VIBRIOS FROM CALVES AND THEIR SEROLOGICAL RELATION TO VIBRIO FETUS.

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Nothing is known of the life cycle of Vibrio fetus beyond its presence in the bovine placenta and the fetus following abortion. In the digestive tract of the fetus the vibrio is usually found in pure culture. If the fetus is not invaded, however, the difficulties encountered in the placenta and uterine discharges, owing to contamination with miscellaneous saprophytic bacteria, have made isolation thus far impossible. This is due to its early very feeble multiplication, restricted to sealed agar tubes containing blood or fresh tissues or to tubes in an atmosphere containing a small per cent of CO₂, and its rapid destruction following injection into laboratory animals. The occasional encounter of vibrios in the intestinal tract of young calves calls attention to a possible locus of Vibrio fetus. These vibrios might be survivors of a fetal infection with Vibrio fetus, or they might represent a different group possibly associated with intestinal inflammation in calves after the 1st week.

The first strain (No. 174) was obtained in pure culture from the spleen of a calf killed when 10 days old. This animal began to scour when 5 days old and when killed was very weak. At autopsy, the middle portion of the small intestine was deeply congested. Fresh villi under the microscope showed the entire capillary network injected and certain groups of epithelial cells undergoing fatty changes. The large intestine contained normal fecal matter but the mucosa was overlaid with stringy elastic masses of mucus. Bacillus fluorescens was isolated from the intestines on agar plates. No special search was made for vibrios. In sections of small and large intestines, vibrios were not found. Agglutination relations of this strain have been briefly described. Sera prepared with three different living cultures of Vibrio fetus failed to act upon this strain, although

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clumping *Vibrio fetus* almost completely at 1:640 dilution. A specific serum prepared with the calf strain clumped it at 1:2,560 completely but failed to clump fifteen strains of *Vibrio fetus* at 1:20.

A second vibrio (No. 321) was isolated from a calf dead when 58 days old and noticed sick for about a week. The autopsy showed small ulcerations of rumen and the traces of early hemorrhages of the fourth stomach mucosa. There was considerable mucus in the small intestine and the large intestine was congested. There was pneumonia of the left lung indicated by numerous, small, partly coalescing foci of a flesh red color. In sections these foci consisted of injected capillaries and alveoli filled with polymorphs. There was a large amount of coagulable protein in the urine.

The vibrio appeared in cultures of the liver, and in a spleen culture of a guinea pig killed 7 days after inoculation with contents of duodenum. The serological relation of this strain to *Vibrio fetus* has been briefly given. It agrees so far as direct agglutination goes with *Vibrio fetus*. Morphological and cultural distinctions could not be demonstrated.

The two strains described were isolated in 1917 and 1918. In 1925 an obscure disease of calves was brought to our attention. No study of the disease was made on the spot and the one suggestive fact reported was that the calves had been bedded in buckwheat hulls. Two calves, one living, one dead, were brought for examination.

*No. 1206.*—Ayrshire female, about 3 weeks old and weighing 64½ pounds. Dried feces on buttocks and tail. Kept under observation 15 days when the calf died. During this period it was alternately constipated and passing watery feces. The temperature fluctuated around 39°C. There were signs of pneumonia. Milk was taken in small quantities.

Autopsy.—The rumen distended during life was about twice normal size and filled with cut straw, buckwheat shells, and hair. There was some congestion of large intestine. The cephalic lobe of the right lung was consolidated, larger than normal, and permeated with numerous grayish foci, 2 to 10 mm. in diameter. The pneumonia was associated with a bipolar organism (*B. bovis*). Cultures with bits of liver, spleen, and kidneys remained sterile with the exception of one liver tube which contained a vibrio.

*No. 1208.*—Guernsey female, brought to the Department about 12 hours after death. Age, 23 days. Weight after death, 59 pounds. The report was that the calf had developed scours. There was pneumonia of the cephalic and ventral lobes of the right lung similar in character to that of No. 1206, and the same bipolar type of organism was isolated. The intestines were more or less congested. Cultures of spleen, liver, and kidneys were negative like those of No. 1206 with

the same exception that in one containing liver tissue a vibrio appeared in culture obviously like that from No. 1206. Films and sections of the intestines did not show vibrios.

At this time a small number of strains of *Vibrio fetus*, some isolated recently, others several years before, were on hand. Strains 174 and 321 had died out. Cultural differences between Strains 1206 and 1208 and the fetal strains were limited to a freer, more rapid multiplication on agar slants of the former. Agglutination tests were therefore undertaken. The vibrio cultures studied included five fetal strains and two calf strains. The cultures were grown on agar slants sealed with sealing wax. The growth was used after 3 to 4 days incubation at 37°C. The immune sera were prepared by injecting rabbits intraperitoneally. The injections were made with living cultures and with cultures heated 2 hours at 100°C. Living cultures, standardized by the Gates instrument to disappear at 2.4 on the scale, were used for the agglutinating and absorbing suspensions. The absorbing dose used was 6 times the concentration as represented by 2.4 on the Gates scale, i.e. 6 times the agglutinating dose. Each serum was absorbed by the same dose of each culture so that the results are comparable.

The experiments include cross-agglutinations with sera prepared by injecting living and heated cultures and reciprocal absorption tests carried out between calf and fetal strains, between fetal strains, and between calf strains. In Table I, the various relationships be-

<table>
<thead>
<tr>
<th>Rabbit serum prepared with</th>
<th>Agglutination titer</th>
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<tbody>
<tr>
<td></td>
<td>Fetal strains</td>
</tr>
<tr>
<td></td>
<td>741</td>
</tr>
<tr>
<td>997 (living)</td>
<td>1:2,560</td>
</tr>
<tr>
<td>997 (heated)</td>
<td>1:20</td>
</tr>
<tr>
<td>1208 (living)</td>
<td>1:2,560</td>
</tr>
<tr>
<td>1208 (heated)</td>
<td>1:20</td>
</tr>
</tbody>
</table>
VIBRIOs FROM CALVES

tween fetal and calf strains tested with the sera of rabbits treated with living and heated cultures are briefly summarized.

With one exception, the fetal strains present no differences among themselves when tested with "living" or "heated" sera of fetal strains. This exception is No. 741. In the "heated" sera of 997 only a little agglutinin for 741 was present. When the fetal strains were acted upon by "living" and "heated" sera of calf strain 1208, only the "living" serum was found to contain agglutinins for them. The calf strain agglutinins were only slightly lower in the "heated" calf strain serum than in the "living" serum. When the calf strains were treated with "living" and "heated" sera of the fetal strain 997, it was found that the agglutinins common between calf and fetal strains were present only in traces in the "heated" serum. There was therefore no cross-agglutination between calf and fetal strains when tested in "heated" sera. This test defines two serological groups based on the habitat of the strains. It indicates the existence of a distinct heat-stable calf-group antigenic factor and a distinct heat-stable fetal-group antigenic factor.

Absorption tests were next tried with results as given in Table II. In making and interpreting the absorption results the statements of Krumwiede, Cooper, and Provost on agglutinin absorption were taken into consideration. These are in substance that direct agglutination may not be a reliable index of the serological relationship of a bacterium. Absorption of common agglutinins cannot be a criterion for likeness but such an absorption may suggest groupings. To determine agglutinative identity it is necessary to demonstrate specific agglutinins. The reciprocal absorption test is therefore the ultimate method available for determining agglutinogenic likeness or unlikeness. A complete reciprocal reaction indicates identity and the absence of such reciprocal reaction indicates dissimilarity. Between these extremes there may be found all degrees of partial reciprocal reactions.

Table II illustrates the different types of reciprocal absorption reactions which occurred between calf and fetal strains, between

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3 These abbreviated terms signify "sera from rabbits injected with living vibrios or vibrios exposed to 100°C. for 2 hours."

individual fetal strains, and between individual calf strains. The readings given are those made after three successive absorptions. The results throughout each series were very similar, so that the examples tabulated cover all the different degrees of reciprocal absorption observed.

A certain serological difference between calf and fetal strains was demonstrated by reciprocal absorption tests using sera prepared with living organisms. The results showed an absence of reciprocal absorptions and suggest distinct calf and fetal group antigenic factors. Thus by two methods serological differences according to source have been demonstrated.

Reciprocal tests between fetal strains acted upon by sera prepared with living cultures show partial absorptions and almost complete
reactions, indicating an essential identity of fetal strains but possibly some individual strain factor as well. Three strains are practically identical according to the reciprocal tests; namely 997, 1217, and 1149. Strain 996, on the other hand, shows more strain individuality. The 996 agglutinins after three successive absorptions by the other strains are not removed beyond a certain point, while the agglutinins for these other strains continue to be reduced. This indicates some individual strain antigenic factor in 996 which produced these specific agglutinins. The other fetal culture, 741, is an exceptional strain. In absorption experiments it fits into the fetal group, but it differs from the other fetal strains as already mentioned by failing to agglutinate in the 997 serum prepared with the heated culture (Table I).

**TABLE III.**

*Absorption of Agglutinins between the Calf Strains in Sera Prepared with Living and Heated Antigens.*

<table>
<thead>
<tr>
<th>Serum prepared with 1208</th>
<th>Absorbed by 1206</th>
<th>Agglutination titer after absorption</th>
<th>Agglutination titer before absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1206</td>
<td>1208</td>
</tr>
<tr>
<td>Living culture</td>
<td>3 successive times</td>
<td>1:40</td>
<td>1:640</td>
</tr>
<tr>
<td>Culture heated at 100°C, 2 hrs.</td>
<td>3 “”</td>
<td>1:20</td>
<td>1:40</td>
</tr>
</tbody>
</table>

The reciprocal tests between the calf strains with sera prepared from living cultures show a definite relation between these strains and also a strain individuality. Each strain absorbs agglutinins for the other up to a certain point, but after three successive absorptions specific agglutinins still remain for the antiserum-producing strain while agglutinins for the absorbing strain are almost wholly removed.

Comparative absorption tests between the two calf strains have been made also with sera prepared with living cultures and cultures heated at 100°C. The results are summarized in Table III. These indicate that the individual strain factor is heat-labile. With the living culture antiserum 1208 repeated absorptions by 1206 left a residue of agglutinins for 1208, while agglutinins for 1206 were wholly removed; but with heated culture antiserum 1208 repeated absorptions by 1206 removed agglutinins for 1208 and 1206 to about the same degree. Thus in the heated culture antiserum there is no spe-
specific strain agglutinin corresponding to that in the living culture antiserum; the individual strain factor is therefore heat-labile.

The relation of the antigenic factors to flagellar and somatic agglutinins has not been demonstrated since no non-motile vibrio form has been isolated. However, since the vibrio strains all show some motility, flagellar and somatic antigens are assumed to be present in all cultures and they may or may not be the basis of the serological difference between calf and fetal strains. Previous work has shown that flagellar antigen in the group of paratyphoid bacilli is heat-labile and somatic antigen heat-stable. The common and the individual strain heat-labile, antigenic factors in the vibrios probably represent the flagellar portion of the antigen and the specific calf and fetal group factors the somatic portion.

In brief these results indicate that at least four types of antigenic factors exist among the vibrio strains studied and any one strain may contain three of these different factors. They may be designated as (1) common vibrio factor, (2) calf group factor, (3) fetal group factor, (4) individual strain factor. The cross-agglutination tests with sera prepared with living cultures give an indication of the common vibrio factor. The cross-agglutination tests with sera prepared from heated cultures and reciprocal absorption tests between calf and fetal strains demonstrate the distinct calf and fetal group factors. The reciprocal absorption tests between fetal strains and between calf strains suggest some individual strain factors. Comparison of results with sera prepared with living and heated cultures indicate that the common vibrio factors and the individual strain factors are heat-labile while the specific calf group and specific fetal group factors are heat-stable. This partial analysis of the antigenic factors demonstrates that the vibrios are of a complex antigenic nature. The serological tests further indicate a close relationship between but not an identity of the calf and the fetal strains.

SUMMARY.

The calf vibrios thus far studied include one strain serologically distinct from the fetal strains. The others are closely related to the fetal strains though not identical with them. The pathogenic characters of the calf vibrios, either as possible descendents of *Vibrio fetus*, or as independent factors in the production of enteritis have not been demonstrated.