THE RECOGNITION OF THE CHOLERA VIBRIO.*[1]

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Curved organisms resembling in many ways the cholera vibrio of Koch are known to occur widely in nature, chiefly as saprophytes externally, and also incidentally in one or more of the fluid secretions of the body. It is only during the prevalence of cholera epidemics that the presence of a comma-shaped organism in the intestinal contents of a person sick with an acute diarrhea may be regarded upon morphology alone as strong presumptive evidence of an infection by the specific vibrio.

THE CHOLERA-LIKE VIBRIOS.

Saprophytic cholera-like vibrios have been found in healthy persons from infected localities, and in those suffering from diseases other than cholera, and in the waters of rivers and wells of cholera-infected districts. The morphology and cultural appearances of these vibrios at times closely approximate those of the cholera organism, differences being usually of degree only; so that we may say with the exception of the negative reaction with anticholera sera, the cholera and non-cholera vibrios appear closely related to a remarkable extent. For this reason the gross appearances, which originally were considered by Koch so distinctive as to make possible a definite diagnosis of cholera alone, have been found to be the characteristic features of a large class of bacteria, many members of which have been found to be able to sustain existence in the fluids of the body as well as to lead a saprophytic life outside.

As far as we know, the cholera-like vibrios are non-pathogenic to man, but how far it is justifiable to assume that all non-aggluti-

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nating vibrios are invariably of a permanent harmless saprophytic type has been for some time a question of conjecture, especially in those instances where cholera cases have occurred in the vicinity within a recent period of time, and where infection by an attenuated variety of the cholera vibrio cannot be ruled out altogether as a possibility. It is certain that wherever cholera cases have occurred proper search has revealed the concurrent existence of the non-agglutinating vibrios.

CHOLERA-LIKE VIBRIOS IN CHOLERA EPIDEMICS.

Gottschlich, at Tor in Egypt in 1905–1906, isolated 48 non-agglutinating vibrios from cases of acute dysentery; and in 1911 on examining 1,160 pilgrims he found 31 cholera bacillus carriers and 23 carriers of non-agglutinating vibrios.

MacLaughlin,[2] at Manila in 1908, made a bacteriological examination of 376 cholera contacts, and from them isolated 27 cholera vibrios, and 46 non-agglutinating cholera-like vibrios.

The presence of non-agglutinating vibrios was particularly remarked during the months preceding the cholera epidemic in St. Petersburg in 1910, during which time cases of gastro-enteritis occurred with great frequency, and from the dejecta of some of the patients vibrios were isolated which did not agglutinate with specific anticholera sera and which had therefore to be classed as of a non-cholera nature. The etiological relation between the cholera-like organisms and the concurrent disease, although significant, was not regarded as definitely established.

VARIATIONS IN CHOLERA STRAINS.

It has been frequently noted that the Asiatic vibrio possesses to a marked degree the tendency of the group to undergo polymorphism, the development of atypical forms, which may occur in recently isolated strains from cholera cases and carriers, as well as in old laboratory cultures. Notable differences in morphology have been observed in cholera vibrios isolated from different cholera cases in the same epidemic.

During the epidemic in St. Petersburg in 1909–1910, Horowitz[3] noticed that the morphology of the cholera vibrios was not constant, and that under varying conditions the same vibrio could present such divergences from type as to become unrecognizable. The tendency to modification from type occurs also in the cultural properties which have been found to vary considerably in different

[2] MacLaughlin, *Philippine Hosp. Rep.*, 1912, xxvii, 381.
[3] Horowitz, A., *Bull. de l'Inst. Pasteur*, 1911, ix, 786.

strains, although some of these may persist more than others. The ability to liquefy gelatin, for instance, is generally present in the cholera vibrio, as it is in most of the cholera-like vibrios.[4] Virulence upon animals is not a constant property and may be altogether absent in recently isolated typical cultures from cholera cases.

Zlatogoroff[5] states that the virulence to guinea pigs of some non-agglutinating vibrios exceeds that of the cholera vibrio. The indol reaction has proved to be so uncertain that it is of little value in a final differentiation. Liefmann and Nieter[6] and others have shown that cholera vibrios possess hemolytic action upon blood media, as well as do the other members of this class of bacteria.

SERUM DIAGNOSIS.

It is by reason of these resemblances in form and culture between the cholera and cholera-like vibrios, that certain biological properties have become of importance for the purpose of diagnosis, and by means of these it is generally assumed that we have been able to recognize fundamental differences in the behavior of the typical Asiatic organism and other members of the same group.

It is well known that the presence of the specific vibrio in the body of cholera patients and of highly immunized animals causes strong activation of those tissues which bring about the formation of cellular antibodies, leading to the phenomena of agglutination, bacteriolysis, complement deviation, and serum precipitation, when the specific antigen is added to the separated immune serum. Of all pathogenic organisms the cholera vibrio is the most susceptible to the action of serum antibodies, positive agglutination taking place in extreme dilutions of the antiserum (1 to 10,000 and 1 to 40,000); and similarly with a strong bactericidal serum, bacteriolysis may be observed by Bordet's method *in vitro* in high dilutions, or *in vivo* by means of Pfeiffer's method. The reactions with the anticholera serum may be said to be specific for the cholera vibrio, and it has been considered that when we are dealing with a typical cholera culture these tests enable us to come to a definite conclusion as to whether or not we have the specific organism or a cholera-like vibrio before us. By the serum reactions, epidemic vibrios have been divided into two classes: the first includes those which agglutinate

[4] Craster, C. V., *Jour. Infect. Dis.*, 1913, xii, 472.
[5] Zlatogoroff, S. J., *Centralbl. f. Bakteriol., 1te Abt., Orig.*, 1908, xlviii, 684.
[6] Liefmann, H., and Nieter, A., *Med. Klin.*, 1906, ii, 254.

with a specific serum in high dilution, give a positive Pfeiffer reaction, and are capable of producing in immunized animals a serum which will agglutinate in a specific manner other cholera cultures (cross agglutinations); the second class includes all the cholera-like vibrios which give a negative agglutination with anticholera sera, or agglutinate in low dilution only 1 to 10 and 1 to 50, and in which the Pfeiffer reaction and cross agglutination are generally negative. The existence of a third class presumes a condition of the cholera vibrio in which certain biological properties have become latent.

THE DEVELOPMENT OF AGGLUTINATION IN CHOLERA-LIKE VIBRIOS.

The virulence of cholera cultures is known to lapse with long continued cultivation, and agglutination power to become considerably modified under the same condition, unless maintained by repeated animal inoculation.

The possibility of a temporary disappearance of the agglutinating power of the cholera vibrio under unusual conditions of environment and association with other bacteria, outside the animal body, and its subsequent reëstablishment by cultural and inoculation methods was first suggested by Zlatogoroff.[7] In the course of a study of eighteen different strains of non-agglutinating vibrios, isolated from the water of rivers and wells during a cholera epidemic in Saratow in 1908, he noted, after a month's culture upon ordinary laboratory media, a spontaneous development of agglutination with anticholera sera on the part of several of the vibrio cultures, which had previously given a negative reaction. Suspecting from this occurrence that with the remainder he might possibly have to deal with cholera vibrios that had become more or less changed by environmental conditions, he originated the following procedures for influencing the return of agglutinating properties in cultures of non-agglutinating vibrios: (1) repeated daily cultivation upon fresh alkaline beef agar with alternate incubation at 37° C. and cool storage at 16° to 18° C; (2) weekly passage through the peritoneal cavity of a series of guinea pigs.

Because of the slight virulence of the majority of the water vibrios, it became necessary to increase this property before successful passage through animals could be carried out, and this was accomplished by injecting with the vibrio cultures a certain quantity of dead typhoid bacilli, colon bacilli, or streptococci. The result of this treatment was that at the end of fifty-four generations, out of the eighteen vibrio strains which did not previously agglutinate ten became possessed of full agglutinating properties with an anticholera serum, in dilution of 1:10,000 and 1:20,000, a very high agglutinating titer. To establish further the cholera nature of his vibrios Zlatogoroff controlled his results by means of the complement fixation test of Bordet, in each case obtaining a con-

[7] Zlatogoroff, S. J., *loc. cit.*

firmation of the positive results with the agglutination reaction. As all these vibrio strains which eventually developed specific agglutination were isolated from the water of rivers and wells in Saratow, Zlatogoroff looked upon them as true cholera vibrios which had become weakened by a direct passage out of the agglutinogen from the bodies of the vibrios into the water, due either to the phenomenon of osmosis or to the action of the chemical constituents of the water upon the external capsule of the organism, changing in this way their biological reaction from a parasitic to a saprophytic type. To test further the theory of the loss of agglutination power of vibrios in water Zlatogoroff allowed a suspension of the cholera vibrio to remain in distilled water for seven days at room temperature. After centrifugalization and washing several times in distilled water, the agglutinating titer was found to have fallen from 1 : 5,000 to 1 : 1,000, and further treatment on these lines reduced the agglutination titer as low as 1 : 300. Barrenscheen,[8] repeating the experiments of Zlatogoroff, cultivated the cholera vibrio, which normally agglutinated in a dilution of 1 : 40,000, for eight days in distilled water, and found the agglutination titer reduced to 1 : 2,000, and after seven days more to 1 : 200. Both Zlatogoroff and Barrenscheen noted that when the cholera vibrio was kept for some days in distilled water there occurred a passing out of a substance (agglutinogen) from the bacterial bodies, which was shown by the appearance of an opalescence and later a flocculent precipitate in the centrifugalized culture liquid, after the addition of cholera immune serum. Horowitz[9] states that about 4 per cent. of vibrios isolated from actual cholera sources eventually regained agglutinating power, either spontaneously or with the use of Metchnikoff's method by cultural symbiosis with the yellow sarcinæ. In confirming the cholera nature of the vibrios by cross agglutinations, Horowitz found that the antisera from animals immunized against the same vibrios did not give the same reactions with different strains of cholera vibrios. At the same time some vibrios which gave a negative agglutination with an anticholera serum were able to produce in animals a serum which had a strong agglutinating reaction upon typical cholera cultures. In the case of other vibrios isolated from the stools of cholera convalescents the cross agglutination was negative at first, but later the appearance of a positive reaction with the specific serum and the Pfeiffer phenomenon proved their cholera nature. Horowitz concludes that among the cholera vibrios were certain strains presenting biological differences which evidently indicate some form of evolutionary change brought about by the reaction of the living cell to its altered environment. Carapelle,[10] in examining a series of sixteen vibrio cultures isolated from water in and around the city of Palermo during the cholera epidemic of 1911, in seven of which direct fecal infection of the water was evident, noted that the specific agglutination was negative or only positive in extremely low dilution of the antiserum, although the bacteriolytic reaction *in vitro* and complement deviation tests pointed to their cholera nature. Twelve cases of cholera occurring at this time in a hospital which had been carefully guarded against outside infection, and from the household water of which non-agglutinating

[8] Barrenscheen, H., *Centralbl. f. Bakteriol., 1te Abt., Orig.*, 1909, 1, 261.

[9] Horowitz, A., *loc. cit.*

[10] Carapelle, *Ann. d'ig. sper.*, 1912, xxii, 497.

vibrios had been isolated, caused this observer to suspect that these vibrios might be of a cholera nature. He accordingly subjected the non-agglutinating vibrios to the procedures suggested by Zlatogoroff, and by passage through a number of guinea pigs alone a high agglutinating titer was developed in all the vibrio cultures (1:4,000). The virulence was also greatly enhanced and bacteriolysis *in vitro* became much more complete. By immunizing animals with the vibrio cultures he was able to obtain antisera which agglutinated typical cholera vibrios in maximum dilution. Carapelle thus corroborates the conclusions of Zlatogoroff that non-agglutinating vibrios obtained from water and subsequently developing agglutination with anticholera sera were really true cholera vibrios which had temporarily lost this property. In order to prove his conclusions of the loss of agglutination in water he exposed suspensions of cholera vibrios in sterile water contained in Berkefeld candles to the action of running water so that the water could wash the vibrios in suspension. After twenty-two days the agglutinating power of the cholera vibrios contained in the candles had been reduced to 1:100.

In a former communication[11] I described the properties of a number of cholera-like vibrios which were isolated from rectal swab cultures during a cholera outbreak at Quarantine, N. Y., in 1911.[12] All were obtained from persons free from any disease, the majority having been isolated some weeks after cases of cholera had ceased to arrive in the port. About 15 per cent. gave a modified positive indol reaction, and the hemolytic power upon blood agar was found to be variable for each strain. At that time the morphological and cultural differences discernible between the cholera and cholera-like vibrios were in many instances so slight that little importance could be attached to them for the purpose of differential diagnosis.

In the preliminary examination for specific agglutination, although negative results were obtained in moderately high dilutions of the antiserum, there were revealed at the time considerable differences in the reactions with many of the vibrio cultures, the differences varying from a complete negative result at any dilution to one showing moderate clumping at dilutions much below that of a typical cholera culture. This was looked upon at the time as due to the presence of group affinities. The Pfeiffer reactions carried out with the cholera-like cultures were found to be negative, and a series of cross agglutination tests with the cholera vibrio and antisera obtained by immunizing rabbits with cultures of the non-agglutinating vibrio gave unsatisfactory results on account of the low titer of the antisera produced.[13]

Some time later when the vibrio cultures had been kept upon laboratory media for several months an examination of the agglutination power showed that three of the cultures which had formerly agglutinated only in very low dilution of the antiserum now gave a positive reaction in slightly greater dilution (1 to 100). This re-

[11] Craster, *Jour. Infect. Dis., loc. cit.*

[12] Craster, *Jour. Am. Med. Assn.*, 1913, lxi, 2210.

[13] Craster, *Jour. Infect. Dis., loc. cit.*

sult, although not high enough to be looked upon as a specific agglutination, suggested the advantage of attempting to increase this property in the cultures and in others in which agglutinations had not so developed, upon the lines pointed out by Zlatogoroff and Carapelle. For this purpose fourteen vibrio cultures, including the three agglutinating strains, were selected. Table I shows that the

TABLE I.

Properties of Cholera-Like Vibrios.

No. of vibrio culture.	Gelatin liquefaction.	Fermentation.				Hemolysis.	Indol reaction.	Pathogenicity for guinea pigs.
		Saccharose.	Glucose.	Lactose.	Maltose.			
98	+	+	+	−	+	−	+	−
103	+	+	+	−	+	+	+	−
109	+	+	+	+	+	+	−	−
125	+	+	+	−	+	−	−	−
151	+	+	+	−	−	+	−	−
219	+	+	+	+	+	+	+	−
269[14]	+	+	+	−	+	+	−	−
899	+	+	+	−	+	+	−	−
2464[14]	+	+	+	−	+	−	−	−
3064	+	+	+	+	+	−	−	−
3832	+	+	+	−	−	+	+	−
5947	+	+	+	+	+	+	−	−
5999	+	+	+	+	+	−	−	−
6061	+	+	+	−	+	+	+	−

Gelatin liquefaction after 72 hours. Hemolysis on rabbit blood agar. Pathogenicity tested with one whole agar slant culture upon a guinea pig weighing 250 gm.

cultural properties differ only slightly; hemolysis in rabbit blood agar is generally positive, the indol reaction is positive in a few cases, and pathogenicity to guinea pigs is absent in doses of one agar slant. The Pfeiffer reaction was negative for all.

At the commencement of experimental work vibrio cultures 103, 899, and 5,999 showed a positive agglutination with specific anticholera serum in dilution of 1 to 100 (table II). Cultures 151, 269, and 3,832 gave a similar reaction in dilutions of 1 to 40, and cultures 98 and 219, in dilutions of 1 to 20 only. The virulence being extremely low, special measures had to be taken to insure successful animal passage. This was attained in some cases by inject-

[14] Pigment former.

TABLE II.

Agglutination of Cholera-Like Vibrios with Anticholera Serum and Pfeiffer Reaction at Commencement of Experimental Work.

No. of vibrio culture.	Normal saline.	Normal horse serum.		Anticholera serum.					Pfeiffer test.
		1:10	1:40	1:20	1:40	1:100	1:500	1:1,000	
98	−	−	−	+	−	−	−	−	−
103	−	+	−	+	+	+	−	−	−
109	−	−	−	−	−	−	−	−	−
125	−	+	−	−	−	−	−	−	−
151	−	+	−	+	+	−	−	−	−
219	−	−	−	+	−	−	−	−	−
269	−	+	−	+	+	−	−	−	−
899	−	−	−	+	+	+	−	−	−
2464	−	−	−	−	−	−	−	−	−
3064	−	−	−	−	−	−	−	−	−
3832	−	+	−	+	+	−	−	−	−
5947	−	+	−	−	−	−	−	−	−
5999	−	−	−	+	+	+	−	−	−
6061	−	−	−	−	−	−	−	−	−

Nos. 103, 899, and 5,999 developed agglutination in 1:100 dilution of antiserum, in laboratory cultivation after isolation.

ing two or more agar slant cultures emulsified in normal saline at one time into the peritoneal cavity of a guinea pig weighing 250 grams. When this method failed cultures of dead typhoid bacilli or *Bacillus coli* were injected at the same time.

After serial passage through five guinea pigs, and daily cultivation upon fresh alkaline agar for seventy generations, considerable increase in the agglutinating power was observed to have taken place (table III). Of the three vibrio cultures formerly agglutinating in serum dilution of 1 to 100, Nos. 899 and 5,999 showed positive results in serum dilution 1 to 2,000, and No. 103 in a dilution of 1 to 1,000.

Vibrios 269 and 3,832 increased in agglutination from dilutions of 1 to 40 to 1 to 100; and cultures 219 and 98 from 1 to 20 to 1 to 400 and 1 to 100, respectively. The response of the agglutinating power to intensive cultivation and animal passage was progressive, although the limit of agglutination was reached in some instances by the second or third animal passage, further inoculation producing no increase in this property.

TABLE III.

Agglutination of Cholera-Like Vibrios with Anticholera Serum and Pfeiffer and Bordet Reactions after Intensive Cultivation and Passage through Guinea Pigs.

No. of vibrio culture.	Normal horse serum.		Anticholera serum.						Pfeiffer test.	Bordet test.
	1:10	1:40	1:20	1:40	1:100	1:400	1:1,000	1:2,000		
98	−	−	+	+	+	−	−	−	−	−
103	+	−	+	+	+	+	+	−	−	+
109	−	−	−	−	−	−	−	−	−	−
125	+	−	−	−	−	−	−	−	−	−
151	+	−	+	+	−	−	−	−	−	−
219	−	−	+	+	+	+	−	−	−	+
269	+	−	+	+	+	−	−	−	−	−
899	−	−	+	+	+	+	+	+	−	+
2464	−	−	−	−	−	−	−	−	−	−
3064	−	−	−	−	−	−	−	−	−	−
3832	+	−	+	+	+	−	−	−	−	−
5947	+	−	−	−	−	−	−	−	−	−
5999	−	−	+	+	+	+	+	+	−	+
6061	−	−	−	−	−	−	−	−	−	−

The virulence upon animals had not been increased to any great extent by these methods. The most virulent culture, No. 5,999, became pathogenic to a guinea pig weighing 250 grams in doses of one platinum loop of a twenty-four hour agar-grown culture, and the others required larger doses than this to produce death of a similar sized guinea pig in twenty-four hours. To this absence of high virulence must be ascribed the failure to give a positive Pfeiffer reaction which was negative for all the vibrio cultures at the end of the period of special treatment.

For this reason the effect of an anticholera bacteriolytic serum upon the agglutinating vibrio cultures was tested *in vitro* by the method of Bordet.[15] The specific bacteriolytic serum was diluted so that when added to a mixture of the bacterial emulsion and fresh guinea pig serum to provide alexin (complement), dilutions of the specific serum of 1 to 200, 1 to 500, and 1 to 1,000 were obtained. Controls were made with the same quantities of the bacterial emulsion and alexin, but without the specific serum.

Hanging drop preparations of these mixtures were made, and they were incubated at 37° C. and examined in two to three hours.

[15] Bordet, J., *Ann. de l'Inst. Pasteur,* 1895, ix, 462.

A positive result showed complete lysis of the vibrios with the immune serum; the controls showed actively motile organisms. If the results after this time were incomplete, a further examination was made in eighteen to twenty-four hours at 37° C.

By this method positive bacteriolysis was observed to occur with cultures 103, 219, 899, and 5,999 (table III) in serum dilution of 1 to 1,000. The remaining vibrio cultures gave negative results even in low dilutions of the serum (1 to 200). Control hanging drop tests with known cholera vibrio cultures were carried out at the same time.

If we consider the results obtained by these methods, it will be seen that certain vibrio cultures which formerly gave so slight an agglutination as to be considered negative, later reacted in a specific manner with an anticholera serum. Similarly a strong bactericidal serum exerts upon them a bacteriolytic power seen only in the case of true cholera vibrios. If it cannot be said definitely that these vibrio cultures are really cholera, we are at least justified in strongly suspecting their cholera nature until other means of proving their true character are found.

Upon this hypothesis it may be agreed that the cholera vibrio in the process of attenuation in the intestinal contents at times loses simultaneously with its ability to produce the disease the power of responding to circulating agglutinins. The loss of agglutination may be due to the production by the bacterial cell of a defensive ferment of an anti-agglutinating nature, probably a necessary function for continued existence in hostile body fluids. Upon removal to an environment away from these serum antibodies the necessity for the formation of defensive substances ceases to exist, and agglutination tendencies slowly return.

Experience has shown that the exact value to be placed upon tests depending for their accuracy upon the possession of biological properties rests upon the appreciation of the margin of error which must be allowed for in their proper manipulation. It is well understood that biological properties are not always constantly manifest and are subject to variation in the same bacterial strain,—such changes being dependent upon conditions of environment, such as cultural media and the presence of other bacteria. Changes of this

character are well exemplified in the modifications observed as taking place in the agglutinating property of *Bacillus typhosus,* an organism which is peculiarly susceptible to the action of agglutinating sera. Many observers have noted that some varieties of this bacillus after isolation from typhoid stools or from infected water have shown at first little or no tendency to agglutinate with an antityphoid serum, but that further cultivation upon laboratory media brings about strong agglutination properties. *Bacillus typhosus* loses its agglutinating property rapidly in water, but it may be restored by cultivation in suitable media. Gay and Claypole[16] have noted variations in the agglutinability of freshly isolated typhoid bacilli, especially the frequent failure of typhoid blood cultures to clump with the antiserum from an animal immunized by means of agar-grown typhoid cultures. A peculiar deviation of agglutination was induced by growing the agar-cultivated organism upon blood agar for two or three generations. These cultures would then agglutinate with the serum from an animal immunized against blood media cultures but not against that of an animal immunized against agar-grown cultures. It is clear then that in the case of *Bacillus typhosus* the property of agglutination with a specific serum may be absent under natural conditions in the stools of typhoid patients, or, if present, it may be modified by growth in different media.

The agglutination property of the cholera vibrio has also been found to become considerably modified or lost under experimental conditions when cultivated upon a medium composed of bouillon and anticholera serum,[17] and the same result may be brought about by the use of earth and water media.[18] Conversely non-agglutinating vibrios if subjected to cultural symbiosis with sarcinæ may develop at times specific agglutination with an anticholera serum.[19]

Great variation in the agglutination of cholera cultures was observed by Pottevin[20] in a study of 127 vibrio cultures isolated at Tor in Egypt in 1912–1913. The agglutination power of many of the

[16] Gay, F. P., and Claypole, E. J., *Jour. Am. Med. Assn.,* 1913, lx, 1141.
[17] Ransom and Kitashima, cited by Zlatogoroff, S. J., *loc. cit.,* p. 694.
[18] Puntoni, V., *Policlinico, sez. med.,* 1913, xx, 385.
[19] Puntoni, V., *Gior. d. r. soc. ital. d'ig.,* 1913, xxxv, 289.
[20] Pottevin, *Bulletin de l'office internationale d'hygiene publique,* 1913, v, 1158.

cultures was seen to disappear or become latent in cultivation, and non-agglutinating vibrios at times apparently spontaneously developed specific agglutination some time after isolation. This observer further states that agglutinating and non-agglutinating vibrios could exist at the same time in one patient.

SUMMARY.

Cholera-like non-agglutinating vibrios are invariably found in the intestinal contents of healthy persons, and frequently in the water of wells and rivers, during epidemics of cholera. Although many of these saprophytic vibrios are indistinguishable in morphology and cultural properties from the cholera vibrio, the negative reaction with an anticholera serum has readily differentiated them from the Asiatic vibrio. The biological polymorphism of the cholera vibrio has been suggested by the development of agglutination, by special methods of culture, in cholera-like vibrios. Confirmatory Pfeiffer reactions have not been obtained, as a rule, in these instances, probably because of the low virulence of the vibrio culture, although positive bacteriolysis *in vitro* (Bordet's test) was observed in some, and in others positive complement fixation and cross agglutination indicated the cholera nature of the vibrios in question.

Although it cannot as yet be definitely proven, we are justified in suspecting that cholera-like vibrios which eventually develop agglutination properties are of a true cholera nature. It is probable that the production of agglutination antibodies in the serum brings about the development by the bacterial cell of defensive anti-agglutinins, resulting in the disappearance of agglutinating power. In the case of the water vibrios, changed physical conditions could bring about a similar alteration in biological properties.

It may be said that the absence of agglutination in a vibrio isolated from a suspected source does not define conclusively its non-cholera nature. In all probability among a number of cholera-like vibrios isolated from suspected sources a certain percentage will eventually be found to develop agglutination either during laboratory cultivation or by means of animal passage, and until subjected to a procedure that will induce the return of agglutination no vibrio can be regarded with assurance as of a truly saprophytic variety.