VIBRIOS (VIBRIO JEJUNI, N.SP.) ASSOCIATED WITH INTESTINAL DISORDERS OF COWS AND CALVES

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PLATE 31

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Although many vibrios were described following the discovery of the cholera organism relatively few have been shown to be pathogenic for man or animals.

Gamaléia (1) obtained vibrios (*V. metchnikovi*) from the blood and intestines of fowls suffering from enteritis. MacFadyean and Stockman (2) succeeded in cultivating vibrios from abortion disease in sheep and succeeded in reproducing the disease by injection of pure cultures into pregnant ewes. Since then such organisms have been obtained from sheep by Carpenter (3), Welch and Marsh (4), and Graham and Throp (5). Theobald Smith (6) isolated from the diseased placentas and aborted fetuses of cows vibrios (*Vibrio fetus*) which on injection into pregnant cows produced placental disease and abortion. In a series of papers (7, 8, 9) the disease and the etiological agent were discussed. A similar organism had been recognized by McFadyean and Stockman (10) and has subsequently been found to affect cattle in various parts of the world.

In addition to the disease-producing type of vibrios, Smith and Orcutt (11) reported vibrios obtained from the livers of young calves which resembled *V. fetus* in morphological and cultural characters but failed to agglutinate with sera specific for *V. fetus*.

The Source of the Cultures

The vibrios in this study were obtained from several sources but all were of bovine origin. The first three, 1629, 1641, and 1655, presumably originated from cases of infectious diarrhea (12) experimentally reproduced by feeding calves feces from spontaneous cases. The second pair comprises Vibrios 1669 and 1686 which were cultivated from the intestinal tracts of calves fed Cultures 1629, 1641, and 1655. The third group is composed of Vibrios 1652, 1699, 1700, 1728, 1731,

1745, and 1748, all obtained from spontaneous enteritis of calves (13). The fourth series includes Cultures 1707, 1709, 1714, and 1721 from enteric cases in calves experimentally produced, and finally Cultures 1208, 1522, 1524, and 1679 kindly furnished to us by Dr. Smith. *Vibrio fetus* 1660 was also furnished by Dr. Smith.

Method of Obtaining Vibrios from the Intestinal Tract

Two methods have been employed throughout the work. The less reliable may be said to consist in the inoculation of the water of condensation of veal infusion agar, to which five drops of defibrinated horse blood have been added, directly with the intestinal content of inflamed segments of the intestine. Immediate inoculations from the primary culture to secondary and tertiary tubes of the same media with the sterile loop will at times give pure cultures in one tube but overgrowth of intestinal bacteria is apt to occur and vibrios fail to develop.

A more complicated procedure but one on the whole more satisfactory is the following:

The seared serosa is cut, the intestinal tube flattened, and the content gently brushed from the inflamed mucosa. Four or five bits of the superficial mucosa, 1 to 2 mm. in diameter, are removed and washed in five or more changes of sterile salt solution. The mucosa is then ground in a Hagan (14) grinder, a little broth added, and the suspension inoculated by means of a loop into the condensation fluid of blood agar. It is customary to set up three series of two tubes. The first tube of each pair receives 1, 2, and 3 loopfuls of suspension, and after flaming the loop the secondary tubes are inoculated with 1, 2, and 3 loopfuls of the condensation fluid from the primary cultures. All tubes are then sealed with sealing wax and incubated. After 3 or 4 days tubes which show relatively little growth are examined microscopically. Even in spite of prolonged washing in many instances *B. coli*, streptococci, etc., may develop in the primary inoculations, but the secondary tubes are apt to contain only vibrios. In some cases vibrios will be found only in the first tube and then mixed with other organisms from which they can with difficulty be separated. In other instances only vibrios develop in the first of the series and the secondary tubes may or may not contain them.

In early cultures relatively little is manifested in the gross in the blood agar tubes. The condensation fluid is slightly turbid within 4 or 5 days and the blood's buffy coat is thickened. Later delicate lines at the border of the agar become visible and after several transfers

the border lines become well defined and a delicate film may spread from the condensation fluid to the lower slant. After prolonged cultivation many strains grow readily on the surface of the slant. From blood agar, cultures may be established in plain agar or in leptospira medium to which a little fresh kidney has been added. After prolonged cultivation on plain agar growth may even be obtained in broth where a heavy tenacious mass is formed on the bottom of the tube.

Certain strains grow well from the start, others adapt themselves slowly. In serum agar shake cultures the growth is on the surface or in a narrow zone just beneath the surface. In leptospira medium the heaviest growth is just beneath the surface (Fig. 1). From these facts it may be inferred that the organisms are not anaerobes.

When some of the condensation fluid of young blood agar is examined by means of the hanging drop actively motile elements are readily detected but it is difficult to ascertain their morphology because of their extremely rapid movement. Three types are frequently visible in the same culture. Short, slightly convoluted forms (Figs. 2 and 3) are the most active, and in suitable preparations one or two flagella situated at the poles may be demonstrated (Figs. 3 and 4). Forms with two or more complete coils as illustrated in Figs. 5 and 6 move more slowly and revolve about the long axis. The extremely long type as encountered in Fig. 7 is rarely motile although the body appears to sway. As the cultures become older clumps occur and cellular fragmentation or granule formation is the rule. In Fig. 5 the edge of a clump of granules resulting from vibrionic degeneration is illustrated, and several reveal early degenerative changes, and in Fig. 7 an atypical granule formation is visible.

The cultures stain well with ordinary stains after a longer time than is usually required. In films prepared from tissues only prolonged staining seems to color them but in fresh, unstained preparations of tissue and exudate they may be readily recognized. They are Gram-negative. The optimum reaction for growth is about pH 7.6. The organisms fail to grow in either slightly acid or definitely alkaline media. Cultures soon die when dried in the air and a temperature of 55°C. for 5 minutes kills them. When autoclaved moist cow feces were inoculated with vibrios and stored in the room the vibrios lived

for 6 days but when the feces became thoroughly dried cultures could no longer be obtained. Distilled water is not especially toxic since actively motile forms may survive for 24 hours. The vibrios resist the soluble action of bile.

Pathogenicity for Laboratory Animals

In accompanying papers we have described (12, 13) our experiments dealing with the pathogenic properties of these organisms for cows and calves. It can be said that for laboratory animals the organisms possess little pathogenicity by the usual standards. Guinea pigs and white rats are refractory when injected intraperitoneally. White mice are more susceptible since two of the strains (1655 and 1686) when injected in small quantities intraperitoneally produced multiple necrotic foci of the liver from which the vibrios were cultivated. Other cultures failed to do so.

Strains 1629 and 1641 possessed no pathogenicity for rabbits, but others (1669, 1686, 1731) when freshly obtained and injected intravenously produced well marked febrile reactions and during this phase vibrios were readily cultivated from the peripheral blood and organs. After the fever declined there was established a catarrhal inflammation of the small intestine, at times accompanied by diarrhea. The vibrios were recovered from the inflamed intestinal mucosa. At this stage the blood and organs were sterile.

Feeding culture to rats resulted negatively. In one mouse of ten fed various cultures vibrios were obtained from the small intestine. Vibrios fed to unprepared rabbits fail to establish themselves in the intestinal tract but when 0.5 gm. of sodium bicarbonate is administered a brief interval before the culture is introduced into the stomach, the vibrios reach the small intestine where they produce catarrhal inflammation and can be recovered after 5 days.

The Immunological Relationship of Various Strains as Judged by Agglutination

It was decided to correlate by means of agglutinin the vibrios obtained from epidemic diarrhea in cows and enteritis in calves with those obtained by Dr. Smith, and with this in view a number of rabbits were immunized with living cultures.

Inasmuch as the cultures grow relatively poorly it was a considerable undertaking to obtain sufficient antigen from plain agar growths for the tests. If, however, to tubes 2.5 cm. in diameter containing 10 to 12 cc. of melted veal infusion agar a fragment of guinea pig or rabbit kidney is added, the tube slanted, and later heavily seeded with young culture and sealed with sealing wax, excellent growth is obtained after 3 or 4 days incubation and suspensions of such growth in NaCl solution afford good antigens.

In Table I the results of the agglutination tests are shown. The same form of arrangement as indicated on page 853 has been followed. The higher serum dilutions in the tests with *Vibrio fetus* antiserum have not been shown since this serum fails to agglutinate the heterologous strains at dilutions higher than those shown.

It is clear from Table I that there exist among the intestinal vibrios at least two well defined immunological groups. Two of the strains presumably originating from cases of infectious diarrhea in adult cows (1629 and 1655) agglutinate well with the same serum and when the serum is absorbed with the heterologous strain the agglutinins are equally removed from both organisms.

The large group, which comprises all but one of the calf enteritis group, one of those presumed to have originated from diarrhea in cows, all the vibrios cultivated from the experimentally induced disease, and the cultures given to us by Dr. Smith, forms what appears to be one immunological group. Vibrio 1728 is not agglutinated by any serum.

The serum specific for *Vibrio fetus* Culture 1660 agglutinates slightly many of the cultures in only the lower dilutions.

Although the members of the large group were strongly agglutinated by both Serums 1641 and 1700 it has been possible to show by absorption that certain vibrios possess a more complex antigen. For instance, if Serum 1641 was absorbed with the homologous strain agglutinin was removed for all strains. If, however, Culture 1700, which was strongly agglutinated by Serum 1641, was used for absorption the agglutinin still remains for the homologous organism and a number of others. This is brought out in Table II.

If Culture 1700 possessed only part of the antigen of strains behaving like 1641, then its antiserum should be completely absorbed by organisms possessing the complete antigen. This proved to be correct. Table III indicates the results of the agglutination tests of Serum 1700

TABLE I

The Agglutination Affinities of I

No. of culture	Source	Antiserum Culture 1629 — Serum dilutions								Antiserum Culture — Serum dilutions				
		1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	1:10,240	1:80	1:160	1:320	1:640	
1629	Presumably originating	C	C	C	C	C	+++	++	+	++	+	+	+	
1641	from diarrhea in cows	−	−	−	−	−	−	−	−	C	C	C	C	
1655		C	C	C	C	C	C	+++	++	+++	++	++	+	
1669	Recovered from calves	−	−	−	−	−	−	−	−	C	C	C	+++	
1686	fed Cultures 1629, 1641, and 1655	−	−	−	−	−	−	−	−	C	C	C	+++	
1652	Obtained from spon-	−	−	−	−	−	−	−	−	C	C	C	C	
1699	taneous enteritis in	−	−	−	−	−	−	−	−	C	C	C	C	+
1700	calves	−	−	−	−	−	−	−	−	C	C	C	C	+
1728		+	+	+	±	−	−	−	−	±	±	−	−	
1731		−	−	−	−	−	−	−	−	C	C	++++	+++	+
1745		−	−	−	−	−	−	−	−	++++	++++	++++	++++	+
1748		+	+	−	−	−	−	−	−	+++	+++	++	++	
1707 Intestine	Recovered from natur-	−	−	−	−	−	−	−	−	C	C	C	C	
1707 Liver	ally induced and arti- ficially infected cases	−	−	−	−	−	−	−	−	C	C	C	C	
1709	of enteritis in calves	−	−	−	−	−	−	−	−	++++	++++	+++	++	
1714		−	−	−	−	−	−	−	−	C	C	C	C	
1721		−	−	−	−	−	−	−	−	C	C	C	C	
1208 Liver	Obtained by Dr. Smith from calves	−	−	−	−	−	−	−	−	C	C	C	+++	
1522 Liver		+	+	±	±	±	−	−	−	C	C	C	++++	+
1524 Liver		−	−	−	−	−	−	−	−	C	C	C	C	
1679 Lung		−	−	−	−	−	−	−	−	C	C	C	++++	+
1660 *Vibrio fetus**	Obtained by Dr. Smith from case of vibrionic abortion	±	−	−	−	−	−	−	−	+++	++	+	−	

* *Vibrio fetus* in homologous serum had the following titer: 1:640 ++++ 1:1,280 ++ 1:2,560 + 1:5,120 −

TABLE I

ıtion Affinities of Intestinal Vibrios

Antiserum Culture 1641						Antiserum Culture 1700							Antiserum Culture *Vibrio fetus* 1660		
Serum dilutions						Serum dilutions							Serum dilutions		
1:320	1:640	1:1,280	1:2,560	1:5,120	1:10,240	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	1:80	1:160	1:320
+	+	±	−	−	−	−	−	−	−	−	−	−	−	−	−
C	C	C	C	++++	+++	C	C	C	++++	+++	++	+	++	+	±
++	+	−	−	−	−	+	+	−	−	−	−	−	++	+	−
C	+++	+	±	−	−	C	C	C	C	+	±	−	−	−	−
C	+++	+	±	−	−	C	C	C	++++	+	+	±	−	−	−
C	C	C	C	++	+	C	+++	++	++	++	±	−	++	++	−
C	C	+++	+	±	−	C	C	C	C	++	+	±	−	−	−
C	C	+++	±	−	−	C	C	C	C	++	±	−	+	−	−
−	−	−	−										++	+	+
++++	+++	++	+	−		C	C	C	++++	++	±	−	+	±	−
++++	++++	+++	++	++	+	C	C	++++	++	++	+	±	++	+	+
++	++	+	−	−	−	+++	+++	+++	++	++	+	−	++	+	±
C	C	C	C	++++	+++	C	++++	++++	+++	++	−	−	++	+	±
C	C	C	C	+++	+	C	C	C	++++	++	+	−	++	+	−
+++	++	+	±	−	−	++++	++++	++++	+	±	−	−	+	−	−
C	C	C	C	++++	+++	C	C	++++	+++	++	+	−	+++	+	−
C	C	C	C	+++	++	C	C	C	++++	++	+	−	+++	+	±
C	+++	+	±	−	−	C	C	C	C	+++	±	−	+	−	−
C	++++	++	++	−	−	C	C	C	C	C	++	+	++	+	±
C	C	C	C	++	+	+++	++	++	+	−	−	−	+++	+	+
C	++++	+++	+++	++	−	C	C	++++	+++	++	+	±	++	+	±
+	−	−	−	−	−	+++	+++	+	±	−	−	−	C	C	C

which had been absorbed with Culture 1641. In the experiments the serum was tested at dilutions as great as 1:20,000 but agglutination

TABLE II

The Effect of Absorption of Serum 1641 with Culture 1700

No. of culture	Dilutions of serum							
	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	1:10,240
1641	C	C	C	C	C	C	++++	++
1700	+	−	−	−	−	−	−	−
1208	++	+	±	−	−	−	−	−
1652	++++	C	C	C	C	C	++++	+++
1699	++	+	+	+	+	−	−	−
1707	C	C	C	C	C	++++	++	++
Intestine								
1707	+	+	±	−	−	−	−	−
Liver								
1709	+++	++	++	+	−	−	−	−
1714	C	C	C	C	C	C	++++	+++

TABLE III

The Effect of Absorption of Serum 1700 with Culture 1641

No. of culture	Dilutions of serum				
	1:80	1:160	1:320	1:640	1:1,280
1641	++	+	±	−	−
1700	+++	++	+	±	−
1208	++	+	+	−	−
1652	++	++	+	±	−
1699	++	+	±	±	−
1707	++++	++++	+++	+	−
Intestine					
1707	+++	++	+	−	−
Liver					
1709	++	+	±	−	−
1714	++	+	±	−	−

stopped at the titer of 1:640. The higher dilutions have been omitted from the table.

The experiment was repeated but this time 1641 was absorbed with

Culture 1707 which, from the data in Tables II and III, was considered to comprise the complete antigen. The results indicated that Culture 1707 contained the complete antigen since the titer of the serum for all vibrios was greatly reduced.

It is true that the vibrios with the exception of one strain fall into two well defined groups, the smaller comprising only two strains which both originated from epidemic diarrhea in cows, the larger embracing the vibrios cultivated from the inflamed intestinal tracts or livers of calves. The latter group may be divided on the basis of agglutinin absorption into two types, one possessing a complete antigenic character and the other possessing only a portion of the antigen. It is also true that none of the vibrios are antigenically similar to the culture of *Vibrio fetus*.

DISCUSSION

The vibrios while presenting certain slight morphological differences, such as the length, the number of coils, and to some extent the depth of coils, nevertheless resemble each other sufficiently to be regarded as a closely related group. Since their first locus in the intestinal tract as judged by their presence in lesions encountered in acute infections is the jejunum, the name *Vibrio jejuni* is proposed.

Their pathogenic properties for cows and calves have been discussed in earlier papers. In the main the disease induced resembles to a certain extent human cholera and the vibrios from the bovine disease often resemble in young cultures the human organism. Both maladies are infections of the small intestine and both are characterized by excessive mucous secretion. However the bovine vibrios differ markedly from the comma vibrio in cultural characters. The bovine group are more difficult to grow, fail to liquefy gelatin or blood serum, and will not survive on strongly alkaline media. Thus far they have not been shown by means of acid or gas production to utilize carbohydrate. Similarities in pathogenic properties for rabbits exist, both organisms when introduced into the circulation produce fever and penetrate the intestinal wall. In both cases it is necessary to neutralize the acidity of the stomach to infect rabbits by mouth. No such procedure is necessary in infection experiments with the cow or the calf since the vibrios readily pass the stomach and frequently are observed in the

fecal mucus after 36 hours. It should be stated that the cultures recovered from the livers of artificially infected mice, and from the peripheral blood, organs, and small intestines of rabbits were proved by agglutination to be similar to those injected.

The grouping according to agglutination affinities has been of assistance to us in certain respects. When Culture 1707, which had been shown to possess the whole antigen of the larger group, was fed to Calf 1714 it gave rise to definite intestinal disease and the vibrio obtained from the jejunum also possessed the whole antigen. When Culture 1714 was fed to Calf 1721 more severe disease was encountered and Vibrio 1721 was shown to possess the complete antigen. Evidently the character is transmitted in spite of considerable variations in the environment. That both types may exist in cultures from different regions in the same animal is also true. The vibrios obtained from the intestine of Calf 1707 possessed the whole antigen while the strain cultivated from the liver failed to possess the complete complex. This has been true in other instances, all the vibrios from the liver studied have failed to contain the complete antigenic complex.

In regard to the relation of the vibrios originating in the intestinal tract to *Vibrio fetus* all that can be said is that they failed to show the same agglutination affinities as the culture of *Vibrio fetus* employed.

SUMMARY

A number of vibrios obtained from the small intestines of calves fed feces from spontaneous diarrhea in cows, natural intestinal disorders of calves, experimentally induced infections of calves, and cultures obtained from Dr. Theobald Smith have been studied. From the close morphological resemblance, similarities in motility, position and number of flagella, tinctorial properties, and the tendency to fragmentation in older cultures, as well as the narrow nutritive requirements, we are led to regard them as a closely allied group and we propose the name *Vibrio jejuni*.

Immunologically as judged by agglutination the organisms have been divided into two groups, the smaller representing two strains originating from diarrhea in cows and the larger comprising one from this source and many from the calf disease. The larger group can be

subdivided by means of agglutination absorption into cultures which do not contain the complete antigenic complex and others which do so.

Certain freshly isolated vibrios when injected into rabbits incite definite reactions terminating in a localization in the small intestine accompanied by catarrhal inflammation.

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EXPLANATION OF PLATE 31

FIG. 1. Vibrios 1629 and 1641. Seven days growth on leptospira medium and guinea pig kidney about natural size.

FIG. 2. 3 day plain agar culture Vibrio 1700, 40th transfer, illustrating slightly convoluted forms. Dilute carbolfuchsin. × 1,500.

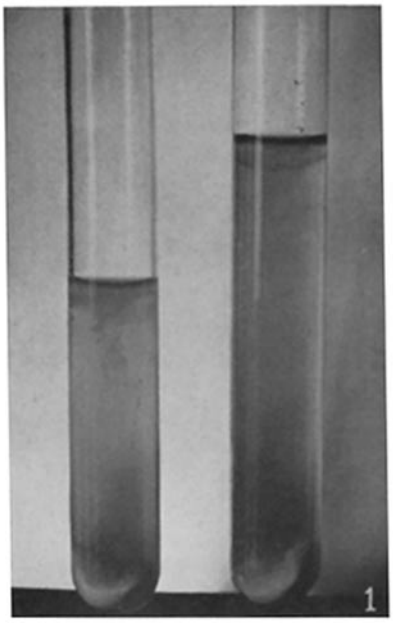
FIG. 3. Young plain agar culture Vibrio 1629 showing flagella. Bailey's flagella stain. × 1,400.

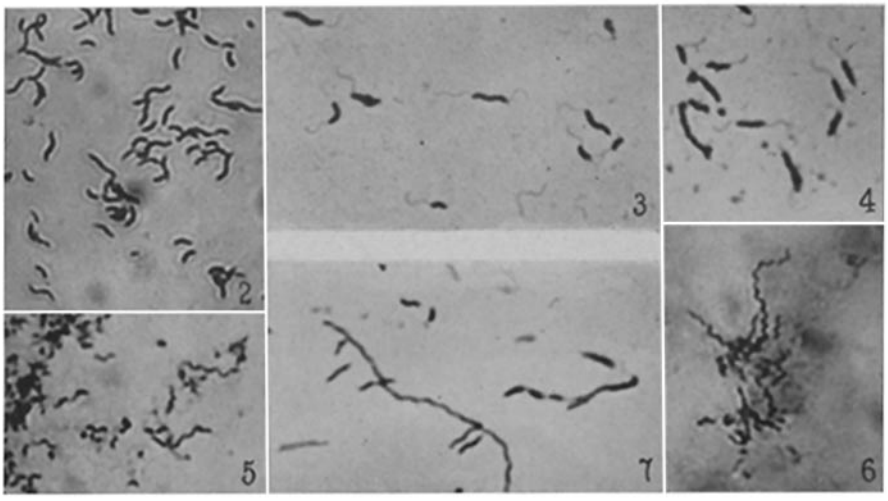
FIG. 4. Young plain agar culture Vibrio 1700. Bailey's flagella stain. × 1,400.

FIG. 5. 3 day blood agar culture Vibrio 1629, sixth transfer, showing longer coiled forms and a portion of a clump in which the vibrios have fragmented. Giemsa stain. × 1,500.

FIG. 6. 4 day blood agar culture Vibrio 1655, first transfer. Long and short coiled forms are illustrated. Giemsa stain. × 1,500.

FIG. 7. 3 day plain agar culture Vibrio 1629, 35th transfer, showing extremely long forms as well as shorter types and illustrating early fragmentation. Giemsa stain. × 2,000.





(Jones *et al.*: Vibrios and intestinal disorders of cattle)