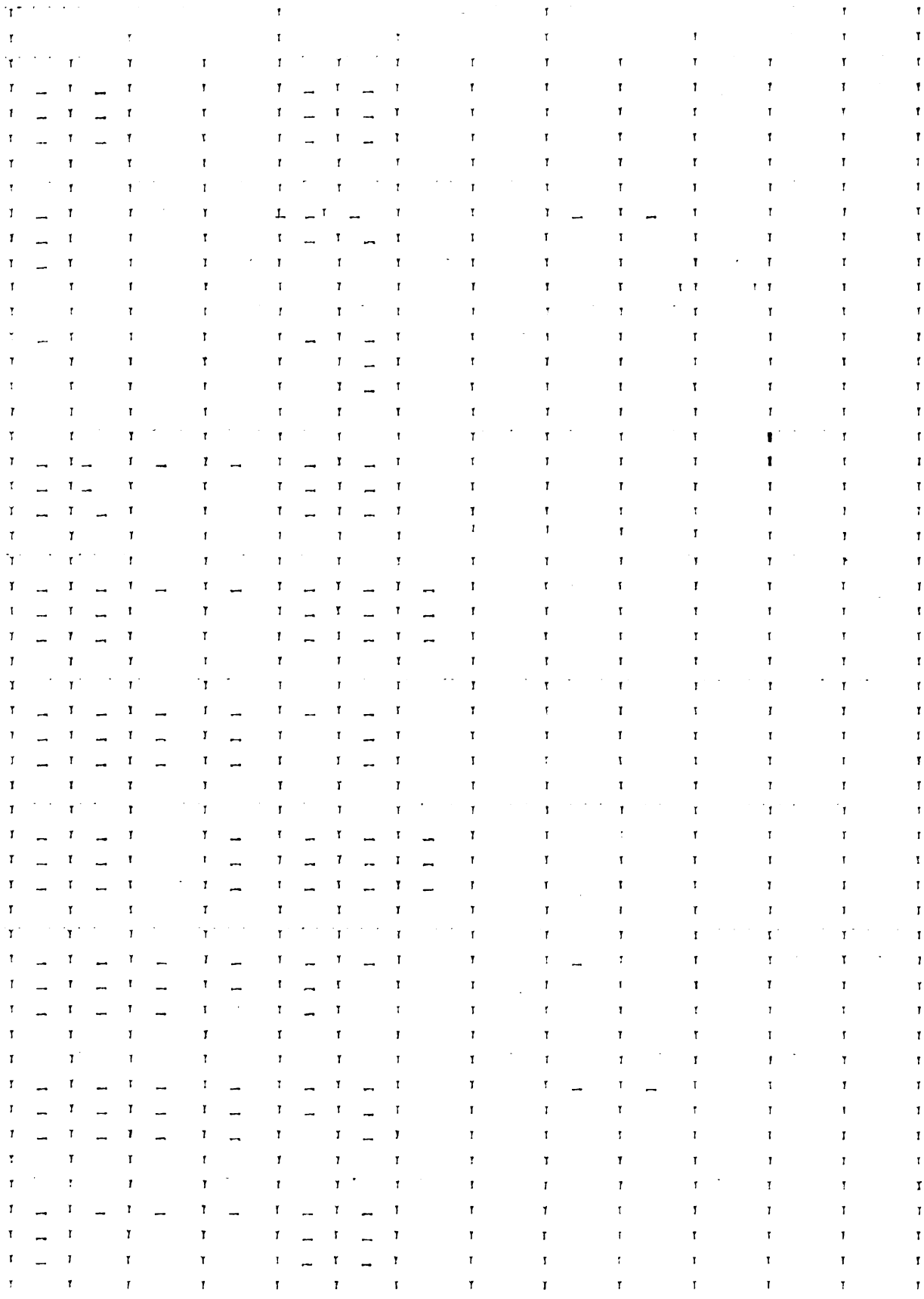


TABLE XI (continued)

| Strain | Days | Dextrose 1922 | | Dextrose 1924 | | Levulose 1922 | | Levulose 1924 | | Mannose 1922 | | Mannose 1924 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 33 | 1 | 5 | 5 | B- | B- | - | B | - | - | | | - | - |
| | 2 | 10 | 10 | B | B | B | 5 | - | - | | | B | B |
| | 3 | 15 | 10 | B | B | B | 5 | - | - | | | B | B |
| 34 | 1 | 20 | B | B | B | B | - | - | - | - | - | - | - |
| | 2 | 30 | 10 | 30 | 10 | B | - | B | - | B | - | - | - |
| | 3 | 30 | 10 | 40 | 10 | B | - | B | - | 5 | B | - | - |
| 35 | 1 | 10 | B | B | B | - | - | - | - | - | - | - | - |
| | 2 | 10 | 10 | B | B | B | B | - | - | - | - | - | - |
| | 3 | 15 | 15 | B | 5 | B | B | - | - | B | B | - | - |
| 36 | 1 | B | 15 | - | - | B | - | - | - | - | - | - | - |
| | 2 | 10 | 20 | 5 | 5 | 5 | B | B | B | - | B | - | B |
| | 3 | 10 | 20 | 5 | 5 | 5 | 5 | B | B | B | B | - | B |
| 37 | 1 | 10 | 10 | B | B | B | B | - | - | - | - | - | - |
| | 2 | 10 | 10 | B | 5 | B | B | - | - | 5 | - | B | B |
| | 3 | 15 | 10 | 5 | 10 | B | B | - | - | 5 | -* | B | B |
| 38 | 1 | 10 | B | B | B | - | - | - | - | - | - | - | - |
| | 2 | 20 | 10 | B | 5 | - | B | B | B | - | - | - | - |
| | 3 | 20 | 10 | 5 | 5 | B | 5 | 10 | B | 5 | -* | - | B |
| 39 | 1 | 10 | B | B | B | - | - | - | - | - | - | - | - |
| | 2 | 15 | 10 | B | 5 | - | - | B | B | - | - | B | B |
| | 3 | 15 | 10 | B | 5 | B | B | B | B | -* | B | B | B |
| 40 | 1 | - | - | B | B | - | - | - | - | - | - | - | - |
| | 2 | B | 15 | 10 | 10 | B | B | - | - | B | B | - | - |
| | 3 | 5 | 15 | 15 | 10 | B | B | - | -* | B | B | - | - |
| 41 | 1 | 10 | B | - | - | - | - | - | - | - | - | - | - |
| | 2 | 15 | B | - | - | - | - | B | B | - | - | - | - |
| | 3 | 15 | 10 | - | - | - | - | 5 | 5 | B | B | - | - |
| 44 | 1 | 10 | 5 | - | - | 5 | 5 | - | - | - | 5 | - | - |
| | 2 | 30 | 30 | - | - | 20 | 30 | B | - | 10 | 10 | - | - |
| | 3 | 30 | 30 | 20 | B | 20 | 30 | B | - | 10 | 10 | - | - |

| Strain | Days | Galactose 1922 | | Galactose 1924 | | Mannite 1922 | | Mannite 1924 | | Arabinose 1924 | | Rhamnose 1924 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 33 | 1 | - | - | B | B | 15 | 15 | - | - | 5 | - | - | - |
| | 2 | B | B | 5 | B | 20 | 20 | B | B | 5 | B | B | B |
| | 3 | 10 | 10 | 10 | B | 20 | 20 | 5 | 10 | 10 | B | B | B |
| 34 | 1 | B | - | B | B | 20 | 10 | B | - | B | B | - | - |
| | 2 | 10 | B | B | B | 20 | 10 | B | 5 | B | B | - | - |
| | 3 | 10 | B | B | B | 20 | 10 | 20 | 10 | B | B | - | - |
| 35 | 1 | - | - | B | B | 20 | 20 | - | - | B | B | - | - |
| | 2 | - | B | B | B | 20 | 20 | B | 10 | B | B | - | - |
| | 3 | 5 | 5 | 10 | B | 20 | 20 | B | 10 | B | B | - | - |
| 36 | 1 | - | - | - | - | 20 | 20 | - | - | B | B | - | - |
| | 2 | 5 | 5 | B | - | 20 | 20 | B | - | B | B | - | - |
| | 3 | 5 | 10 | B | B | 20 | 20 | 10 | 5 | B | B | - | - |
| 37 | 1 | - | - | - | - | 20 | 20 | - | - | - | B | B | - |
| | 2 | 5 | - | - | - | 20 | 20 | B | B | B | B | B | - |
| | 3 | 5 | 5 | -* | - | 20 | 20 | 10 | 15 | B | B | B | - |
| 38 | 1 | - | - | - | - | 20 | 10 | - | - | - | B | - | - |
| | 2 | B | - | 5 | B | 25 | 10 | 10 | B | B | B | - | - |
| | 3 | 10 | 5 | 5 | B | 25 | 10 | 20 | 10 | B | B | - | - |
| 39 | 1 | - | - | - | - | 10 | 10 | - | - | B | B | - | - |
| | 2 | - | 10 | B | - | 10 | 10 | - | - | B | B | - | - |
| | 3 | 10 | 10 | B | B | 10 | 10 | B | -* | B | B | - | - |
| 40 | 1 | - | - | - | - | 20 | 40 | - | - | - | B | - | - |
| | 2 | 5 | 5 | - | - | 20 | 40 | B | B | B | B | - | - |
| | 3 | 10 | 5 | - | - | 20 | 40 | 5 | 5 | B | B | - | - |
| 41 | 1 | - | - | - | - | 15 | 10 | - | - | B | B | - | - |
| | 2 | - | - | - | - | 20 | 20 | - | - | B | B | - | - |
| | 3 | B | B | - | - | 20 | 20 | - | - | B | B | - | - |
| 44 | 1 | B | B | - | - | 5 | B | - | - | B | B | - | - |
| | 2 | 5 | 5 | - | - | 10 | 5 | - | - | B | B | - | - |
| | 3 | 5 | 5 | - | - | 10 | 5 | 30 | 30 | B | B | - | - |

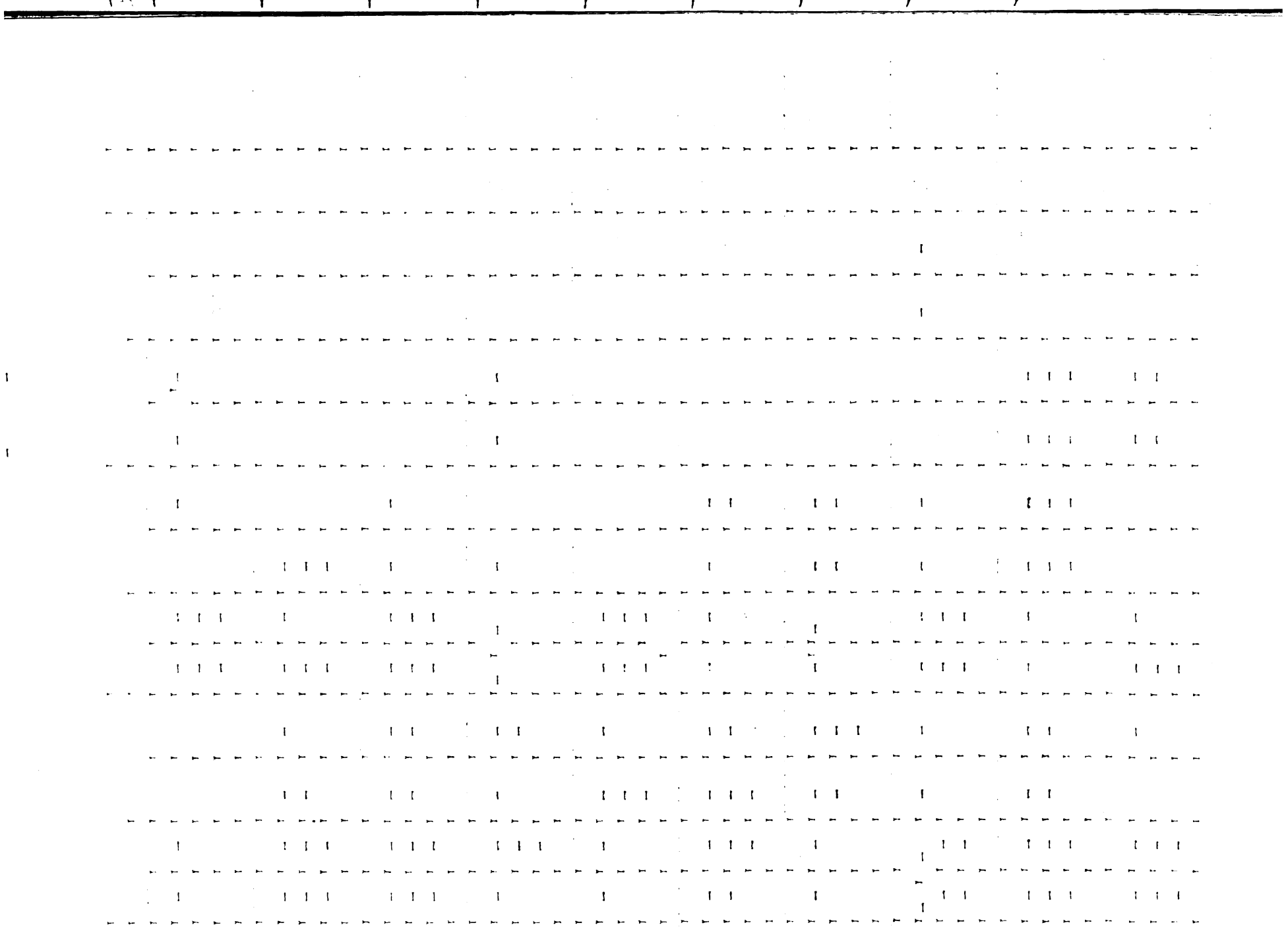


TABLE XI (continued)

| Strain | Days | Dextrose 1922 | | Dextrose 1924 | | Levulose 1922 | | Levulose 1924 | | Mannose 1922 | | Mannose 1924 | | Galactose 1922 | | Galactose 1924 | | Mannite 1922 | | Mannite 1924 | | Arabinose 1924 | | Rhamnose 1924 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 46 | 1 | 5 | 5 | B | B | 5 | B | - | - | 10 | 5 | - | - | 5 | 5 | B | - | - | - | - | B | - | - | B | - |
| | 2 | 10 | 15 | B | B | 10 | 20 | - | - | 10 | 5 | - | B | 5 | 5 | B | - | - | - | 15 | 40 | B | B | B | B |
| | 3 | 10 | 15 | 5 | 5 | 10 | 20 | B | B | 15 | 10 | -* | B | 5 | 5 | 5 | -* | - | - | 25 | 50 | B | B | B | B |
| 47 | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | 2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | 3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 48 | 1 | 30 | 15 | B | B | 10 | 10 | - | - | 10 | 10 | - | - | - | - | - | - | 30 | 40 | B | B | B | B | - | - |
| | 2 | 40 | 20 | 15 | 15 | 40 | 40 | - | - | 10 | 10 | - | - | 5 | 5 | - | - | 40 | 40 | 30 | 20 | B | B | - | - |
| | 3 | 40 | 20 | 25 | 25 | 40 | 40 | B | - | 10 | 10 | -* | - | 5 | 5 | B | -* | 40 | 40 | 50 | 30 | B | B | - | - |
| 49 | 1 | 15 | 10 | B | B | 10 | 5 | - | - | 10 | 10 | - | - | B | B | - | - | 5 | 10 | - | - | B | - | - | - |
| | 2 | 50 | 20 | 10 | 10 | 20 | 10 | - | - | 10 | 10 | - | - | 5 | 5 | - | - | 10 | 10 | B | B | 10 | B | - | - |
| | 3 | 50 | 20 | 10 | 10 | 20 | 10 | B | B | 10 | 10 | - | - | 5 | 5 | B | B | 10 | 10 | 10 | 10 | B | B | - | - |
| 50 | 1 | 20 | 15 | B | B | B | B | - | - | 10 | 10 | - | - | B | B | - | - | 20 | 10 | - | - | - | - | - | - |
| | 2 | 40 | 30 | 5 | B | B | B | - | - | 10 | 10 | - | - | 5 | 5 | - | - | 30 | 10 | 15 | 5 | B | B | - | -? |
| | 3 | 40 | 30 | 10 | 10 | B | B | - | - | 10 | 10 | - | - | 5 | 5 | - | - | 30 | 10 | 20 | 10 | B | B | - | -? |
| 51 | 1 | 15 | 15 | - | B | B | B | - | - | 10 | 10 | - | - | B | B | - | - | 10 | 20 | - | - | B | - | - | - |
| | 2 | 20 | 20 | B | B | 10 | 10 | - | - | 10 | 10 | - | - | 5 | 5 | B | B | 20 | 30 | - | - | B | B | - | - |
| | 3 | 20 | 20 | 10 | B | 10 | 10 | - | - | 10 | 10 | - | B | 10 | 10 | B | B | 20 | 30 | B | 10 | B | B | - | - |
| 52 | 1 | - | - | B | - | B | - | - | -' | B | B | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | 2 | B | B | B | B | 10 | B | - | - | 5 | 5 | - | - | - | - | - | - | - | B | - | - | - | - | - | - |
| | 3 | 5 | B | B | B | 10 | B | - | - | 5 | 5 | - | - | - | - | - | - | B | 5 | -B | - | - | - | - | - |
| 53 | 1 | 20 | 10 | B | B | B | B | - | - | 10 | 10 | - | - | B | B | - | - | 10 | 20 | - | - | - | B | B | B |
| | 2 | 30 | 20 | 15 | 10 | 15 | 15 | B | - | 10 | 10 | - | B | 5 | 5 | - | - | 20 | 30 | - | - | B | B | B | B |
| | 3 | 30 | 30 | 15 | 10 | 15 | 15 | 5 | - | 10 | 10 | -* | B | 5 | 5 | 5 | 5 | 20 | 30 | B | B | B | B | B | B |

* = gas produced later.

TABLE XII

Acid Production from Carbohydrates.

* = acid in 21 days

| | | Dextrose | | | | Levulose | | | | Mannose | | | | Galactose | | | |
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| | | Glycerin | | | | Mannite | | | | Lactose | | | | Sucrose | | | | Dextrin | | | |
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| | | Inulin | | | | Starch | | | | Dulcite | | | | Adonite | | Raffinose | | Arabinose | | Rhamnose | | Xylose | | Maltose | |
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Table XII Continued.

| | | Dextrose | | | | Levulose | | | | Mannose | | | | Galactose | | | |
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| | | 1922 | | 1924 | | 1922 | | 1924 | | 1922 | | 1924 | | 1922 | | 1924 | |
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| | | Glucerin | | | | Mannite | | | | Lactose | | | | Sucrose | | | | Dextrin | | | |
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 Table XII Continued

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Table XII continued.

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| 49 | 1 | ± | ± | - | - | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| | 2 | ± | ± | - | - | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| | 7 | ± | + | - | - | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| | 14 | + | + | - | ± | + | + | + | + | - | - | - | - | | | - | - | - | - | - | - |
| 50 | 1 | ± | ± | - | - | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| | 2 | ± | ± | - | - | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| | 7 | - | - | - | - | + | + | + | + | - | - | + | - | - | - | - | - | - | - | - | - |
| | 14 | + | + | -* | -* | + | + | + | + | - | - | + | - | | | - | - | - | - | - | - |
| 51 | 1 | - | - | - | - | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| | 2 | - | - | - | - | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| | 7 | - | - | - | - | + | ± | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| | 14 | + | ± | - | -* | + | + | + | + | - | - | - | - | | | - | - | - | - | - | - |
| 52 | 1 | - | - | - | - | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| | 2 | - | - | - | - | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| | 7 | - | ± | - | - | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| | 14 | + | + | -* | -* | + | + | + | + | - | - | - | - | | | - | - | - | - | - | - |
| 53 | 1 | - | ± | - | - | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| | 2 | - | ± | - | - | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| | 7 | + | + | - | - | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| | 14 | + | + | -* | -* | + | + | + | + | - | - | - | - | | | - | - | - | - | - | - |

| | | Inulin | | | | Starch | | | | Dulcite | | Adonite | | Raffinose | | Arabinose | | Rhamnose | | Xylose | Maltose |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1922 | | 1924 | | 1922 | | 1924 | | 1922 | | 1924 | | 1924 | | 1924 | | 1924 | | 1924 | 1924 |
| 48 | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | ± | ± | - | - |
| | 2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - | - | - |
| | 7 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | 14 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 49 | 1 | ± | ± | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | + | - | - |
| | 2 | ± | ± | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | ± | - | - |
| | 7 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | 14 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 50 | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | - | - |
| | 2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | ± | - | - |
| | 7 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | 14 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 51 | 1 | - | ± | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | 2 | - | ± | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - | - | - |
| | 7 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | 14 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 52 | 1 | - | ± | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - | - | - |
| | 2 | - | ± | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | - | - |
| | 7 | - | ± | - | - | - | - | - | - | - | - | - | - | - | - | ± | - | - | - | - | - |
| | 14 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 53 | 1 | ± | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | - | - |
| | 2 | ± | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | ± | - | - |
| | 7 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | 14 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

## FERMENTATION STUDIES

### Dextrose

The fermentation of this sugar appears to be fairly constant. A strong acid reaction was observed in all tubes, both in the 1922 series and the 1924 series. Only one organism was slow in the formation of acid. Culture 44 in the 1924 series did not show acid until after 48 hours incubation. (Table XII) Gas formation did not appear until after 7 days so the organisms in question apparently at the time of the test was undergoing one of its typical variations, which will be discussed later.

Cultures 11 and 47 failed to show any gas formation on dextrose in either of the series of tests. (Table XI)

In the 1924 series, culture 41 failed to show gas formation and culture 10 showed gas production, which is just the opposite of the 1922 series.

In general, the quantity of gas produced was much less in the 1924 series than in the 1922 series. Very frequently, only a bubble of gas was produced. In these same tubes acid production was delayed. In cultures, 50, 51 and 52 where only a slight amount of gas was produced, the acid produced was slight and disappeared in 7 days.

### Mannite.

The fermentation of mannite, like dextrose, appears to be fairly constant. No variations were observed in the acid production in either series. Acid production was strong in

24 hours and remained constant through the 21 days of observation.

The gas production did vary. In both series of tests cultures 11 and 47 failed to produce gas. In the 1924 series culture 41 in addition to 11 and 47 failed to produce gas, which is similar to the results obtained on dextrose.

The amount of gas produced in the 1924 series, as in dextrose, was limited in amount and frequently confined to only one of the two tubes used, and this tube showing perhaps only a bubble of gas. The time of appearance of the gas varied. Instead of appearing in 24 hours, as occurred in the 1922 series, it frequently would not appear until after 48 hours incubation.

Levulose.

The fermentation of levulose was quite variable as compared with dextrose and mannite. In the 1922 series, gas production was variable as to quantity produced and time of production. However, in summing up, all culture showed gas production with the exception of 11, 41 and 47. In the 1924 series, a decided variation was noted, cultures 4, 8, 9,10, 11, 13, 14, 22, 23, 26, 27, 29, 32, 35, 37, 47, 50, 51 and 52 not showing any gas in 21 days incubation. In those cultures showing gas, the amount was generally confined to a bubble.

The formation of acid was especially constant in the 1922 series. In the 1924 series, although the cultures showed acid, the amount of acid produced varied somewhat. Cultures 4, 8, 9, 10, 11, 12, 13, 14, 19, 40, 41, 44, 46, 47, 48, 49, 50 and 51 showed less acid production at first than the others, but in 7 days all tubes showed about the same reaction.

Mannose.

Due to the cost of this sugar, only 0.1 percent was used instead of the regular one percent as used with the cheaper sugars. As the amount of sugar was limited, the fermentation was less marked, particularly the acid fermentation. The variability of this sugar was similar to levulose in that some strains produced no gas while others produced considerable.

Cultures 28 and 47 were the only strains showing absence of gas in the 1922 series. Strain 11, which has been negative on all sugars discussed, showed a bubble in one tube. This might be an error as the 1924 series showed no gas.

In the 1924 series, an extremely large number failed to produce gas. Cultures 6, 7, 8, 9, 10, 11, 13, 16, 17, 20, 21, 22, 23, 27, 28, 29, 30, 31, 34, 35, 40, 41, 44, 47, 49, 50 and 51 failed to produce gas. Where gas was produced, it was confined generally to a small bubble. Frequently no gas was produced until after 3 days incubation had elapsed.

Acid production was decidedly variable in both series. In 1922, cultures 16, 27, 28, 30, 31, 32, 34, 35, 36 and 39 failed to show acid. It is interesting to notice that the cultures that failed to produce acid in 1922 with the exception of 2 organisms produced gas in 1924. This is just one example of how these organisms vary.

Acid production, when present, was generally slight, so the absence of acid has no diagnostic value, even though it were a constant property of the organism affected. The fact that

gas was produced in a number of cases where acid was not produced indicates that the sugar was attacked and that the acid production was masked or neutralized by ammonia or alkali production from other sources.

Galactose.

This sugar was also used in amounts of 0.1 percent instead of the usual 1 percent.

The fermentation of galactose in the 1922 series compares very favorably with mannose. All strains produced gas except cultures 11, 47 and 52. In 1924 the gas production like the rest of the 1924 series, showed much less gas where gas was produced and a large number of cases where no gas was produced. Cultures 4, 6, 11, 29, 40, 41, 44, 47, 50 and 52 produced no gas.

Acid production was quite regular on this sugar in both series. In 1922, cultures 8, and 18 did not show gas production, while in 1924 all strains produced acid.

Arabinose.

Arabinose was used in 0.1 percent amounts. This sugar was used in only the 1924 determinations. Here the gas production was decidedly variable. Strains 6, 8, 11, 9, 14, 15, 16, 17, 18, 47 and 52 failed to produce gas. If this sugar had been run in the 1922 series, it would probably have acted as mannose, galactose and the others, producing gas in all of the strains.

Acid production was quite constant, as all strains produced acid in approximately equal amounts.

Rhamnose.

Rhamnose or iso-dulcite was used in 0.1 percent amounts and was used in only the 1924 determinations. This carbohydrate, according to Goldberg and Hadley, is always fermented with gas and acid. Strains 5, 12, 13, 19-33, 37, 46 and 53 produced gas; however, only one set of determinations was made and these were made in the 1924 series, at which time practically all of the cultures were very slow in producing gas and as previously stated, many organisms refused to produce gas at all, although this property was not constant.

Only 3 strains, cultures 36, 44 and 51 showed absence of acid formation. However, none of the strains produced very much acid. In every case the acid disappeared after the third or fourth day.

Glycerine.

Glycerine, according to Hadley, Goldberg and others, is not attacked by Bacterium bullorum. In both the 1922 and 1924 series, glycerine was fermented with acid production. The acid production never occurred until after the 7 days incubation, and occasionally not until the 14th day.

In the 1922 series, all strains produced acid while in the 1924 series, strains 7, 14, 15, 17, 24, 26, 28, 29, 31, 32, 35, 34, 36, 37, 40, 41, 44 and 46 failed to show acid in 21 days. This might be explained by the fact that the broth used in the sugar fermentations in 1924 had a titre of pH 7, while in 1922 the titre was pH 6.8.

The glycerine broth was sterilized in both cases in the autoclave at 15 pounds pressure for 20 minutes. It may be possible that the glycerine was broken down into compounds, that Bacterium pullorum is able to ferment with acid production. A study of the effect of heat on glycerine is planned for future studies in order to check these results. The glycerine was not distilled before using.

Adonite.

Adonite was used in 0.1 percent amounts. The results obtained on this carbohydrate confirm previous work. No acid or gas was produced in any strains.

Raffinose.

Raffinose was also used in 0.1 percent amounts. This trisaccharide was constant on all strains, producing neither gas nor acid in 21 days incubation.

Dulcite.

Dulcite was used in 0.1 percent amounts. In the 1922 series, strain 33 caused slight acid production, but in the 1924 series, all strains showed negative acid and gas.

Inulin.

This polysaccharide was used in 1 percent amounts. It was sterilized by autoclaving at 15 pounds pressure for 20 minutes, due to the fact that inulin sometimes carries resistant spore formers. All tubes were kept at room temperature for a week to eliminate any contamination that might develop. In the 1922 series, strains 11, 13, 14, 15, 16, 17, 18, 20, 25 26, 27, 28, 29, 30, 31, 32, 33, 35, 38, 46, 47, 49, 51, 52 and 53 showed slight amounts of acid. In many cases only one of the

two tubes inoculated showed acid production.

In the 1924 series no strains showed acid production, perhaps because the titre of the broth was pH 7.0 instead of pH 6.8 as in the 1922 series.

Starch.

The starch broth was prepared in the same manner as the glycerine and the inulin, using the same amount of the carbohydrate.

In the 1922 series, strains 5, 24, 27, 28-30, 31, 32, 33, 34, 35, 36, 37, 38, 40 and 41 showed slight acid production. In most cases, the tubes showed a slight acidity on the second day which disappeared in a day or so.

In the 1924 series, all of the strains showed negative acid production, which shows that this property of attacking starch, if such is the case, is transitory.

Maltose.

Due to the fact that lactose saccharose and maltose are very susceptible to heat, these broths were prepared by filtering the sugar through a Berkefeld filter and adding aseptically to sterile broth.

The susceptibility of maltose was demonstrated quite early in these studies. Maltose broth fermentation tubes were prepared by sterilizing in the autoclave. These tubes upon inoculation showed slight acid and gas production. Using the same sugar, the broth was prepared without heating and none of these tubes showed gas or acid formation.

Lactose.

With lactose, acid was produced in strains 6, 8 and 11 in the 1922 series. In the 1924 series, strains 13, 31, 36, 41 and 50 showed acid. With the exception of strain 36, only one of the tubes in each set showed acid.

Saccharose.

In saccharose, strains 6, 9, 10, 11, 12, 15, 16 and 29 showed acid production in 1922, while in 1924 strains 6 and 36 showed acid. Strain 36 showed pronounced acid production.

Dextrin.

The dextrin broth was prepared by autoclaving for 20 minutes at 15 pounds pressure. A one percent solution was used.

In the 1922 series, acid production was shown in strains 13, 14, 15, 16, 27, 29, 31, 32, 33, 34, 35, 37, 38, 39 and 41. In the 1924 series only strain 36 showed acid.

Xylose.

Xylose was used in 0.1 percent amount and was sterilized by autoclaving at 15 pounds pressure for 20 minutes. This carbohydrate was used in only the 1924 series. Most of the strains produced no change, but strains 4, 5, 6, 12, 13, 14, 17 and 18 showed slight acid production. Hadley, Goldberg and others state that Xylose is always fermented with acid formation.

## EXPERIMENTS TO DETERMINE THE FACTORS RESPONSIBLE FOR THE IRREGULAR GAS PRODUCTION PROPERTIES OF BACTERIUM PULLORUM

### The Inconsistencies of Gas Production of Bacterium pullorum upon Fermentable Sugars.

Bacterium pullorum, as a careful study of Tables XI and XII shows, does vary considerably in its fermentative properties. The accompanying table (Table XIII) is a list of the 1924 determinations showing those strains that failed to produce gas. A careful study of this table will show that although all of these sugars were inoculated at the same time, incubated at the same time and all, still a strain will attack dextrose vigorously and then refuse to attack mannose, or it will attack mannose and not levulose. If an organism would at one stage of development attack all sugars of similar molecular structure and then at another stage not attack any of these same sugars the complexity of the problem would not be as great and an explanation might be advanced. On the six fermentable sugars studied, only a few strains were constant. Strains 11 and 47 are anaerogenic types while strains 5, 46 and 53 are aerogenic types. All of the remainder of the 47 strains showed some variation.

### Experiment 1. To Determine Whether the Medium For Invigoration Has Any Influence on Gas Oriduction.

Three media were selected for investigation, namely, plain agar, liver agar and infusion agar. The cultures were transferred every day for a week, when they were transferred

TABLE XIII

Inconsistencies in Gas Production of *Bacterium pullorum* Upon Fermentable Sugars in the 1924 Series.

| Sugar | Strains Studied | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 |
| Levulose | - | + | + | + | - | - | - | - | + | - | - | + | + | + | + | + | + | + | - | + | + | + | - | - |
| Mannose | + | - | - | - | - | - | - | - | + | - | + | + | - | - | + | + | - | - | - | - | + | + | + | - |
| Galactose | - | + | - | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Arabinose | + | + | - | + | - | - | + | - | + | + | - | - | - | - | - | + | + | + | + | + | + | + | + | + |
| Rhamnose | - | + | - | - | - | - | - | - | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Dextrose | + | + | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Mannite | + | + | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

| Sugar | Strains Studied | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 44 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | |
| Levulose | + | - | + | + | - | - | + | - | + | - | + | + | + | + | + | + | - | + | + | - | - | - | + | |
| Mannose | - | - | - | - | + | + | - | - | + | + | + | + | - | - | - | + | - | + | - | - | + | - | + | |
| Galactose | + | - | + | + | + | + | + | + | + | + | + | + | - | - | - | + | - | + | + | - | + | - | + | |
| Arabinose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + | - | + | |
| Rhamnose | - | - | - | - | - | + | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | - | + | |
| Dextrose | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | - | + | + | + | + | + | + | |
| Mannite | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + | |

to dextrose and mannite extract broth.

The data in Table XIV show that with dextrose, the three different invigorating media check in gas production, but on mannite considerable variation is evident. Although a variation occurs, still the data do not point to any one medium as giving constant results. It appears that invigoration, as far as the medium is concerned, plays no part.

Experiment 2. To Determine the Influence of Invigoration on Gas Production.

Experiment 1 shows that invigoration apparently plays no part in determining gas production. To determine the effect of lack of invigoration, transplants were made directly to dextrose and mannite extract broth from the stock gelatin cultures which had not been transferred for several months. The results obtained (Table XV) where no invigoration was used were also variable; the dextrose showed gas where the organism was aerogenic, but in the mannite no gas was produced. Further study here would be of interest, although the results obtained on mannite after invigoration showed variation, and the fact that variations also occurred before invigoration would tend to show that invigoration plays no part in deciding gas production.

Experiment 3. Influence of Kind of Broth on Gas Production.

Hadley states that frequently when an aerogenic strain refuses to produce gas in extract broth it will produce it in infusion broth.

At the time of the 1922 series of tests with extract broth two sets of infusion broth containing dextrose and mannite respectively were also run.

TABLE XIV

The Relation of Gas Production to the Invigorating Medium

| Strain | Mannite Extract Broth | | | | | | | | Dextrose Extract Broth | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Plain Agar | | | | Liver Agar | | | | Infusion Agar | | | |
| | Gas | | Acid | | Gas | | Acid | | Gas | | Acid | |
| 47 | - | - | + | + | - | - | + | + | - | - | + | + |
| 41 | B | - | + | + | - | - | + | + | - | - | + | + |
| 11 | - | - | + | + | - | - | + | + | - | - | + | + |
| 10 | - | - | + | + | B | - | + | + | B | - | + | + |
| 47 | - | - | + | + | - | - | + | + | - | - | + | + |
| 41 | 10 | 10 | + | + | 5 | 10 | + | + | 10 | 15 | + | + |
| 11 | - | - | + | + | - | - | + | + | - | - | + | + |
| 10 | 5 | 10 | + | + | B | 10 | + | + | 15 | B | + | + |

TABLE XV

The Effect of Lack of Invigoration on Gas Production

| Strain | Dextrose Extract Broth | | | | Mannite Extract Broth | | | |
|---|---|---|---|---|---|---|---|---|
| | Gas | | Acid | | Gas | | Acid | |
| 47 | - | - | + | + | - | - | + | + |
| 41 | 5 | C | + | C | - | | + | |
| 11 | - | - | + | + | - | - | + | + |
| 10 | 5 | 10 | + | + | - | - | + | + |

The variations obtained are shown in Table XVI. Invigoration used for these tubes was similar to that used for the extract broth. The time between running the two sets of tests was approximately two weeks.

The results, as shown in Table XVI do not show any marked difference. It will be noted that on dextrose, the amount of gas produced was slightly greater on infusion broth than on the extract broth. Although strain 41 showed gas on infusion broth and not in extract broth, still strain 52 was exactly the opposite.

On mannite, gas production was very frequently negative on infusion broth, while on extract, it was positive. From all outward appearances the extract broth was the better. This might not be borne out in repeated studies, which should be carried out.

In summarizing all the results obtained, it appears that gas production in Bacterium pullorum is a variable characteristic, and, further, it appears that it is some inherent factor that we know nothing about at present.

Table XVI

Relation of Infusion Broth and Extract Broth in Determining Gas Production.

| | | Extract Broth | | | | Infusion Broth | | | |
|---|---|---|---|---|---|---|---|---|---|
| : | : | dextrose | | mannite | | dextrose | | mannite | |
| 4 | 1 | B | B | - | - | B | - | - | - |
| | 2 | 5 | 5 | B | B | 20 | 10 | - | - |
| | 7 | | | | | 30 | 10 | - | - |
| 5 | 1 | B | B | - | - | - | - | - | - |
| | 2 | 5 | 3 | 10 | 10 | 5 | B | B | B |
| | 7 | | | | | 20 | 15 | 5 | B |
| 6 | 1 | B | B | - | - | - | - | - | - |
| | 2 | 5 | 5 | - | - | B | - | - | - |
| | 7 | | | | | 10 | 10 | 10 | - |
| 7 | 1 | B | B | - | - | - | - | - | - |
| | 2 | 5 | 5 | - | - | B | 5 | - | B |
| | 7 | 8 | 10 | 10 | - | 15 | 15 | - | 15 |
| 8 | 1 | B | B | B | 5 | - | - | - | - |
| | 2 | 5 | 5 | 20 | 40 | B | B | B | - |
| | 7 | 10 | 15 | 40 | 50 | 15 | 15 | 10 | 5 |
| 9 | 1 | B | B | 5 | 5 | B | - | - | - |
| | 2 | 10 | 5 | 30 | 30 | 10 | 5 | B | B |
| | 7 | 30 | 10 | 30 | 30 | 25 | 25 | 10 | 10 |
| 10 | 1 | B | B | - | - | - | - | - | - |
| | 2 | 5 | 5 | - | - | B | B | - | - |
| | 7 | 10 | 10 | 5 | 5 | 10 | 15 | - | - |

Table XVI continued.

| | | Extract Broth | | | | Infusion Broth | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | dextrose | | mannite | | dextrose | | mannite | |
| 11 | 1 | - | - | - | - | - | - | - | - |
| | 2 | - | - | - | - | - | B | - | - |
| | 7 | B | B | - | - | - | B | - | - |
| 12 | 1 | B | B | B | B | B | B | - | - |
| | 2 | 5 | 10 | 30 | 60 | B | 10 | - | - |
| | 7 | 10 | 15 | 40 | 60 | 15 | 30 | - | - |
| 13 | 1 | B | B | - | - | - | - | - | - |
| | 2 | 3 | B | 40 | B | B | B | - | - |
| | 7 | 10 | B | 40 | 10 | 10 | 10 | - | - |
| 14 | 1 | B | B | - | - | - | - | - | - |
| | 2 | 5 | 5 | 3 | 10 | B | 5 | - | - |
| | 7 | 10 | 10 | 10 | 30 | 20 | 30 | - | 5 |
| 15 | 1 | B | B | B | B | - | - | - | - |
| | 2 | 3 | 3 | 10 | 10 | B | B | - | - |
| | 7 | 10 | 10 | 20 | 20 | 10 | 15 | - | - |
| 16 | 1 | B | B | - | - | - | - | - | - |
| | 2 | 10 | 5 | 15 | 20 | B | 5 | - | - |
| | 7 | 20 | 10 | 20 | 30 | 15 | 20 | - | - |
| 17 | 1 | B | B | - | - | B | B | - | - |
| | 2 | 10 | 25 | B | - | 10 | 10 | - | - |
| | 7 | 20 | 30 | 10 | 10 | 30 | 40 | - | - |
| 18 | 1 | B | B | - | B | - | B | - | - |
| | 2 | 15 | 15 | 20 | 30 | 5 | 20 | - | - |
| | 7 | 20 | 20 | 40 | 40 | 20 | 25 | - | - |

Table XVI continued.

| | | Extract Broth | | | | Infusion Broth | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | dextrose | | mannite | | dextrose | | mannite | |
| 19 | 1 | B | - | - | - | - | - | - | - |
| | 2 | 10 | 5 | 25 | 25 | B | 5 | - | - |
| | 7 | 15 | 15 | 40 | 50 | 20 | 20 | - | - |
| 20 | 1 | B | B | - | B | - | - | - | - |
| | 2 | 10 | 5 | 15 | 10 | 5 | 5 | - | - |
| | 7 | 15 | 15 | 20 | 15 | 30 | 20 | - | B |
| 21 | 1 | B | B | - | - | - | - | - | - |
| | 2 | B | 15 | B | B | - | B | - | - |
| | 7 | 10 | 30 | 5 | 5 | 10 | 15 | - | - |
| 22 | 1 | B | B | - | - | - | - | - | - |
| | 2 | B | B | - | 3 | B | B | - | - |
| | 7 | 5 | 5 | 5 | 5 | 15 | 20 | - | - |
| 23 | 1 | B | B | - | - | - | - | - | - |
| | 2 | 10 | 5 | B | B | B | B | - | B |
| | 7 | 20 | 10 | 20 | 10 | 15 | 15 | - | B |
| 24 | 1 | - | - | - | - | - | - | - | - |
| | 2 | 10 | B | - | - | B | - | - | - |
| | 7 | 10 | 15 | 5 | 15 | 20 | 20 | - | - |
| 25 | 1 | B | B | - | - | - | B | - | - |
| | 2 | B | B | - | - | 5 | 5 | - | - |
| | 7 | 5 | 5 | 5 | 5 | 30 | 15 | - | B |
| 26 | 1 | B | B | - | - | - | - | - | - |
| | 2 | 20 | 10 | B | B | B | B | - | - |
| | 7 | 20 | 10 | 15 | B | 20 | 20 | - | - |

Table XVI continued.

| | | Extract Broth | | | | Infusion Broth | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | dextrose | | mannite | | dextrose | | mannite | |
| 27 | 1 | B | B | - | - | - | - | - | - |
| | 2 | 10 | 10 | B | B | B | B | - | - |
| | 7 | 10 | 10 | 20 | 15 | 10 | 15 | - | - |
| 28 | 1 | B | B | - | - | B | - | - | - |
| | 2 | B | B | B | B | 10 | B | - | - |
| | 7 | 5 | B | 20 | 70 | 30 | 15 | - | - |
| 29 | 1 | B | B | - | - | - | - | - | - |
| | 2 | B | B | - | B | B | B | - | - |
| | 7 | 5 | 5 | - | B | 5 | 5 | - | - |
| 30 | 1 | B | - | - | - | - | - | - | - |
| | 2 | 10 | 10 | B | B | B | B | B | - |
| | 7 | 10 | 10 | B | B | 20 | 25 | B | - |
| 31 | 1 | - | - | - | - | - | - | - | - |
| | 2 | B | B | - | - | B | - | - | - |
| | 7 | 5 | 5 | 20 | 10 | 5 | 15 | - | - |
| 32 | 1 | B | B | - | - | - | - | - | - |
| | 2 | 5 | 5 | - | - | B | B | - | - |
| | 7 | 10 | 10 | 5 | B | 15 | 10 | - | - |
| 33 | 1 | B | B | - | - | - | - | - | - |
| | 2 | B | B | B | B | - | - | - | - |
| | 7 | B | B | 10 | 15 | B | B | - | - |
| 34 | 1 | B | B | B | - | - | - | - | - |
| | 2 | 30 | 10 | B | 5 | - | B | - | - |
| | 7 | 50 | 10 | 30 | 10 | 15 | 15 | - | - |

Table XVI continued.

| | | Extract Broth | | | | Infusion Broth | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | dextrose | | mannite | | dextrose | | mannite | |
| 35 | 1 | B | B | - | - | - | - | - | - |
| | 2 | B | B | B | 10 | B | B | - | - |
| | 7 | B | 5 | 5 | 10 | 10 | 15 | - | - |
| 36 | 1 | - | - | - | - | - | - | - | - |
| | 2 | 5 | 5 | B | - | 10 | B | - | - |
| | 7 | 5 | 5 | 15 | 15 | 40 | 20 | - | - |
| 37 | 1 | B | B | - | - | - | - | - | - |
| | 2 | B | 5 | B | B | B | B | - | - |
| | 7 | 5 | 10 | 20 | 20 | 30 | 30 | - | - |
| 38 | 1 | B | B | - | - | - | - | - | - |
| | 2 | B | 5 | 10 | B | - | - | - | - |
| | 7 | 5 | 10 | 30 | 10 | 10 | 15 | - | 10 |
| 39 | 1 | B | B | - | - | - | - | - | - |
| | 2 | B | 5 | - | - | B | B | - | B |
| | 7 | B | 10 | 30 | 30 | 10 | 15 | - | B |
| 40 | 1 | B | B | - | - | B | - | - | - |
| | 2 | 10 | 10 | B | B | 10 | 5 | - | - |
| | 7 | 15 | 10 | 10 | 10 | 40 | 40 | - | - |
| 41 | 1 | - | - | - | - | - | - | - | - |
| | 2 | - | - | - | - | B | B | - | - |
| | 7 | - | - | - | - | 30 | 20 | - | - |
| 42 | 1 | - | - | - | - | - | - | - | - |
| | 2 | - | - | - | - | 10 | 15 | - | - |
| | 7 | 20 | B | 40 | 40 | 30 | 30 | - | - |

Table XVI continued.

| | | Extract Broth | | | | Infusion Broth | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | dextrose | | mannite | | dextrose | | mannite | |
| 46 | 1 | B | B | - | B | B | - | - | - |
| | 2 | B | B | 15 | 40 | B | - | - | - |
| | 7 | 5 | 5 | 30 | 60 | B | - | - | - |
| 48 | 1 | B | B | B | B | - | - | - | - |
| | 2 | 15 | 15 | 30 | 20 | 10 | 5 | - | - |
| | 7 | 25 | 25 | 50 | 30 | 30 | 20 | - | - |
| 49 | 1 | B | B | - | - | - | - | - | - |
| | 2 | 10 | 10 | B | B | B | B | - | - |
| | 7 | 10 | 15 | 20 | 20 | 10 | 10 | - | - |
| 50 | 1 | B | B | - | - | - | - | - | - |
| | 2 | 5 | B | 15 | 5 | B | B | - | - |
| | 7 | 15 | 10 | 20 | 10 | 15 | 10 | - | - |
| 51 | 1 | - | B | - | - | - | - | - | - |
| | 2 | B | B | - | - | B | B | B | - |
| | 7 | 10 | 5 | 10 | 15 | 15 | 15 | B | - |
| 52 | 1 | B | - | - | - | - | - | - | - |
| | 2 | B | B | - | - | - | - | - | - |
| | 7 | B | B | B | - | - | - | - | - |
| 53 | 1 | B | B | - | - | B | - | - | - |
| | 2 | 15 | 10 | - | - | 10 | B | B | B |
| | 7 | 15 | 10 | B | B | 30 | 20 | - | B |

## AGGLUTINATING POWER OF VARIOUS STRAINS OF *BACTERIUM PULLORUM*

In 1922 at the time of the first sugar fermentation studies, antigens of the 47 strains studied were prepared. These were prepared by washing the growth from a 48 hour liver agar slant culture with physiological salt solution which had 0.5 percent phenol added as a preservative. These antigens were then standardized to a nephelometer reading of 1. A strong agglutinating *Bacterium pullorum* serum was selected and run against the 47 antigens separately, using dilutions of 1-50, 1-100 and 1-200. Readings were made in 24 hours. The results obtained are given in Table XVII.

TABLE XVII

Agglutinating Power of Various Strains of Bact. pullorum.

| Strain No. | Control | 1-50 | 1-100 | 1-200 | Strain No. | Control | 1-50 | 1-100 | 1-200 |
|---|---|---|---|---|---|---|---|---|---|
| 4 | cloudy | 4 | 4 | 4 | 28 | cloudy | 3 | 4 | 3 |
| 5 | " | 3 | 4 | 4 | 29 | " | 4 | - | 4 |
| 6 | " | 2 | 3 | 3 | 30 | " | 3 | 3 | 1 |
| 7 | " | 3 | 4 | 4 | 31 | " | 4 | 1 | 1 |
| 8 | " | 3 | 3 | 3 | 32 | " | 4 | 4 | 4 |
| 9 | " | 2 | 3 | 4 | 33 | " | 4 | 2 | 2 |
| 10 | " | 3 | 3 | 4 | 34 | " | 3 | 3 | 2 |
| 11 | settled out | 2 | 3 | 2 | 35 | " | 4 | 4 | 4 |
| | | | | | 37 | " | 4 | 4 | 4 |
| 12 | cloudy | 3 | 3 | 3 | 38 | " | 3 | 3 | 1 |
| 13 | " | 4 | 3 | 2 | 39 | " | 2 | 1 | 1 |
| 14 | " | 3 | 4 | 4 | 40 | " | 2 | 1 | 1 |
| 15 | " | 4 | 4 | 4 | 41 | " | 4 | 4 | 1 |
| 16 | " | 3 | 3 | 4 | 44 | " | + | - | - |
| 17 | " | 4 | 4 | 4 | 46 | " | 4 | 4 | 4 |
| 18 | " | 1 | 3 | 4 | 47 | settled out | - | - | - |
| 19 | " | 3 | 4 | 4 | | | | | |
| 20 | " | 3 | 4 | 4 | 48 | cloudy | 4 | 4 | 4 |
| 21 | " | 4 | 4 | 4 | 49 | " | 4 | 4 | 4 |
| 22 | " | 3 | 4 | 4 | 50 | " | 3 | 4 | 4 |
| 23 | " | 2 | 4 | 4 | 51 | " | 3 | 2 | 1 |
| 24 | " | 3 | 4 | 4 | 52 | " | 4 | 4 | 4 |
| 25 | " | 4 | 4 | 4 | 53 | " | 3 | 4 | 4 |
| 26 | " | 3 | 4 | 3 | | | | | |
| 27 | " | 3 | 3 | 3 | | | | | |

These tests as shown by the table indicate a variation in the agglutinating power. Both strains 11 and 47 settled out in the control tubes and made it nearly impossible to determine whether any agglutination occurred. Considerable variation was also evident in those cultures showing agglutination. The variation was so pronounced that cross-agglutination studies were made.

The antigens were prepared as before and birds were immunized to cultures 4, 5, 6, 7, 8, 9, 10, 12, 13, 41 and 50 respectively, two birds being used for each culture. Only one injection was made, using 1 c.c. of a 24 hour suspension in physiological salt solution.

The method of setting up the test was as follows: Ten dilutions were used. Ninety-five hundredths of a cubic centimeter was pipetted into the first tube and five-tenths of a cubic centimeter into the other nine tubes. Five hundredths c.c. of undiluted serum was added to the first tube, making the total one c.c. and giving 1-20 dilution. After shaking, 0.5 c.c. of this 1-20 dilution was transferred to tube 2, giving a 1-40 dilution. Using the same pipette, 0.5 c.c. was transferred from tube 2 to tube 3 and so on, giving dilutions of 1-80, 1-160, 1-320, 1-640, 1-1280, 1-2560, 1-5120 and 1-10240.

The 0.5 c.c. amounts per tube were used, as the quantity of serum available was limited. This amount of antigen allowed a maximum number of dilutions to be used. Before adopting this amount, experiments were carried on to determine the accuracy

as compared with 1 c.c. and 2 c.c. portions. No difference existed provided small diameter Wasserman tubes were used.

The tubes were incubated at 37° C. for 48 hours before reading.

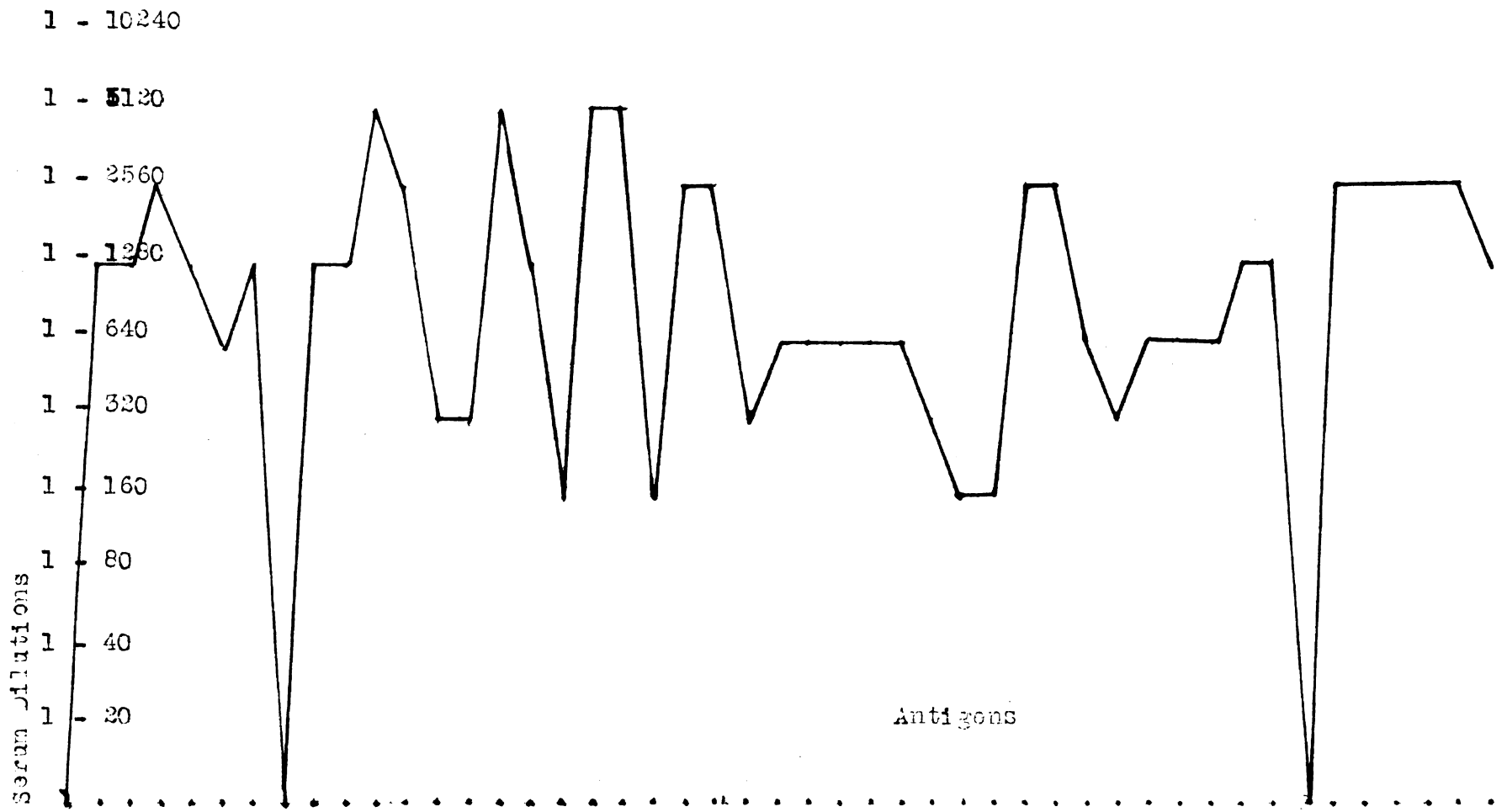
The data obtained are given in the accompanying graphs. (Fig. )

As also shown in the accompanying graphs, all the antigens were active against all the sera except cultures 11 and 47. These two cultures are, according to Hadley and Rettger, Bacterium pullorum Beta. Although these were the only two Beta type organisms used, and although their antigenic power is apparently low, still the data are insufficient to state that the Beta type will not agglutinate Alpha type serum.

The graph of culture 11 serum shows very poor agglutination properties on all antigens. It was highest against its own antigen, and here it was positive in only the high dilutions.

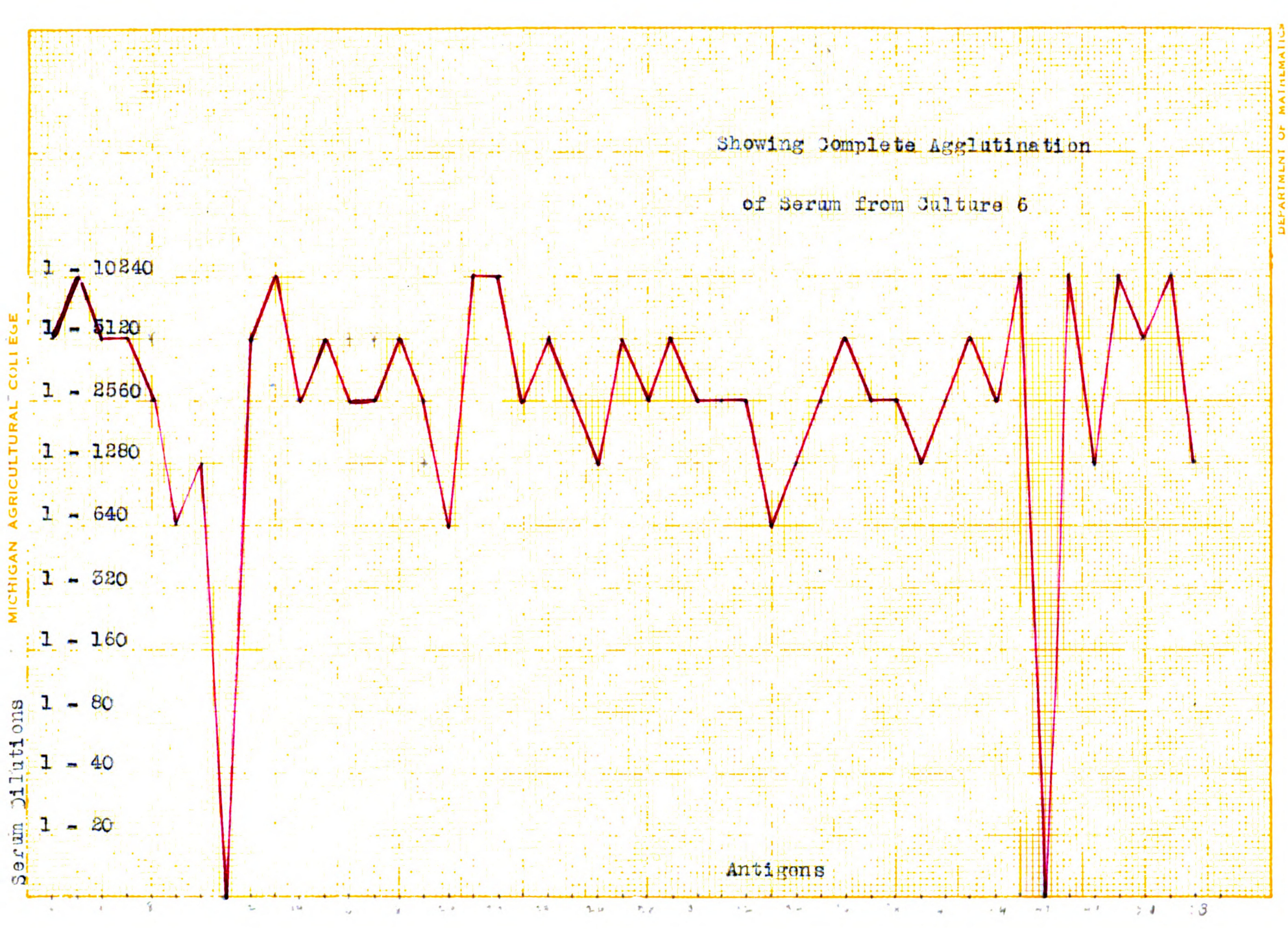
Showing Complete Agglutination

of Serum from Culture 4



Showing Complete Agglutination

of Serum from Culture 5


Showing Complete Agglutination
of Serum from Culture 6
1 - 10240
1 - 5120
1 - 2560
1 - 1280
1 - 640
1 - 320
1 - 160
1 - 80
1 - 40
1 - 20
Serum Dilutions
Antigens
MICHIGAN AGRICULTURAL COLLEGE
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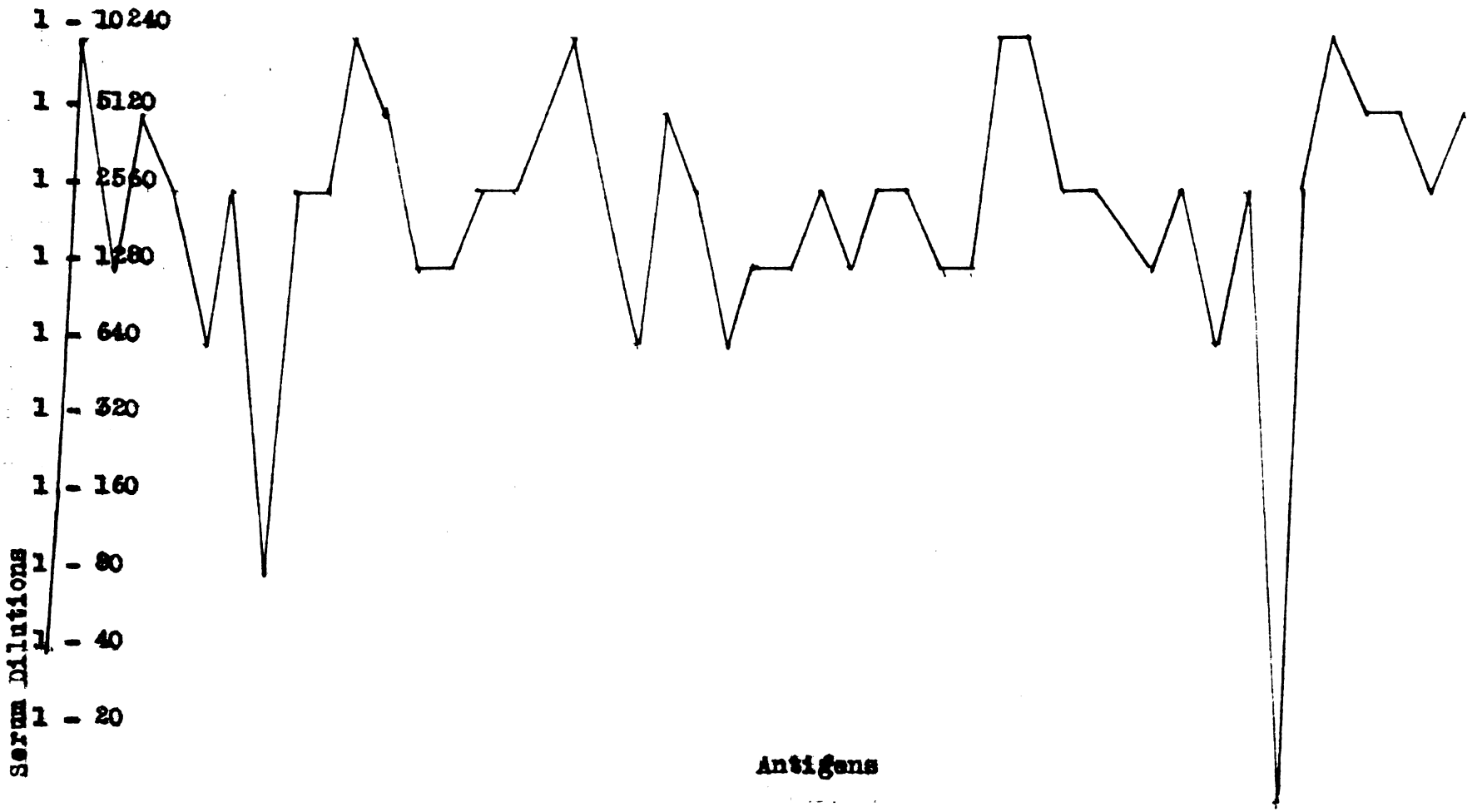
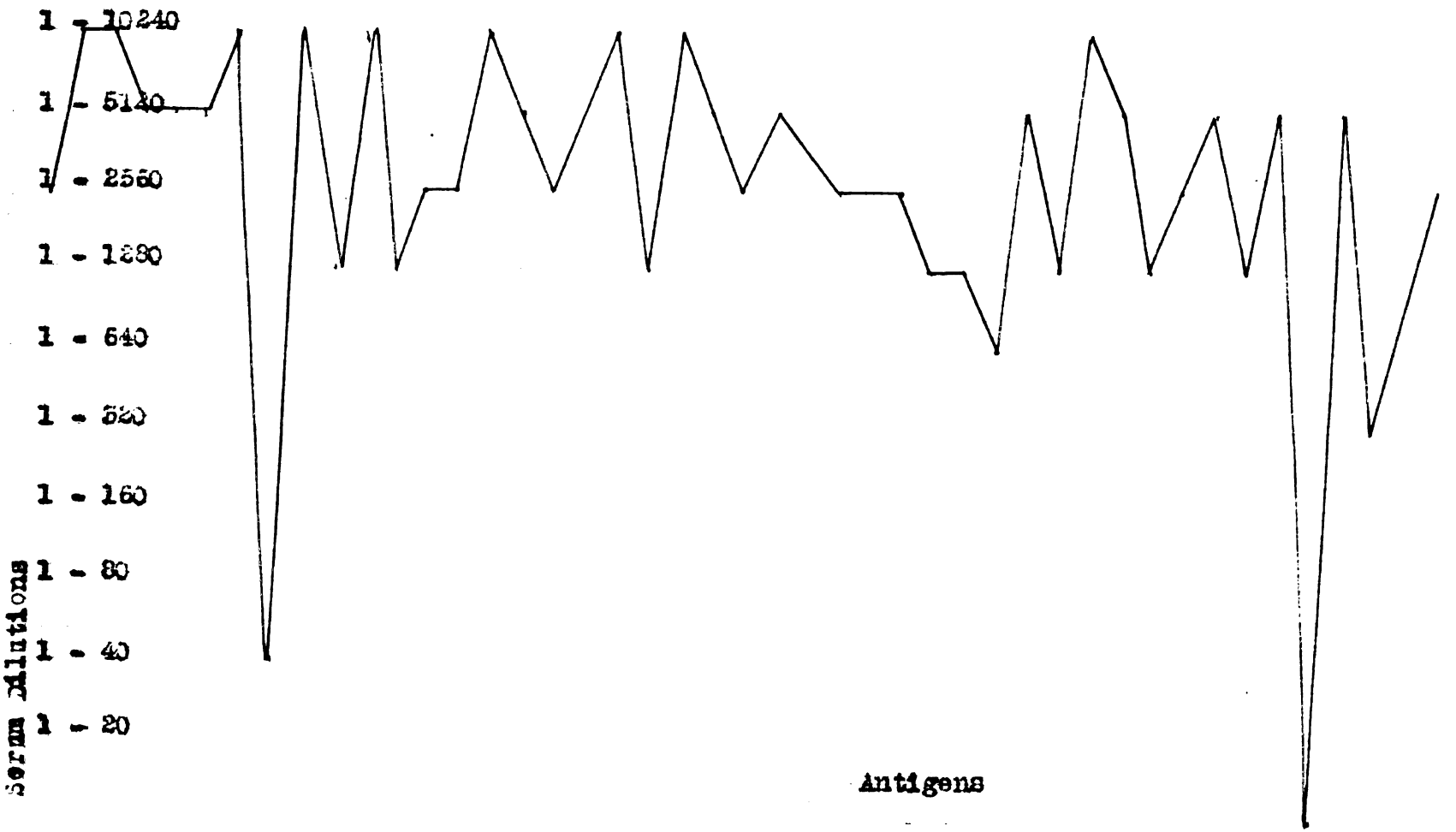
Showing Complete Agglutination

of Serum from Culture 7



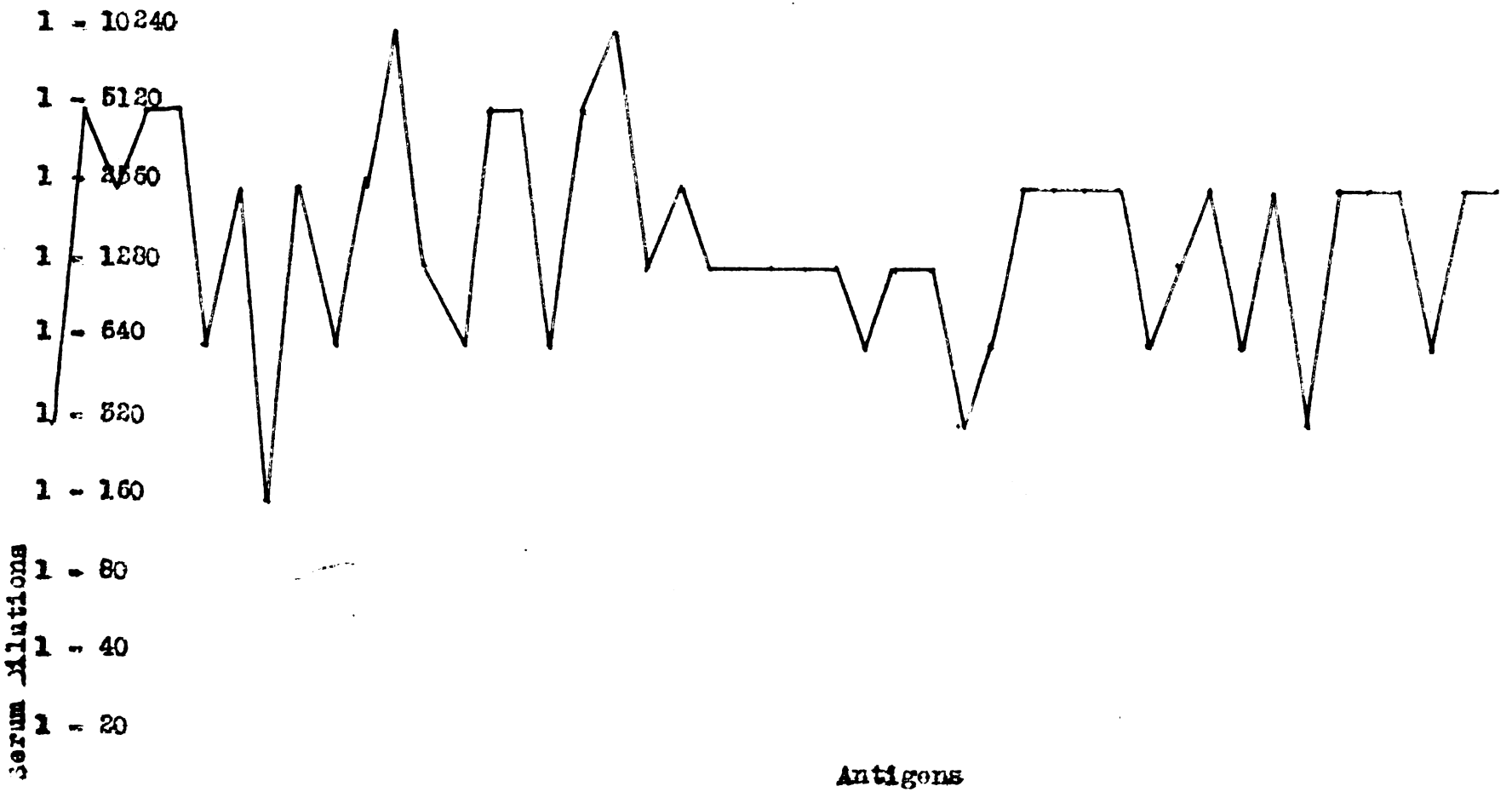
Showing Complete Agglutination

of Serum from Culture 8



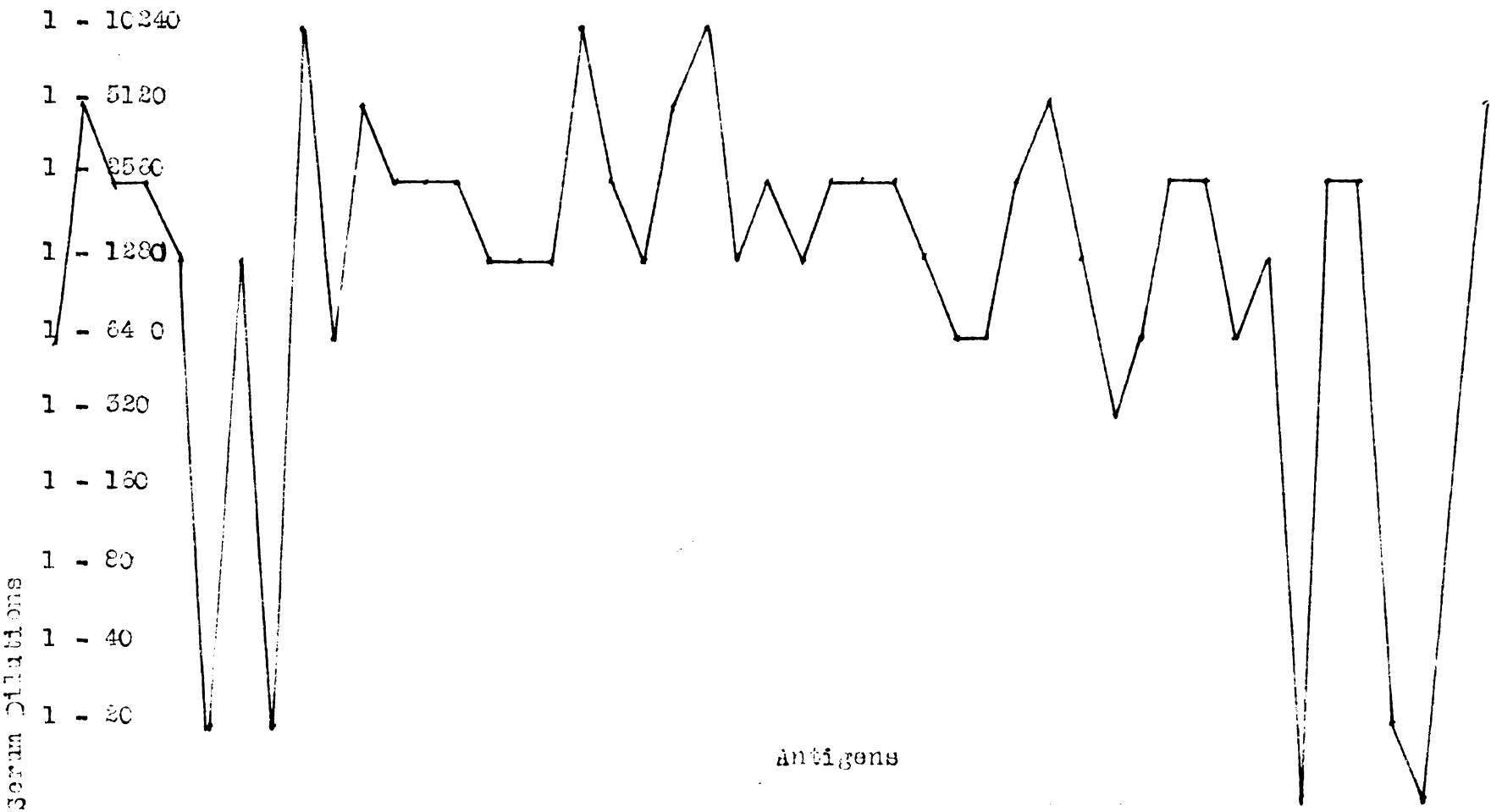
Showing Complete Agglutination

of Serum from Culture 9



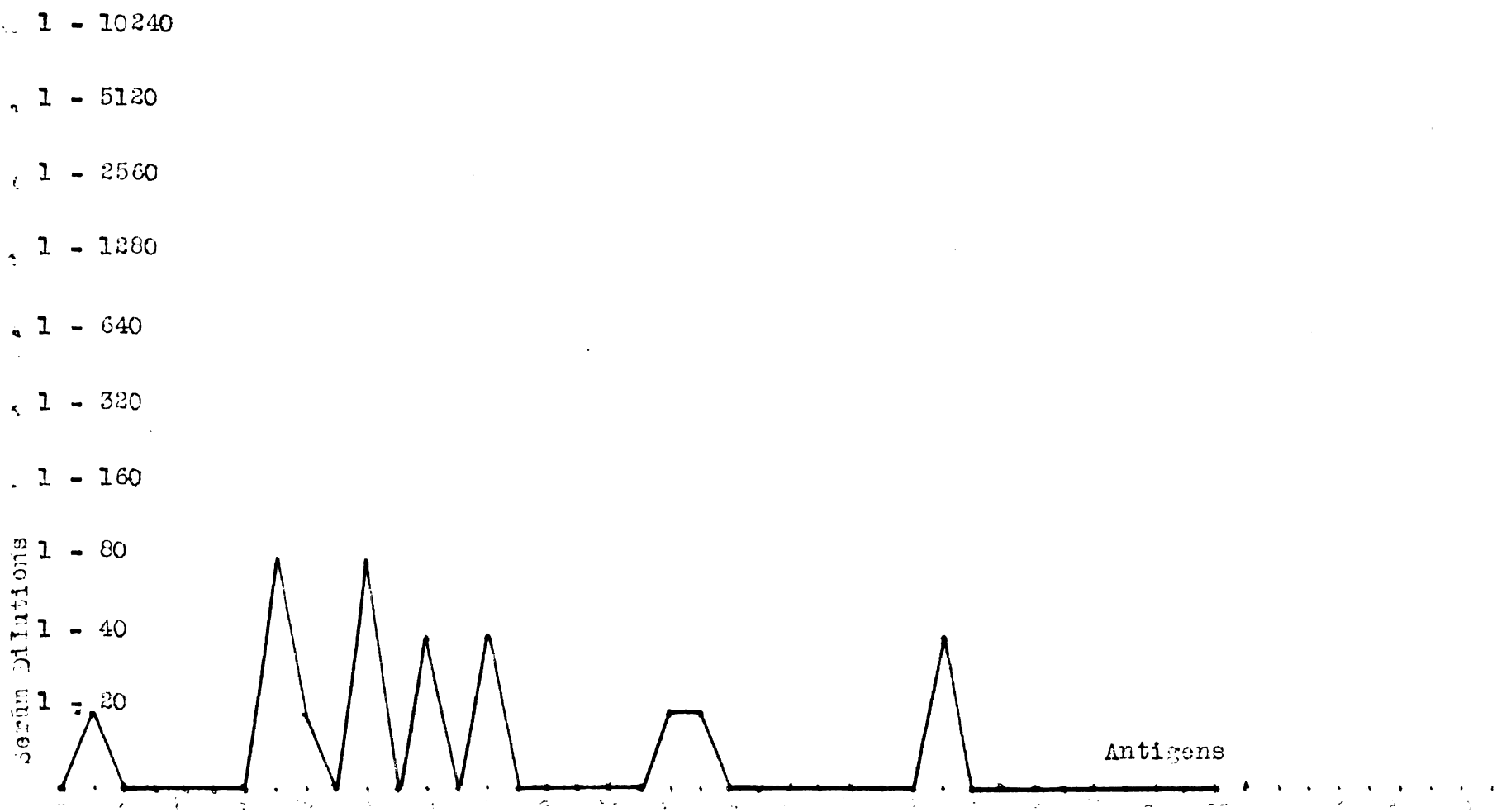
Showing Complete Agglutination

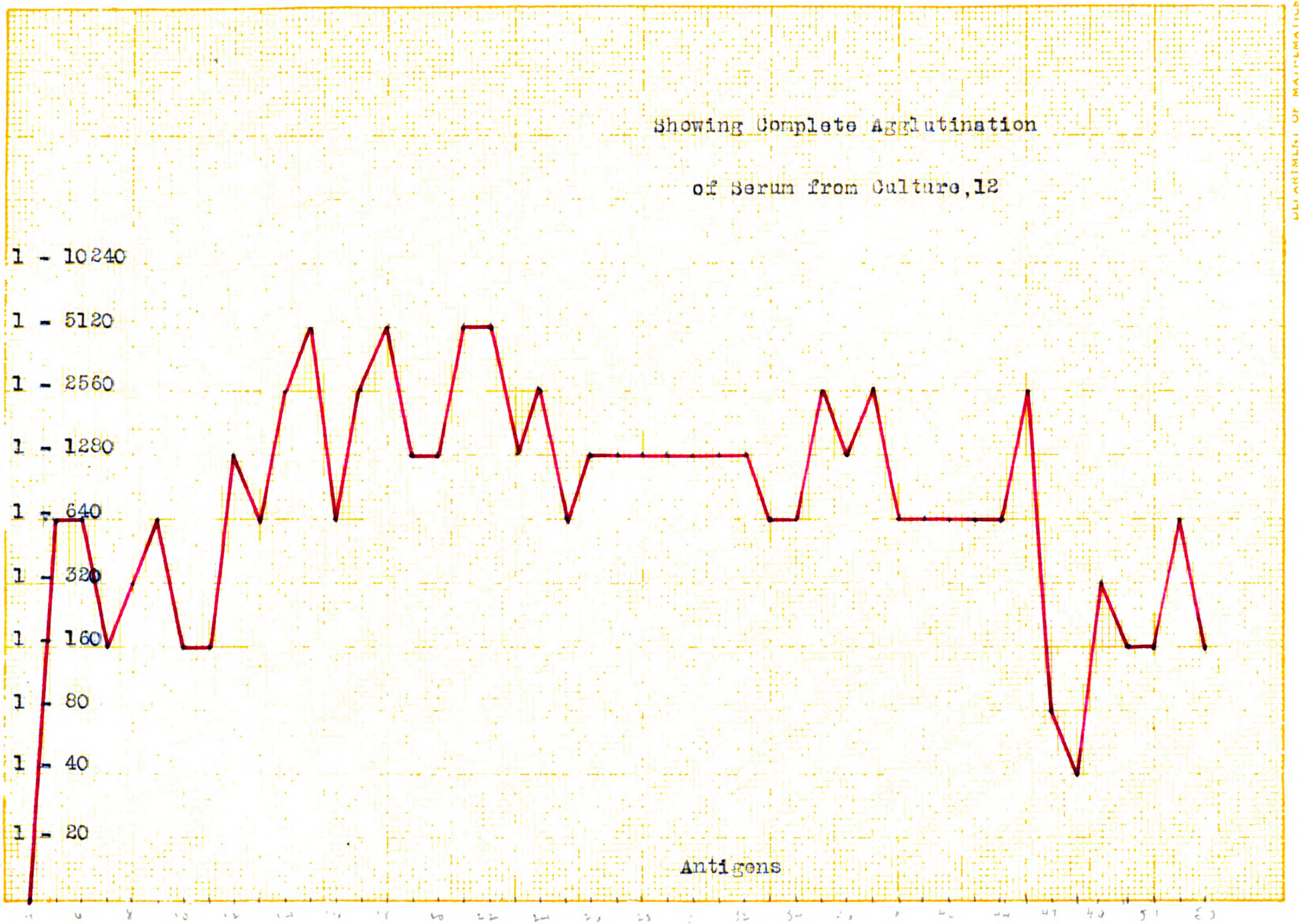
of Serum from Culture 10.

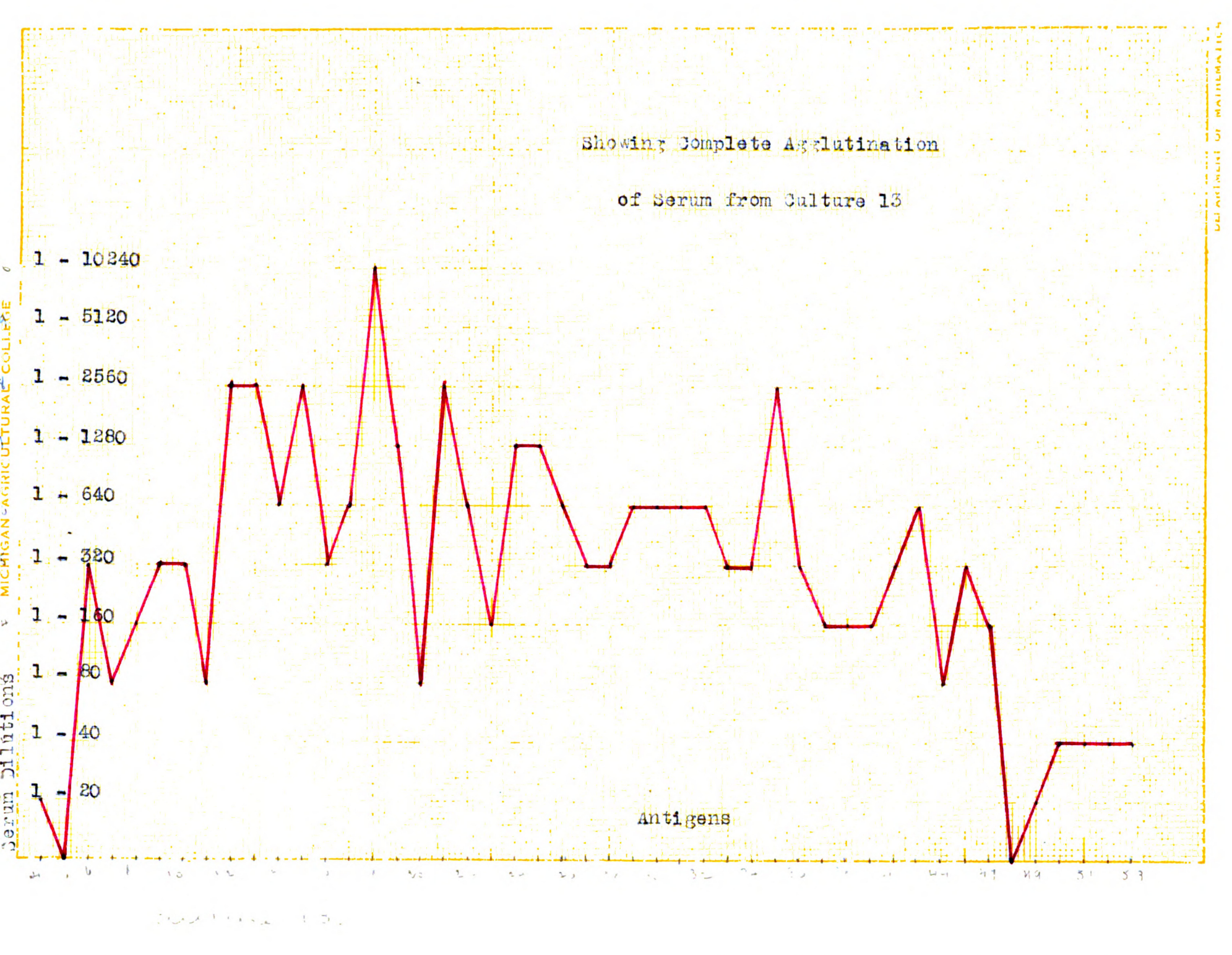


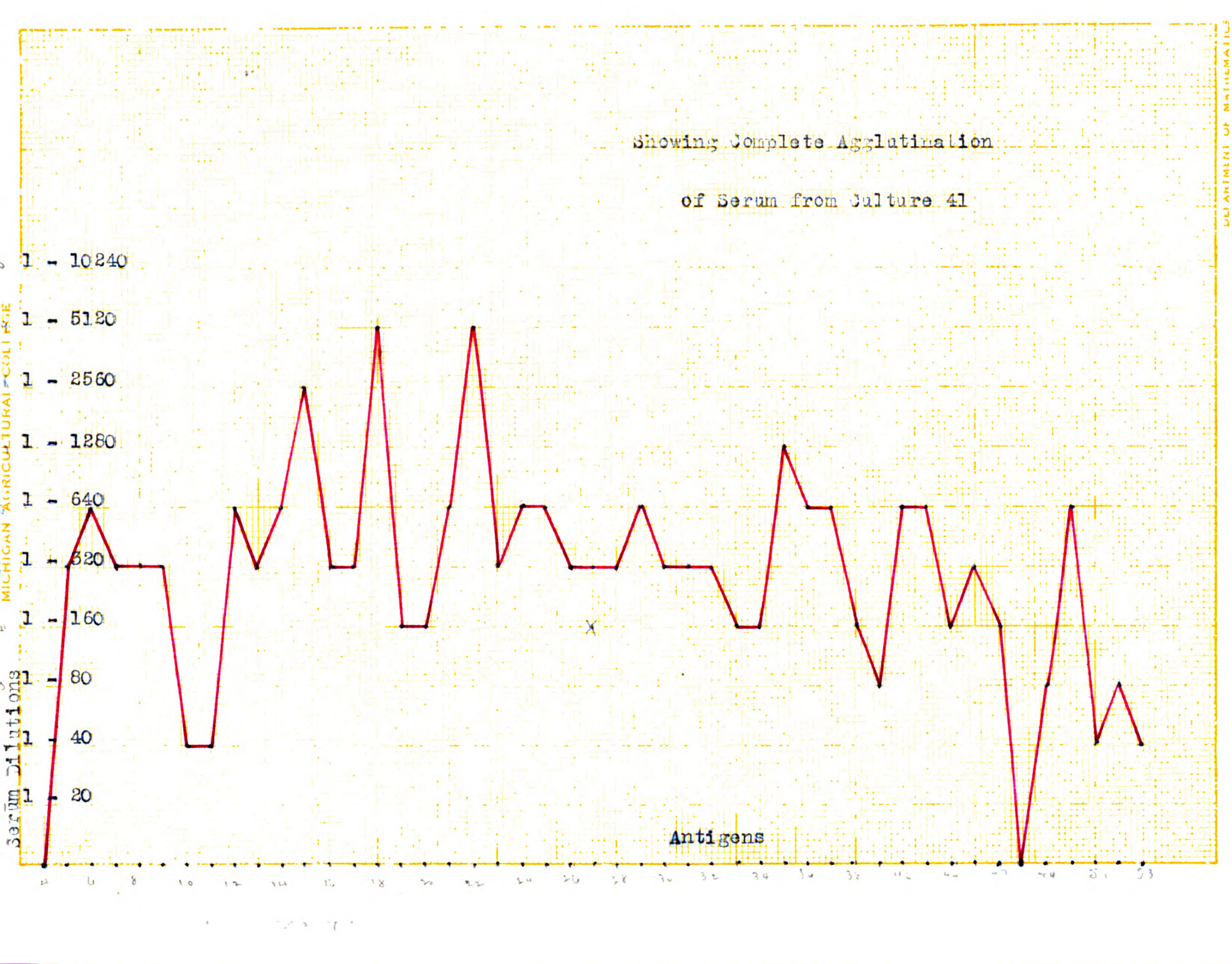
Showing Complete Agglutination

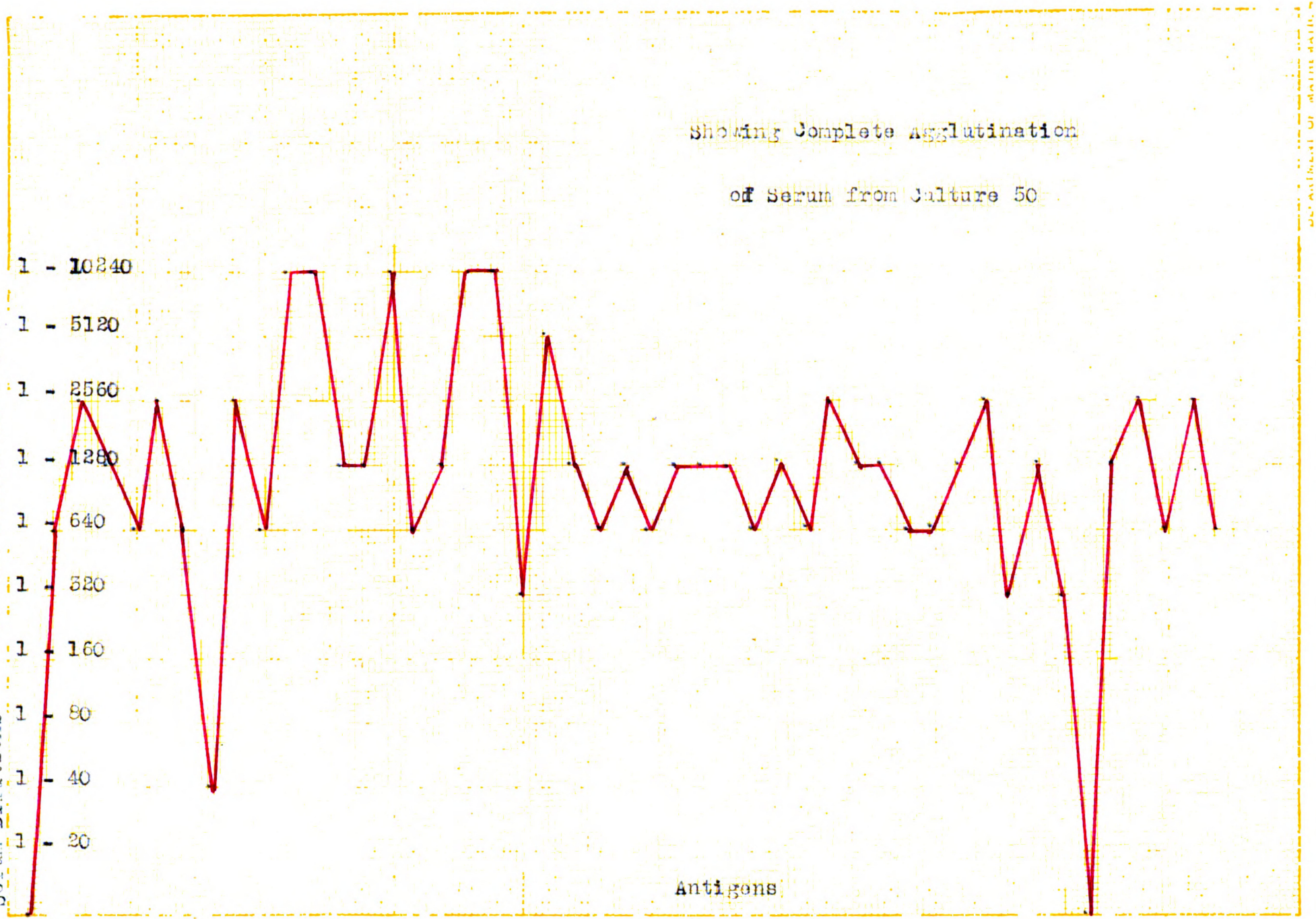
of Serum from Culture 11




Showing Complete Agglutination
of Serum from Culture,12
1 - 10240
1 - 5120
1 - 2560
1 - 1280
1 - 640
1 - 320
1 - 160
1 - 80
1 - 40
1 - 20
Serum Dilutions
Antigens
MICHIGAN AGRICULTURAL COLLEGE
DEPARTMENT OF MATHEMATICS


Showing Complete Agglutination
of Serum from Culture 13
1 - 10240
1 - 5120
1 - 2560
1 - 1280
1 - 640
1 - 320
1 - 160
1 - 80
1 - 40
1 - 20
Serum Dilutions
Antigens


Showing Complete Agglutination
of Serum from Culture 41
1 - 10240
1 - 5120
1 - 2560
1 - 1280
1 - 640
1 - 320
1 - 160
1 - 80
1 - 40
1 - 20
Serum Dilutions
Antigens
MICHIGAN AGRICULTURAL COLLEGE
DEPARTMENT OF MATHEMATICS


Showing Complete Agglutination
of Serum from Culture 50
1 - 10240
1 - 5120
1 - 2560
1 - 1280
1 - 640
1 - 320
1 - 160
1 - 80
1 - 40
1 - 20
Serum Dilutions
Antigens

## SUMMARY.

Of the 47 strains studied, two distinct types were found which differ in their gas producing powers. One strain attacks dextrose and mannite with gas and acid formation, while the other produces acid and no gas. These results confirm the work of Rettger and Hadley.

Cross agglutination of strains failed to show any marked differences with the exception of strains Nos. 11 and 47, which were anaerogenic strains. Because of the fact that only two strains of the anaerogenic type were studied, the above results obtained in the cross-agglutination tests are insufficient for drawing any conclusions.

The morphology of the Bact. pullorum changed considerably when grown on liver agar medium.

Gas production on the fermentable sugars varied considerably. Experiments carried out to determine the cause of this variation failed to show any results.

Action on the non-fermentable sugars with few exceptions was quite constant.

Biochemical tests were also quite constant with the exception of the hydrogen sulphide test.

Reaction on litmus milk varied considerable with some stains. Most strains, however, were quite constant.

No particular strain appeared to be variable at all times. Practically all of the strains seemed to vary at one time or another.

## Acknowledgements

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