On Variation and Adaptation in Bacteria, Illustrated by Observations upon Streptococci, with Special Reference to the Value of Fermentation Tests as Applied to these Organisms.

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(Communicated by Prof. Francis Gotch, F.R.S. Received February 18,—Read March 30, 1911.)

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The fermentation tests introduced by M. H. Gordon (1) in his study of streptococci isolated from saliva and some other sources were proposed by him as a means of differentiating among these organisms and separating them into definite varieties.

Gordon's methods were applied by A. C. Houston (2) in observations upon streptococci obtained from normal human fæces and from milk, and they were largely relied on by Andrewes and Horder (3) in their investigation of pathogenetic streptococci from disease in man.

The tests in question concern the reaction (acid or alkaline) produced by any given streptococcus during three days' growth at 37° C. in a series of tubes of alkaline sugar-free litmus broth to which 1 per cent. of saccharose, lactose, raffinose, inulin, salicin, coniferin, and mannite respectively have been added. The production of acid in sufficient amount to change the colour of the medium from blue to red constitutes a positive reaction in each case, and is believed to be due to the fermentation of the added carbohydrate substance by the streptococci.

To these culture tests in the seven different carbohydrates two others were added by Gordon, namely, the presence or absence of clotting in litmus milk within three days at 37° C., and the reduction or non-reduction of neutral red broth in anaërobic culture at 37° C. within two days.

In Houston's observations the coniferin test was omitted, and so far as I am aware this glucoside can now no longer be obtained commercially. It was, however, employed by Andrewes and Horder, who, as the result of their investigations, maintained their ability to distinguish well-marked differences of type among their streptococci. These observers elaborated a method of classification based on Gordon's tests, but taking into consideration in addition the length of the streptococcal chains produced in ordinary broth

(at 37° C.), and the power of growth in gelatin at 20° C. with or without the liquefaction of that medium. It would appear that they regard their method as enabling them to subdivide the morphologically indivisible type, Streptococcus, into a number of distinct and independent groups or "provisional species."

It is clear that so simple a method of distinguishing definite varieties (if such exist) among the streptococci would possess the greatest value, not only from a theoretical and taxonomic standpoint, but also from its enormous practical utility and most important bearing both on the diagnosis, the prognosis, and the treatment of streptococcal infections. But while a method of such great potential value cannot be lightly set aside or disregarded in dealing with the organisms in question, neither can it be accepted with reliance without the most thorough and complete investigation.

The problem of the identity or diversity of the streptococci which invade the human body is one whose importance is almost equalled by its difficulty. Many observers have attacked the problem without conclusive result, and Marmorek's (4) contention for the unity of human streptococci still held the field successfully at the period when Gordon's tests were introduced.

The question had been prominently before my own mind, owing to the necessity of endeavouring to determine whether the micrococcus which (following Triboulet and Paine and Poynton) I found in a number of cases of acute rheumatism, and spoke of as the *Micrococcus rheumaticus*, was or was not distinguishable from other members of the group Streptococcus. Such definite distinction it was eventually found impossible to establish, since even the remarkable production of formic acid by the streptococcus from rheumatism which Ryffel and I (5) observed proved to be only quantitative and not qualitative in character. I was therefore anxious to determine whether Gordon's tests would afford a reliable means of differentiation.

Clearly the first requisite for test reactions such as those proposed is that they shall yield constant and definite results with any given strain of streptococcus. Gordon himself fully recognised that "stability of the reactions" was important, and gave some attention to the question, but regarded it as sufficiently decided when he had ascertained that the reactions remained unchanged in observations extending over a fortnight, and were only modified in two cases out of eleven (and then only in respect of one or two of the reactions) by one passage of the micro-organisms through the mouse.

In their preliminary criticism of Gordon's tests, Andrewes and Horder also emphasised the fact that "constancy of the tests is clearly a cardinal point," and they went on to state that as the result of their own observa-

tions they had "no hesitation in asserting that the reactions are remarkably constant" for any particular streptococcus examined. They admitted that the neutral red and salicin reactions showed some tendency to variation, and mentioned two instances in which the mannite reaction was lost after a week of culture on gelatin. But on the whole they found the tests appreciably constant. They stated that they had in a number of cases found them unchanged after so long as a year, quoting one instance in which the interval of observation was nine months. Other evidence which they gave of constancy is open to the criticism that it consisted in part of examples in which the reactions actually showed some degree of variation, and they accordingly suggested that "slight differences in the composition of the media may possibly affect the series of reactions to some little extent." Evidence is offered here that greater differences in the media do actually affect the reactions to a remarkable extent.

Using these test reactions Gordon found as many as 48 different chemical types among 300 streptococci from saliva, though it is to be noted that more than one-third of the number fell under three of the types. Houston found 40 types among 300 streptococci from fæces.

Andrewes and Horder, examining 228 strains of streptococci from man, referred them all to five types (one of which consists of pneumococci), but this was only accomplished by classifying no less than 173 of the strains as variants by defect and excess of one or other of the five main types selected. Their streptococcal types are named respectively pyogenes, salivarius, anginosus, and fæcalis.

The remarkable frequency of variants and the numerous gradations of the type forms are explained by Andrewes (if I understand him rightly) as due to the survival and persistence of intermediate biological links, connecting those main types in the "dominant genus" constituted by the streptococci, which will become distinct species if (and when) these variants disappear in the course of evolution. This view entails the supposition that the types and variants in question have definite and relatively fixed identities, and that the characters relied on for their differentiation have to some extent at any rate a specific significance. But it would seem to be an equally satisfactory alternative in an *a priori* explanation to suppose that the differences observed in the chemical reactions now under consideration are due to merely temporary and casual modifications of metabolism. It might further be suggested that the features of streptococcal activity which they concern are so extremely variable, and are so easily varied under the influence of varying environment as to be almost accidental in character and to afford no criteria of any differential value, nor any basis for a classification

of these micro-organisms. This is the attitude which my experiments appear to justify.

Abundant grounds exist in the known variability of chemical activity, and even of morphological characters which bacteria present, for regarding any simple test for specificity among these organisms with some suspicion, until it has been fully investigated under appropriate conditions. Under the influence of altered environment a considerable degree of adaptation would naturally be anticipated in these primitive organisms where the successive generations follow one another with such great rapidity as to reach in many cases even two or three per hour. But the remarkable degree of variability which bacteria do actually exhibit is perhaps not always kept sufficiently in mind.

The great difficulties met with by Dreyer and FitzGerald (6) in ascertaining the precise conditions under which neutral red lactose bouillon could be made to yield uniform results in the differentiation of the *B. typhosus*, *B. paratyphosus*, and *B. coli* showed incidentally that slight alterations in the composition of the culture medium are frequently sufficient to modify and even to *reverse* the chemical changes produced by these organisms in the medium in question.

Again, Twort (7) has shown that the *B. typhosus* can be trained (in about two years) to ferment lactose in lactose peptone water. More recently Penfold (8) has noted a morphological change in the colonies associated with the development of this faculty, and has made important observations on the development or loss under suitable conditions of a number of other fermenting properties of the same organism. My own earlier observations (9) on the "immunisation" of the same bacillus against its own specific immune serum (of the horse) by continued cultivation and sub-cultivation in that fluid demonstrated an adaptation of much greater complexity. This was associated with an increased virulence of the micro-organism, an increased resistance to the specific action of the anti-serum, and what appeared to be an actual production of specific anti-antibodies, showing a high degree of adaptability in the organism in question.

Morphological variations of a greater or less degree are also common, and like the chemical variations they can be induced experimentally. Almquist (10) has described them both in *B. typhosus* and in *Vib. cholerœ*; and Murray and I (11) have shown that in special media the *B. typhosus* can be made to assume a variety of novel appearances, in some of which it might readily be mistaken for a streptothrix.

Both morphological and chemical variations of greater or less permanence are, therefore, very readily produced among bacteria. Accordingly a claim

put forward that particular strains of any given type of micro-organism, such as Streptococcus, can be regarded as distinct and fixed varieties on the results of any given series of culture tests requires to be most carefully scrutinised before it can be accepted, and the constancy of the reactions concerned must be thoroughly assured.

As regards the streptococci, however, the observations which I have carried out appear to show that this is not the case. If cultures of these organisms are examined at considerable intervals of time their reactions in the test media are by no means constant, but on the contrary are found to undergo remarkable changes. Further it is found that such comparatively simple manipulations as continued culture in a particular medium may entirely alter the reactions of these organisms not only to that medium itself, but (it may be) to others also of the carbohydrate media.

Methods.

The streptococci to be examined were first plated out twice to ensure as far as possible that the culture obtained arose from a single individual or chain of individuals. With the exception of the one named E below (cultivated from horse-dung) they had all been isolated from the human subject.

The stock cultures of these organisms were propagated in stab agar, and sub-cultures were established every seventh day.

The test media were prepared as directed by Gordon, at first with ordinary bouillon freed from sugar, but later, and for the bulk of the results recorded below, from "lemco." With each series of cultures in a particular test medium an uninoculated control tube of the same medium was put up, and incubated with them for comparison. Whenever a negative reaction was obtained, a sub-culture from the negative tube was prepared in ordinary bouillon in order to make sure that the organism had actually grown in the test medium. If growth was not obtained in this sub-culture, the test was repeated.

In the tables given below, the sign + indicates the production of an acid reaction under the test conditions (and where milk is concerned, the formation of a clot), while the sign 0 indicates that the reaction remained alkaline (or milk unclotted, whether it remained alkaline or became acid in reaction). The former may be spoken of as a positive, and the latter as a negative reaction. The neutral red test, which is admitted to be somewhat variable, was usually omitted; and coniferin, which could not be obtained commercially, was not used.

Results.

In the earlier observations I examined a considerable number of streptococci of known origin which were available at different times, and noted their reactions, subsequently comparing the results thus recorded with those obtained after a longer or shorter period of cultivation in stab agar as laboratory stock. Three sets of observations illustrate the character of the results thus obtained :—

Table I.—Streptococcus B. Showing Six Changes of Reaction between June, 1907, and May, 1908.

Date of testing.	Milk clot.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.	Changes.	Total of changes.
June, 1907	0	+	0	0	0	0	+		
January, 1908	0	+	+	+	0	0	0	3	
May, 1908	+	+	0	+	+	0	0	3	6

Streptococcus B.—This was isolated from a case of acute rheumatism by Dr. Bushnell, in 1907, and sent to me for examination of its formic acid production.

Table II.—Streptococcus E. Showing Two Changes of Reaction after Three Months' Cultivation in Stab Agar.

Date of testing.	Milk clot.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.	Changes.
August 8, 1910	0	+	+	0	+	+	+	
November 15, 1910	0	+	+	+	0	+	+	2

Streptococcus E, tested when freshly isolated from horse-dung, and again after 14 weeks.

Table III.—Streptococci L, P, S, G, M, H and V. Showing the Changes of Reactions (20 out of a possible 49) which had taken place after the lapse of Two Years.

Streptococcus.	Date of testing.	Milk clot.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.	Reactions gained.	Reactions lost.	Total of changes.
L	June, 1908 ...	+	+	0	0	0	0	0			
	„ 1910 ...	0	0	+	0	0	+	0	2	2	4
P	„ 1908 ...	+	0	+	+	0	+	0			
	„ 1910 ...	0	0	+	0	0	+	0	0	2	2
S	„ 1908 ...	+	+	+	+	0	+	+			
	„ 1910 ...	0	+	+	0	0	+	0	0	3	3
G	„ 1908 ...	+	+	+	0	+	+	+			
	„ 1910 ...	+	+	+	+	0	+	0	1	2	3
M	„ 1908 ...	+	+	+	+	0	+	0			
	„ 1910 ...	0	+	+	0	0	+	0	0	2	2
H	„ 1908 ...	0	+	+	+	+	+	0			
	„ 1910 ...	0	+	+	0	0	+	0	0	2	2
V	„ 1908 ...	+	+	0	0	+	+	0			
	„ 1910 ...	0	0	+	0	0	+	0	1	3	4

Seven streptococci selected as giving each a different series of reactions when first tested in 1908. L and P had been under cultivation in the laboratory for more than a year, while S, G, M, H, and V had recently been introduced from Copenhagen.

These results show that, when examined after longer or shorter intervals of time, the reactions of the streptococci tested exhibited marked changes under the conditions of ordinary cultivation.

The seven strains of streptococci shown in Table III were now chosen for further investigation.

P, which had never fermented saccharose, was grown in the saccharose medium.

L and V, which had never fermented raffinose, were grown in the raffinose medium.

M, V, and H, which had never fermented mannite, were grown in the mannite medium.

The cultures were sub-cultivated every three days in the appropriate medium. The course of the experiments was as follows :—

P in Saccharose.

June 14—17.....	1st passage	Reaction	0
„ 17—20.....	2nd „	„	+
„ 20—23.....	3rd „	„	0
„ 23—26.....	4th „	„	+
„ 26—29.....	5th „	„	+

The negative reaction reappeared occasionally until the middle of July, after which the organism remained a strong saccharose fermenter until the end of the observation (August 13). It was then tested through all the media, but showed no change in any of its other test reactions.

L and V in Raffinose.

Begun June 14.

V became a raffinose fermenter on the fifth passage and so remained as long as it was kept under observation (several weeks).

L refused to ferment raffinose, though grown continuously in this medium until October 7. It was then carried on *anaërobically* in the raffinose medium, and began to change the colour to red on the third passage. This reaction continued to be obtained in successive passages, though until October 27 the *blue colour was found to return on admitting air.* From October 27, the red colour produced anaërobically remained permanent even when air was admitted. But on continuing the cultures aërobically from November 10 to December 5, the organism still refused to ferment the raffinose in the presence of oxygen.

The change of colour to red produced anaërobically, which speedily changed back again to blue on admitting air, has frequently been met with in other instances, and appears to be quite common. Still more frequently the blue colour is simply discharged in the anaërobic cultures. In a large number of cases the blue colour rapidly returns on the admission of air, reappearing first at the surface of the medium and travelling slowly downwards to the foot of the tube. The medium will frequently be found to be still quite strongly alkaline in reaction.

The change concerned in this discharge of colour must apparently be one involving some deoxidation process, as the result of which the litmus is converted into a leuco-body, but I am at present quite unable to throw any light upon the actual nature of the change. It is, however, important that it should not be mistaken for an anaërobic fermentation of the test carbohydrate.

As Andrewes pointed out, such fermentation may often be obtained quite

readily in anaërobic cultures, when it entirely fails to appear under aërobic conditions of cultivation.

M, V, and H in Mannite.

H became a mannite fermenter on the third passage, and so remained. M and V were carried on in mannite from June 14 until August 1 without any change in the reaction appearing.

These three sets of observations show that by continued passage through a particular medium some of the streptococci easily acquire the faculty of fermenting a special carbohydrate, to which they had always previously given a negative reaction. In other cases similar changes could not be obtained within the period of investigation.

Inulin Test.

In the next set of experiments inulin was selected as the medium for observation, as being apparently the most difficult for streptococci to ferment. Andrewes and Horder found that only 3 per cent. of their streptococci could ferment this polysaccharide.

Five streptococci, L, P, S, G, M, were selected for observation, none of which fermented inulin, and only one of which (G) had ever done so within my experience. Table IV shows the result of growing these organisms for certain periods in inulin medium. All became eventually (eight weeks) strong inulin fermenters. They were then put back into stab agar for three months, after which all but M were found to have lost their action upon

Table IV.—Streptococci L, P, S, G and M. Showing the Reactions in Inulin Medium at Different Stages of the Experiment.

Streptococcus.	Original reaction in inulin.	After 14 days in inulin medium.	After 24 days in inulin medium.	After 5 weeks in inulin medium.	After 8 weeks in inulin medium.	After return for 3 months to stab agar.	After 4 weeks' anaërobic culture and 2 weeks' aërobic culture in inulin medium.
L	0	0	+	+	+	0	+
P	0	0	0	+	+	0	+
S	0	0	+	+	+	0	+
G	0	0	0	0	+	0	0
M	0	+				+	+

inulin. On again growing them, first anaërobically (four weeks) and then aërobically (two weeks) in inulin medium, all regained the power of acting anaërobically on inulin, and all but G the power of acting on it in the presence of air.

At intervals during the course of this experiment the organisms from inulin culture were sub-cultivated, and tested through the series of test media, with the results given in Table V below. They showed marked variations at different stages of the experiment.

Table V.—Streptococci L, P, S, G and M. Showing the Reactions of the Five Streptococci in the Test Media at Different Stages of the Inulin Experiment.

Streptococcus.	Time of testing.	Milk clot.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.	Changes.	Total of changes.
L	1. Original reactions, June, 1910	0	0	+	0	0	+	0		
	2. After 5 weeks in inulin	0	+	+	0	+	+	0	2	
	3. After 8 weeks in inulin	+	+	+	+	+	+	+	3	
	4. After 3 months in stab agar	0	0	+	0	0	+	0	5	
	5. After 2 weeks in inulin (anaërobic)	+	0	+	0	0	+	+	2	
	6. At end of experiment	0	0	+	0	+	+	0	3	15
P	1. Original reactions, June, 1910	0	0	+	0	0	+	0		
	2. After 5 weeks in inulin	0	0	+	0	+	+	0	1	
	3. After 8 weeks in inulin	0*	0	+	0	+	+	0	0	
	4. After 3 months in stab agar	0	0	+	0	0	+	0	1	
	5. After 2 weeks in inulin (anaërobic)	+	0	+	0	0	+	+	2	
	6. At end of experiment	+	0	+	0	+	+	0	2	6
S	1. Original reactions, June, 1910	0	+	+	0	0	+	0		
	2. After 5 weeks in inulin	0	+	+	+	+	+	0	2	
	3. After 8 weeks in inulin	+	+	+	+	+	+	+	2	
	4. After 3 months in stab agar	0	0	+	0	0	+	0	5	
	5. After 2 weeks in inulin (anaërobic)	+	0	+	0	0	+	0	1	
	6. At end of experiment	+	+	+	+	+	+	0	3	13
G	1. Original reactions, June, 1910	+	+	+	+	0	+	0		
	2. After 5 weeks in inulin	0	+	+	+	0	+	0	1	
	3. After 8 weeks in inulin	+	+	+	+	+	+	+	3	
	4. After 3 months in stab agar	0	+	+	0	0	+	0	4	
	5. After 2 weeks in inulin (anaërobic)	0	+	+	0	0	+	+	1	
	6. At end of experiment	+	+	+	+	0	+	+	2	11
M	1. Original reactions, June, 1910	0	+	+	0	0	+	0		
	2. After 2 weeks in inulin	0	+	+	+	+	+	+	3	
	3. After 3 months in stab agar	0	+	+	+	+	+	+	0	
	4. After 2 weeks in inulin (anaërobic)	0	+	+	+	+	+	+	0	
	5. At end of experiment	0	+	+	+	+	+	+	0	3

* After another week of cultivation in inulin medium this reaction became positive.

It will be noted that, after eight weeks of growth in inulin, L, S, and G gave identical reactions which only differed in respect of the milk-clotting from the reactions given by M after a fortnight's growth in the same medium. The number and variety of the other changes which appeared is very striking.

Mannite Test.

An attempt was also made to teach the organisms, L, P, S, G, M, V, H, to ferment mannite by growing them in the mannite medium. This was unsuccessful after four months' trial, but is interesting as showing (Table VI) that although the mannite reaction itself refused to change, some of the other reactions were modified in some cases by the continued cultivation in mannite.

The following is the protocol of the experiment :—

L, P, S, G, M, V, H, were grown from August 1 to October 1 as follows: Three days in mannite medium, sub-culture in ordinary bouillon one day, back to mannite medium for three days, and so on. No change of reaction had occurred by October 1.

The organisms were now grown three days in mannite anaërobically, one day in bouillon aërobically, back to anaërobic culture in mannite for three days, and so on. By October 15 all but G and H discharged the colour in mannite, but the blue colour returned on admitting air. This state of things continued in the successive cultures until November 10, when G and H were abandoned. L, S, P, V, M, were now grown in mannite

Table VI.—Streptococci L, P, S, V and M. Showing the Changes of Reactions which had taken place at the end of the Mannite Experiment.

Streptococcus.	Time of testing.	Milk clot.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.	Changes.
L	1. June, 1910.....	0	0	+	0	0	+	0	
	2. After 4 months in mannite	0	0	+	0	0	+	0	0
P	1. June, 1910.....	0	0	+	0	0	+	0	
	2. After 4 months in mannite	0	0	+	0	0	+	0	0
S	1. June, 1910.....	0	+	+	0	0	+	0	
	2. After 4 months in mannite	0	+	0	0	0	0	0	2
V	1. June, 1910.....	0	0	+	0	0	+	0	
	2. After 4 months in mannite	+	0	+	0	0	+	0	1
M	1. June, 1910.....	0	+	+	0	0	+	0	
	2. After 4 months in mannite.....	0	0	+	0	0	+	0	1

aërobically, sub-cultures being made every three days directly from the mannite cultures to fresh mannite media.

On December 5 all were still negative to mannite. The organisms were now tested through the other test media, with the results shown above in Table VI.

Milk Test.

The streptococci L, P, S, G, M, were grown continuously in milk for well over four months, from June 17 until October 31, fresh subcultures being made in litmus milk once a week.

When the experiment was begun only G clotted milk (on the second day).

On July 15 S and P had begun to clot milk, while G now only clotted the milk on the fourth day of growth.

On July 21 G had ceased to clot milk at all, and had not regained this power by October 31, when the experiment was brought to an end.

On August 19 S and P no longer clotted milk. On September 20 S gave a feeble clot. On October 2 L and P clotted the milk on the sixth day. On October 19 L, P, and S clotted the milk firmly in three days, and this condition still held on October 31. M never clotted milk throughout the experiment.

Thus, of the five streptococci, one lost the power of clotting milk, three gained it, and one remained unchanged. These five organisms were now put

Table VII.—Streptococci L, P, S, G and M. Showing the Changes of Reactions which had taken place after Four Months' Cultivation in Litmus Milk.

Streptococcus.	Time of testing.	Milk clot.	Saccharose.	Lactose.	Raffinose.	Inulin.	Salicin.	Mannite.	Changes.
L	1. June, 1910	0	0	+	0	0	+	0	
	2. After 4 months in milk	+	0	+	+	+	+	0	3
P	1. June, 1910	0	0	+	0	0	+	0	
	2. After 4 months in milk	+	0	+	0	+	+	0	2
S	1. June, 1910	0	+	+	0	0	+	0	
	2. After 4 months in milk	+	0	+	+	0	0	0	4
G	1. June, 1910	+	+	+	+	0	+	0	
	2. After 4 months in milk	0	+	+	+	0	+	0	1
M	1. June, 1910	0	+	+	0	0	+	0	
	2. After 4 months in milk	0	+	+	0	0	+	+	1

through the test media, and showed other changes of reaction as exhibited in Table VII above.

The general bearing of the results obtained in the experiments recorded above on the problem of the identity or diversity of human streptococci, and on the value of test media in the diagnosis and differentiation of these organisms may now briefly be indicated.

The streptococci shown in Tables I, II, and III undergo such great changes under cultivation in stab agar as to exhibit collectively 28 alterations of individual reactions out of a possible 70, namely, nine gains out of a possible 28, and 19 losses out of a possible total of 42.

Among the seven streptococci in Table III which each gave a different series of reactions in 1908, and could therefore at the date have been classified as different varieties, L, P, and V give identical reactions in 1910, and S, M, and H are also identical, but give a slightly different series of reactions from the former, while G still stands alone, although itself somewhat changed. Examined for the first time at this date these seven streptococci might, therefore, have been regarded as representing only three varieties.

At the end of the mannite experiment (Table VI) M is no longer identical with S, but has assumed the reactions of the L, P, V group, while V has left this group and now clots milk.

Streptococcus G, after three months in agar following eight weeks in inulin (Table V), gives the same reactions as M (vi, 1910) in Table III. And L, S, and G, in Table V, give identical reactions at the third stage—all positive, and only differ from M in that the latter still lacks the power of clotting milk, which it possessed when first observed in 1908.

It is unnecessary to adduce further instances, though a number occur in the tables, that streptococci, which are at one time different, may at another give identical reactions, while those which are apparently identical at a given date may later on exhibit totally different series of reactions in the test media. But it is clear that no trace of specificity has been found in the tests in question in the present observations.

Looking through the tables one finds that Streptococcus L exhibits no less than seven different series of reactions in different circumstances, P exhibits six different series, S exhibits nine, G exhibits seven, and M exhibits four. These five streptococci have, in fact, given rise to 32 different varieties or variants in the course of this investigation. It follows that the method of identifying varieties of streptococci by means of the series of test media which have been employed in these experiments rests on no fixed or specific differences in the organisms themselves. The differences observed cannot in

any sense be regarded as permanent, but would seem to be due to merely temporary changes in the metabolism of the organisms concerned.

Lest it should be thought that the alterations in reactions which have been here described are not of absolutely fundamental character, I have compared a number of my induced varieties as nearly as possible with the types and variants described by Andrewes and Horder. For this purpose it is necessary to omit their neutral red and coniferin tests, which I have not used, and to disregard the questions of growth in gelatin and length of chains in bouillon. These latter do not appear to form very important features in their system of classification. The facts, however, must be fully borne in mind in estimating the value of the present criticism. Their streptococcal types are numbered (1) pyogenes, (2) salivarius, (3) anginosus, and (4) fæcalis; the variants being indicated by the letters *a*, *b*, *c*, *d*, etc.

Among my streptococci derived from L are to be found (with the provision just mentioned) the following types and variants of Andrewes and Horder in succession:—3 *c*, 1 *d*, 3 *g*, 4 *b*, 1 *d*, 4 *e*, 3 *z*, 1 *d*, and a form not met with by these writers. That is to say, L appears to correspond at different times in my experiments with variants of *S. pyogenes*, *S. anginosus*, and *S. fæcalis* in their classification. P gives 1 *a*, 3 *h*, 1 *d*, 3 *e*, of Andrewes and Horder, again pyogenes, anginosus, and fæcalis, as well as other forms not found by them, and similarly with others of the streptococci.

The conclusion, therefore, is apparently unavoidable that, in spite of the very extended observations of Gordon, Houston, and Andrewes and Horder on the streptococci, there is still no evidence of the existence in the human subject of more than one micro-organism, Streptococcus, though this may vary as greatly in its chemical reactions in different cases as it is known to do in virulence. This view agrees with those consistently advanced by Marmorek and others.

A paper has quite recently appeared by C. O. Jensen giving an account of investigations carried out by him and his co-worker Holth on a number of micro-organisms, including streptococci, by the application of a long series of chemical and biological tests. Jensen states that Holth has examined 150 different strains of streptococci in relation to *all* the carbohydrates and polyvalent alcohols which he could obtain, and to a number of glucosides in addition. The conclusion arrived at is that culture in bouillon containing these substances, followed by *titration* of the reaction affords a valuable means of identification, differentiation, and classification of these organisms (the italics are mine). These observers regard lactose, trehalose, cellobiose, and gentobiose as of great value for the purpose of classification,

and find that further differentiation is possible by the use of various polyvalent alcohols and glucosides.

Using these methods Holth and Jensen find, even in "closely similar conditions of disease," that the strains of streptococci present in different cases are different, and they ask the question whether these differences are really stable and fixed characters or are merely casual variations. Jensen states that Holth's experiments (not yet published) lead to the conclusion that the differences are actually fixed, and that the organisms concerned are distinct and well-defined forms, but possessing similar pathogenetic action.

A description of the methods which Holth has employed to prove the stability of the differences in question will be awaited with interest, since it is evident from the experiments described above that such observations need to be prolonged or the conditions of experiment sufficiently varied before definite conclusions can be safely drawn.

One conclusion of great interest follows from the observation of these authors that closely similar conditions of disease may be associated with quite different strains of streptococci. It is that the differences brought out in carbohydrate, alcohol, and glucoside containing media are in no way closely related to the virulence and pathogenetic action of the streptococci examined. Accordingly no reliable inferences as regards these most important questions can legitimately be drawn from the results obtained in the media at present under discussion. The claim of Andrewes and Horder that the results so obtained were such as to enable one the better to form a prognosis and to initiate treatment therefore appears to lack sufficient evidence.

In continuing his communication Jensen next proceeds to discuss a special streptococcal disease of horses, namely, strangles. After pointing out the fact that the course and pathology of this disease varies remarkably in different cases, he states that Holth, who has examined about 40 strains of streptococci from strangles of Danish, German, English, and Swedish origin, has by the use of a very large number of his test media definitely proved them all to be identical. And the constancy of the reactions thus found is not attributable to the residence of the organisms in the horse, since this animal harbours many other streptococci which present quite different series of reactions in the same media.

It would appear, however, that the fact that organisms so completely identical as these are found to be in the test media are admitted to vary greatly in their pathogenicity in horses only shows again how small is the assistance to be looked for from this method of identification in dealing with the practical problems of disease. It is quite possible, and indeed even

probable, that the causal agent of strangles is an independent and specific organism; but this would only prove that Marmorek was correct in his conclusions as regards the streptococci, and that this organism is not a true streptococcus at all, in the usual sense, any more than is the *Streptococcus lanceolatus* of pneumonia.

Jensen makes the suggestion that when a single streptococcal form (like strangles) shows so great a constancy in the test media, it is the most probable conclusion that other streptococci also possess constant reactions. It may be pointed out that a generalisation of this character from a single instance is extremely apt to be fallacious. But a consideration of the facts adduced, namely, that on the one hand strangles presents different pathological events, the organisms being identical to the test media, while on the other hand similar conditions of disease may be occasioned by streptococci which give quite different series of reactions in these media, suggests that the reactions of true streptococci possess no fundamental relation to their pathogenicity, and present no evidence of permanence.

Although the test media have been shown to afford no evidence of the diversity of human streptococci, it is still possible that they may be made to serve a useful purpose in supplying indications as to the probable recent or habitual environment of particular strains.

A study of the very numerous and careful observations made by other workers and notably by Gordon, Houston, and Andrewes and Horder, suggests that on continued residence in a fairly uniform environment, as for example in the human mouth, the streptococcus tends to assume a moderately uniform series of reactions. But there is no satisfactory evidence as to the length of time such a process may require.

From early in 1908 our laboratory media have been prepared in a fairly uniform manner save for the natural variations in "meat extract." And it might be thought from an inspection of Table III above that the streptococci cultivated in stab agar were actually tending to a definite series of reactions such as that exhibited in 1910 by L, P, and V, and almost reached by S, M, and H, G for the time remaining refractory.

Again in Table V it is very striking how after eight weeks in inulin medium L, S, and G reach an identity which M falls short of only in the matter of clotting milk, while P appears to be moving slowly in the same direction. On returning to stab agar for three months L and P re-assume and S now assumes the reactions seen before from agar cultures.

This evidence, however, does not amount to more than a suggestion of what might prove to be the fact upon an extended trial and the use of suitably selected media.

On the other hand it is apparent that the organisms did not *always* respond very readily to changed environment in my experiments, and little or nothing is at present known of the conditions which determine such response.

As regards the investigation of the practical problems of pathogenicity and virulence it would seem more appropriate to employ media of known and definite composition instead of media based upon material so indefinite as meat extract, and to use proteins and protein derivatives as test substances rather than carbohydrates, alcohols, and glucosides.

Conclusions.

1. The reactions of any given strain of streptococcus in Gordon's media vary considerably under the conditions of ordinary laboratory cultivation, and by suitable manipulation of the culture media they can readily be made to vary very greatly.

2. The results obtained entirely oppose the view that these reactions afford a means of distinguishing fixed and definite varieties among streptococci isolated from the human subject.

3. Such differences as are observed are of a temporary and accidental character, and are not in any sense specific, though they may perhaps afford some evidence of the natural habitat or previous environment of the organisms concerned.

REFERENCES.

1. M. H. Gordon, "Report on some Characters by which various Streptococci and Staphylococci may be Differentiated and Identified," 'Reports Med. Officer Local Gov. Board,' 1905, p. 388.
2. A. C. Houston, "Report on the Bacteriological Examination of (1) the Normal Stools of Healthy Persons; (2) the Intestinal Contents of Sea-fowl and Fish; and (3) Certain of our Public Water Supplies," 'Reports Med. Officer Local Gov. Board,' 1905, p. 472.
3. Andrews and Horder, "A Study of the Streptococci pathogenic for Man," 'Lancet,' 1906, II, pp. 708, 775, 852.
4. Marmorek, "Die Arteinheit der für den Menschen pathogenen Streptokokken," 'Berliner Klin. Wochenschr.,' 1902, vol. 39, p. 299.
5. Ainley Walker and Ryffel, "The Pathology of Acute Rheumatism and Allied Conditions," 'Brit. Med. Journ.,' 1903, II, p. 659.
6. Dreyer and FitzGerald, "The Unreliability of the Neutral Red Method as generally employed for the Differentiation of *B. typhosus* and *B. coli*," 'Festskrift to Celebrate the Inauguration of the Danish State Serum Institute, Copenhagen,' 1902.
7. Twort, "The Fermentation of Glucosides by Bacteria of the Typhoid-coli Group and the Acquisition of New Fermenting Powers by *B. dysenteriæ* and other Micro-organisms," 'Roy. Soc. Proc.,' 1907, B, vol. 79, p. 329.

8. Penfold, "Variations of the Fermentation Properties of the *B. typhosus*," 'Brit. Med. Journ.,' 1910, II, p. 1672.
9. Ainley Walker, "Immunisation against Immune-serum," 'Journ. Pathol. and Bacteriol.,' 1903, vol. 8, p. 34.
10. Almquist, "Neue Entwickelungsformen des Choleraspirills und der Typhusbakterie," 'Centralbl. f. Bakt. Parasitenk. u. Infektionskrankheiten,' 1904, Originale 37, p. 18; "Cultur von pathogenen Bakterien in Düngerstoffen," 'Zeitschr. f. Hygiene und Infektionskrankheiten,' 1905, vol. 52, p. 179.
11. Murray and Ainley Walker, "The Effect of Certain Dyes upon the Cultural Characters of the *B. typhosus* and some other Micro-organisms," 'Brit. Med. Journ.,' 1904, vol. 2, p. 16.
12. C. O. Jensen, "Adskillelse og Gruppering of nærstaaende Bakterieformer ved Forhold til kemiske Forbindelser," 'Bulletin de l'Académie Royale des Sciences et des Lettres de Danemark,' 1910, No. 2, p. 155.