PROPERTIES OF QUINOXALINE OXIDE-RESISTANT RICKETTSIA TYPHI

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Received for publication August 28, 1961

ABSTRACT

WEISS, EMILIO (Naval Medical Research Institute, Bethesda, Md.) AND HARRY R. DRESSLER. Properties of quinoxaline oxide-resistant Rickettsia typhi. J. Bacteriol. 83: 415-417. 1962.—An effort was made to apply to the Wilmington strain of Rickettsia typhi the observation that in certain strains of rickettsiae and viruses of the psittacosis group quinoxaline oxide resistance is easily obtained and is often accompanied by other changes. Resistance to quinoxaline oxide was obtained in R. typhi after 14 serial egg passages by a procedure somewhat more elaborate than the one used previously for R. prowazekii. Three limit-dilution isolates manifested an appreciable level of drug resistance (were unaffected by 0.5 mg/egg), but changes for unselected characteristics, such as "toxicity" for the mouse or growth or virulence for the chick embryo, were small and different in each case, and did not clearly resemble those obtained with the other microorganisms.

Hurst et al. (1953) and Greenland and Moulder (1958) have shown that viruses of the psittacosis group are highly susceptible to the chemotherapeutic action of 2,3-dimethyl-quinoxaline-1,4-dioxide (hereafter called quinoxaline oxide), but resistant strains developed after a few serial passages in mice or eggs in the presence of the drug. Woodroofe and Moulder (1960) obtained a quinoxaline oxide-resistant strain of feline pneumonitis virus after a single drug passage in eggs. Furthermore, when a penicillin-resistant strain of feline pneumonitis virus was used, quinoxaline oxide resistance was accompanied by other changes: the virus was no longer neutralized by specific antiserum, was completely unaffected by penicillin, and produced an irregular pattern of embryo deaths (death occurred at 4 to 14 days instead of the original 4 to 6). Somewhat similar results were obtained with the Madrid E strain of Rickettsia prowazekii. Weiss Dressler, and Suitor (1959) obtained a quinoxaline oxide-resistant substrain after 20 serial egg passages in the presence of the drug; in later work (Weiss, 1960), resistance was obtained in a single passage. The resistant substrains appeared to have lost some virulence for the cotton rat and the chick embryo. The latter property was expressed as the ratio between the number of ID<sub>50</sub> and LD<sub>50</sub> in a given dose. The quinoxaline oxide-susceptible substrains had ratios well below 10, and those of the resistant substrains were appreciably above 10.

The work to be presented was prompted by the above-described suggestions that mutation to quinoxaline oxide resistance is easily obtained in some strains and is often accompanied by other changes. It appeared of special interest to investigate whether these findings would apply to a strain that has retained its virulence for man and animals, such as the Wilmington strain of R. typhi.

MATERIALS AND METHODS

The first series of egg passages was carried out with the Wilmington strain of R. typhi, obtained from the Viral and Rickettsial Registry of the American Type Culture Collection. The rest of the work was done with a strain obtained from Henry S. Fuller, Department of Rickettsial Diseases, Walter Reed Army Institute of Research. A highly reproducible "toxic" LD<sub>50</sub> was obtained by the intravenous inoculation of mice, weighing approximately 20 g, with twofold dilutions of the rickettsial suspensions. "Toxic" deaths, occurring during the first 24 hr, could be clearly separated from deaths due to infection starting 2 days later. The source of quinoxaline oxide was described in a previous publication (Weiss et al., 1959). Other materials and methods are described in the accompanying paper (Weiss and Dressler, 1962).

RESULTS

Contrary to our previous experience with the Madrid E strain of R. prowazekii (Weiss et al., 1959; Weiss, 1960) quinoxaline oxide resistance
TABLE 1. Properties of quinoxaline oxide-resistant substrains of Rickettsia typhi (Wilmington strain)

<table>
<thead>
<tr>
<th>Substrain</th>
<th>Effect of quinoxaline oxide on chick embryo survival*</th>
<th>No. egg ID₅₀ per mouse &quot;toxic&quot; LD₅₀</th>
<th>No. egg ID₅₀ per egg LD₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Survival (mean days), treated (mg/egg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.25</td>
<td>0.50</td>
</tr>
<tr>
<td>Parent</td>
<td>6.3</td>
<td>1.4</td>
<td>16 × 10⁴</td>
</tr>
<tr>
<td>WQ1</td>
<td>5.0</td>
<td>0.9</td>
<td>180 × 10⁴</td>
</tr>
<tr>
<td>WQ2</td>
<td>5.5</td>
<td>−0.1</td>
<td>1 × 10⁴</td>
</tr>
<tr>
<td>WQ3</td>
<td>6.1</td>
<td>0.3</td>
<td>16 × 10⁴</td>
</tr>
</tbody>
</table>

* Increase in survival time of drug-treated embryos by 1 day or longer was considered as indicating significant protection.
† Only mice dying during first 24 hr were included in the calculation of the "toxic" LD₅₀.

was not easily obtained using the Wilmington strain of R. typhi. Two extended series of passages of the rickettsiae in eggs in the presence of moderately inhibitory doses of the drug failed to elicit the appearance of resistant substrains. Success was finally obtained in a third series of 14 passages, in which the procedure was changed somewhat. During the first passage, unselected growth was allowed to proceed for 5 days, then a moderately inhibitory concentration of drug (0.25 mg/egg) was added, at a time when it was no longer protective. Serial passages were then continued as in previous experiments in the presence of 0.25 to 0.5 mg of drug per egg, but additional drug was added on the 4th day after inoculation during the third (0.25 mg) and ninth (1.0 mg) passages. The harvest from the 14th passage was passed at limit-dilution and three isolates were recovered, which will be designated WQ1 (from a group having 2.5% incidence of infection), WQ2, and WQ3 (both 25%).

In Table 1 some of the properties of the three isolates are compared to those of the parent strain. Although the parent strain was markedly inhibited by 0.5 mg of quinoxaline oxide per egg, the three isolates were completely or nearly completely resistant. Unselected changes were small, however. The ratio between the egg ID₅₀ and mouse "toxic" LD₅₀ was slightly above 10⁶ in the case of the parent strain, as expected. This ratio was higher for WQ1 and lower for WQ2 by factors slightly greater than 10, suggesting that small changes in "toxicity" for mice or infectivity for eggs had taken place. WQ3 did not undergo such changes, but its virulence for the chick embryo was slightly reduced as evidenced by the ratio between the egg ID₅₀ and LD₅₀, which was somewhat greater than 10. The ID₅₀ to LD₅₀ ratios remained below 10 in the other two drug-resistant substrains.

DISCUSSION

Substrains WQ1–3 developed an appreciable level of quinoxaline oxide resistance. The other changes, affecting their "toxicity" for mice, growth, and virulence for chick embryos, although significant, were small; each substrain appeared to have undergone somewhat different change. Because of the wide range of physiological variation which is known to occur in rickettsiae (Weiss, 1960), it is not certain that these changes were in any way associated with the acquisition of quinoxaline oxide resistance.

The Wilmington and Madrid E strains, although similar in their susceptibilities to the chemotherapeutic effects of quinoxaline oxide, differed in their responses to serial egg passages in the presence of the drug. Drug-resistant substrains were produced more easily, and an association between drug resistance and loss of virulence for the chick embryo could be demonstrated more consistently with the Madrid E strain (Weiss et al., 1959; Weiss, 1960). The diversity of results is not surprising, since the two strains belong to two distinct species. However, an explanation for this phenomenon may also lie in the difference of the passage histories of the two strains. The Madrid E has undergone a series of egg passages which has greatly reduced its virulence for laboratory animals and man (Perez Gallardo and Fox, 1948; Everitt, Bhatt, and Fox, 1954), but the Wilmington strain has remained relatively unchanged. It is plausible that certain mutational steps facilitate the selective action of quinoxaline oxide. A parallel is found in the previously cited experiments of Woodroffe and Moulder (1960), who produced changes unrelated to quinoxaline oxide resistance in a penicillin-resistant, but not in a penicillin-susceptible, strain of feline pneumonitis virus.

ACKNOWLEDGMENT

The able technical assistance of L. W. Newman and O. L. Stewart is gratefully acknowledged.
LITERATURE CITED


