THYMIDINE antagonises the antibacterial action of trimethoprim (Miovic and Pizer, 1971), and thus media containing this nucleoside are unsatisfactory for trimethoprim-sensitivity tests. With the exception of Wellcome Nutrient Agar, most laboratory media contain significant amounts of thymidine (Bushby and Hitchings, 1968; Koch and Burchall, 1971). Consequently, bacterial sensitivity to trimethoprim can be reliably assessed on Wellcome Nutrient Agar, whereas other media may be unsatisfactory for this purpose (Waterworth, 1969).

Thymineless mutants lack the enzyme thymidylate synthetase and cannot grow unless thymine or thymidine is provided (Barner and Cohen, 1959). Such mutants are resistant to trimethoprim (Stacy and Simson, 1965; Bertino and Stacy, 1966; Wilson, Farmer and Rothman, 1966). Under normal circumstances wild-type (trimethoprim-sensitive) bacteria cannot utilise exogenous thymine (Siminovitch and Graham, 1955; Crawford, 1958; Kammen, 1967) although they can incorporate exogenous thymidine. It therefore seemed possible that thymine could be incorporated into laboratory media to detect the presence of thymineless mutants without simultaneously antagonising the effects of trimethoprim upon sensitive bacterial strains. However, we found that thymine did antagonise the antibacterial action of trimethoprim on wild-type bacteria; thus, neither thymine nor thymidine could be added to culture media when trimethoprim sensitivity was being investigated.

To overcome these difficulties we developed a two-stage approach to trimethoprim sensitivity testing. (1) Primary isolation was done on a nutrient agar containing thymidine or thymine, thus allowing the isolation of thymineless mutants as well as normal bacteria. (2) The sensitivity of the bacteria to trimethoprim was then tested on a nutrient medium (such as Wellcome Nutrient Agar) which is deficient in thymine and thymidine (T⁻ N/A). This second
S. G. B. AMYES AND J. T. SMITH

test gave a (MIC) value for trimethoprim on wild-type bacteria. However, because thymineless mutants cannot grow in this test, the second stage required additional subcultivation of the primary isolates on to T⁻ N/A and on to nutrient agar containing thymine or thymidine. Thymineless mutants, which by their nature are resistant to trimethoprim, can grow only on the nutrient-agar plate containing thymine or thymidine, whereas wild-type bacteria grow on both types of nutrient agar.

Experiments were done to check these procedures on Wellcome Nutrient Agar because this medium is the least antagonistic to the action of trimethoprim. As expected, thymineless mutants grew on Wellcome Nutrient Agar containing added thymidine, but unexpectedly they failed to grow when thymine, rather than thymidine, was added. Similar results were obtained with Wellcotest Agar, and it appeared that both these media contain a compound preventing the bacterial utilisation of thymine. We therefore recommend that thymidine, but not thymine, be added to Wellcome media when the two-stage method for the identification of thymineless mutants and the determination of bacterial sensitivity to trimethoprim is used.

MATERIALS AND METHODS

Organisms. The bacteria used were Escherichia coli no. NCTC10418, Staphylococcus aureus no. NCTC6571 and Pseudomonas aeruginosa no. NCTC10662, which were kindly supplied by Miss Pamela Waterworth. Other strains were E. coli no. 114 and its thymineless (thy⁻) mutant, Aerobacter cloacae no. 53, and its thymineless mutant (Smith, 1967), and P. aeruginosa strain D8, a trimethoprim-resistant organism isolated from a clinical specimen provided by Dr D. M. MacLaren, Stepping Hill Hospital, Stockport, Cheshire. Except for the two thymineless mutants, all the other bacteria grew on media lacking thymidine or thymine.

Media. The various laboratory media used are listed in table I and were made according to the manufacturers' instructions. Davis-Mingioli (DM) minimal medium (Davis and Mingioli, 1950) was supplemented with 0.28% w/v glucose and solid DM was made as described by Smith (1967). Blood-agar plates were made by autoclaving the basal medium, cooling it to 50°C and then adding sufficient lysed horse blood (Wellcome, Beckenham, Kent) to give a 4% solution.

Reagents. Thymidine, thymine and deoxyadenosine were purchased from Koch-Light, Colnbrook, Bucks, and sterile aqueous solutions made by autoclaving at 115°C for 20 min. Control tests showed that all three compounds were stable during this process. A sterile aqueous solution of uridine (Calbiochem, Los Angeles, USA) was prepared by membrane filtration. Trimethoprim, as trimethoprim lactate, was kindly given by the Wellcome Research Laboratories, Beckenham, Kent, and aqueous solutions were sterilised by membrane filtration. Results are expressed in terms of the concentration of trimethoprim base.

Methods for MIC estimations. Cultures were grown overnight at 37°C in Oxoid No. 2 Nutrient Broth containing 60 μg per ml of thymine, diluted in DM lacking glucose, and between 50 and 300 cells were plated on to media containing concentrations of trimethoprim at two-fold intervals from 1.25 to 80 μg per ml. All plates, except for DM, were incubated at 37°C for 18 hours, the DM plates being incubated for 66 hours. The results were recorded as the minimum concentration required to prevent growth.

Method for trimethoprim antagonism experiment. An overnight culture of E. coli strain 114 was diluted 1 in 100 in Wellcome Nutrient Broth containing the supplements listed in the figure. The tubes were incubated at 37°C and the viable count was estimated by diluting in DM base and plating on Oxoid MacConkey Agar. The plates were incubated for 18 hours at 37°C.
RESULTS

Minimum inhibitory concentrations of trimethoprim in various media

The MIC of trimethoprim for the eight bacterial strains were determined on a variety of solid media (table I). Three different classes of organisms were investigated. The first class comprised four strains of trimethoprim-sensitive wild-type bacteria (Tm\(^{+thy}\)). The second class consisted of two strains of

### Table I

Results of trimethoprim-sensitivity testing on various media

<table>
<thead>
<tr>
<th>Medium</th>
<th>MIC values (µg per ml) for trimethoprim on</th>
<th>Tm(^{+thy}) organisms</th>
<th>Tm(^{-thy}) organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxoid Blood Agar Base</td>
<td></td>
<td>E. coli no. NCTC10418</td>
<td>E. coli no. 114</td>
</tr>
<tr>
<td>Difco Nutrient Agar</td>
<td></td>
<td>S. aureus no. NCTC6571</td>
<td>A. cloacae no. 53</td>
</tr>
<tr>
<td>Oxoid MacConkey Agar</td>
<td>80</td>
<td>1⋅25</td>
<td>20</td>
</tr>
<tr>
<td>Oxoid Diagnostic Sensitivity Test Agar</td>
<td>10</td>
<td>1⋅25</td>
<td>5</td>
</tr>
<tr>
<td>Oxoid Diagnostic Sensitivity Test Agar</td>
<td></td>
<td></td>
<td>80</td>
</tr>
<tr>
<td>Oxoid Diagnostic Sensitivity Test Agar</td>
<td></td>
<td></td>
<td>80</td>
</tr>
<tr>
<td>Davis-Mingioli Agar</td>
<td></td>
<td></td>
<td>80</td>
</tr>
<tr>
<td>Wellcome Nutrient Agar</td>
<td></td>
<td></td>
<td>80</td>
</tr>
<tr>
<td>Wellcotest Agar</td>
<td></td>
<td></td>
<td>80</td>
</tr>
<tr>
<td>Wellcotest Agar +4% lysed horse blood</td>
<td></td>
<td></td>
<td>80</td>
</tr>
</tbody>
</table>

NG = The organism failed to grow on the medium without trimethoprim.

P. aeruginosa that do not require thymine but are trimethoprim-resistant (Tm\(^{-thy}\)). The third class consisted of two trimethoprim-resistant thymineless mutants (Tm\(^{-thy}\)).

The results for trimethoprim-sensitive bacteria showed that Wellcome Nutrient Agar, Wellcotest Agar with or without lysed horse blood and Oxoid Diagnostic Sensitivity Test Agar containing lysed horse blood were the only four media that provided meaningful results with all four strains. Presumably the other media, with the exception of DM, gave erroneously high MIC value for trimethoprim because they contain a derivative of thymine. Wellcome Nutrient Agar does not contain any significant amount of thymidine (Koch and Burchall, 1971), and the results in table I suggest that the same applies to Wellcotest Agar.
The results on Oxoid Diagnostic Sensitivity Test Agar with *E. coli* no. 114 and *A. cloacae* no. 53 were interesting, because it appeared that although some thymine derivative was present in the plain medium, its antagonistic effects could be abolished by the addition of lysed horse blood. However, with the two NCTC strains, the addition of lysed horse blood did not affect their sensitivity to trimethoprim on Oxoid Diagnostic Sensitivity Test Agar. Thus, although these two strains are commonly used as standard sensitive bacteria in trimethoprim-sensitivity testing, their failure to detect the presence of thymine derivatives in test media means that they are not as suitable as *E. coli* no. 114 or *A. cloacae* no. 53 as reference strains.

DM medium did not support the growth of *S. aureus*, and since there are many other pathogenic bacteria that fail to grow on this medium, it cannot be recommended for routine use. However, it was included in this study because it is a completely synthetic medium in which the thymine and thymidine content can be precisely controlled.

The *P. aeruginosa* strains (Tm\(^R\)thy\(^+\)) were trimethoprim resistant on all media tested, although with no NCTC10662, it can be seen that some variations in the level of resistance occurred, especially in media containing little or no thymidine. The resistance of pseudomonads to trimethoprim has been attributed to their impermeability to the drug (Hitchings, Burchall and Ferone, 1966).

The results obtained with the thymineless strains were straightforward; on some media, no growth occurred even in the absence of trimethoprim, whereas on media on which growth did occur the strains were resistant to trimethoprim at all concentrations tested. The absence or availability, respectively, of thymine derivatives in the media probably provides an explanation for these results.

The thymineless strains grew on Oxoid Diagnostic Sensitivity Test Agar in the absence but not in the presence of lysed horse blood. This indicated that lysed horse blood contains some compound antagonistic to the thymine derivatives present in the Oxoid Diagnostic Sensitivity Test Agar.

**Trimethoprim antagonism**

The presence of derivatives of thymine thus reduces the efficacy of trimethoprim against trimethoprim-sensitive, wild-type bacteria. Therefore the viability of a representative of this class, *E. coli* no. 114, was followed in liquid suspension to determine the effect of trimethoprim in the presence and absence of added thymine. The effects of thymidine were also investigated in the system for comparative purposes. Wellcome Nutrient Broth was chosen because preliminary experiments had shown that this medium, like Wellcome Nutrient Agar and Wellcotest Agar, seemed to lack completely any derivative of thymine.

The results (figure) show that the addition of trimethoprim produced a rapid bactericidal effect. However, thymine was able to reverse this effect completely because bacteria treated with trimethoprim in the presence of
thymine multiplied almost to the same extent as those in the control culture. This is similar to the antagonistic effect of thymidine. These results with thymine were unexpected because Siminovitch and Graham (1955), Crawford (1958) and Kammen (1967) have presented evidence indicating that wild-type bacteria cannot utilise exogenous thymine. In conclusion, it would seem

![Graph](https://via.placeholder.com/150)

**FIGURE.**—An overnight culture of *Escherichia coli* strain 114 was diluted 1 in 100 in Wellcome Nutrient Broth containing the supplements listed below. The tubes were incubated at 37°C and the viable counts estimated by dilution in DM base and plating on Oxoid MacConkey Agar.  
★ = No supplements; ○ = +trimethoprim (5 µg per ml); ▲ = +thymine (60 µg per ml);  
△ = +thymine (60 µg per ml) and trimethoprim (5 µg per ml); ■ = +thymidine (60 µg per ml);  
□ = +thymidine (60 µg per ml) and trimethoprim (5 µg per ml)

that, in the presence of trimethoprim, wild-type bacteria are able to use thymine to overcome the inhibitory effects of the drug.

**The availability of thymidine or thymine to thymineless mutants in various laboratory media**

Because the thymine or thymidine content of media used in trimethoprim-sensitivity testing appear to be critical, we investigated the presence of these compounds by determining whether various media could support the growth of *thy*<sup>+</sup> and *thy*<sup>-</sup> strains of bacteria. The effect of added thymine or thymidine to these media was also studied. It was found that the addition of thymine
or thymidine to the media listed in table II had no significant effect on the growth of any of the Tm*thy* or Tm*thy+ strains.

However, these two compounds influenced the growth of the thymineless mutants (table II). Oxoid Diagnostic Sensitivity Test Agar, Oxoid Blood Agar Base, Difco Nutrient Agar and Oxoid MacConkey Agar supported the growth of thymineless mutants without either additive, suggesting that these four media contained sufficient thymine or thymidine for the growth of these bacteria. This conclusion confirms the findings (table I) which indicated that these media were also unsatisfactory for trimethoprim-sensitivity testing.

**TABLE II**

**Effect of adding thymine or thymidine to various media on the growth* of Escherichia coli strain 114thy− and Aerobacter cloacae strain 53thy−**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Growth on the stated medium† with the addition of 60 μg per ml thymine</th>
<th>60 μg per ml thymidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxoid Blood Agar Base</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Difco Nutrient Agar</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oxoid MacConkey Agar</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Oxoid Diagnostic Sensitivity Test Agar +4% lysed horse blood</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Davis-Mingioli Agar</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Wellcome Nutrient Agar</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Wellcotest Agar</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Wellcotest Agar +4% lysed horse blood</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

* From an inoculum of an overnight broth culture diluted 10⁻⁶.
† + = Unrestricted growth, and − = a reduction of >95% in the number of colonies of both organisms.

Thymineless mutants grew on DM medium and Oxoid Diagnostic Sensitivity Test Agar with added lysed horse blood only if thymine or thymidine was added (table II). These media gave the same result probably because neither contained an effective amount of either. However, the mechanism by which this outcome is achieved must differ, because DM completely lacks thymidine and thymine, whilst Oxoid Diagnostic Sensitivity Test Agar must have contained at least one of these compounds, for in the absence of lysed horse blood it supports the growth of thymineless mutants. Hence lysed horse blood seems to contain a component that inactivates the quite small amounts of thymine derivatives available in Oxoid Diagnostic Sensitivity Test Agar. The concentration of this component is apparently insufficient to deal with amounts of thymine or thymidine of the order of 60 μg per ml, because Oxoid Diagnostic Sensitivity Test Agar with lysed horse blood and added thymine or thymidine supported the growth of the thymineless mutants.

Neither thymineless mutant grew on Wellcome Nutrient Agar, Wellcotest
Agar or Wellcotest Agar containing lysed horse blood (table II). This was to be expected, because earlier results (table I) had indicated that these media do not contain significant amounts of trimethoprim antagonists, i.e., they seem to be deficient in derivatives of thymine. When thymidine was added to any of the Wellcome media, both the thymineless mutants grew. However, added thymine (60 μg per ml) unexpectedly failed to support the growth of either thymineless mutant in any of the Wellcome media.

The Wellcome media might be deficient in a component essential for the efficient utilisation of thymine by thymineless mutants, but because these mutants grew readily on DM medium with added thymine this is improbable. A more likely explanation is that the Wellcome media contain a component antagonistic to the utilisation of thymine but not of thymidine. Such an antagonism might result from the activity of a substance that either inactivates thymine directly or prevents the bacterial assimilation of thymine. The latter hypothesis was subsequently investigated by testing Wellcome media for the presence of substances that interfered with the utilisation of thymine by thymineless mutants.

The effect of deoxyadenosine on the utilisation of thymine by thymineless mutants in Wellcome media

The assimilation of exogenous thymine by bacteria is dependent on its conversion to thymidine by the enzyme thymidine phosphorylase. The activity of this enzyme is therefore crucial for thymine uptake. It is known that thymidine phosphorylase is induced by deoxyadenosine, whereas uridine both represses and inhibits the enzyme (Budman and Pardee, 1967; Munch-Petersen, 1967).

Thus, if the Wellcome media contained a compound acting like uridine, this would account for the finding that thymineless mutants could not grow even in the presence of added thymine; the conversion of thymine to thymidine would be blocked and the assimilation of thymine inhibited. However, added thymidine, because it is not dependent on the action of thymidine phosphorylase for uptake, would still be assimilated and support the growth of thymineless mutants. The presence of a uridine-like compound in media could be checked by testing whether deoxyadenosine, which induces thymidine phosphorylase, permits the growth of thymineless mutants in the presence of added thymine.

Tests were made to ensure that there was no residual thymine in Wellcome Nutrient Agar for, although thymidine is known to be absent from this medium (Koch and Burchall, 1971), there remains a possibility that it may contain some thymine, which would remain undetected because of the presence of the thymine antagonist. This was investigated by plating A. cloacae no. 53thy<sup>−</sup> on Wellcome Nutrient Agar containing no added thymine, but with various concentrations of deoxyadenosine up to 1 mg per ml. No growth appeared on any plate, so it is unlikely that a significant amount of thymine is present in this medium.

The thymine requirement for A. cloacae no. 53thy<sup>−</sup> on Wellcome Nutrient
Agar was then determined in the presence and absence of deoxyadenosine and compared with the behaviour of this strain on DM agar. The results (table III) show that thymine even at 200 µg per ml was insufficient to promote the growth of the thymineless mutant on Wellcome Nutrient Agar, although the thymine requirement on DM was only 10 µg per ml. However, when 50 µg per ml of deoxyadenosine was added to Wellcome Nutrient Agar, the thymine requirement was reduced to 5 µg per ml, whereas in DM medium the thymine requirement in the presence of 50 µg per ml deoxyadenosine was only 0.4 µg per ml. Consequently, although deoxyadenosine overcame the effects of the antagonist of thymine assimilation present in Wellcome Nutrient Agar, the reversal was incomplete. Various concentrations of deoxyadenosine were tested to determine if the concentration had any effect on the extent of reversal. It was found that maximum reversal by deoxyadenosine occurred at a concentration of 25 µg per ml. Higher concentrations did not reduce the thymine requirement below 5 µg per ml in Wellcome Nutrient Agar.

The effect of uridine on the thymine requirement of the thymineless mutant Aerobacter cloacae 53thy⁻

To investigate whether uridine prevents the uptake of thymine by thymineless mutants, the effects of adding various supplements to Oxoid Blood Agar Base and DM agar on the growth of A. cloacae strain 53thy⁻ were studied (table IV). Uridine inhibited the utilisation of thymine by thymineless mutants on Oxoid Blood Agar Base and on DM agar. This inhibition was overcome by the further addition of deoxyadenosine. However, as expected, uridine did not prevent the utilisation of thymidine in these media. The thymineless mutant grew on Oxoid Blood Agar lacking any supplement, but this growth was abolished by the addition of uridine, so it seems that this medium contains some thymine, but not thymidine.

The growth of the thymineless mutant on Wellcome Nutrient Agar was also studied (table IV). The growth responses on this medium were similar to those found on DM agar containing added uridine. It would thus appear that Wellcome Nutrient Agar contains an antagonist to the utilisation of

---

**TABLE III**

*Requirement for thymine, with and without deoxyadenosine, of Aerobacter cloacae strain 53thy⁻*

<table>
<thead>
<tr>
<th>Substance</th>
<th>Minimum additions of the stated substance (µg per ml) necessary for growth* on</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wellcome Nutrient Agar</td>
</tr>
<tr>
<td>Thymine</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Thymine + 50 µg per ml deoxyadenosine</td>
<td>5</td>
</tr>
</tbody>
</table>

* As visible colonies from an inoculum of an overnight broth culture diluted 10⁻⁶.
TRIMETHOPRIM SENSITIVITY

thymine by thymineless mutants; the effect of this antagonist is similar to that of uridine.

DISCUSSION

Most laboratory media are unsuitable for the sensitivity testing of trimethoprim and sulphonamides, because of the presence of antagonists (Harper and Cawston, 1945; Waterworth, 1969). Koch and Burchall (1971) showed that thymidine is an important antagonist to trimethoprim in laboratory media. When thymidine monophosphate is synthesised by bacteria, equivalent

TABLE IV

Effect of uridine on the thymine requirement of Aerobacter cloacae strain 53thy-  

<table>
<thead>
<tr>
<th>Supplement*</th>
<th>Growth† with and without supplement on</th>
<th>Supplement*</th>
<th>Growth† with and without supplement on</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oxoid Blood Agar Base</td>
<td>DM Agar</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Thymidine</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Thymine</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Uridine</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Uridine+ thymidine</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Uridine+ thymine</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Uridine+ deoxyadenosine</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Uridine+ thymine+ deoxyadenosine</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Thymine and thymidine 60 μg per ml; uridine and deoxyadenosine 200 μg per ml.
† From an inoculum of an overnight broth culture diluted 10^-6; + = visible growth after 66 hours on DM agar and after 24 hours on the other media; - = no visible growth.

amounts of uridine monophosphate and a tetrahydrofolate derivative are utilised in the reaction; the tetrahydrofolate is oxidised to dihydrofolate in the process. Trimethoprim prevents the re-synthesis of tetrahydrofolate from dihydrofolate by inhibiting the enzyme dihydrofolate reductase (Hitchings, 1969). The resultant depletion of the tetrahydrofolate pool therefore prevents the production of thymidine monophosphate and consequently the synthesis of deoxyribonucleic acid ceases. If the bacteria are supplied with exogenous thymidine, this compound can be converted directly into thymidine monophosphate, and thus channelled into DNA synthesis, so largely overcoming the action of trimethoprim.

Thymine, unlike thymidine, is not normally incorporated by wild-type organisms (Siminovitch and Graham, 1955; Crawford, 1958; Kammen, 1967), and thus it was thought possible that thymine would not antagonise the action of trimethoprim. However, we have found that thymine reversed the effect of trimethoprim on wild-type trimethoprim-sensitive bacteria. Thus thymine, as well as thymidine, is an antagonist to the action of the drug.

The MIC of trimethoprim for four trimethoprim-sensitive strains indicated
that only four laboratory media gave an accurate representation of trimethoprim sensitivity when compared with trimethoprim activity in a minimal medium (DM) known to contain no derivatives of thymine. Two of these satisfactory media contained lysed horse blood, which Garrod and Waterworth (1971) advise against because it is inconvenient to use and is not available in some countries. Thus Wellcotest Agar and Wellcome Nutrient Agar are the only two media that, in the absence of lysed horse blood, lack trimethoprim antagonists.

For trimethoprim-sensitivity testing there appears to be no difference between the two Wellcome media. The MIC of trimethoprim for each of eight strains was identical on both, and neither permitted the growth of thymineless mutants even if thymine was added. This peculiarity, unique to the Wellcome media, was thought to be due to inhibition of the enzyme thymidine phosphorylase, which is required for the uptake of thymine. Exogenous thymidine added to either medium permitted the growth of thymineless mutants. Uridine, which inhibits thymidine phosphorylase, prevented the uptake of thymine and it is suggested that a uridine-like inhibitor is present in both Wellcome media. The inhibition of thymine uptake in Wellcome medium was overcome by deoxyadenosine. This compound induces thymidine phosphorylase and thus circumvents the effect of a uridine block.

Therefore, if the Wellcome media are used for testing trimethoprim sensitivity, a two-stage process has to be employed. The initial isolation of the bacteria may be performed on any suitable nutrient medium, provided that it contains a suitable concentration of added thymidine (i.e., 60 \( \mu \)g per ml). This permits the growth of thymineless mutants as well as wild-type bacteria. Subcultures from these primary isolates should then be made on to three plates of Wellcome media: one containing no supplements, a second containing trimethoprim 5 \( \mu \)g per ml, and a third containing thymidine 60 \( \mu \)g per ml. A trimethoprim-sensitive thymine-independent strain will not grow on the trimethoprim plate, a trimethoprim-resistant thymine-independent strain will grow on all three plates and a trimethoprim-resistant thymine-dependent strain will grow only on the thymidine-containing plate.

**Summary**

Various laboratory media were examined for use in trimethoprim-sensitivity testing. The most suitable were Wellcotest Sensitivity Test Agar and Wellcome Nutrient Agar, because these were the least antagonistic to the action of trimethoprim. Thymine as well as thymidine were found to antagonise trimethoprim activity against wild-type bacteria, and both these compounds are absent from the above media.

A procedure was devised for the identification of thymineless mutants, which are inherently resistant to trimethoprim. The two Wellcome media were found to contain an inhibitor that prevented the uptake of thymine but not of thymidine by thymineless mutants. The action of this inhibitor could be partially reversed by the addition of deoxyadenosine to the medium.
Because thymine and thymidine both antagonise the action of trimethoprim, a two-stage testing scheme was devised to distinguish between three types: trimethoprim-sensitive thymine-independent, trimethoprim-resistant thymine-independent, and trimethoprim-resistant thymine-requiring organisms.

We are grateful to the Wellcome Trust for a Research Training Scholarship to S. G. B. Amyes.

REFERENCES


