A Method for the Large-scale Production of Streptomycin by Surface Culture

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SUMMARY: Working details are given of a method for producing streptomycin by the surface culture of Streptomyces griseus in pint milk bottles on a papain digest of beef + meat extract + glucose + mineral salt medium. Streptomycin titres in the crude culture filtrates of 250 µg./ml. or more were obtained after 10–14 days' growth.

Evans's peptone, papain digest of spent pancreas from insulin manufacture, papain digest of yeast, and a proprietary casein-meat hydrolysate were found to be possible alternative sources of organic nitrogen. Growth was good on media containing peptic digest of beef or peptic-tryptic casein digest, but the streptomycin titre was low.

The utilization of glucose and the production of streptomycin depended on the relative amount of nitrogen present in the medium.

During the latter half of 1945 attempts were made in these laboratories by Mrs Ursula Wilson to produce streptomycin from Streptomyces griseus on the lines advocated by American workers (see review by Waksman & Schatz, 1945). Great difficulty was experienced in obtaining growth on the surface of the medium, the investigations were hampered by the lack of a streptomycin standard by which to measure the yield, and in spite of much experimentation the results were unsatisfactory. At the beginning of 1946 the work was continued by two of us (G. C. A. and A. R. B.) with indifferent results, until a particularly suitable medium for the growth of the organism was found and the conditions required for promoting good surface growth were recognized. At the same time, the streptomycin assay was standardized by the receipt of a sample of streptomycin sulphate from America. During the early summer the opportunity arose for a short period of experimental large-scale streptomycin production using the plant at the Wellcome Penicillin Unit. This paper summarizes aspects of the experimental work and outlines the production method devised.

METHODS AND MEDIA

Strain. The strain of Streptomyces griseus employed was Waksman's 'strain 4' (National Collection of Type Cultures no. 6961) but, because of an impression that the strain had undergone slight variation while in these laboratories, a culture of the organism actually used in the large-scale trials has been returned to the National Collection of Type Cultures where it is catalogued as no. 7187.

Method of assay. The method of assay for streptomycin, a dilution test against Escherichia coli, is described by Brown & Young (1947); it is only necessary to state here that the unit employed is 1.0 µg. of streptomycin base.
Belmont medium

The unsuccessful experiments on media and methods of culture will not be
detailed. The best and most used of the earlier media was the one advocated
by Waksman & Schatz (1945), which had the following composition: glucose
10.0 g.; NaCl 5.0 g.; peptone (Evans's)* 5.0 g.; meat extract (Wilson's or Lab
Lemco) 5.0 g.; water to 1 l.; pH 7.0. Surface growth on this medium was
frequently fair although the streptomycin titre was invariably low. At other
times, for reasons still not fully understood, the growth was wholly submerged
and streptomycin production negligible. Waksman emphasized the importance
of meat extract and peptone for streptomycin production, and when these
ingredients were substituted for casein hydrolysate in the Wellcome modifica-
tion of Czapek-Dox solution, a synthetic medium devised for penicillin pro-
duction by Dr C. G. Pope (Clayton, Hemis, Robinson, Andrews & Hunwicke,
1944), it was at once apparent that a very favourable medium for the growth of
S. griseus and the production of streptomycin had been found. The composi-
tion of this medium, which will be referred to as ‘Belmont medium’, was, in %
(w/v): NaNO₃, 0.4; KH₂PO₄, 0.1; KCl, 0.05; MgSO₄.7H₂O, 0.5; FeSO₄.7H₂O,
0.005; CuSO₄.5H₂O, 0.00175; sodium citrate, 0.2; sodium acetate, 0.2;
glucose, 4.4; peptone (Evans's), 1.0; meat extract (Wilson's), 0.5; water to
100 ml. The pH was adjusted to 7.0 with NaOH, and sterilization was effected
by autoclaving for 15 min. at 15 lb./sq.in.

Glaxo flasks (Clayton et al. 1944) containing 200 ml. amounts of medium
were found to be convenient culture vessels; later pint milk bottles were sub-
stituted for the Glaxo flasks except for cultures to be used as inoculum, when
the larger surface area given in a Glaxo flask was advantageous.

Inoculation

The conidia of S. griseus are difficult to wet and tend to aggregate in clumps
(Carvajal, 1946); this is probably the reason that, to establish good surface
growth, a large inoculum and the addition of a wetting agent are essential.
The routine procedure finally adopted was to add a 1:10⁴ dilution of sterile
'calsolene'† to a freely sporing 7-14-day liquid culture, and after vigorous
shaking to break up the mycelial mat, using the resulting suspension of spores
and mycelial fragments as inoculum at the rate of 3-4 ml./200 ml. of the
medium to be inoculated. By this method a film of surface growth was
apparent after 24 hr. at 28°, and was complete after 48 hr.

Incubation temperature

Experiments showed 28° to be near the optimum for growth of S. griseus and
for streptomycin production. At this temperature the surface of the medium
was completely covered by the second day, and the thick wrinkled mycelial mat
remained white. At 25° growth and streptomycin production were somewhat

* Evans, Sons, Lescher and Webb Ltd., London and Liverpool.
† Obtained from Imperial Chemical Industries Ltd.
Large-scale production of streptomycin

slower, but a satisfactory surface coverage and a final titre equal to that obtained at 28° resulted. At 31° the optimum had been passed; the surface of the medium became covered slightly more rapidly, but the thinner mycelial mat, at first greyish in colour, turned brown and became waterlogged and the streptomycin titre fell. The browning of the mycelium at higher temperatures could, however, be counteracted by lowering the temperature to the optimum or, better, to slightly suboptimal (25°).

Using Belmont medium and the methods outlined above titres of 200–300 μg. streptomycin/ml. at 10–12 days were regularly obtained, and on occasion titres of over 400 μg./ml. resulted (see Table 1).

Table 1. Streptomycin production on Belmont medium and analysis of metabolism fluid

<table>
<thead>
<tr>
<th>Period of incubation (days)</th>
<th>0</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomycin titre (μg./ml.)</td>
<td>0</td>
<td>18</td>
<td>45</td>
<td>95</td>
<td>105</td>
<td>215</td>
</tr>
<tr>
<td>pH</td>
<td>---</td>
<td>6-75</td>
<td>6-82</td>
<td>6-78</td>
<td>6-52</td>
<td>6-17</td>
</tr>
</tbody>
</table>

Composition of medium as % of original:

<table>
<thead>
<tr>
<th>Glucose</th>
<th>100</th>
<th>90.5</th>
<th>85.5</th>
<th>71.0</th>
<th>58.0</th>
<th>38.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total N</td>
<td>100</td>
<td>81.5</td>
<td>81.0</td>
<td>70.5</td>
<td>74.0</td>
<td>75.5</td>
</tr>
<tr>
<td>Amino-N</td>
<td>100</td>
<td>100.0</td>
<td>100.0</td>
<td>89.0</td>
<td>86.0</td>
<td>66.5</td>
</tr>
<tr>
<td>Ammonia-N</td>
<td>100</td>
<td>55.0</td>
<td>31.0</td>
<td>25.0</td>
<td>24.5</td>
<td>130.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Period of incubation (days)</th>
<th>8</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomycin titre (μg./ml.)</td>
<td>305</td>
<td>390</td>
<td>445</td>
<td>360</td>
<td>345</td>
<td>360</td>
</tr>
<tr>
<td>pH</td>
<td>6-8</td>
<td>7-48</td>
<td>7-75</td>
<td>7-78</td>
<td>8-25</td>
<td>8-3</td>
</tr>
</tbody>
</table>

Composition of medium as % of original:

<table>
<thead>
<tr>
<th>Glucose</th>
<th>33.5</th>
<th>16.5</th>
<th>10.0</th>
<th>7.0</th>
<th>5.0</th>
<th>4.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total N</td>
<td>69.0</td>
<td>66.5</td>
<td>72.0</td>
<td>72.5</td>
<td>71.5</td>
<td>73.0</td>
</tr>
<tr>
<td>Amino-N</td>
<td>61.0</td>
<td>44.5</td>
<td>39.0</td>
<td>39.0</td>
<td>37.5</td>
<td>37.5</td>
</tr>
<tr>
<td>Ammonia-N</td>
<td>40.0</td>
<td>180.0</td>
<td>300.0</td>
<td>400.0</td>
<td>500.0</td>
<td>490.0</td>
</tr>
</tbody>
</table>

Experimental media

The prospect of a large-scale production trial in the penicillin plant necessitated modifications in the Belmont medium because of the difficulty and expense of obtaining sufficient peptone and meat extract under the prevailing conditions. Various ad hoc experiments were therefore undertaken to find substitutes for these ingredients, and at the same time a more systematic study of S. griseus was made to elucidate its nutritional requirements and to explain the success of Belmont medium. The results of the former experiments are indicated below and an account of the latter investigation is given by Spilsbury (1947).

Organic nitrogen. The effects on streptomycin production of various substitutes for Evans's peptone are summarized in Table 2. In general, the vigour of growth on the different media was directly correlated with the streptomycin titre, but although growth was quite heavy on media containing peptic digest of beef or peptic-tryptic casein digest, the titres were very low on such media. Papain digest of beef, digest of spent pancreas from insulin manufacture,
papain digest of yeast, and ‘Casydrol’ (a casein-meat hydrolysate; Bengers Ltd., Holmes Chapel, Cheshire) all showed promise. Papain digest of beef was adopted for the production medium. In addition to the materials tabulated, a papain digest of Penicillium felts was tried but found worthless, and no success was obtained with cornsteep liquor either as a nitrogen source or in place of meat extract.

Table 2. Effect on streptomycin production of replacing casein hydrolysate in Wellcome modification of Czapek-Dox solution by other organic nitrogen sources, with or without meat or yeast extract

<table>
<thead>
<tr>
<th>Nitrogen source</th>
<th>Equivalent total N (µg./ml.)</th>
<th>Streptomycin titre (µg./ml.) at 10–12 days</th>
<th>+0.5% meat extract</th>
<th>+yeast extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein hydrolysate 0.25% (w/v)</td>
<td>0.4</td>
<td>90*</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>Peptone (Evans’s) 1.0% (w/v)</td>
<td>1.5</td>
<td>—</td>
<td>250–400†</td>
<td>—</td>
</tr>
<tr>
<td>Papain digest of beef (concentrated):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% (v/v)</td>
<td>1.5</td>
<td>75</td>
<td>270</td>
<td>—</td>
</tr>
<tr>
<td>5% (v/v)</td>
<td>0.75</td>
<td>65–70</td>
<td>150–250</td>
<td>—</td>
</tr>
<tr>
<td>2.5% (v/v)</td>
<td>0.4</td>
<td>25–60</td>
<td>75–160</td>
<td>—</td>
</tr>
<tr>
<td>Peptic digest of beef:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20% (v/v)</td>
<td>0.86</td>
<td>40</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>10% (v/v)</td>
<td>0.43</td>
<td>30</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Spent pancreas digest</td>
<td>1.2</td>
<td>160</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>1.06</td>
<td>105</td>
<td>—</td>
<td>160–200</td>
</tr>
<tr>
<td></td>
<td>0.53</td>
<td>50</td>
<td>—</td>
<td>35–60</td>
</tr>
<tr>
<td></td>
<td>0.21</td>
<td>30</td>
<td>—</td>
<td>20–55</td>
</tr>
<tr>
<td>Papain digest of yeast</td>
<td>1.0</td>
<td>190</td>
<td>240</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>160</td>
<td>140</td>
<td>—</td>
</tr>
<tr>
<td>Papain digest of soya flour</td>
<td>1.2</td>
<td>150</td>
<td>150</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>120</td>
<td>140</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>40</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>Casein digests:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papain</td>
<td>—</td>
<td>25</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tryptic</td>
<td>—</td>
<td>25</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Peptic–tryptic</td>
<td>1.06</td>
<td>30–50</td>
<td>110</td>
<td>—</td>
</tr>
<tr>
<td>‘Casydrol’:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0% (w/v)</td>
<td>2.6</td>
<td>100</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1.0% (w/v)</td>
<td>1.3</td>
<td>120–200</td>
<td>—</td>
<td>80–220</td>
</tr>
<tr>
<td>0.5% (w/v)</td>
<td>0.65</td>
<td>45–100</td>
<td>—</td>
<td>45–55</td>
</tr>
<tr>
<td>0.25% (w/v)</td>
<td>0.325</td>
<td>20–25</td>
<td>—</td>
<td>20–25</td>
</tr>
</tbody>
</table>

* Wellcome modification of Czapek-Dox solution. † Belmont medium.

Meat extract. The beneficial effect of meat extract on streptomycin production was repeatedly confirmed. For example, in a large factorial experiment, certain results of which are given in Table 4, the streptomycin titres at 12 days for 5% papain digest + 4% glucose with the addition of 0.5, 0.25 and 0.0% meat extract were 150, 100 and 70 µg./ml. respectively. In a limited series of trials Marmite and lentil extract proved to be of no value as a replacement for meat extract, but some success was obtained with yeast extract (see Table 2).
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LePage & Campbell (1946) used 1-0 % (w/v) Bacto yeast extract as a substitute for peptone and meat extract. The yeast extract used in the present work was prepared by adding 100 g. of dried yeast to 1 l. of boiling water, allowing to cool, and using the filtered liquid (which had a total N of c. 2-5 mg./ml.) at the rate of 1-0 or 2-0 % (v/v), i.e. at a total N level of 0-025 or 0-05 mg./ml. in the finished medium.

Table 3. Streptomycin titre and glucose utilization at 12 days in Belmont and in Waksman’s media

<table>
<thead>
<tr>
<th>Initial concentration of glucose (%) in medium</th>
<th>4-0</th>
<th>2-0</th>
<th>1-0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belmont medium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose utilization (µg./ml.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptomycin</td>
<td>310</td>
<td>190</td>
<td>70</td>
</tr>
<tr>
<td>Glucose utilization (%)</td>
<td>98</td>
<td>97-5</td>
<td>97-5</td>
</tr>
<tr>
<td>Waksman’s medium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose utilization (µg./ml.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptomycin</td>
<td>115</td>
<td>115</td>
<td>60</td>
</tr>
<tr>
<td>Glucose utilization (%)</td>
<td>60</td>
<td>99</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 4. Streptomycin titre and glucose utilization at 11 days in Belmont medium with peptone replaced by papain digest of beef

<table>
<thead>
<tr>
<th>Initial concentration of glucose (%) in medium</th>
<th>9-0</th>
<th>6-0</th>
<th>4-0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Papain digest (%, v/v)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose utilization (µg./ml.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptomycin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose utilization (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-0</td>
<td>190</td>
<td>160</td>
<td>150</td>
</tr>
<tr>
<td>2-0</td>
<td>60</td>
<td>&gt;70</td>
<td>75</td>
</tr>
<tr>
<td>1-0</td>
<td>40</td>
<td>40</td>
<td>&lt;30</td>
</tr>
</tbody>
</table>

Carbohydrate. Most of the experimental work on the carbohydrate component of the medium was directed towards exploring the possibility of replacing glucose by lactose and ascertaining the most efficient glucose level. When lactose was substituted for all or part of the glucose in Belmont medium, or was combined with papain or cornsteep liquor, growth was poor and titres low. Glucose had a marked effect on streptomycin production. The results of experiments on varying the percentage glucose in Belmont and modified Belmont medium and in Waksman’s medium are summarized in Tables 3 and 4.

Increasing the glucose in Waksman’s medium from 1-0 to 2-0 % increased the streptomycin titre, but a further increase had no effect. Decreasing the glucose in Belmont medium had the reverse effect (see Table 3), and though 6-0 or 9-0 % glucose in modified Belmont medium increased the streptomycin titre the percentage glucose utilized decreased (see Table 4). It is clear from these results (and those of Spilsbury, 1947) that the C/N ratio is important. Nitrogen is the limiting factor in Waksman’s medium containing 4-0 % glucose, and 9 % glucose is supra-optimal for the modified Belmont medium containing 2-0 or 1-0 % papain digest.
THE PRODUCTION PROCESS*

Inoculum

A culture of *S. griseus* (N.C.T.C. 7187) was subcultured on 2% agar slopes of Belmont medium in 6 x 1 in. culture tubes. After 7 days' incubation at 28-29° the tubes were sealed by dipping the cotton plugs into molten paraffin wax and stored in the dark at 5° until required.

The medium used for the production of conidia (spore medium) was a modification of Moyer's medium, having the following composition: papain digest of beef (see below) 6.0% (v/v); all % (w/v): molasses, 1.5; glycerol, 0.58; NaCl, 0.5; FeSO₄.7H₂O (A.R.), 0.0015; CuSO₄.5H₂O (A.R.), 0.009; (NH₄)₂MoO₄.4H₂O (A.R.), 0.0013; MnSO₄.4H₂O (A.R.), 0.00028; KH₂PO₄, 0.006; MgSO₄.7H₂O, 0.005; water to 100 ml.

This medium was prepared in 60 l. batches in a steam-heated enamelled cauldron. After adjusting to pH 7.0 ± 0.2 by the addition of pellet sodium hydroxide (B.P. quality), the unfiltered medium was distributed in 300 ml. quantities into carefully washed 2.5 l. bottles of the Roux type (Thompson bottles), autoclaved for 15 min. at 15 lb./sq.in., and allowed to stand overnight before inoculation.

About 10 ml. of a 1:10,000 sterile aqueous 'calsolene' solution were added to a slope culture (6 x 1 in. tube) and the growth rubbed off the agar with the pipette. The whole of the suspension so obtained was used to inoculate one bottle of spore medium which was then incubated at 28.5°. After 7 days 3.0 ml. of 1% 'calsolene' solution were added, the cotton plug replaced by a sterile rubber bung, and the bottle thoroughly shaken. The heavy spore suspension so obtained was used to inoculate a further fifty Thompson bottles of spore medium at the rate of 4.0 ml./bottle; the transfer being effected by means of sterile glass tubes (internal diam. approx. 5 mm.) plugged at one end with cotton-wool and calibrated to hold 4.0 ml. This larger batch of inoculated spore medium was also incubated for 7 days at 28.5° and then used as inoculum for the production medium. A small proportion of each batch was reserved as inoculum for more spore medium, and the process was repeated until it was considered necessary to revert to a sealed culture.

Production medium

The production medium was a modification of Belmont medium in which the peptone was replaced by papain digest of beef, the meat extract was decreased in quantity, and 10 p.p.m. of manganese substituted for the trace of iron (Spilsbury, 1947). It had the following composition (all % (w/v) unless otherwise stated): NaNO₃ (Chilean), 0.4; KH₂PO₄, 0.1; KCl, 0.05; sodium citrate, 0.2; sodium acetate (recryst.), 0.2; MnSO₄.4H₂O, 0.004; CuSO₄.5H₂O.

* The large-scale production was carried out in the penicillin surface culture plant designed by Dr C. G. Pope and Dr W. B. Hawes; it is hoped that a fuller description of this plant will be published.
Large-scale production of streptomycin

0.00175; glucose monohydrate, 4.0; Wilson's* pure extract of beef, 0.25%;
 papain digest of beef (see below), 5.0% (v/v); water to 100%.

A papain digest of beef was prepared by the method used by Mr J. G. C. Campbell in the W.P.R.L. Media Production Unit:

120 lb. beef muscle, finely minced, trimmed from fat and suspended in 90 l. of
water previously heated to 80° and contained in a steam-heated cauldron fitted with
a 1 h.p. motor-driven propeller stirrer. The temperature was raised to 60°, 190 g. of
powdered papain added, and the temperature kept constant for 4 hr. At the end of
this period the digest was brought to the boil and boiled for 5 min., allowed to cool,
and then filtered through a bag filter. The digest was usually prepared the day before
it was required, and after storage at 5.0° overnight, any fat on the surface was
removed by skimming. This operation was important because the thin film of fat
which otherwise covered the surface of the final medium had an adverse effect on the
growth of the actinomycete.

The whole of the digest from 120 lb. beef was incorporated in 1700 l. of
medium, the ingredients for which were measured out and placed in a stainless
steel mixing tank fitted with a motor-driven propeller stirrer. Water at
a temperature of 80° was added to make up the required volume, 3 kg. of celite
(Johns Manville Ltd.) was stirred in to aid the subsequent filtration, and the
pH was adjusted to 7.0 with sodium hydroxide pellets. The hot medium was
passed through two bag filters each containing a further 3 kg. of celite, cooled
to 50°, and then stored in a stainless steel holding tank which supplied the
filling machine.

Before autoclaving, the production medium gave a chemical analysis of
the following type: total N, 2.8–1.8 mg./ml.; amino-N, 0.84–0.5 mg./ml.;
ammonia-N, c. 0.05 mg./ml.; nitrate (as NaNO₃), 0.2%; total reducing sugars
(as glucose), 4.0–3.6%; total solids, 80–60 mg./ml. The specific gravity was
about 1.03.

After autoclaving a slight increase in reducing substances was noted, the
pH fell to 6.3–6.2, and a deposit was observed along the bottom of the bottles.

Bottling and sterilization

Pint milk bottles (‘crown cork milk bottles’) were charged with production
medium at the rate of 200 ml./bottle by means of an automatic bottle-washing
and filling machine. After filling, the bottles were plugged by hand with non-
absorbent cotton-wool, fitted with loose-fitting aluminium caps (Wellcome
Penicillin Unit Type 2, see Pl. 1, fig. 2), and arranged in twelves in wire baskets
to facilitate handling. The aluminium caps were those previously used for
penicillin production, and they had been designed to permit a similar passage of
air to that allowed by the cotton-wool plugs. When a closer fitting cap was tried
it was found that the diminished aeration gave substantially lower titres.

The medium was very suitable for bacterial growth, and it was found
necessary to plug as well as cap the bottles previous to inoculation because
most contamination was found to occur during the cooling after autoclaving.
Sterilization of the medium was effected by autoclaving at 2.5 lb./sq.in. for
30 min. This was not entirely satisfactory because on incubation a small amount

of contamination by a *Bacillus subtilis*-like organism occurred in uninoculated, plugged and capped bottles. Autoclaving at 15 lb./sq.in. for 15 min. proved satisfactory, but extension of the time to 1 hr. rendered the medium useless for the growth of *S. griseus*.

After autoclaving, the baskets of hot bottles were packed on to the mono-rail carriers, which supported the baskets in an almost horizontal position, and passed through a cooling tunnel in which the temperature of the bottles was brought down to that of the incubators (28.5°) by a blast of air. The carriers were then removed to the spray-gun room.

Empty canisters with the spray-guns attached were returned to the spore preparation section where they were cleaned by rinsing in warm water and the cotton-wool air filters were renewed. Experience during the developmental stages showed that traces of detergents such as 'teepol' or soap and the use of dilute phenol solutions for rinsing out spray-guns after washing had a deleterious effect on the inoculum.

**Inoculation**

The spray-guns used to inoculate the production medium were Acrograph guns (type MP). Each gun was fitted with a cotton-wool air filter and a 1 l. canister containing a filter of 50/in. mesh wire gauze and connected to the gun by 6 ft. of rubber tubing. The nozzle of the gun was covered with a cap of cotton-wool which was tied in place and the whole gun put into a small calico bag. The canister lid was covered with a square of cotton cloth which was tied down round the canister. The gun and canister were then carefully packed into a specially constructed galvanized iron box and the whole sterilized by autoclaving for 1 hr. at 15 lb./sq.in.

The sterilized guns were taken to the sterile room where the canisters were filled with inoculum and from thence to the spray-gun room where the nipple of the air filter was attached to the air line (compressed air at 5 lb./sq.in.), the canister being suspended 2–3 ft. above the bottles to be inoculated. The gun was then removed from the bag and the nozzle flamed. The operator removed the metal cap from the bottle to be inoculated and discarded the cotton plug (see next section). The mouth of the bottle and the nozzle of the gun were flamed. Inoculum was injected into the bottle as a fine spray for 8 sec. The mouth of the bottle was again flamed and the cap replaced. The volume of inoculum introduced into each bottle was 3.0 ± 0.5 ml., and this amount was checked at intervals by similarly delivering inoculum into a 10 ml. measuring cylinder. It was found that 192 bottles could be inoculated from one canister containing about 900 ml. of inoculum by one person in 20 min.

**Incubation**

The inoculated medium was incubated for 10–14 days in two large incubators each 165 ft. long × 14 ft. wide and capable of holding four rows of thirty-three carriers (Pl. 1, fig. 1). The temperature was maintained at 28.5 ± 1.0° by air-conditioning plant.
G. C. Ainsworth, A. M. Brown, P. S. S. F. Marsden, P. A. Smith and J. F. Spilsbury—
A method for the large-scale production of streptomycin by surface culture.
Plate 1
Large-scale production of streptomycin

Harvesting

This was effected in a mechanical harvester in which bottles were inserted and drained of their contents for 3–4 min. before being transferred to the input end of the bottle-washing and filling machine. The crude filtrate was filtered and then pumped to the extraction department.

Extraction

The streptomycin was extracted from the culture fluid by a method involving charcoal adsorption, elution with dilute aqueous phosphoric acid, readsorption on charcoal followed by elution with acidified methanol from which the streptomycin was isolated as the hydrochloride. This process is described in detail in the next paper (Woodthorpe & Ireland 1947).

We wish to express our gratitude to our Beckenham colleagues, too numerous to mention individually, whose advice and help were always put freely at our disposal. We are also indebted to all those laboratory technicians, and particularly to Mr J. Elson, Miss E. Howard, Mr Y. Jaulmes and Miss Jean M. Weston, who helped with the large amount of routine and assay work which this investigation involved.

REFERENCES


EXPLANATION OF PLATE

Fig. 1. General view of the incubator.

Fig. 2. Detailed view, showing bottles arranged in wire baskets, and methods of capping.

(Received 21 February 1947)