VCN–Inhibited Strains of *Neisseria gonorrhoeae*

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WITH great success we have been using Thayer-Martin (T-M) medium with vancomycin, colistimethate, and nystatin (VCN) inhibitor (*1,2*) for the culture of *Neisseria gonorrhoeae* (*3*). Specimens from a number of patients with purulent discharges containing large numbers of Gram-negative diplococci, however, have yielded no growth of gonococci on this medium. Our suspicions that some gonococci were suppressed by one of the three antibiotics of T-M medium were confirmed by the work of Reyn (*4*) who reported that 3 percent of gonococcal strains from 171 cultures were suppressed on a medium containing 3 μg per milliliter of vancomycin.

We thereupon embarked on a study to examine the sensitivity of various wild strains of gonococci to the VCN inhibitor used in the T-M medium and to determine to a limited extent whether vancomycin was the inhibitory substance.

Methods and Materials

Our basal medium (T-M) was composed of Difco GC (gonococcal) medium base, Difco hemoglobin, Baltimore Biological Laboratory (BBL) VCN, and BBL IsoVitaleX, used as recommended by the manufacturers. We poured biplates, using the T-M medium on one side of the plate (VCN^+) and T-M medium prepared without the antibiotics (vancomycin, colistimethate, and nystatin) on the other side (VCN^-). The biplates were then stored at 4° C. until used, for up to 2 weeks.

In the clinic the plates were immediately streaked twice across each half of the biplate with a cotton swab that had been touched to the urethral orifice of male patients. At the end of the clinic (less than 2 hours), the plates were carried to the laboratory and streaked with a bacteriological loop at right angles to the inoculation lines.

Late in the study, a vancomycin antibiotic sensitivity disk at 30μg concentration was placed on each side of the plate. The plates were then incubated in polyethylene bags in 8 percent CO_2 at 36° C. If there was visible growth the next day, the zone of inhibition around each vancomycin disk was measured and recorded. Whether growth was observed or not, a few drops of oxidase reagent were added to each side of the plate along the line of original inoculation.

Oxidase-positive colonies were picked and examined for reaction to Gram's stain and specific fluorescence. In addition, several colonies were picked and inoculated onto quadrant plates with four sugars. Each quadrant, respectively, contained in a phenol red agar base 0.5 percent glucose, maltose, or sucrose or 1 percent lactose. The inoculated plates were then incubated overnight at 36° C.

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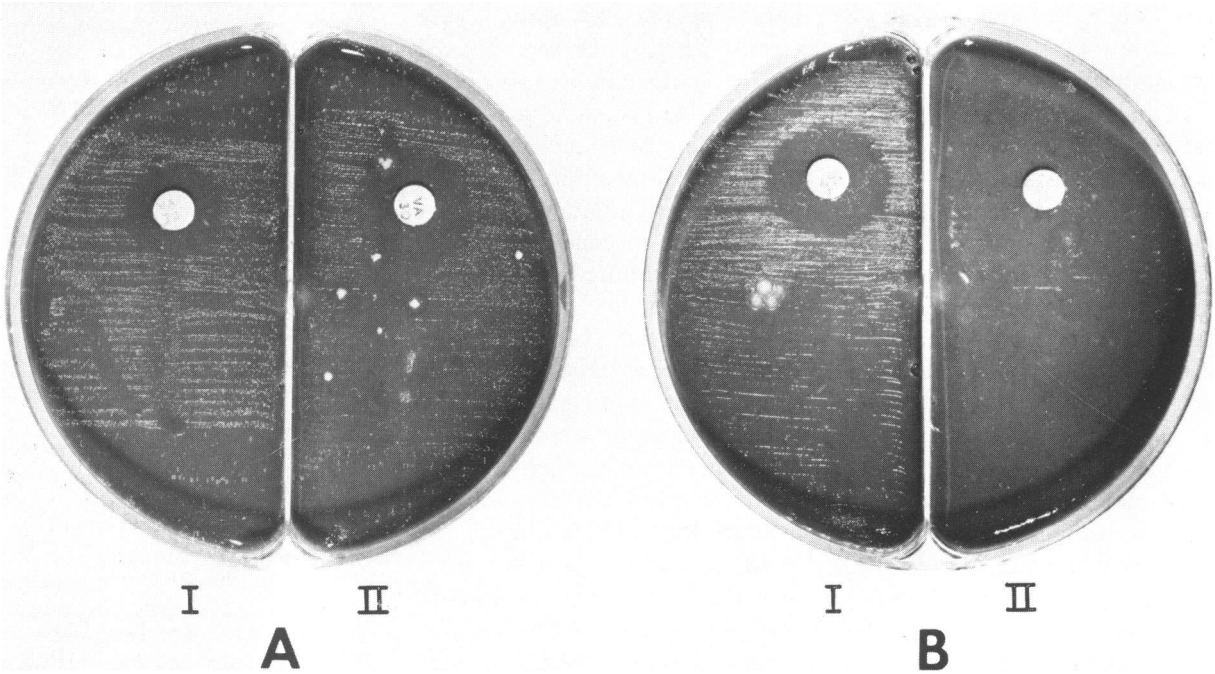
Plate showing inhibition of a strain of *Neisseria gonorrhoeae* by vancomycin. (See text for explanation)



To be considered positive for *N. gonorrhoeae,* cultures had to contain Gram-negative, oxidase-positive diplococci that produced acid from only the glucose of the four sugars tested and fluoresced when treated with a specific fluorescent antibody.

Results

Specimens from 371 men were cultured for *N. gonorrhoeae.* A total of 152 cultures showed growth that met our criteria for this organism on both the VCN^+ and VCN^- media; an additional 13 showed growth only on the VCN^- side. These results are summarized in the following tabulation:

Test Results	
Number of men submitting specimens	371
Number of cultures positive for *N. gonorrhoeae*	165
Number of positive cultures that grew on VCN^- side only	13
Percent of positive cultures that grew on VCN^- side only	7.9

We were subsequently able to culture specimens from three female contacts of these men, and in all three a VCN^- gonococcus was recovered.

Late in the study we added 30 μg vancomycin sensitivity disks to 49 cultures, which subsequently grew *N. gonorrhoeae,* to determine if any correlation existed between the size of the inhibition zone around a vancomycin disk and whether or not the wild strain was inhibited by the VCN component. We determined that the average wild strain of gonococcus (43 cultures of VCN^+) showed an inhibition zone of approximately 14 mm. Three strains showed sparse growth on the VCN^+ side of the plate and yielded zones of 16, 16, and 21 mm. on the VCN^- side of the plate. For three VCN wild strains, the zones of inhibition were 18, 18, and 25 mm. (see photographs).

The left photograph (biplate A) shows a culture of the usual wild strain of gonococcus after 20 hours of incubation. The organism grew well on both sides of the plate. The zone of inhibition on side I was approximately 14 mm.

The right photograph (biplate B) is a VCN^- culture after 20 hours of incubation. No growth of gonococci occurred on side II of the plate. The zone of inhibition around the vancomycin disk (I) was approximately 18 mm.

Discussion

The results of this study were obtained through the initial outgrowth of wild strains of gonococci as isolated from the men attending a venereal disease clinic. We found that 13 of 165 or approximately 8 percent of the men with positive cultures carried gonococcal strains that were inhibited by the VCN component of Thayer-Martin medium. A single lot of VCN additive was used as supplied by the manufacturer, and was not checked by us for ex-

cessive potency or for the presence of toxic impurities. However, subsequent studies with different lots of VCN have yielded similar results.

In an attempt to find out if vancomycin was the inhibiting antibiotic, we measured the zone size surrounding the sensitivity disks in normal and inhibited strains. We determined for three strains completely inhibited by VCN (VCN^-) that the zone of inhibition around a 30 μg vancomycin disk was 18 mm. or greater as compared with apparently uninhibited strains showing a zone of 14 mm. That vancomycin was in some instances inhibitory to gonococcal culture agrees with the results reported by Reyn (*4*).

Thayer-Martin medium has greatly simplified the culturing of gonococci from specimens with highly varied bacterial populations, such as cervical and rectal cultures. On the other hand, the inhibitory type of media, in some instances, inhibit the growth of the gonococcus. Thus Caldwell and associates (*5*) and Schmale and associates (*6*), using the T-M medium, reported that the gonococcus was not recovered from women (15 percent in one instance and 6 to 8 percent in another) who were thought to be harboring the organism. These results can perhaps be explained by the occurrence of vancomycin-sensitive strains of gonococci.

Since the male patient should be the focal point in an epidemiologic study of gonorrhea (*7*), it seems that specimens from men should be cultured additionally on a medium that is not of the inhibitory type. In our study, specimens from three female contacts of the male patients with VCN^- gonococci were cultured. All three harbored strains of gonococci that did not grow on the medium with VCN inhibitor.

We are collecting information of a more quantitative nature in studying these VCN-sensitive strains. Limited results suggest that VCN-resistant gonococci can be recovered from strains that are routinely observed as VCN-sensitive. It is of interest to speculate on the natural history of these strains. Could they be organisms that have had no antibiotic "experience"? Could "normal" strains, organisms that grow on Thayer-Martin medium, have been selected over the years by partial antibiotic therapy?

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Using a direct plating technique for the processing of clinical specimens from 371 male patients, researchers found that 8 percent of 165 men carried strains of *Neisseria gonorrhoeae* that were inhibited by the vancomycin, colistimethate, and nystatin (VCN) component of Thayer-Martin (T-M) medium. Specimens were plated on biplates, one-half of which contained VCN, the other half without VCN. Inhibition zones around 30 μg vancomycin disks placed on the original culture plates were larger for six partially or totally inhibited strains than for the normal wild strain of gonococcus.

Specimens from three female contacts were subsequently cultured, and a VCN-inhibited gonococcus was recovered in each instance. It has been suggested that in male patients, at least, a noninhibitory growth medium be used for detection of *N. gonorrhoeae* instead of, or in addition to, an inhibitory medium of the T-M type.