STUDIES ON URINARY CARRIAGE OF ENTERIC GROUP ORGANISMS

VII.—THE VALUE OF DIFFERENT CULTURAL METHODS FOR ROUTINE CLEARANCE TESTS AND FOR FOLLOW-UP INVESTIGATION OF CARRIERS

BY

Colonel G. T. L. ARCHER, M.B., M.R.C.P.I.

late Royal Army Medical Corps

Methods of urine culture for enteric group organisms and for the concentration of organisms of this group in specimens of urine were quantitatively evaluated by Archer and Ritchie (1950) in the first paper of this series. It was concluded that enrichment was probably unnecessary and could not be used exclusively, that direct plating, oxalate precipitation and primary fluid culture in an indicator medium ("MacConkey-mannite") should be used in carrier investigation work, and that assessment of the best methods for future routine use should be based on the results observed.

Williams Smith (1952) found selenite and tetrathionate superior to liquid desoxycholate-citrate, liquid Wilson and Blair, cacotheline broth and brilliant green peptone water for isolation of Salmonellae from faeces. The use of either selenite or tetrathionate might reveal the presence of ten Salmonella organisms, while it was necessary to add several thousand Salmonellae to faeces before they could be recovered by direct culture on desoxycholate-citrate-agar or Wilson and Blair solid medium. In general, isolation was easier from human and animal than
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from bird faeces, being easiest from horse faeces and most difficult from duck's, by each method. Different species of Salmonella varied in the ease with which they were recovered. *Salm. cholerae suis* and *Salm. abortus-ovis* were isolated more satisfactorily by direct plating than after enrichment. The combination of selenite and solid Wilson and Blair was sometimes too inhibitory, and there was little advantage in the substitution of mannite for lactose in selenite medium. Selenite was slightly better than tetrathionate; prolongation of incubation beyond 30 hours was detrimental with tetrathionate but not with selenite. Anderson and Richards (1948), however, found that under 18 hours' incubation was optimal for selenite, and that when this period was exceeded the number of colonies obtained on plate subcultures might fall. Browning *et al.* (1933) report direct culture as more successful than brilliant green enrichment for isolation of *Salm. typhi* from faecal carriers. They attribute this to antagonism between carriers' coliform strains and *Salm. typhi*. Brilliant green was, however, found superior to direct culture for *Salm. paratyphi B*. These workers consider that the likelihood of recovering bacilli (late in enteric fever or during convalescence) when a few loopfuls of urine are cultivated daily for 7-10 days is so considerable as to make this a valuable method of investigation. As regards carriers, they quote Garbat as recommending 24-hour specimens owing to intermittency of excretion.

Taylor (1947) refers to the use of tetrathionate broth and desoxycholate-citrate-agar for urine culture in enteric.

Direct plating, selenite, and simple primary culture in an indicator medium, with subculture to desoxycholate citrate or MacConkey plates, were used in the detection and follow-up of carriers described in the second (Archer, Goffe and Ritchie, 1952) and fourth (Archer and Naylor, 1952) papers of this series. For a short time oxalate precipitation was also used. The comparative value of methods used in primary screening and in follow-up tests is recorded and discussed below.

**PART I—Routine Screening**

The routine detection of carriers was attempted, as earlier described, by culture of a pool of three specimens taken on consecutive days from each individual. Thus each inoculum consisted of urine from one individual only but was a mixture of three of his specimens. In an effort to determine whether this was liable to cause failure of isolation due to intermission (or variation in numbers passed) leading to over-dilution of positive specimens, the individual specimens from approximately every tenth person were cultured in addition to the pool. Such single specimens were sometimes not submitted to all the methods of culture in current use, however, and the results of their culture will be referred to only if of special significance in any case. The results obtained by the different methods are shown on Table 1. Direct plating was usually to MacConkey but sometimes to desoxycholate-citrate-agar (D.C.A.). Culture of an oxalate precipitate was either direct or after passing through selenite. (Enrichment was suggested by Archer and Ritchie if the simple direct plating showed contamination, and this was generally done.) For a time both ordinary fluid
MacConkey, containing lactose (MacC-L), and MacConkey mannite (MacC-M) were used, but the former was later discarded. Subcultures from either of these media were commonly made to both MacConkey and D.C.A. plates, but subculture was generally omitted if there was no evidence of fermentation of the mannite in the primary culture containing it. Cultures from cases IX, XXI, XXIV and XXVI (notes (k), (t) and (q) to Table I) showed the inconstant production of gas by aerogenic species discussed by Archer (1953) in the fifth paper of this series.

**TABLE I.—RESULTS OF ROUTINE CULTURE FOR DETECTION OF CARRIERS BY THE METHODS SHOWN**

(Case numbers in Roman numerals in Column 7 refer to this section of the paper only and bear no relationship to strain or carrier numbers used in previous papers or in Part III of this paper.)

<table>
<thead>
<tr>
<th>Series</th>
<th>No. of Persons Tested (by culture of a pool of 3 specimens)</th>
<th>Method (D=simple direct plating, O=oxalate precipitation)</th>
<th>No. of positive cultures</th>
<th>Isolations</th>
<th>Comment</th>
<th>Case Nos.</th>
<th>Note reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>69</td>
<td>D and O</td>
<td>Nil</td>
<td>T=A=Salm. typhi isolated</td>
<td>From one case</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>124</td>
<td>D</td>
<td>1</td>
<td>T=A=Salm. paratyphi A isolated</td>
<td>From a second case</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>O</td>
<td>1</td>
<td>T=A=Salm. paratyphi C isolated</td>
<td>From case I</td>
<td>I</td>
<td>(a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MacC-L</td>
<td>3</td>
<td>T=A=Salm. paratyphi C isolated</td>
<td>From case II and a third case</td>
<td>II III (b) (c)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MacC-M</td>
<td>3</td>
<td>T=A=Salm. paratyphi C isolated</td>
<td>From cases II and III</td>
<td>I</td>
<td>(a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>II III (b) (c) (d)</td>
<td></td>
</tr>
</tbody>
</table>

**NOTES TO TABLE I**

(a) Numerous suspicious colonies on subculture, but sugar reactions atypical on first test. Plate from lactose medium further studied, with positive result, when direct culture found positive. Non-lactose-fermenting colonies from mannite medium not tested further. *Salm. paratyphi A* isolated was aerogenic, but MacC-M, though acid, contained no gas; further tests on the plate from it might therefore have revealed *Salm. typhi*. The lactose medium, however, was also acid (without gas) and it is possible that both media were in fact mannite, one having been labelled lactose in error (see also note (c)). Otherwise, acidity of the urine specimen may have caused the effect in the lactose medium, and also have led to the failure of the oxalate method by lethal action during prolonged contact.

(b) Case II: MacC-L remained neutral, and MacC-M was acid. The former was positive on subculture to D.C.A., the latter on subculture to both MacConkey and D.C.A. This was the primary isolation from chronic carrier No. 4 (q.v. under Part III and Table II).

(c) Case III: Neither of the MacConkey media was acid after 48 hours (possibly a container of lactose medium was marked mannite in error (see note (a)) or this too may perhaps have been an inoculum effect, in this case strong buffering action of the urine). This was the primary isolation from chronic carrier No. 2 (q.v. under Part III and Table II).

(d) Direct cultures from cases II, III, VI and VIII were contaminated.
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Table 1—continued

<table>
<thead>
<tr>
<th>Series</th>
<th>No. of Persons Tested (by culture of a pool of 3 specimens)</th>
<th>Method (D=simple direct plating, O=oxalate precipitation)</th>
<th>No. of positive cultures</th>
<th>Pathogens isolated</th>
<th>Comment</th>
<th>Case Nos.</th>
<th>Note reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>166</td>
<td>D</td>
<td>2</td>
<td>A</td>
<td>From two cases</td>
<td>IV, V</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MacC-L</td>
<td>2 A</td>
<td></td>
<td></td>
<td></td>
<td>IV, V</td>
<td>(e)</td>
</tr>
<tr>
<td></td>
<td>MacC-M</td>
<td>3 A</td>
<td></td>
<td></td>
<td></td>
<td>IV, V</td>
<td>(e)</td>
</tr>
<tr>
<td>D</td>
<td>508</td>
<td>D</td>
<td>1</td>
<td>A</td>
<td>From one case</td>
<td>VII</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MacC-M</td>
<td>4 A</td>
<td></td>
<td></td>
<td></td>
<td>VII, VIII</td>
<td>(g), (d)</td>
</tr>
<tr>
<td>E</td>
<td>534</td>
<td>D</td>
<td>5</td>
<td>T</td>
<td>From four cases</td>
<td>XI, XII, XIII, XIV</td>
<td>(m)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td>XV</td>
<td>(n)</td>
</tr>
<tr>
<td>F</td>
<td>682</td>
<td>D</td>
<td>8</td>
<td>A</td>
<td>From one case</td>
<td>XVI</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MacC-M</td>
<td>11 A</td>
<td></td>
<td></td>
<td></td>
<td>XVI, XXI, XXII</td>
<td>(p)</td>
</tr>
</tbody>
</table>

Comment:
- Salm. paratyphi A (poorly aerogenic) from MacConkey plate from both. Colonies on D.C.A. were minute and not further examined.
- Salm. typhi from subcultures on both MacConkey and D.C.A. plates.

Note:
- (e) Case IV: Acid and gas in both MacConkey media. Salm. paratyphi A (poorly aerogenic) from MacConkey plate from both. Colonies on D.C.A. were minute and not further examined.
- (f) MacC-L no change and no record of subculture, MacC-M acid. Salm. typhi from subcultures on both MacConkey and D.C.A. plates.
(g) Case VII: MacC-M acid and gas but poor growth on D.C.A. (only three colonies), missed until direct culture positive. No subculture to MacConkey plates. Case VIII: MacC-M acid and gas.

(h) MacC-M acid and gas: no gas in glucose (slight gas in mannite) inoculated from one colony; no gas in mannite or dulcite (but slight gas in glucose) inoculated from another colony.

(i) MacC-M acid only. Colonies from D.C.A. produced acid only in glucose and mannite: growth rough. No record of serological tests.

(m) Case XI: Oxalate culture apparently omitted. Case XII: Investigated by both pool and individual cultures of three specimens. All four were found positive by oxalate precipitation, but one separate specimen was negative on direct plating. Case XIV: Plate from oxalate accidentally broken.

(n) Case XV: Negative by oxalate method.

(p) Non-lactose fermenting colonies; not further tested.

(q) MacC-M showed acid and gas in cases XVI, XXIII and XXV. Acid only in case XXVI and alkali in case XXIV.

(r) MacC-M showed acid in case XVIII, but was neutral in case XXVII and weakly alkaline in case XVII. Case XX gave an acid culture in MacC-M but lactose fermenters only on subculture.

(s) MacC-M acid, no further record.

(t) MacC-M acid only from case XXI, neutral with gas from case XXII.

**SUMMARY:**

<table>
<thead>
<tr>
<th>Persons examined</th>
<th>...</th>
<th>...</th>
<th>2,083</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enteric group strains isolated</td>
<td>...</td>
<td>...</td>
<td>27</td>
</tr>
</tbody>
</table>

Proportion missed by—

<table>
<thead>
<tr>
<th>Direct culture</th>
<th>...</th>
<th>...</th>
<th>10/27</th>
<th>T 5/14, A 4/11 and one doubtful, C 1/3. Both A and T isolated from double carrier. See also note (m).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxalate</td>
<td>...</td>
<td>...</td>
<td>3/6</td>
<td>T 2/5, A 2/2. Both A and T missed from double carrier. (This failure therefore shown under each species). But see note (m).</td>
</tr>
<tr>
<td>Fluid MacConkey</td>
<td>...</td>
<td>...</td>
<td>1/22</td>
<td>T 1/9; one other T doubtful; T missed in double carrier; in one of the other ten specimens from A excretors the organism was scanty and missed until direct examination was found positive.</td>
</tr>
</tbody>
</table>

Specimens from 192 cases were cultured in MacC-M only. Four yielded cultures of *Salm. paratyphi* A and four cultures of *Salm. paratyphi* C, bringing the incidence to 35 excretors among 2,275 persons (1.5 per cent.).

Fermentation by the former was indicated by acid twice; the two other cultures were alkaline. Slight gas was present in three, more abundant gas in the fourth (an acid culture). *Salm. paratyphi* C produced acid in three of four cultures (two being only slightly acid); the fourth culture was neutral but contained gas. Gas was absent from one of the weakly acid cultures.

**PART II.—INVESTIGATION FOLLOWING AN OUTBREAK**

Eighteen foodhandlers were involved. The specimens came from a distance. A first examination was made on triple pools by direct, oxalate and fluid MacConkey cultures. One case was apparently positive by all methods. The MacConkey media used for this case both showed gas. The lactose was alkaline, but the mannite acid. Non-lactose fermenters grew from the latter on both
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D.C.A. and MacConkey plates. The strain was too rough to identify, but later isolations from the same carrier, though also rough, were established as *Salm. paratyphi C* (strain 6 of the sixth paper of this series). It chanced that this individual was one from whom single specimens were also examined separately. Direct and oxalate cultures were used for these separate examinations. Two of the three were positive by the former and only one of the three by the latter. The next two examinations were on incomplete batches of specimens cultured by direct and MacConkey methods only. In one the *Salm. paratyphi C* carrier was missed, his specimen being omitted from the other. Seven further batches of specimens were cultured singly, not pooled, by direct and MacConkey methods (MacC-M only, on the last three occasions). The *Salm. paratyphi C* carrier was missed four of seven times by direct culture, and one of seven times by MacConkey. With regard to the latter, the lactose medium showed gas, gas with alkali, and no change on the three occasions when it was used in positive tests; the mannite medium showed acid and gas in all such tests. D.C.A. plate-subcultures were positive on five of the six occasions. Subcultures to MacConkey were positive on the only two occasions when it was used (which included the one on which D.C.A. was negative, showing fermenting colonies only). On one occasion only, an organism provisionally identified as *Salm. paratyphi A* was isolated in MacC-M (but not on direct culture) from a different person in the group. The MacC-M contained acid and gas; MacC-L was unchanged and the strain was agglutinated by A "O" (but not A "H") serum.

**PART III.—FOLLOW-UP CULTURES ON KNOWN CARRIERS**

1. *In an Investigation of Regularity and Weight of Excretion* (Archer et al., 1952)

The results obtained by the use of direct plating, selenite and MacC-M in the follow-up of nine of the ten carriers classified as chronic in the second paper in this series are recorded in Table II, which shows a higher proportion of positive cultures from direct plates than from either of the fluid media.

This can partly, but not entirely, be accounted for by the failure of both fluid media in the majority of tests carried out on carrier No. 2, though direct inoculation of plates from the same specimen was successful. Archer, Goffe and Ritchie (1952) considered this apparent self-sterilization as possibly due to phage action. Later observations on the acid tolerance of carrier strains and the acidity produced by fermentation (Archer, 1953) suggest that a heavy inoculum of acid urine was, perhaps, a more likely cause. This, however, would hardly explain a peculiar abrupt termination of growth from serial dilutions in a viable count (when the number of colonies present on the last positive plate led to an expectation of at least one further dilution showing some), a finding which was also recorded as twice observed by them among five attempts to carry out viable counts on specimens from this person. The tabulated results for successful isolations are very probably too small since positive findings may have been reduced by failures of serological identifications due to antigenic degradation. This is suggested by failures of this kind sometimes occurring with cultures isolated only by one or two of the methods used, the remaining culture (or
### Table II.-Comparative Results in Follow-up Cultures of Urine of Chronic Carriers by Different Methods

<table>
<thead>
<tr>
<th>Carrier's No.</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>5</th>
<th>9</th>
<th>10 (a)</th>
<th>19</th>
<th>20</th>
<th>21</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Specimens isolated</strong> (T = Salm. typhi, A = Salm. paratyphi A)</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>A</td>
<td>A</td>
<td>T</td>
<td>A</td>
<td>T</td>
<td>A</td>
<td>All T's</td>
</tr>
<tr>
<td><strong>Direct Culture</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number positive</td>
<td>32</td>
<td>17</td>
<td>19</td>
<td>20</td>
<td>26</td>
<td>20</td>
<td>28</td>
<td>30</td>
<td>18</td>
<td>118</td>
</tr>
<tr>
<td>Number examined</td>
<td>37</td>
<td>27</td>
<td>22</td>
<td>23</td>
<td>35</td>
<td>27</td>
<td>30</td>
<td>30</td>
<td>18</td>
<td>143</td>
</tr>
<tr>
<td>Percentage positive</td>
<td>86</td>
<td>63</td>
<td>86</td>
<td>87</td>
<td>74</td>
<td>74</td>
<td>93</td>
<td>100</td>
<td>100</td>
<td>82.5</td>
</tr>
<tr>
<td><strong>Selenite</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number positive</td>
<td>12</td>
<td>17 (c)</td>
<td>17</td>
<td>18</td>
<td>11 (d)</td>
<td>22</td>
<td>6</td>
<td>74</td>
<td>44</td>
<td>118</td>
</tr>
<tr>
<td>Number examined</td>
<td>21</td>
<td>26</td>
<td>21</td>
<td>17</td>
<td>22</td>
<td>26</td>
<td>11</td>
<td>22</td>
<td>6</td>
<td>116</td>
</tr>
<tr>
<td>Percentage positive (when more than 10 tests)</td>
<td>57</td>
<td>19</td>
<td>81</td>
<td>58</td>
<td>77</td>
<td>69</td>
<td>100</td>
<td>100</td>
<td>63.8</td>
<td>78.6</td>
</tr>
<tr>
<td><strong>MacConkey mannite</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number positive</td>
<td>16</td>
<td>8</td>
<td>15</td>
<td>13</td>
<td>14</td>
<td>16</td>
<td>4</td>
<td>55</td>
<td>31</td>
<td>86</td>
</tr>
<tr>
<td>Number examined</td>
<td>23</td>
<td>25</td>
<td>20</td>
<td>17</td>
<td>22</td>
<td>23</td>
<td>4</td>
<td>91</td>
<td>43</td>
<td>134</td>
</tr>
<tr>
<td>Percentage positive (when more than 10 tests)</td>
<td>70</td>
<td>32</td>
<td>75</td>
<td>76</td>
<td>64</td>
<td>70</td>
<td></td>
<td>60.4</td>
<td>72.1</td>
<td>64.2 (f)</td>
</tr>
<tr>
<td><strong>Results by any method</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Number positive</td>
<td>33</td>
<td>18</td>
<td>25</td>
<td>23</td>
<td>34</td>
<td>25</td>
<td>30</td>
<td>30</td>
<td>18</td>
<td>131</td>
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<tr>
<td>Number examined</td>
<td>39</td>
<td>29</td>
<td>26</td>
<td>25</td>
<td>37</td>
<td>29</td>
<td>30</td>
<td>30</td>
<td>18</td>
<td>153</td>
</tr>
<tr>
<td>Percentage positive</td>
<td>85</td>
<td>62</td>
<td>96</td>
<td>92</td>
<td>91</td>
<td>86</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>85.6</td>
</tr>
</tbody>
</table>

### Notes on Table II

(a) Results shown are those following the first course of unsuccessful treatment recorded by Archer and Naylor (1952).

(b) Direct examinations were more frequent than those using either fluid medium. A reduced series, however, consisting of cultures of specimens for which one or both of the additional methods were also used, yielded 82 per cent. (141/172) positive and "presumed positive" cultures.

In this series a "presumption of identity" of the organisms present with those identified in the other medium has been made twelve times when serological tests on the plate culture failed (see text), or when identification is not recorded. If these results are regarded as negative, or omitted from the series, the positive findings fall to 75 per cent. (129/172) or 80.6 per cent. (129/160) respectively.

(c) In four other tests (three positive and one negative) tetrathionate was used in place of selenite.

(d) First isolation was in selenite, the direct plate being negative.

(e) This percentage rises to 77 positive (137/178) if certain doubtful, and a few later, results are included.

(f) Rising to 75.4 per cent. positive (104/138) if certain doubtful results are included.

(g) The figures here are, in the main, smaller than the corresponding ones in the second paper in this series. Most of the examinations here listed, however, formed part of the larger numbers then reported. Even so, sometimes only one or two methods, not all three, were used for this reduced series, and the figures for the positive results by all methods are therefore probably lower than they would have been had all three been used on each occasion.
# Table III: Success of Different Culture Methods in Cases Under Treatment

<table>
<thead>
<tr>
<th>Case No. (Pathogens carried)</th>
<th>Nature and stage of treatment</th>
<th>Isolation of Pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Direct culture</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>9 (Salm. paratyphi A)</td>
<td>Preliminary cultures and cultures between courses</td>
<td>33 (a)</td>
</tr>
<tr>
<td></td>
<td>For 3 days after Hexamine ... Sulphanilamide Course ...</td>
<td>2 (e)</td>
</tr>
<tr>
<td></td>
<td>For 7 days after Sulphanilamide ... ... ...</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>For 3 days after Streptomycin ... ... ...</td>
<td>1</td>
</tr>
<tr>
<td>10 (Salm. typhi—second series of courses)</td>
<td>Preliminary cultures and cultures between courses</td>
<td>75 (q)</td>
</tr>
<tr>
<td></td>
<td>Hexamine Course ... Sulphanilamide Course ... Streptomycin Course ...</td>
<td>1 (w)</td>
</tr>
<tr>
<td>20 (Salm. typhi)</td>
<td>Preliminary Cultures ...</td>
<td>12</td>
</tr>
<tr>
<td>Totals (percentage by each method)</td>
<td></td>
<td>135 (81.3)</td>
</tr>
</tbody>
</table>

* The results of all cultures between courses are not here recorded, but the numbers given for each method refer to cultures made between the same dates, from portions of the same specimens.

**Notes on Table III**

(a) After sulphanilamide course plates sometimes showed only a few colonies, but acid and gas were regularly present in MacConkey-mannite.
(b) The ten positive selenites include the specimen negative by direct plate. Three of the ten were negative in 6-hour subcultures though positive after 18 hours; seven were positive after 6 hours.
(c) These were tested at 6 hours only, not after 18 hours.
(d) Presumptive.
(e) One colony and three colonies only on respective direct plates; the former was also positive in the fluid media, but there was no acid or gas in MacC-M, and only the 18-hour subcultures were positive from selenite.
(f) After 18 hours; negative on 6-hour subculture.
(g) One showed no acid or gas in MacC-M, another showed gas only.
(h) Two positives at 18 hours only (both negative at 6 hours). One was direct negative but also MacC-M positive. Two of the five negatives were only tested after 6 hours, not after 18.
(k) Two (both negative by direct test) showed only poor fermentation (one slight acid and gas, the other slight gas only). The three other positives were presumptive.
(m) At 18 hours, negative at 6 hours.
(n) The positives include the five which were negative by direct test; 66 others were presumptive.
(p) Four contaminated.
(q) One to eight colonies only on each plate.
(r) Includes one negative by direct plating.
(s) One was from the other specimen negative by direct plating. The other seven were presumptive.
(t) Same specimen; contaminated in MacC-M.
(u) Two showed no acidification of MacC-M.
(w) One colony only.
(x) The specimen producing the positive culture was negative by direct plating, and one of the five negatives was positive by direct plating.
(y) The specimens producing the positives were both negative by direct plating, one was positive in selenite. The four negatives included the direct positive.
(z) Presumptive.
cultures) being identifiable. Where this occurred an assumption of identity has commonly been made for all, but when all isolations from a specimen were inagglutinatable a negative result was recorded. Later, biochemical tests were mainly used, and the presumptive evidence afforded by them accepted, serological confirmation of identity being only occasionally sought.

These three methods were also used to examine specimens from carriers 13, 14, 17 and 18 (Archer et al., 1952). Direct culture was positive 29 times in 33, selenite 19 times in 31, MacC-M 21 times in 30. All 33 specimens were positive by one or more methods. Here again the comparative failure of the fluid methods for carrier 18 (direct 12/12, selenite 6/12, MacConkey 6/11) largely accounts for the observed differences in effectiveness.

2. In Control of Treatment reported by Archer and Naylor (1952)

The same three methods were used for the culture of specimens before, during and after treatment of cases. Selenite was frequently subcultured twice—after 6, and again after 18, hours. When direct cultures were positive, biochemical and serological identification tests were generally confined to them, and positive results with other media were “presumptive” only; when direct cultures were negative, however, identity was established by similar tests on organisms isolated by the fluid methods. Results are given in Table III.

They show again the high value of direct culture, though here even better results attended the use of MacConkey-mannite, probably largely due to the relative success of the latter when the number of pathogens was low. Comparatively poor results with selenite are due to the number of negative results when subculture was made after 6 hours only. An inhibitory concentration of the excreted drug may have caused direct cultures of some specimens to be negative, while inoculation into a fluid medium diluted the drug content of the same specimen below an effective level so that growth occurred. (Para-aminobenzoic acid was, however, used in plates inoculated during sulphanilamide treatment.) It will be seen from notes (g), (k) and (v) to Table III that fermentation in MacConkey-mannite was sometimes not evident though the organism was present. Such findings will be discussed in general in a further paper, but in these instances they may have been due to a carry over in the inoculum of semi-inhibitory drug concentrations.

The suitability of 18 hours in selenite and its superiority to 6 hours is confirmed by notes (b), (f), (h) and (m) to Table III, though for most specimens the latter period is quite satisfactory.

Analysis of small numbers shows little difference in success of subculture to MacConkey or D.C.A. plates from either MacConkey-mannite or selenite.

PART IV.—DISCUSSION, SUMMARY AND CONCLUSION

Discussion

Before attempting to make recommendations regarding the best practicable methods of routine screening by urine culture to exclude enteric carriers, an attempt must be made to account for Table I’s showing fluid MacConkey to be
Urinary Carriage of Enteric Group Organisms

considerably better than direct culture, while Table II shows the reverse. Two possibilities suggest themselves: the type of carrier concerned, and the type of specimen.

The Type of Carrier Concerned.—Table I records the results of routine screening; the carriers are unselected and may be presumed to include a proportion who are transient or intermittent. The larger inocula possible when using fluid media were presumably responsible for the success of 13/24 cultures in selenite or tetrathionate, as compared with 8/24 positive direct plates, from the intermittent carrier No. 12 recorded in the second paper in this series. Intermittent carrier No. 14 showed five cultures positive in MacC-M as compared with four positive by direct plating, and the results in Table III also suggest the superiority of MacC-M when pathogens are scanty (due to treatment in the results there recorded, but as may occur from time to time even in chronic carriers (Archer et. al., 1952)). The passage of the pathogens in small numbers may also have caused the relatively poor results of direct culture of specimens from the \emph{Salm. paratyphi C} carrier recorded in Part II above, though contamination was also present.

All carriers listed in Table II, on the other hand, were chronic persistent. Nevertheless the possible occurrence of transient or intermittent carriers in the series analysed in Table I does not wholly explain the discrepancy, since two of the carriers missed by direct examination in routine screening were chronic carriers No. 2 and No. 4 (\emph{vide} notes (b) and (c) to Table I), and the frequency with which direct cultures from them were positive (\emph{vide} Table II) shows that the chance against this occurring is approximately 19 to 1. Carrier No. 19 was also missed by direct culture (\emph{vide} note (d) to Table II) and the chance against this should have been 14 to 1, while the chance against all three being negative by direct culture (as they were) would be 296 to 1, \textit{if in all cases cultures were similar}.

The Type of Specimen.—Cultures for routine screening (Table I) and for carrier follow-up (Table II) were not similar, however, but differed in the nature of the inoculum, that for routine screening being a pool of three specimens taken on consecutive days, that for follow-up, a fresh specimen passed that day. Though three specimens were taken and used by the former method, a negative result on direct culture occurred three times (33 per cent.) in the primary (screening) tests on the nine cases listed in Table II. The over-all chance of a single specimen being negative by direct plating in the examinations there recorded, however, is only 15.7 per cent. and that of two direct plates both, and that of three direct plates all, being negative 2.5 and 0.4 per cent. respectively. Similarly, while the screening plates of carriers No. 2 and No. 19 actually were negative, the chance of the former (the least frequently positive of the chronic carriers) being so by all of one, two or three direct plates was 37, 13.3 and 5.1 per cent. and of the latter being similarly negative 6.7, 0.4 and 0.03 per cent. respectively.

It thus appears that the method of specimen pooling used for screening was not good; that, although three samples were taken, better direct plating results
might have been achieved by the immediate culture of a single one from each case, and would almost certainly have followed the separate culture of two; and that it was this difference of inoculum which accounted for the discrepancies found between the relative efficiency of direct plating and MacC-M in the two series of tests recorded in Tables I and II respectively. The manner in which pooling might diminish the chances of positive culture may include:

(1) The possibility of contaminants over-growing the pathogen in the first and second specimens while waiting for the third; as stated in note (d) to Table I, four of the ten direct negatives were recorded as contaminated. This chance may be diminished in fluid culture by pooling in the medium, rather than pooling before inoculation—adding to the medium a portion of each specimen when passed, and refrigerating (rather than incubating) until the last portion is added.

(2) The possibility of over-dilution of one (or two) specimens containing a few pathogens by additions of two (or one) containing none. When dealing with chronic persistent carriers this seems only likely to arise if there is—

(3) Killing of the pathogens in the first and second specimens by acid (or some other factor) in one or both of them, while the third is awaited. Such a possibility is suggested by the observed occurrence of rapid death in media, on the supposition that death of the culture is then due to the lethal action continuing in a large urine inoculum. Large inocula of this type may also have accounted in whole or in part for the inferiority of MacC-M and selenite to direct plating shown in Table II, the adverse effect being perhaps enhanced by acid products of fermentation in the former. The poor results of oxalate precipitation recorded in Table I may have had a similar cause. This supposition and the absence of visual evidence of "sugar" fermentation sometimes noted in MacC-M will be considered in the next paper in this series.

(4) Development of bacterial antagonism in specimens awaiting culture.

Recent Results.—While separate specimens are thus probably the inocula of choice for direct plating, recent results since the use of two such inocula was adopted (Nagington, 1953) do not, unfortunately, show that degree of improvement that the above calculations might suggest. Direct plating remained inferior to selenite, which was used in parallel with it for local specimens. (The use of MacC-M was now confined to specimens obtained at a distance and sent in it to the laboratory.)

Thus Nagington obtained positive culture in selenite from 31 of 32 excretors in 1952, while only 25 of the 32 yielded positive direct plates, a direct/selenite ratio of 80.6 per cent. If, however, this is compared with the direct/MacC-M ratio for those series in Table I (B, C, D and F) where both were used, which was 12/21 (57.1 per cent.), some improvement in direct plating results would seem to have resulted from the change of method. Such a deduction calls for an assumption that the selenite used in the 1952 series was as efficient as the MacC-M in the earlier one. This seems more than justified by results shown in Table II, but of doubtful validity on considering those in Table III, where treatment had, in some
instances, apparently given rise to inocula containing scanty organisms. Moreover, if irregular, doubtful, or retrospectively positive results in MacC-M (notes (a), (c), (g), (l), (r) and (s) to Table I) are left out, the direct MacC-M ratio for Table I becomes 12/15 (80 per cent.).

**Numbers Excreted.**—When comparing these recent results with those in Table II it must be emphasized that the former, as with those in Table I, may be presumed to include positive isolations from transient or intermittent carriers. Those missed in the recent series by either method were the only excretor of *Salm. paratyphi B*, which was negative by selenite; and five excretors of *Salm. paratyphi A*, one of *Salm. typhi* and one of *Salm. paratyphi C* missed by direct plating. The proportion of *Salm. paratyphi A* to *Salm. typhi* direct-plating failures was thus 5/1, while the ratio of positive isolations of these species in selenite was less than 2/1. This might be due to a general greater likelihood of missing *Salm. paratyphi A* than *Salm. typhi* by direct plating, or to the incidence in this series of a higher proportion of excretors of scanty organisms of the former than of the latter species. To assess the first of these possibilities the numbers of organisms found by viable counts on specimens passed by chronic carriers of each may be compared. Of the 38 successful viable counts recorded by Archer et al. (1952), 21 were on six chronic carriers of *Salm. typhi*, 12 were on four chronic carriers of *Salm. paratyphi A*, and 4 on an intermittent and two unclassified excretors of the latter species. Sixteen of the *Salm. typhi* counts (from four carriers) were over a million per ml. and, though nine of the sixteen were from one person, the average of the mean counts on each of the six was five million per ml.

On the other hand, only two of the counts (from different carriers) on the chronic carriers, and one of the counts on an unclassified carrier, of *Salm. paratyphi A* were over a million. The average of mean counts on the chronic carriers was 440,000; on the others, one million; and on all *Salm. paratyphi A* excretors, 650,000. These figures, however, cannot be correlated with a higher failure rate of direct culture for the latter than for the former species, since all these specimens submitted to viable counts save two should, on the evidence of the counts, have produced considerable growth, while the remaining two should not have been missed, on plating a loopful of urine. Further, though wide fluctuation is also shown by a colony record of 8, 1, 1, 3, 14 and "scanty" colonies from loop-inocula from four of the chronic carriers of *Salm. typhi*, and 2, "scanty" and "scanty" from three of the *Salm. paratyphi A* excretors, carriers No. 1 and No. 19, who had the two lowest average viable counts (21,500 *Salm. typhi*—average of two counts, and 29,000 *Salm. paratyphi A*—average of five counts, respectively) were, as will be seen from Table II, generally positive by direct plating. Table II also shows a slightly higher proportion of positive results of direct plating from the four chronic carriers of *Salm. paratyphi A* than from the five of *Salm. typhi*. The observed difference in viable counts may well be due to chance. If confirmed as significant in a larger series it might afford a possible explanation of the apparently greater existing risk of infection with *Salm. typhi* than with *Salm. paratyphi A*, but Chadwick (1952) found carriers of
both species to be infective, "there being slight and possibly unimportant differences in the behaviour of the two species."

**Volume of Plating Inoculum.**—In view of the above, and since direct-culture failures shown in Table I are evenly distributed between species, it seems likely that the recent high failure rate of *Salm. paratyphi A* may reflect the incidence at that time of habitual excretors of few organisms. Habitual scanty excretion by chronic persistent carriers is uncommon. Hence the excretors missed by direct plating in 1952 may have been non-persistent. The fact that, in respect of six of them, only one of the two specimens cultured in selenite from each was positive, gives a little support to this idea. Transient or intermittent carriers would be liable to be missed by any method, and should be less dangerous if employed, but in the absence of direct evidence that these only were, or are likely to be, missed by direct plating, and in an endeavour to increase the probability of finding excretors of relatively scanty organisms of all types by this method, it seems desirable to try the use of larger inocula. The possibility of thus favouring overgrowth of the enteric species by other organisms also present must not be overlooked, but, as will be shown in a further paper, this is unlikely to be great if fresh specimens are used. Larger inocula could be obtained by the use of drops from a pasteur pipette which could then be spread more widely over the plate (Vogelsang and Boe, 1948), by the use of a swab dipped in the specimen and rubbed over the plate, by preparing pour-plates as used by Archer *et al.* (1950), or by the use of centrifuged specimens. The last two, of course, add to the work involved and the equipment required and hence should be avoided if possible. Use of pour-plates was soon stopped as being unnecessary for carriers' follow-up. Centrifuging may be possible without a great increase of work if a suitable centrifuge, in which a large number of specimens may be rapidly spun down in the containers in which they were collected, is available. It is hardly practicable on the scale required, however, in simple laboratory centrifuges, and the use of precipitation was investigated by Archer and Ritchie (1950) as a practical substitute.

**Oxalate Precipitation Method.**—Oxalate precipitation was subsequently advocated as almost the method of choice. It proved somewhat laborious in routine practice, however, particularly the removal of the supernatent fluids from the large number of specimens involved. This entailed the attachment to a suction pump and reservoir of a fresh sterile pasteur pipette for each specimen. Further, as noted above and in Table I, results were disappointing. It was therefore discontinued.

**Summary and Conclusions**

1. The results of direct plating, and of plating after oxalate precipitation and after culture in MacConkey-mannite, in routine carrier clearance tests are tabulated, described and discussed. More recent results, using direct plating and plating after culture in selenite medium, are also considered.

The relative failure shown by direct plating is suggested as partly due, in the earlier series, to the use of pooled specimens, since it is at variance with its
success when using fresh single specimens in carrier follow-up. An additional cause in that series, and one also affecting the more recent results, however, seems to lie in specimens containing scanty pathogens. Such specimens are considered as more likely to be commonly passed by transient or intermittent, than by chronic persistent, carriers.

2. Recently *Salm. paratyphi* A has been, both relatively and absolutely, more frequently missed by direct plating than was *Salm. typhi*. Chronic carriers of *Salm. typhi* would appear, on the limited evidence available, to be more liable to pass organisms in very large numbers than carriers of *Salm. paratyphi* A, the average viable counts for the two species in a small series being about eight times greater for the former. There is no correlation, however, between these sets of findings.

3. Results of direct plating and of preliminary culture in selenite medium or MacConkey-mannite medium in the follow-up of chronic carriers are similarly considered.

4. It is thought that—

(i) Direct plating of two fresh specimens on different days should give better results than the pooling of three specimens before culture. When specimens can reach the laboratory fresh it is the single method of choice.

(ii) Culture of a third specimen gives, of course, an even better chance of success, but an improvement in detection of chronic persistent carriers of 2 to 10 per cent. is all that can be expected from it. As some Egyptian carriers may be transient or intermittent and, as such, be missed by any feasible screening plan, complete elimination of all carriers among these people is not practicable. Such a gain is therefore not commensurate with the 50 per cent. increased work involved, when working in Egypt, if facilities are limited.

(iii) The use of a single culture method is the ideal, since each additional method is liable to cause 50 to 200 per cent. extra work for a relatively small gain. If a single method is to be relied upon, however, it should achieve 90 per cent. of the success to be gained by the combined use of any two. Its more frequent use, rather than the rarer use of multiple methods, then seems fully justified. Such a degree of success has not yet attended the use of direct plating in routine screening. It has been found in 1952 for selenite, but the high proportion of failures of cultures containing large fluid inocula from certain carriers, and the lower general success of fluid culture from chronic persistent carriers studied, renders the sole use of such culture methods inadvisable, until the causes of such failure, and means to overcome them, are known.

(iv) Thus the examination of two fresh specimens both by direct plating and by plating after primary fluid culture seems indicated pending trial of larger inocula for the former, which might so improve results as to warrant its use alone.

**ACKNOWLEDGMENTS**

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G. R. E. Naylor, and Captain J. Nagington. I am also greatly obliged to Captain Nagington for detailed information on the work done since I left the Laboratory early in 1952. I wish also to thank the Director of Pathology, Brigadier A. Sachs, C.B.E., M.D., M.Sc., Q.H.P., for permission to publish this work.

REFERENCES


CORRIGENDUM

The following correction should be made in the fifth paper in this series entitled "Cultural Abnormality and Variation in Isolated Strains." (This Journal, Vol. 99, pp. 55-65.)

Page 60: Under "Least acid reaction at which a culture died," second line, before "(Salm. paratyphi C in glucose)," for "pH 4.63" substitute "pH 4.80."

THE LATE DR. MERVYN GORDON

Dr. Mervyn Gordon, C.M.G., C.B.E., D.M., F.R.S., who died on 26th July, 1953, at the age of 81, had been a member of the Army Pathology Advisory Committee since its inception in 1919, and in these thirty-four years he attended every one of the seventy meetings of the Committee. Dr. Gordon had previously been, from 1915 to 1919, a Consulting Bacteriologist to the Army, with the honorary rank of Lieutenant-Colonel.

The Army Medical Services, in mourning an able and stimulating adviser, honour a record of loyal and devoted service which will not readily be equalled.
Studies on Urinary Carriage of Enteric Group Organisms: VII. —The value of Different Cultural Methods for Routine Clearance Tests and for follow-UP Investigation of Carriers

G. T. L. Archer

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