THE SENSITIVITY OF PROTEUS TO NITROFURANTOIN IN VITRO

BY

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(RECEIVED FOR PUBLICATION MAY 26, 1956)

Dodd and Stillman (1944) described the antibacterial activity of the nitrofurans, and nitrofurantoin was synthesized by the Eaton Laboratories in 1952. Since that time this substance has been given extensive clinical trials in the United States of America in infections of the urinary tract. Limited clinical trials were recently reported in this country (Heffernan, Kippax, and Pamplin, 1955), and arising from this work it was decided to make a more careful study of the sensitivity in vitro of Proteus strains to nitrofurantoin.

Method

A tube dilution method was used, employing urease activity as an index of growth.

Media and Dilutions.—The first medium tried was "oxoid" urease broth (Maslen, 1952). It was found impossible, however, to determine the solubility of nitrofurantoin in this medium, since precipitation occurred between that substance and some unknown constituent in the medium. At the suggestion of Messrs. Duncan, Flockhart and Co. Ltd. a simple "oxoid" phenol red peptone water with added urea was substituted. This medium was modified as follows: One hundred millilitres of medium were prepared according to manufacturers' instructions. This gave a reddish solution with a pH of about 7.4. Since Maslen's modification of Christensen's medium depends for its end-point on a change to alkaline in the medium, the pH of the phenol red peptone water was adjusted by adding 2.0 ml. N/10 HCl to each 98.0 ml. This gave an orange yellow colour and a pH of 6.5. After autoclaving at 15 lb. for 20 min., 5.0 ml. of sterile 40% urea solution was added to 100 ml. of this acidified solution. Since the resulting medium gave satisfactory results when inoculated with strains of Proteus, and since no precipitation was observed when nitrofurantoin was added to it, it was used in all tests described here.

A saturated solution of nitrofurantoin was prepared in the medium (by solution in the autoclave before the addition of urea) and this was used as stock.

A gravimetric estimation of the solubility of nitrofurantoin showed that this stock solution contained 43 mg. per 100 ml. Serial doubling dilutions of this solution were employed in 0.5 ml. amounts, from saturated down to 1/32, and a control row was included using medium without nitrofurantoin.

Inoculum.—Strains of Proteus to be tested were grown overnight in peptone water at 37° C. When turbidity fell, as it usually did, between Brown's tubes 2 and 3, the culture was used for preparing dilutions. If the culture was over-turbid, it was adjusted by dilution. Where turbidity fell short of this standard, the culture was not used until subculture had given satisfactory overnight growth. Serial tenfold dilutions of satisfactory cultures, from "neat" to 1 in 1,000,000 were prepared in peptone water.

The Test.—Seven rows of doubling dilutions of nitrofurantoin were prepared in cotton-wool-plugged tubes with 0.5 ml. amounts from saturated to 1 in 32. Each row was inoculated with a different dilution of the Proteus culture, starting with 1 in 1,000,000, to avoid carry over, and using 2 drops from a 30-dropper pipette as inoculum.

The entire series was incubated at 37° C. and observed every half-hour for six hours for change to a reddish colour, indicating production of ammonia by urease activity. A further final reading was made after 24 hours' total incubation. A tube of medium made alkaline by the addition of a few drops of ammonia was used as a standard.

Results

Four strains of Proteus were tested by the above method. One result is shown in Table I.

<table>
<thead>
<tr>
<th>Nitrofurantoin dilutions</th>
<th>Neat</th>
<th>1/10</th>
<th>1/100</th>
<th>1/1,000</th>
<th>1/10,000</th>
<th>1/100,000</th>
<th>1/1,000,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated</td>
<td>4-0</td>
<td>2-5</td>
<td>2-5</td>
<td>2-5</td>
<td>2-5</td>
<td>2-5</td>
<td>2-5</td>
</tr>
<tr>
<td>1/2</td>
<td>1/0</td>
<td>1/0</td>
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<td></td>
</tr>
<tr>
<td>1/4</td>
<td>2-5</td>
<td>2-5</td>
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<td>2-5</td>
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<td></td>
</tr>
<tr>
<td>1/8</td>
<td>3-5</td>
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<td>3-5</td>
<td>3-5</td>
<td>3-5</td>
<td></td>
</tr>
<tr>
<td>1/16</td>
<td>4-5</td>
<td>4-5</td>
<td>4-5</td>
<td>4-5</td>
<td>4-5</td>
<td>4-5</td>
<td></td>
</tr>
<tr>
<td>1/32</td>
<td>5-5</td>
<td>5-5</td>
<td>5-5</td>
<td>5-5</td>
<td>5-5</td>
<td>5-5</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1/0</td>
<td>2-0</td>
<td>2-0</td>
<td>2-0</td>
<td>2-0</td>
<td>2-0</td>
<td></td>
</tr>
</tbody>
</table>

Numbers show time in hours to colour change. Where tubes are shown "positive" at 24 hours they were negative at six hours; no readings being made in between.
Twenty-eight further strains of Proteus were therefore tested using a shortened series of culture dilutions, "neat," 1/10, and 1/100, and nitrofurantoin dilutions from saturated to 1/16.

The results of the first of these tests, carried out in triplicate on the same culture to check consistency, are shown in Table II.

It is suggested that an arbitrarily chosen strain of Proteus of known sensitivity should be introduced with each batch of tests as a standard.

There appears to be no reason why the test described should not be applied to other antibacterial agents to give a rapid estimate of the sensitivity of Proteus.

Summary

A test for determining the sensitivity of Proteus to nitrofurantoin is described.

The substance appears to be bacteriostatic and shows a pronounced inoculum-size effect. Sensitivities of 28 strains of Proteus are summarized.

Thanks are due to Mr. R. J. Parris, who carried out the nitrofurantoin solubility estimations, and to Messrs. Duncan, Flockhart and Co. Ltd. for a supply of pure "furadantin" and advice on solubility estimation. The work was carried out under a research grant from the South West Metropolitan Regional Hospital Board.

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The Sensitivity of *Proteus* to Nitrofurantoin *in vitro*

P. W. Kippax

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