# *In the Laboratory*

## Saccharose-fermenting Diphtheria Bacilli

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In the routine laboratory examination of throat cultures for *Corynebacterium diphtheriae,* it has been customary (*2*) to discard as diphtheroids those organisms which ferment both dextrose and saccharose. Possible error due to this practice has more recently been pointed out (*1, 3*).

It is to be noted that each of the strains was recovered from a case of diphtheria, from a contact of a case, or from a convalescent contact who was suspected of having had the disease before being seen by the physician. It is probable that, had not the specimens come from sources which might have been expected to yield positive cultures, they would have been discarded after saccharose fermentation had been demonstrated. As a result of these few experiences we now routinely check for virulence all corynebacteria which show typical morphology on Loeffler slants, typical colony formation on chocolate tellurite agar plates, and dextrose fermentation with or without saccharose fermentation. This would be an advisable procedure for other public health laboratories to adopt until the prevalence of the saccharose-fermenting strains has been determined.

TABLE 1

SOME CHARACTERISTICS OF 8 STRAINS OF SACCHAROSE-FERMENTING DIPHTHERIA BACILLI

| Strain No. | Acid formation from* | | | | H† | Virulence test in | | Colony type‡ | P§ | Final pH in broth | Clinical Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | D | S | St | Glyc | | rabbits | chicks | | | | |
| 8–16 | + | + | – | – | – | + | + | Smooth | – | 8.0 | When first seen, patient had sore throat and remnants of membrane. |
| 8–1 | + | + | + | – | – | + | + | Smooth | – | 7.6 | Patient severely ill; had typical membrane; good response to antitoxin. |
| 7–9 | + | + | – | – | + | + | + | Almost smooth | – | 7.2 | Patient severely ill; had typical membrane; good response to antitoxin. |
| 14–1 | + | + | | | | + | | | | | Contact of case; had sore throat before seen by physician. |
| 8–10 | + | var. | + | – | – | + | + | Smooth | – | 7.4 | Contact of case; had severe sore throat before seen by physician. |
| 17–4 | + | var. | + | + | | + | + | | | | Severe case; typical membrane first noted on tonsillar region on one side, then on other side, finally spreading toward uvula; good response to antitoxin. |
| 11–5 | + | var. | + | + | – | + | + | Almost smooth | + | 7.8 | Severe sore throat regarded by one physician as typical clinical diphtheria and by another as not typical; good response to antitoxin. |
| 12–1 | + | var. | + | + | – | + | + | Almost smooth | – | 7.7 | Mild case; typical membrane; good response to antitoxin. |

* D = dextrose; S = saccharose; St = starch; Glyc = glycogen.
† H = hemolysin production.
‡ On chocolate tellurite agar plate after 48-hour incubation at 37° C.
§ P = pellicle formation.

Several throat cultures received by this laboratory in the past two years have yielded organisms which were typical and virulent *C. diphtheriae* except that they more or less consistently produced acid from saccharose. The fermentative properties of these organisms were checked in this laboratory and in two others. Data on the strains isolated appear in Table 1.

### References

1. Frobisher, Martin, Jr., Adams, Martha L., and Kuhns, William J. *Proc. Soc. exp. Biol. Med.*, 1945, **58**, 330–334.
2. ———. *Diagnostic procedure and reagents.* New York: American Public Health Association, 1941.
3. ———. *Diagnostic procedure and reagents.* New York: American Public Health Association, 1945.


## A Method for Immunological and Chemical Investigations of Body Fluids by Means of Purified Gelatin


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Gelatin sheets of good quality are placed for 24 hours or longer in running water and then for three changes (several hours each) in distilled water; the sheets are then dried by means of a stream of air and stored, protected from moisture and dust.

Strips of purified gelatin are placed into serum and allowed to swell until the liquid outside the gelatin has been reduced to some desired volume. The serum is analyzed for certain constituents before and after the addition of the strips. These constituents may be divided into two classes: those which penetrate the swelling gelatin, such as water and other small molecules, and those which are incapable of entering, such as proteins and lipids. The concentration of the second class increases in proportion, with the fraction of water which serves to swell the gelatin. Thus, the class to which a certain constituent belongs may be determined by analysis of the serum before and after the swelling.

### Results

Experiments were made with respect to the behavior of chlorides, calcium, icterus index, cholesterol, protein, and certain antibodies, as well as syphilis "reagin," with the following results:

*Chlorides*—Nearly unchanged but found slightly lower in the concentrate. (Original, 0.58 per cent; after gelatin treatment, 0.56 per cent. Original, 0.52 per cent; concentrate, 0.49 per cent. Method: Shales and Shales.)

*Protein*—Concentration increased after gelatin treatment. (Original, 5.0 per cent; after gelatin treatment (volume reduced $2\frac{1}{2}\times$), 11.0 per cent. Original, 6.0 per cent; concentrate, 13.1 per cent.[1] Method: Greenberg.)

*Icterus index*—Increases with gelatin procedure. (Original, 11.8; $2\frac{1}{2}\times$ concentrate, 23.0.)

[1] The increase in protein concentration would explain the slight reduction in chlorides because of adsorption at the occasion of protein precipitation in the chloride method. The Greenberg method may determine to a minor degree other substances than proteins, in particular those which smaller molecules may diffuse into gelatin.

*Cholesterol*—Increases with procedure. (Original, 0.19 per cent; $2\frac{1}{2}\times$ concentrate, 0.42 per cent. Original, 0.16 per cent; concentrate, 0.33 per cent. Method: Bloor.)

*Calcium*—Increases like nondiffusible substances. In this respect the procedure is different from ultrafiltration, where only a fraction is found to be nondiffusible. (Original, 10.0 mg. per cent; $2\frac{1}{2}\times$ concentrate, 24.2 mg. per cent. Original, 10.2 mg. per cent; concentrate (about $3\times$), 27.0 mg. per cent. Method: Kramer-Tisdal.)

*Agglutinin against typhoid bacilli*—Rabbit serum. (Original, titer 1:1,400; 1:1,600 and higher, negative. $2\frac{1}{2}\times$ concentrate, titer increased to 1:3,000; 1:4,000 and higher, negative.)

*Isoagglutinin*—B serum against A cells. (Original, titer 1:10; 1:15 and higher, negative. $3\times$ concentrate, titer 1:25; 1:30 and higher, negative.)

Anti-Rh serum, monkey. (Original, titer 1:30; 1:40, negative. $3\times$ concentrate, titer 1:80; 1:100, negative.)

*Quantitative Kahn test*—(Original, 40 Kahn units; $3\frac{1}{2}\times$ concentrate, 120 units. Original, 60 Kahn units; concentrate (about $2\frac{1}{2}\times$), 160 Kahn units.) The reagin appears to be nondiffusible.

*Vernes test*—Behavior quite different from Kahn test; reagin appears to be diffusible. (Originals and concentrates, respectively, as follows: 119, 120; 52, 51; 10, 10; 42, 40; 14, 17; 28, 29; 28, 25; 15, 13; 31, 32; 91, 90.

*Controls*—Numerous controls were employed throughout. Not only did these testify to the chemical purity of the gelatin, in particular with respect to calcium, but in no instance did an antibody or reagin appear in any concentrate unless it was present in the original.

### Discussion

The described method permits the separation of smaller, diffusible molecules from larger, nondiffusible ones; in this respect it resembles the methods of dialysis, ultrafiltration, and ultracentrifugalization. However, there are some important differences. As is well known, only a fraction of serum calcium is capable of traversing a dialysis membrane or an ultrafilter, while none of it enters swelling gelatin. Swelling gelatin seems to act in a different manner than dissolved gelatin; at any rate, it appears somewhat less permeable. The method permits the concentration of certain antibodies. In the determination of antibodies, dilutions of serum are commonly employed. The present method permits in a simple way extension of a series of dilution in a reciprocal direction, which might be useful in the case of weak concentrations of