MECHANISMS INVOLVED IN THE RESISTANCE OF MYCOBACTERIUM TUBERCULOSIS TO PARA-AMINOSALICYLIC ACID

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That para-aminobenzoic acid antagonizes competitively the inhibitory effect of para-aminosalicylic acid on the growth of Mycobacterium tuberculosis is well known. In a previous communication (Hedgcock, 1956) it was reported that inhibition of M. tuberculosis (strain H37Rv) by para-aminosalicylic acid was reversed noncompetitively by methionine and biotin when the concentration of the inhibitor did not exceed 0.5 μg per ml. At higher concentrations of para-aminosalicylic acid, reversal of inhibition occurred in the presence of methionine and biotin following the addition of certain fatty acids or purines as well as specified amino acids. In the present paper competitive and noncompetitive reversal of para-aminosalicylic acid-inhibition was investigated in susceptible and resistant strains of M. tuberculosis which had been isolated from patients and also in resistant strains selected in vitro. Studies of the organisms were made with respect to the mechanisms involved in the development of resistance to para-aminosalicylic acid.

MATERIALS AND METHODS

The original susceptible and resistant strains of M. tuberculosis utilized in this investigation had been isolated from patients. The resistant strains were resistant to isoniazid (100 μg per ml) as well as to para-aminosalicylic acid. Para-aminosalicylic acid-resistant tubercle bacilli were also selected in vitro from certain of the susceptible strains which had been cultured repeatedly on Lowenstein medium to which varied levels of para-aminosalicylic acid had been added. In each experiment, transfers were made from the stock cultures to Kirchner medium which contained “tween 80” and bovine albumin, fraction V. After incubation for 10 to 12 days the organisms were washed 3 times and diluted in Kirchner medium containing tween 80 until an optical density of 0.04 was attained. The inoculum consisted of 0.05 ml of the diluted suspension per 2 ml of media. All inoculated cultures were incubated for 17 to 19 days. Growth was then determined by measurement of the turbidity of each culture with the Rouy photometer.

All compounds were sterilized by filtration through sintered glass filters. Appropriate amounts were then added to 1 ml of sterile 2% Kirchner medium which contained either tween 80 or “triton WR 1339” (0.05 per cent). The final volume was adjusted to 2 ml with sterile distilled water.

Resistance of the tubercle bacilli to para-aminosalicylic acid was determined by inoculation of 0.05 ml of a suspension having an optical density of 0.04 on the surface of slants of Lowenstein media to which varied amounts of the inhibitor had been added. The results were recorded after incubation at 37 C for 4 weeks.

RESULTS

A determination was made of noncompetitive antagonism of para-aminosalicylic acid-inhibition in normal susceptible tubercle bacilli, in resistant organisms selected in vitro and in resistant tubercle bacilli isolated from patients. Tests which involved the susceptible organisms were conducted in both Kirchner-tween 80 and Kirchner-triton media. The noncompetitive antagonists

1 This work was supported by a grant from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, Public Health Service E-914 (R).

2 Preliminary reports of this work were presented at the 16th Conference on the Chemotherapy of Tuberculosis held in St. Louis, 1957, and at the meeting of the Society of American Bacteriologists at Detroit, Michigan, 1957.

3 Polyoxyethylene sorbitol mono-oleate obtained from Hill Top Laboratories, Inc.

4 Oxyethylated tertiary octyl phenol formaldehyde polymer, obtained from Winthrop Laboratories, Inc.
TABLE 1
Noncompetitive antagonism of inhibition by para-aminosalicylic (PAS) acid in susceptible strains of Mycobacterium tuberculosis

<table>
<thead>
<tr>
<th>Antagonists*</th>
<th>PAS</th>
<th>Strains of Tubercle Bacilli</th>
<th>1S</th>
<th>2S</th>
<th>3S</th>
<th>4S</th>
<th>5S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>optical density X 10³</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>µg/ml</td>
<td>Triton Tween 80 Triton Tween 80 Triton Tween 80 Triton Tween 80 Triton Tween 80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>886 1097 854 886 796 854 824 824 854 886</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>100</td>
<td>0 0 0 0 0 0 0 0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>100</td>
<td>4 51 51 13 61 41 92 9 76 86</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Biotin</td>
<td>100</td>
<td>0 0 0 0 0 0 0 0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>100</td>
<td>0 0 0 0 0 0 0 0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine, biotin</td>
<td>100</td>
<td>9 638 201 886 108 377 328 699 168 523</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine, hypoxanthine</td>
<td>100</td>
<td>569 143 523 4 284 86 482 4 398 149</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine, biotin, and hypoxanthine</td>
<td>100</td>
<td>569 854 553 796 293 377 569 699 854 886</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimal PAS for complete inhibition (µg/ml)</td>
<td>0.1</td>
<td>0.2</td>
<td>0.5</td>
<td>0.2</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The concentrations of antagonists were: DL-methionine, 20 µg/ml; biotin, 1 µg/ml; hypoxanthine, 20 µg/ml.

TABLE 2
Noncompetitive antagonism of para-aminosalicylic acid in selected strains of Mycobacterium tuberculosis of minimal resistance

<table>
<thead>
<tr>
<th>Antagonists*</th>
<th>PAS</th>
<th>Strains of Tubercle Bacilli</th>
<th>1R</th>
<th>2R</th>
<th>3R</th>
<th>4R</th>
<th>5R</th>
<th>6R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/ml</td>
<td>optical density X 10³</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Triton Tween 80 Triton Tween 80 Triton Tween 80 Triton Tween 80 Triton Tween 80 Triton Tween 80</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>854 921 921 770 1097 1155</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>100</td>
<td>0 0 0 0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine</td>
<td>100</td>
<td>260 301 620 432 796 638</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biotin</td>
<td>100</td>
<td>0 0 0 0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>100</td>
<td>0 0 0 0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine, biotin</td>
<td>100</td>
<td>379 469 638 432 824 824</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine, hypoxanthine</td>
<td>100</td>
<td>745 824 638 553 824 824</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biotin, hypoxanthine</td>
<td>100</td>
<td>0 0 0 0 0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methionine, biotin, and hypoxanthine</td>
<td>100</td>
<td>745 824 638 569 854 824</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimal PAS for complete inhibition (µg/ml)</td>
<td>1</td>
<td>0.75</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
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<td></td>
</tr>
</tbody>
</table>

* The antagonists were added to triton-media in the following concentrations: DL-methionine, 20 µg/ml; biotin, 1 µg/ml; hypoxanthine, 20 µg/ml.

were added singly and in combination to the para-aminosalicylic acid-medium in the following concentrations: DL-methionine, 20 µg/ml; biotin, 1 µg/ml; hypoxanthine, 20 µg/ml. The results are recorded in tables 1 and 2. In the susceptible tubercle bacilli inhibition by para-aminosalicylic acid was reversed by methionine, biotin and tween 80 or by methionine and hypoxanthine in the triton-medium. When both hypoxanthine and tween 80 were present in the medium only partial reversal of inhibition was effected. A small amount of growth occurred in the presence of para-aminosalicylic acid when the medium contained only methionine as an antagonist.
The addition of biotin to methionine in the triton-medium resulted in increased growth. The minimal amount of para-aminosalicylic acid required for complete inhibition of the susceptible organisms ranged from 0.05 to 0.5 μg per ml.

With respect to the selected strains of M. tuberculosis which had acquired a minimal amount of resistance to para-aminosalicylic acid in vitro (completely inhibited by 0.75 and 1.00 μg of para-aminosalicylic acid per ml) the extent of growth indicated that approximately 30 per cent of the organisms in each of the strains required only methionine for growth in the presence of para-aminosalicylic acid. In strains for which 2 μg para-aminosalicylic acid per ml represented the minimal inhibitory amount, 76 to 97 per cent of the cells required only methionine for reversal of para-aminosalicylic acid-inhibition.

All of the resistant tubercle bacilli which had been isolated from patients (complete inhibition at 200 μg para-aminosalicylic acid per ml) grew in the presence of para-aminosalicylic acid when only methionine was added to the medium.

The groups of susceptible tubercle bacilli, resistant organisms selected in vitro and the resistant bacilli isolated from patients were characterized further by a determination of the minimal amount of para-aminobenzoic acid required to completely reverse inhibition by para-aminosalicylic acid. The inhibition index was then calculated (Beerstecher and Shive, 1946). The results are recorded in table 3. With one exception, the inhibition index of each susceptible organism was 0.66. The determinations were made at a level of 20 μg para-aminosalicylic acid per ml. The values could not be obtained in the presence of 100 μg para-aminosalicylic acid per ml since amounts of para-aminobenzoic acid in excess of 40 μg per ml were inhibitory for M. tuberculosis. In the group of resistant organisms selected in vitro the inhibition index was dependent upon the resistance of the organism and was greatest in the tubercle bacilli which were most resistant to para-aminosalicylic acid. The resistant organisms which were isolated from patients exhibited inhibition indices of 40 or 100.

Further studies were made of the resistant tubercle bacilli isolated from patients in view of the altered relationships between para-aminosalicylic acid and the competitive and noncompetitive antagonists. It was found that these organisms often failed to grow in Kirchner medium which contained triton WR 1339 although growth was readily obtained when tween 80 was incorporated into the medium. Therefore, the effects of the following compounds on the growth of the resistant tubercle bacilli were determined in Kirchner medium: triton, 0.05 per cent; tween 80, 0.05 per cent; oleic acid, 0.005 per cent; bovine albumin, fraction V, 0.5 per cent. The results are tabulated in table 4. Three of ten resistant organisms grew in Kirchner medium which contained triton (growth 31 to 42 per cent maximal). The remaining 7 strains produced only a small amount

TABLE 3

The relation of the sensitivity of Mycobacterium tuberculosis to para-aminosalicylic acid (PAS) to the inhibition index

<table>
<thead>
<tr>
<th>Type of Tubercle Bacilli</th>
<th>Minimal PAS for complete Inhibition</th>
<th>Minimal PARA for complete Reversal</th>
<th>Inhibition Index</th>
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<tr>
<td>Normal, susceptible</td>
<td>μg/ml</td>
<td>μg/ml</td>
<td></td>
</tr>
<tr>
<td>strains</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>1S</td>
<td>0.1</td>
<td>20</td>
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<tr>
<td></td>
<td>2S</td>
<td>0.2</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>3S</td>
<td>0.5</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>4S</td>
<td>0.2</td>
<td>20</td>
</tr>
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<td></td>
<td>5S</td>
<td>0.05</td>
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<td></td>
<td>6S</td>
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<td></td>
<td>7S</td>
<td>0.2</td>
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<td></td>
<td>8S</td>
<td>0.2</td>
<td>20</td>
</tr>
<tr>
<td>Resistant</td>
<td>1R</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>strains selected in vitro</td>
<td>2R</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>3R</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>4R</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>5R</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>6R</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>7R</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>8R</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>9R</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Resistant</td>
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<td>200</td>
<td>200</td>
</tr>
<tr>
<td>strains isolated from patients</td>
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<td>200</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>3RP</td>
<td>200</td>
<td>200</td>
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<tr>
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<td>4RP</td>
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<td>200</td>
</tr>
<tr>
<td></td>
<td>6RP</td>
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<td>200</td>
</tr>
<tr>
<td></td>
<td>7RP</td>
<td>200</td>
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<tr>
<td></td>
<td>8RP</td>
<td>200</td>
<td>200</td>
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<tr>
<td></td>
<td>9RP</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>10RP</td>
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</table>
The effect of oleic acid on the growth of para-aminosalicylic acid (PAS)-resistant strains of Mycobacterium tuberculosis isolated from patients

<table>
<thead>
<tr>
<th>Medium*</th>
<th>Strains of Tubercle Bacilli</th>
<th>1RP</th>
<th>2RP</th>
<th>3RP</th>
<th>4RP</th>
<th>5RP</th>
<th>6RP</th>
<th>7RP</th>
<th>8RP</th>
<th>9RP</th>
<th>10RP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kirchner, triton</td>
<td>optical density × 10^6</td>
<td>22</td>
<td>13</td>
<td>215</td>
<td>201</td>
<td>9</td>
<td>22</td>
<td>51</td>
<td>22</td>
<td>367</td>
<td></td>
</tr>
<tr>
<td>Kirchner, triton, bovine albumin</td>
<td></td>
<td>86</td>
<td>208</td>
<td>585</td>
<td>409</td>
<td>108</td>
<td>181</td>
<td>347</td>
<td>276</td>
<td>181</td>
<td>560</td>
</tr>
<tr>
<td>Kirchner, triton, albumin, oleic acid</td>
<td></td>
<td>456</td>
<td>432</td>
<td>678</td>
<td>523</td>
<td>367</td>
<td>432</td>
<td>569</td>
<td>432</td>
<td>409</td>
<td>658</td>
</tr>
<tr>
<td>Kirchner, tween 80</td>
<td></td>
<td>276</td>
<td>482</td>
<td>125</td>
<td>620</td>
<td>509</td>
<td>367</td>
<td>585</td>
<td>538</td>
<td>337</td>
<td>796</td>
</tr>
<tr>
<td>Kirchner, tween 80, albumin</td>
<td></td>
<td>824</td>
<td>620</td>
<td>699</td>
<td>658</td>
<td>509</td>
<td>553</td>
<td>796</td>
<td>585</td>
<td>460</td>
<td>854</td>
</tr>
<tr>
<td>Kirchner, triton, catalase</td>
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<td>4</td>
<td>260</td>
<td>137</td>
<td>13</td>
<td>51</td>
<td>32</td>
<td>13</td>
<td>292</td>
<td></td>
</tr>
</tbody>
</table>

* The concentration of each compound added to the medium was as follows: triton WR 1339, 0.05 per cent; bovine albumin, fraction V, 0.5 per cent; tween 80, 0.05 per cent; oleic acid, 50 μg/ml; catalase, 3 μg/ml.

of growth in the medium (growth 11 per cent or less maximal). While the addition of bovine albumin increased the growth of all organisms, the addition of both albumin and oleic acid resulted in near maximal growth. Maximal growth was obtained when bovine albumin and tween 80 were added to the medium. The addition of 3 μg per ml of catalase to Kirchner-triton media did not improve growth.

Since certain of the amino acids, purines, and nucleosides had previously been found capable of replacing tween 80 (an olate) as an antagonist of para-aminosalicylic acid (Hedgecock, 1956), a determination was made of the ability of these compounds to support the growth of the strains of resistant tubercle bacilli which had failed to grow in Kirchner medium. The following compounds were added to Kirchner-triton medium and examined with respect to growth of the resistant strains: L-valine, 10 μg/ml; L-norvaline, 10 μg/ml; L-isoleucine, 25 μg/ml; L-norleucine, 25 μg/ml; L-leucine, 10 μg/ml; L-phenylalanine, 35 μg/ml; hypoxanthine, 5 μg/ml; xanthine, 5 μg/ml; adenine, 5 μg/ml; guanine, 5 μg/ml; inosine, 50 μg/ml; adenosine, 10 μg/ml. None of the compounds supported growth of the strains of tubercle bacilli which had failed to grow in Kirchner-triton medium.

A comparison was made of the methionine-requirements of the susceptible tubercle bacilli and of the para-aminosalicylic acid-resistant organisms which had been isolated from patients. For the susceptible organisms the determinations were made in media containing 1 μg of biotin, 500 μg of tween 80, and 100 μg of para-aminosalicylic acid per ml. The medium used for establishing the methionine-requirements of the resistant tubercle bacilli contained only 200 μg of para-aminosalicylic acid per ml. The requirements of the susceptible tubercle bacilli for dl-methionine varied from 9 to 12 μg per ml and for the resistant organisms, 5 or 6 μg per ml (table 5).

Since it was possible that para-aminosalicylic acid was inactivated by the resistant bacilli during growth, three strains of the resistant tubercle bacilli (4 RP, 8 RP, 9 RP) were examined in this respect. The three organisms were cultured in the presence of varied amounts of para-aminosalicylic acid for 17 days after which each culture was centrifuged then sterilized by filtration through a sintered glass filter. Appropriate dilutions of the para-aminosalicylic acid-containing culture fluid and of a standard solution of para-aminosalicylic acid were made in Kirchner medium. After inoculation with a susceptible strain of H37Rv and incubation for 17 days, growth was measured photometrically. There was no significant difference in the degree of inhibition (complete at 0.2 μg per ml) whether the calculated amount of para-aminosalicylic acid had been added from the sterile culture supernatant of the resistant tubercle bacilli or from the standard solution of para-aminosalicylic acid.

Determinations were also made of the amount of para-aminobenzoic acid produced by the susceptible and resistant tubercle bacilli during
**TABLE 5**
The amount of methionine required for reversal of para-aminosalicylic acid (PAS)-inhibited normal and resistant strains of Mycobacterium tuberculosis

<table>
<thead>
<tr>
<th>Normal Organisms</th>
<th>Resistant Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>Level of PAS (µg/ml)</td>
</tr>
<tr>
<td>1S</td>
<td>100</td>
</tr>
<tr>
<td>2S</td>
<td>100</td>
</tr>
<tr>
<td>3S</td>
<td>100</td>
</tr>
<tr>
<td>4S</td>
<td>100</td>
</tr>
<tr>
<td>5S</td>
<td>100</td>
</tr>
</tbody>
</table>

* In a system containing biotin (1 µg/ml) and tween 80 (500 µg/ml) wherein methionine represents a third component essential for reversal of PAS-inhibition.

growth. Ten strains of susceptible tubercle bacilli and ten strains of para-aminosalicylic acid-resistant organisms isolated from patients were cultured in Kirchner medium for 17 days then sterilized by autoclaving. Free and combined para-aminobenzoic acid (Thompson et al., 1943) were determined in each of the cultures by the method of Bratton and Marshall (1939). The amount of para-aminobenzoic acid produced by the tubercle bacilli did not exceed 0.1 µg per ml in any of the cultures. There was no significant difference in the small amounts of para-aminobenzoic acid produced by the susceptible and the resistant bacilli.

**DISCUSSION**

The analysis of noncompetitive antagonism in normal susceptible strains of *M. tuberculosis* revealed that methionine, biotin, and tween 80 or methionine and hypoxanthine were necessary for complete reversal of para-aminosalicylic acid inhibition. The small percentage of tubercle bacilli of the normal strains which grew in the presence of para-aminosalicylic acid with only methionine as the antagonist may represent variants that were present in the normal cell population. The findings that only methionine was required for reversal of para-aminosalicylic acid inhibition in strains of tubercle bacilli resistant to as little as 2 µg of para-aminosalicylic acid per ml demonstrates that elimination of the need for antagonists other than methionine is an initial step in the development of resistance to para-aminosalicylic acid. These findings suggest that the para-aminobenzoic acid enzyme responsible for the synthesis of methionine in *M. tuberculosis* is much more susceptible to para-aminosalicylic acid than the para-aminobenzoic acid enzyme which may be involved in synthesis of the other antagonists (Beerstecher and Shive, 1946; Winkler and de Haan, 1948). The methionine requirements of the resistant organisms at high levels of para-aminosalicylic acid were similar to that of the susceptible H37Rv strain when exposed to concentrations of 0.4 to 0.6 µg of para-aminosalicylic acid per ml (Hedgecock, 1956).

The growth requirement of certain of the resistant tubercle bacilli for oleic acid may represent the elimination of a biosynthetic mechanism blocked by para-aminosalicylic acid. This biochemical alteration would result in a decreased requirement for para-aminobenzoic acid.

The increase in the competitive ratio of para-aminosalicylic acid to para-aminobenzoic acid appeared to be a function of increase in resistance of the tubercle bacilli to para-aminosalicylic acid. Increased resistance accompanied by a proportionate increase in the competitive ratio of analogue to metabolite was observed by Ivanovics (1941) in sulfonamide-resistant staphylococci and by Davis and Maas (1952) in strains of *Escherichia coli* resistant to p-nitrobenzoic acid. Increases in the inhibition index (para) aminosalicylic acid/para-aminobenzoic acid might result from either a decrease in the permeability of the bacterial cell to para-aminosalicylic acid or from an alteration in the apoenzyme affected by para-aminosalicylic acid such that it has a greater affinity for the metabolite (para-aminobenzoic acid) than for the inhibitor (Davis and Maas, 1952). Barclay (1955) found that para-aminosalicylic acid labeled by C14 was adsorbed to both para-aminosalicylic acid-susceptible and para-aminosalicylic acid resistant tubercle bacilli although much larger amounts were adsorbed by the resistant organisms. The para-aminosalicylic acid-susceptible tubercule bacilli lost 50 per cent of their radioactivity after exposure to washing for one week while the radioactivity of the resistant organisms remained unchanged. The same investigator (Barclay,
1953) found that far less radioactive isoniazid was bound by tubercle bacilli resistant to the inhibitor than by strains of susceptible organisms. While the studies of Barclay do not deal directly with cell permeability the findings suggest that resistance to para-aminosalicylic acid may depend upon factors other than adsorption and entry into the bacterial cell. Increased destruction of the drug would appear to be eliminated as a mechanism of resistance to para-aminosalicylic acid since complete para-aminosalicylic acid activity was demonstrated in cultures of resistant organisms. Likewise, measurements of the amount of para-aminobenzoic acid in cultures of resistant organisms indicated that increased synthesis of the reversing metabolite was not a mechanism of resistance to para-aminosalicylic acid in M. tuberculosis. It would appear that resistance to para-aminosalicylic acid could not be due to increased formation of the inhibited enzyme since the great increase in resistance to para-aminosalicylic acid (400- to 2000-fold) could not be accounted for by this mechanism (Davis and McDermott, 1952). The development of an alternate metabolic pathway as a mechanism of acquiring resistance to para-aminosalicylic acid is not compatible with the existence of varying degrees of resistance (Davis and McDermott, 1952).

SUMMARY

Reversal of para-aminosalicylic acid-inhibition in susceptible strains of Mycobacterium tuberculosis was effected by methionine, biotin, and tween 80 or by methionine and hypoxanthine. Inhibition of growth of resistant strains of tubercle bacilli by high concentrations of para-aminosalicylic acid was reversed by methionine alone. Resistant strains of Mycobacterium tuberculosis which had been isolated from patients exhibited either a partial or an absolute growth requirement for oleic acid. Increased resistance to para-aminosalicylic acid was accompanied by a proportionate increase in the competitive ratio of para-aminosalicylic acid to para-aminobenzoic acid.

REFERENCES