A method for studying antibiotic concentrations in inflammatory exudate

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SYNOPSIS  A new technique is described for studying antibiotic concentrations in an experimental inflammatory exudate in vivo. In most exudates concentrations of four antistaphylococcal drugs which were of potential therapeutic significance could be assayed, and fucidin appeared to diffuse best. Higher concentrations of all antibiotics gained access to the lesion in the first two hours of inflammation, suggesting that their mobility did not depend on binding to cells during the inflammatory response. The technique may ultimately help to elucidate the relationship between antibiotics and host defences at the primary sites of infection.

Measurement of the antibiotic concentration achieved in a specific tissue after oral or parenteral drug administration is a guide to suitable therapy when that tissue is infected. However, acute inflammation may change some tissue antibiotic levels, for higher concentrations can be demonstrated in sputum which is purulent (May and Delves, 1965). Also it has been known for many years that, during the acute inflammatory stages of bacterial meningitis, more penicillin crosses the blood-brain barrier into the cerebrospinal fluid than in normal subjects (Rosenberg and Sylvester, 1944). Taken together, these findings suggest the possibility that some antibiotics pass into any inflamed area, aided by the acute inflammatory response (May, 1968). A possible mechanism can be argued from the studies in vitro of Sagers and Lawson (1970), who suggested that penicillin antibiotics enter the respiratory tract bound to leucocytes.

To investigate the concentration of antibiotics likely to occur in inflammatory exudates in vivo some modifications have been made to the 'skin window' technique originally described by Rebeck and Crowley (1955). This communication describes the method, which is suitable for comparing the kinetics of different antibiotics. The initial results with four antistaphylococcal drugs are summarized.

Methods

Healthy adult volunteers received antibiotic orally after a light meal and serial serum levels were assayed at half, one-and-a-half, two-and-a-half, and three-and-a-half hours. Just before the half-hour sample a small abrasion was made on the flexor aspect of the forearm using a high speed motor (25,000 rpm) and a sterile buff as used by Senn, Holland, and Banerjee (1969). After skin preparation using methylated spirit the subject tensed the skin of the test area with his free hand. A few light touches with the rotating buff made the abrasion over an area of about 25 sq mm. At half-an-hour after the antibiotic had been ingested a 12 mm diameter, sterile, weighed assay disc (Mast Laboratories Ltd) was applied to the abrasion and covered with a sterile glass slide which was firmly strapped in position. In the subsequent hour exudate was absorbed by the disc which, having been reweighed, was placed directly on a seeded assay plate. In all three discs were applied to the abrasion at hourly intervals so

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Dose (mg)</th>
<th>Assay Organism</th>
<th>pH for Assay</th>
<th>Incubation Temperature (°C)</th>
<th>Mean Peak Serum Level (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalexin</td>
<td>500</td>
<td>Sarcina lutea</td>
<td>6-5</td>
<td>28</td>
<td>13-8</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>500</td>
<td>Sarcina lutea</td>
<td>6-5</td>
<td>37</td>
<td>9-0</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>300</td>
<td>Sarcina lutea</td>
<td>7-4</td>
<td>37</td>
<td>3-75</td>
</tr>
<tr>
<td>Fucidin</td>
<td>500</td>
<td>Corynebacterium xerosis</td>
<td>6-0</td>
<td>37</td>
<td>24-4</td>
</tr>
</tbody>
</table>

Table I  Details of antibiotics studied
that exudate antibiotic concentrations could be measured for the periods half to one-and-a-half hours, one-and-a-half to two-and-a-half hours, and two-and-a-half to three-and-a-half hours, with a serum level immediately before and after each exudate collection. All assays were carried out using the plate diffusion technique with the 12 mm assay discs as reservoirs for test or standard solutions. Subsequently two hours prediffusion time was allowed before overnight incubation. Table I summarizes details of the antibiotics studied including the dose given, the assay organism, and the average of the peak serum levels achieved.

Results

The serum levels obtained agreed with previous studies of each antibiotic (Braun, Tillotson, Wilcox, and Finland, 1968; McGhee, Smith, Wilcox, and Finland, 1968; Saggars, Harwood, and Day, 1968; Sutherland, Croydon, and Rolinson, 1970). The mean weight of exudate available for assay was 10-17 mg with a range from 2-55 mg to 24-97 mg. Figure 1 summarizes the information obtained in one individual after fucidin. On this occasion duplicate skin abrasions and exudate assays were performed, demonstrating the degree of reproducibility in one subject. Table II summarizes the results for all antibiotics by recording the means of each exudate level as a percentage of the average serum level during each collection. For cloxacillin experiments, the results are the mean of studies on only three subjects; the results for the other antibiotics were based on four experiments. There was considerable individual variation in the concentration of antibiotic assayed in the exudate and in part this must reflect variations in lesion size. However, there was some evidence of individual factors operating since one subject consistently achieved higher levels in the exudate with all antibiotics than did his colleagues.

Discussion

The present study was performed in an attempt to investigate the relationship between antibiotic and leucocyte kinetics. Other workers have used the skin window technique to quantitate the cellular and protein changes during inflammation in differing experimental situations (Sheldon, Mildvan, and Allen, 1967; Fekety, 1969; Hutchins and Sheldon, 1970; Senn and Holland, 1970). Clearly, antibiotics can be studied in the same model and this provides an opportunity to compare their role with that of host defences.

It is of interest that the present studies show higher proportions of the serum concentration in exudate produced during the first two hours of the inflammatory response. This occurred with all four anti-staphylococcal drugs studied. Other workers have shown that during the first two hours of inflamma-

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Time of Inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First Hour</td>
</tr>
<tr>
<td>Fucidin</td>
<td>42</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>38</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>34</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>10</td>
</tr>
</tbody>
</table>

Table II  Mean of antibiotic concentration in skin exudates as a percentage of the average serum level

Fig. 1  Results in one individual after fucidin.
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I would like to acknowledge my medical colleagues who were the subjects of these experiments and Mr J. D. Devine and Miss E. J. Hanson for skilfully assisting in the assays. Above all, Professor R. H. Girdwood's continual advice and encouragement has been invaluable.

References
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doi: 10.1136/jcp.24.7.633

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