A STUDY OF TWO ATYPICAL STRAINS OF E. TYPHOSA

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Received for publication May 18, 1942

In 1937, two atypical strains of Eberthella typhosa were isolated twenty-five days apart from patients presenting clinical symptoms of typhoid fever. Blood and feces from one patient, and blood from the other, yielded organisms atypical of E. typhosa in that they showed delayed fermentation of lactose and sucrose, but failed to produce hydrogen sulfide. Both patients gave a history of having eaten raw oysters at the same restaurant.

In reviewing the literature for reports of biochemically-atypical strains of E. typhosa one finds that as early as 1907 Twort artificially induced the fermentation of lactose by cultivation in lactose broth. This additional fermentative power, however, was quickly lost upon reinoculation to lactose-free media. Teague and Marchima (1920) studied particularly the variability of xylose and arabinose fermentation, and more recently Graf (1937) has strongly emphasized the frequency and extent of biochemical variation within the Eberthella group.

The discovery of biochemically irregular strains at primary isolation is, of course, not uncommon. Meyer and Neilson (1920) reported atypical reactions with dulcitol, rhamnose, milk and indole. Bacterial types intermediate between Eberthella and some other genus have also been reported. An example is that of Lazare and Breaks (1932) whose organism biochemically resembled the Hiss-Y type of dysentery bacillus, but possessed flagella and was agglutinated by both E. typhosa and Shigella dysenteriae antisera.

A case of temporary variation following primary isolation is that of Poston (1938). Her strain fermented sucrose, but not lactose, and produced indole. It was at first weakly agglutinated (1:20) by typhoid antiserum, but became readily agglutinable at the same time that the biochemical reactions returned to normal. Poston’s experience somewhat resembled ours in that culturally the atypical behavior was temporary and accompanied by partial agglutinability. In our case, however, it was sucrose and lactose fermentation and hydrogen sulfide production which were abnormal.

Bergey’s Manual (1939) lists three species2 which resemble our isolations in that they ferment lactose and mannitol, but fail to liquefy gelatin. Each one of them, however, differs from our strains in at least one important respect. The sendai type of E. typhosa, like our strains, does not produce H2S; but that organism differs in its fermentation of rhamnose and, of course, its inability to attack lactose or sucrose. To our knowledge the biochemical pattern herein described has not been reported previously.

1 This paper is a summary of the senior author’s Master of Science degree thesis.
2 Eberthella oxyphila, E. belfastiensis and E. pyogenes.

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EXPERIMENTAL

Description of organisms upon isolation. The cell morphology and smooth colonies were typical of *E. typhosa*. Few of the cells exhibited definite motility. Both strains (Jo and Tr) rapidly fermented glucose, maltose and mannitol. Xylose, arabinose, and salicin were negative. On the third day lactose and sucrose were acidified. Evidence of H₂S production was completely lacking; colonies on or in bismuth sulfite media (Difco) were uniformly colorless. Bromcresol-purple milk was temporarily acid without coagulation, but later reverted to slight alkalinity. Gelatin was not liquefied. Indole was not formed; nitrates were reduced.

Five months after isolation. The cultures had been transferred monthly on extract agar, incubated for 24 hours and stored at 4°C. Table 1 shows the results of biochemical tests at this time.

It will be noted from table 1 that both organisms were still atypical with respect to lactose, sucrose and hydrogen sulfide. Motility was now vigorous. Detection of hydrogen sulfide was attempted by the inoculation of the following media: lead acetate agar stabs; bismuth sulfite (Difco) medium, both as pour and streak plates; 0.03 per cent cysteine agar and broth. All were consistently negative, although the Rawlings strain (control) was uniformly positive. It

### TABLE 1

Biochemical reactions of Jo and Tr strains five months after isolation

<table>
<thead>
<tr>
<th>TEST SUBSTANCES</th>
<th>READINGS</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1 day</td>
</tr>
<tr>
<td>Glucose</td>
<td>A</td>
</tr>
<tr>
<td>Maltose</td>
<td>A</td>
</tr>
<tr>
<td>Xylose</td>
<td>A</td>
</tr>
<tr>
<td>Galactose</td>
<td>A</td>
</tr>
<tr>
<td>Mannitol</td>
<td>A</td>
</tr>
<tr>
<td>Levulose</td>
<td>A</td>
</tr>
<tr>
<td>Lactose*</td>
<td>–</td>
</tr>
<tr>
<td>Sucrose*</td>
<td>–</td>
</tr>
<tr>
<td>Glycerol</td>
<td>–</td>
</tr>
<tr>
<td>Dulcitol</td>
<td>–</td>
</tr>
<tr>
<td>Trehalose</td>
<td>A</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>A</td>
</tr>
<tr>
<td>Indole</td>
<td>–</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
</tr>
<tr>
<td>Hydrogen sulfide</td>
<td>–</td>
</tr>
<tr>
<td>Methyl red.</td>
<td>+</td>
</tr>
<tr>
<td>Voges-Proskauer</td>
<td>–</td>
</tr>
<tr>
<td>B-C P milk.</td>
<td>N</td>
</tr>
<tr>
<td>Gelatin</td>
<td>–</td>
</tr>
</tbody>
</table>

* The final pH of 1.0 per cent sucrose and lactose broths was 4.8 to 5.0.
A denotes acid, no gas. N denotes neutral reaction.
was further found that glycerol and dulcitol showed delayed fermentation. On
the other hand arabinose, salicin, dextrin, inulin, cellobiose, inositol, rhamnose
and raffinose were not attacked.

Eight months after isolation. Both strains had now lost the ability to ferment
lactose, sucrose and dulcitol. Glycerol was still acidified. As yet neither
strain produced H2S; indeed, it was a little more than a year after isolation before
these tests became positive. Both strains reverted to a typical biochemical
pattern in about the same length of time.

Attempts to induce lactose fermentation. Since both strains had continued to
attack lactose for at least five months after isolation, it seemed of interest to
attempt reactivation of that enzyme. In spite of 19 months of bi-weekly sub-
culturing in lactose broth at 37°C, both aerobically and anaerobically, lactose
fermentation did not reappear. Believing that the utilization of lactose may
originally have been associated with virulence, serial passage through mice was
resorted to. Both strains killed mice regularly within 24 hours. Serial inocula-
tions of peritoneal exudate were made at 24-hour intervals. After eighteen such
passages lactose was not attacked, and further effort along this line was dis-
continued.

SEROLOGICAL STUDIES

Sera from patient Jo during the course of illness showed, as was expected, a
gradual increase in somatic and flagellar typhoid antibodies. On the other hand,
the second patient (Tr) during her entire hospital stay failed to develop a titer
above 1:20 against typhoid-901 antigen.

Formalinized suspensions of the freshly-isolated strains were only partially
agglutinated by high-titer OH-901 antiserum; Jo antigen was positive through
1:100 dilution, and the Tr suspension through 1:50 only. No agglutination of
living bacilli by O-901 serum could be detected. Reactions with various
Salmonella sera were minimal or entirely absent. Pure “Vi” serum was not
available. The inhibition of flagellar agglutination was compatible with the
few flagella seen by appropriate staining procedures. Absence of “O” agglutina-
tion, on the other hand, was interpreted as being due to the presence of consider-
able “Vi” antigen.

Two months after isolation both strains were readily agglutinated by “H”
and “O” sera. It may be of interest to note that the biochemical reactions were
still atypical. About this time rabbit antisera were produced with formalinized
suspensions and the serological relationships with several Salmonella and Shigella
species determined. Since originally both strains had shown feeble motility and
delayed fermentation of lactose, it seemed advisable to test particularly for any
antigenic relationship with Shigella sonnei. In brief, “H” and “O” reciprocal
agglutination tests revealed the anticipated cross reactions with certain Salmon-
ella species, but none with Shiga, Flexner or Sonne dysentery bacilli. By
reciprocal absorption the serological similarity of cultures Jo and Tr with typhoid
strain 901 was established. Therefore, we felt that at this time we were dealing
with fully antigenic typhoid bacilli and no attempt was made to determine their
antigenic structure or to type them by bacteriophage.
When the biochemical behavior of the strains eventually became typical, new antisera were prepared and the agglutination studies repeated. No essential differences were noted. Somewhat later it was decided to compare our strains with Eberthella sp. (Sendai type) which is also H₂S-negative, but ferments rhamnose. Sendai strain #6968 from the American Type Culture Collection was accordingly tested against the various Jo and Tr sera and against stock typhoid sera. No relationship could be found between the “H” agglutinogens of the sendai and several typhoid strains.

Studies on carrier state. Patient Jo, unfortunately, was not available for follow-up study, but the other patient (Tr) became a carrier and recently was still eliminating “E. typhosa” in the feces and urine. It may be of interest that the isolations obtained up to approximately one year after onset were biochemically identical with the original isolation but usually reverted quickly to the lactose-negative, sucrose-negative, H₂S-positive type. One year later both typical and atypical types were present. But when the cultures were again repeated after an additional six months, three biochemical types were recovered. In addition to the typical and atypical varieties previously encountered, there appeared a transitional H₂S-positive form fermenting sucrose, but not lactose. Three years after onset only typical organisms could be found.

Several times during the series of periodic feces and urine examinations blood was drawn from this individual and tested for antibody. It is of interest that at no time could a titer above 1:50 against the homologous or 901 typhoid antigens be demonstrated. In addition, each new isolation was for a time poorly agglutinated by typhoid “O” sera.

Both the partial inagglutinability of strain Tr at isolation and its persistence in the patient after clinical recovery suggested the presence of “VI” antigen. In fact, it was demonstrated as long as three years after isolation that both strains contained a small amount of this antigen. Their virulence for mice was approximately one-half that of the high-“VI” Watson strain (Eliot, 1940), and following mouse passage living suspensions were agglutinated (1:320) by pure “VI” antiserum. In addition, the patient’s serum consistently agglutinated “Vi” antigen in 1:40 dilution.

DISCUSSION

The isolations described in this communication appear to represent a single variant type. Their origin was the same and their entire behavior during the period of study was remarkably similar.

Because H₂S was not produced upon primary isolation, neither strain could be detected in bismuth sulfite agar. It is not unlikely that similar variants occasionally occur among the H₂S-positive Salmonella group. Therefore, sole reliance upon this type of medium for the primary isolation of H₂S-positive enteric bacilli is undesirable.

That markedly atypical strains may cause the classical form of typhoid fever is illustrated by these two cases. Both patients presented the usual symptoms at onset and subsequently pursued the customary course of typhoid fever.
STUDY OF TWO ATYPICAL STRAINS OF E. TYPHOSA

SUMMARY

1. Two biochemically atypical strains of *Eberthella typhosa* from clinical typhoid fever were observed for a period of three years.

2. Upon isolation lactose and sucrose were fermented, but H₂S was not produced. Only after one year of artificial cultivation did they become entirely typical.

3. All attempts to reinduce lactose fermentation failed.

4. Serologically both strains possessed considerable "Vi" antigen, but when isolated only a small amount of flagellar antigen. No other unusual antigenic property was observed.

5. Periodic feces isolations from one case, a carrier, revealed a gradual transition from the abnormal biochemical pattern first observed to a completely typical one three years later.

REFERENCES


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