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## THE PROPERTIES AND AGGLUTINATIONS OF SOME NON-PATHOGENIC VIBRIOS.\*

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In routine examinations for cholera many observers have noted the occurrence of certain organisms morphologically resembling the cholera vibrio, but differing from it in negative agglutination with an anticholera serum, and in the slight or non-pathogenicity to animals. They are usually found in individuals free from any symptoms of disease, and do not give rise to any known lesion in man. These aberrant types are known as the non-cholera vibrios, and their importance rests not so much upon any intrinsic properties of their own as in the fact of their association with the cholera organism in outbreaks of that disease.

During the routine examination carried out at Quarantine, N.Y., in 1911, a great number (over 100) of the non-cholera vibrios were found. With one exception those were all obtained from individuals in good health, the exception being one case in which mild choleraic symptoms were present, but the cholera vibrio was not isolated at the same time. A somewhat suggestive incident noted was that the passengers from one ship, among whom two cholera carriers were detected, also provided the greatest number of non-cholera vibrios isolated at one time.

A certain significance attaches to this event from the point of view both of some relationship between the cholera and the non-cholera vibrios, and of a possible predisposing action upon the intestinal mucosa, the latter a factor which might influence the development of an attack by the cholera vibrio. There is, however, no direct evidence of any such predisposing action at our disposal.

The presence of this type of vibrio in persons from infected ports and from ships upon which cholera carriers were found was in itself a reason to regard them with peculiar interest.

\*Received for publication March 17, 1913.

## MORPHOLOGY.

In describing the morphology of the non-cholera forms isolated at Quarantine, it may be said that the greater number resembled the cholera organism so closely that a separate description of them would be difficult, yet others presented slight or striking peculiarities, which were of interest as negative evidence upon a suspected organism.

For comparison the cholera organism may be described as crescent shaped, broader at the center, and with blunt ends, and from 1.5-2.  $\mu$  in length. The non-cholera vibrios often showed many departures from this type. These differences were frequently of size, and particularly noticeable were long forms, up to 8  $\mu$  in length with blunt ends, and short forms of extreme curvature with pointed ends. These atypical forms in size and shape are so characteristic of the non-pathogenic vibrios that their occurrence in a smear from a fresh culture may well give grounds for the belief that the observer is not dealing with the organism of cholera. At the same time it must not be forgotten that a number of the non-pathogenic vibrios resemble the cholera organism so closely that no difference can be discerned in microscopical examination. The cholera vibrio is very liable to heteromorphism when grown upon new or unsuitable media. Since the new forms thus produced are not of a permanent character, however, replanting upon suitable media will transform them again into the usual shape. These atypical non-cholera vibrios do not show any tendency to conversion to the cholera shape when grown on ordinary media.

## MOTILITY.

Koch, in his original account of the cholera vibrio, emphasized its characteristic motility, and indeed in no other organism but these with the terminal flagella is seen the extraordinary lightning-like darting to and fro as in this type of spirillum. The non-pathogenic vibrios isolated at Quarantine were found to be monoflagellates, and their motility was typically cholera-like.

## CULTURAL CHARACTERS.

Culturally they resemble the cholera vibrio very closely. In alkaline broth and peptone, clouding occurs through the medium with

more or less pellicle formation. In some the presence of floccules is observable. In gelatin stabs, liquefaction occurred with all, forming bubble or stocking-shaped liquefaction. The rapidity varied with each strain, this being frequently faster than that of the cholera vibrio.

On blood agar, hemolytic power was present to a variable extent with the cultures tested, differing in this way markedly from the cholera vibrio.

The fermentation of sugars was in the main similar to that of the cholera vibrio. Saccharose, glucose, and maltose were fermented freely, while lactose was not so generally acted upon (30 per cent).

The indol reaction was not present to any extent, although some gave a delayed color. In no instance was a typical cholera-red obtained with any non-cholera vibrio isolated at Quarantine.

On alkalin agar plates the colonies of the non-cholera vibrios show certain differences from those of cholera. As a rule they appear more globular, and not so flat, are more granular in substance, and with more coarse granules in the centers. The cholera colony is moist and when touched with a platinum needle tends to flow. The non-cholera colony, on the other hand, has not so much this appearance of fluidity, and tends to roll up when touched with the needle. The fecal odor so generally found in cholera cultures is not so pronounced in those of the non-cholera type. In describing the colonies of the non-cholera vibrios, a hard-and-fast line dividing them from the cholera organism cannot be drawn. Some may differ in one or more, or all of the specific characters described above.

Besides this cultural similarity to the organisms of cholera, there were also some striking special properties. Three vibrio strains showed considerable pigment formation and another was a gas former. The latter was of interest, gas formation not being a characteristic of any other member of the cholera-like group isolated at Quarantine.

#### PATHOGENESIS.

The pathogenicity to animals of the non-cholera vibrios isolated during various epidemics has differed widely. The pathogenicity

of our non-cholera vibrios was tested by simple feeding and with Pfeiffer's peritoneal inoculation test with living cultures upon guinea-pigs and rabbits. No pathogenic power was demonstrated, even though large doses up to a whole agar culture at one time were given to a 200-gram guinea-pig.

#### AGGLUTINATION.

In the tests for the differential agglutination of the cholera and non-cholera vibrios, a serum of high agglutinating power was used. The cholera vibrio is particularly sensitive to the action of a strong immune serum and will agglutinate in serum dilution up to 1-10,000. The non-cholera vibrios showed a slight tendency to agglutinate with the anticholera serum only at very low dilution, 1-10, and so forth, but none at higher dilutions. A difference was also observed in the reactions with an anticholera human and anticholera horse serum. A good agglutination with a serum dilution of 1-200 was considered a positive reaction for the cholera vibrio. For purposes of differential diagnosis, no matter what cultural or morphological resemblances there were to the cholera vibrio, a negative agglutination with the antiserum absolutely ruled out any vibrio from the cholera class. In earlier times a microscopical finding was considered sufficient evidence upon which to base a positive decision for cholera if the special cultural tests were also positive. The recognition of the existence of the non-cholera vibrios has made such a means of diagnosis of tentative value only, reliance being placed now mainly upon a positive agglutination with an anticholera serum.

The morphological and cultural resemblances between these various vibrio strains suggested the possibility of a relationship between them. It was thought that some light could be thrown upon the question by preparing immune sera from a number of them and testing each vibrio strain with the heterologous agglutinating sera. The preparation of antisera from a great number would have been a long and laborious procedure, so that to bring the attempt within reasonable bounds, from 20 laboratory cultures showing good growth and morphological characters, rabbit agglutinating sera were prepared. A preliminary examination of the normal serum

from each rabbit did not disclose the presence of any agglutinating power upon the vibrio cultures used. The technic employed in the preparation of the immune serum was modified somewhat from the usual method. It was found that four weekly inoculations with three dead cultures and one living one in increasing doses did not produce a serum with any degree of agglutinating power. So that four further intraperitoneal, immunizing doses with living cultures were given at weekly intervals. This yielded a better specific serum, but even this was of low titer. Some degree of agglutination was obtained in dilution of 1-80, a better working strength

TABLE I.  
AGGLUTINATION TABLE.

Anti-sera	Vibrio Cultures																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.....	+	-	+	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-
2.....	+	+	+	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-	-	-
3.....	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
4.....	-	-	-	+	+	-	+	-	-	-	-	-	+	+	-	+	-	-	-	-
5.....	-	-	-	+	-	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-
6.....	-	-	-	+	-	+	+	+	-	-	-	-	-	+	-	+	-	-	-	-
7.....	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
8.....	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
9.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
10.....	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+
11.....	-	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+
12.....	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+
13.....	-	-	-	+	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-
14.....	-	-	-	+	+	-	+	+	-	-	-	-	+	+	-	-	-	-	-	-
15.....	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
16.....	-	-	-	+	+	-	-	+	-	-	-	-	-	+	-	+	-	-	-	-
17.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-
18.....	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
19.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-
20.....	-	+	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+

+ = positive agglutination.

- = negative agglutination.

was found to be one of 1-40. This low agglutinating power of the antisera prepared from the non-cholera vibrios may be compared with the powerful agglutinating serum obtained by similar methods from the cholera vibrio. It would seem that the pathogenic organism is capable of producing a greater activation of the tissue cells of the body, and the corresponding response is a production of immune serum of high potency.

The agglutination tests with the immune sera are represented in Table I, and show a great number of cross-agglutinations with the vibrio cultures. All the antisera but one agglutinated not only their

own homologous cultures, but also cultures of other vibrio strains. The number of interagglutinations is variable for each serum; there may be as many as six or as few as one. A point of interest in these



FIG. 1.—Cholera vibrio from direct smear.  $\times 1000$ .

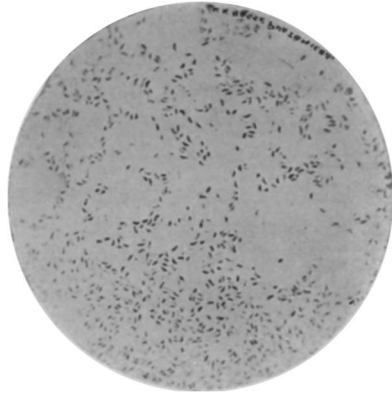


FIG. 2.—Cholera vibrio from 24-hour agar culture (Dr. J. Drennan).  $\times 1000$ .

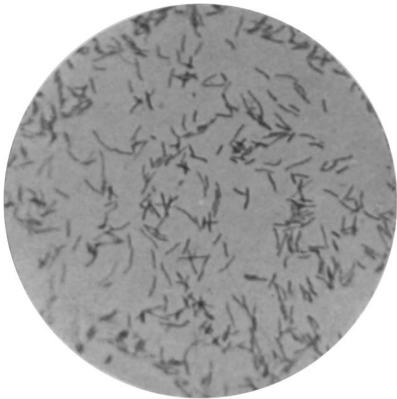


FIG. 3.—Non-cholera vibrio. Long form from 24-hour agar culture.  $\times 1000$ .

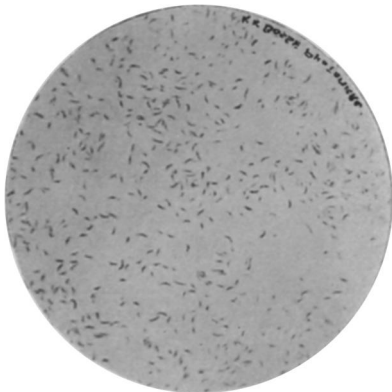


FIG. 4.—Non-cholera vibrio. Short form from 24-hour agar culture.  $\times 1000$ .

reactions is that although the majority are reciprocal in nature, this is not always a rule.

Out of 400 tests represented in Table 1, 75 are positive, made up of 19 homologous and 56 heterologous agglutinations. Thirty-six of the latter were reciprocal.

On careful observation of the table, the presence of certain group agglutinations is indicated by these results, and the various vibrios, by a kind of chain of positive interagglutinations, are seen to arrange themselves into groups, the members of which react only among themselves. Furthermore, the grouping, as indicated above, was accentuated by similar peculiar agglutinative reactions with the anticholera human and horse sera. One vibrio culture did not develop an immune serum of any strength in the animal used, although it was positively agglutinated by other heterologous sera.

It may be justifiable to assume from these results that in the great number of cholera-like non-pathogenic vibrios found we are dealing with the closely allied members of several large groups, and not a great number of separate species of organisms of this nature.

There naturally arises at this time the question of the probable relationship between the non-pathogenic vibrios isolated during cholera epidemics, and the cholera vibrio. From the morphological and cultural characters of some of them, evidences of near relationship could be deduced. Against these we have to place the negative agglutination with the specific immune serum, the non-pathogenicity to animals, and the hemolytic powers.

Some grounds for belief in the distinct individuality of these varieties was furnished by a series of tests with the cholera vibrio and the antisera prepared from the non-cholera cultures, for in every case a negative agglutination reaction was obtained.

As a causative factor of disease in man, the position of the non-cholera vibrios is still in doubt. They have been regarded by some authorities as aberrant or "transitional" forms of the cholera vibrio. Zlatogoroff<sup>1</sup> by his experiments has endeavored to prove that a cholera-like vibrio may acquire agglutinative powers by various methods of cultivation and passage through animals. He maintains that the vibrios found in association with the cholera vibrio may be attenuated varieties, capable at any time of taking on pathogenic powers. McLaughlin and Whitmore,<sup>2</sup> repeating Zlatogoroff's experiments with non-cholera vibrios found in the Philippines,

<sup>1</sup> *Centralbl. f. Bakteriol.*, I, Orig. 1908, 48, p. 684.

<sup>2</sup> *Philippine Jour. Sci.*, 1910, 5, p. 403.



state: "Our vibrios isolated from the human intestine, from water or other sources, which are negative to agglutination with anti-cholera serum, and classified as non-cholera, do not develop agglutinability to cholera serum when treated as directed by Zlatogoroff." The fact remains that the non-cholera vibrios do exist in the human body without the manifestation of any untoward symptoms. A similar rôle is played by the cholera organisms in the case of cholera carriers. The non-cholera organisms are not, however, totally devoid of pathogenic power, for under experimental conditions of lowered vitality, such as were produced by Koch<sup>1</sup> in his experiments upon animals with the non-pathogenic vibrios of Deneke and Miller, it was shown that a rapid and fatal illness with dissemination by the blood and a general septicemia could readily result.

TABLE 2.  
COMPARISON.

	Cholera Vibrio	Non-Cholera Vibrios
Morphology . . . . .	Sickle-shaped crescents, blunt ends 1.5-2 $\mu$	Same as cholera Frequently long forms 8 $\mu$ with blunt ends, and short forms with pointed ends
Motility . . . . .	Characteristic torpedo-like	Cholera-like
Cultural properties	Good growth on alkaline media; fecal odor	Frequently cholera-like (exceptions: pig- ment, and fecal odor sometimes absent, gas formation)
Pathogenicity . . . . .	Pathogenic to animals	Non-pathogenic to animals
Hemolysis . . . . .	Not hemolytic	Hemolytic powers variable
Sugar fermentation	Saccharose, etc., fermented	Saccharose, glucose, and maltose fermented; lactose sparingly
Gelatin liquefaction	Liquefies gelatin	Liquefies gelatin at various rates, but usually more rapidly
Indol reaction . . . . .	Constant with pure cultures	Absent or slight
Agglutinations . . . . .	Positive agglutination with anti- cholera serum Negative with non-cholera sera	Negative agglutination with anticholera serum. Heterologous agglutinations with non-cholera sera

SUMMARY.

The non-pathogenic vibrios are monoflagellate organisms at times found associated with the cholera vibrio in cholera outbreaks. They resemble morphologically the cholera organism, the exceptions being long forms with blunt ends, and short forms with pointed ends.

*Culturally* there are only slight differences from the cholera vibrio. These are noticeable in the colonies on agar plates, and

<sup>1</sup> *Ztschr. f. Hyg. u. Infektionskrankh.*, 1893, 14, p. 319.

in the absence of the indol reaction. The odor of the cultures is not constantly fecal in character. The action upon sugars is very similar to that of cholera. They have distinct hemolytic powers, and gelatin is readily liquefied. Two distinctive characters were observable. These were pigment formation, and gas in saccharose gelatin media. They are non-pathogenic to animals in even large doses, and show peculiar agglutinative reactions with antisera from other like vibrios, suggesting a measure of relationship between the various vibrio strains.

In carrying out this investigation I am indebted for valuable assistance to Dr. E. C. Baldwin, director of the Quarantine Laboratory.