A new Salmonella type, var. concord, whose antigenic formula is VI, VII:1, v-1,2,3 . . . was described. It was represented by four cultures of which three were isolated from fatal infections in chicks and one from the stools of a person affected with gastroenteritis.

A STAINING METHOD FOR CORYNEBACTERIUM DIPHTHERIAE

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The following method is recommended for the staining of Corynebacterium diphtheriae. It provides a good contrast between the body of the organism and the granules in it. Cover the fixed and dried film with Loeffler's methylene blue, leave for five minutes, wash with water, decolorize rapidly with 1/1000 sulfuric acid. The acid should not stay on the film for more than a few seconds, wash with water, treat rapidly with Gram's iodine, wash again and counterstain with 1 per cent aqueous solution of eosine for half a minute, wash with water and dry.

The body of the organism takes a pinkish color, the granules a blue black one. The contrast is quite clear.

While more complex than the methods used in routine diagnostic laboratories, this method produces slides which are very clear and satisfactory for classroom demonstration.

SPECIES DIFFERENTIATION WITHIN THE GENUS SHIGELLA BY TEST FOR THE REDUCTION OF TRIMETHYLAMINE OXIDE

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Species differentiation within the genus Shigella by cultural methods is often slow and by no means free of pitfalls (Neter, Bact. Rev. 6, 1–36; Weil, J. Immunol. 46, 13–44). This is particularly true for the differentiation of S. paradysenteriae (Flexner) from S. sonnei and S. alkalescens. With this in mind, we...
have investigated a method recently announced by Wood et al. (J. Bact. 46, 106–107). It is based on the observation that S. sonnei and S. alkalescens reduce trimethylamine oxide to trimethylamine, whereas Flexner's organisms do not have this ability.

The procedure described by Wood and Baird (J. Fish Research Bd. Can. 6, 194–201) may be summarized as follows: The medium (trimethylamine oxide, 0.1 g; glucose 0.25 g; Difco's bactopeptone, 0.5 g; NaCl, 0.1 g; MgSO₄, 0.1 g; K₂HPO₄, 0.1 g; water, 100 ml; pH 7.2), tubed in 5-ml amounts, is inoculated and incubated for 24 or 48 hours at 37 C. At the end of the incubation period, each tube receives 1.5 ml of 40 per cent formaldehyde, is shaken, allowed to stand for 3 min. and further receives 3 ml of saturated K₂CO₃.

The tube is immediately closed with a one-hole rubber stopper which has been fitted with a short glass tube (0.5 × 4 cm), slightly constricted at the lower end, and containing a small piece of absorbent cotton which has been impregnated with brom-thymol-blue (Clark) adjusted to pH 4.0 with 0.1 N sulphuric acid. The tubes are then incubated in a water bath at 45 C for 30 minutes. If trimethylamine is present in the culture, it distills from the broth and condenses in the cotton causing a shift of the indicator colour to the alkaline side. The formalin serves to bind any ammonia that may be present.

Trimethylamine oxide was prepared according to Dunstan and Goulding (J. Chem. Soc. 1899; 75, 1004–1011). We used commercial trimethylamine (purchased as 33% solution in water). To each 9 g of this solution, 60 ml of 3% hydrogen peroxide were added. After 24 hours standing at room temperature this solution was concentrated by vacuum distillation at 45 C; the remaining water was removed in the desiccator. Duplicate tests were made with 277 strains of Shigella as specified in table 1. One tube of each strain was tested after 24 hours, and the second after 48 hours of incubation at 37 C. Identical results were obtained after 24 and 48 hours of growth. Thus, for the purpose of Shigella differen-

### TABLE 1

**Species differentiation by Wood's test**

<table>
<thead>
<tr>
<th>SHIGELLA SPECIES</th>
<th>PRODUCTION OF TRIMETHYLAmine OXIDE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative cultures</td>
</tr>
<tr>
<td>S. dysenteriae (Shiga)</td>
<td>5</td>
</tr>
<tr>
<td>S. ambiguæ (Schmidt)</td>
<td>8</td>
</tr>
<tr>
<td>S. paradysenteriae (Flexner)*</td>
<td>185</td>
</tr>
<tr>
<td>S. sonnei</td>
<td>0</td>
</tr>
<tr>
<td>S. alkalescens</td>
<td>0</td>
</tr>
<tr>
<td>S. dispar</td>
<td>0</td>
</tr>
</tbody>
</table>

* Including Newcastle bacillus.
† 6 cultures of type P 143 of Boyd obtained from different collections but quite possibly all derived from the same original strain. Two strains of the same type—one of them recently isolated in this country—were negative.
tiation, testing after 24 hours of incubation appears to be satisfactory. Blanks (not inoculated, but incubated tubes) are only necessary for checking new batches of medium. Positive controls were included in each test in accordance with Dr. Wood's suggestion (personal communication). These were aqueous solutions of 0.01, 0.001 and 0.0005 n trimethylamine, which turns positive in 2, 15 and 30 minutes respectively.

As will be seen, our experience confirms completely the data of Wood and his co-workers. We believe that, in view of the difficulties in the practical differentiation of the dysentery organisms, this test will prove to be a valuable addition to our present methods of species identification in *Shigella*. 