ON THE PHEROGRAM OF COMMERCIAL PENICILLINS WITH HIGH-POTENTIAL PAPER-ELECTROPHORESIS

BY

Shō Miyamoto, Kazuo Shibata, Masayuki Morita, Tatsuo Shimamoto and Kishiro Sugiura

The authors had been studying the pherogram of high-potential paper-electrophoresis of some commercially available antibiotics in an attempt to select and identify antibiotics which are contained in microorganisms.1)

One of the interesting findings was that Crystal Penicillin G potassium salt (Kaken Co., Ltd.) was composed of not only one but of several different fractions. The difference of these fractions in biological effects is now under the investigation and it will be reported elsewhere.

The question arises whether the number of fractions might be different or not with the individual product of each manufactures. In order to clarify this program different products of penicillin were analyzed by high-potential paper-electrophoresis.

MATERIALS

The samples of penicillin used in this study were supplied from seven manufacturers to which the authors are indebted. Those are all commercially available and are listed below.

1. Crystal Penicillin G sodium salt
   (Meiji Seika Co., Ltd., Lot # G 386)
2. Crystal Penicillin G potassium salt
   (Sankyo Co., Ltd., Lot # 18523)
3. Crystal Penicillin G potassium salt
   (Takeda Yakuhin Co., Ltd., Lot # G 360)
4. Crystal Penicillin G potassium salt
   (Nihon Kayaku Co., Ltd., Lot # K 238)
5. Crystal Penicillin G sodium salt
   (Banyu Seiyaku Co., Ltd., Lot # G 419)
6. Crystal Penicillin G potassium salt
   (Fujisawa Yakuhin Co., Ltd., Lot # G 1085)
7. Crystal Penicillin G potassium salt
   (Kaken Yakukako Co., Ltd., Lot # G 149)

富本瑞, 柴田一雄, 泰田正之, 岛本達夫, 杉浦賢四郎: Dept. of Biochemistry (Chief. Prof. S. Miyamoto) School of Medicine. Received for publication, March 31, 1960.
METHOD

The apparatus of high-potential paper electrophoresis (The horizontal type of Tokyo Medical and Dental University model) was applied as previously reported. As a cooler insulation kerosin was used. The buffer solution was consisted of acetic acid, formic acid and water (15:5:80, pH 1.5). A strip of Toyo-Roshi No. 51 filter-paper measuring 5 × 50 cm was used.

The sample was dissolved in a small quantity of destilled water and was spotted with a painting brush on the original line of the filter-paper, 12 cm apart from the anodic end. At 4 kV the electrophoresis was performed for 20 minutes. The electrophoresis was immediately brought to dryness by adding the heat below 50°C.

The detection of spots was performed by the fluorescent with the ultraviolet rays having a maximum wave length of 2536 A. In addition, the filter-paper was stained with 0.25% Ninhydrin in acetone, and spots were estimated by means of the photoelectric densitometer.

RESULT AND DISCUSSION

The pherograms of a series of penicillin with ultraviolet rays were illustrated in Fig. 1 (A, B). Samples No. 1, 3 and 7 were founded to have three fluorescent bands respectively, whereas two fluorescent bands were recognized in cases of Sample No. 2, 4, 5, and 6. All these bands showed whitish yellow green except for Sample No. 2 in which one of the bands located nearer the starting line showed pink color.

While, Ninhydrin positive bands in each samples were showed as Fig. 2. Namely, Sample No. 1 were founded to have five bands, No. 3, 4 were nine, No. 2 and 7 were eight, No. 5 was six, and No. 6 was seven bands. To study whether the fluorescent bands and the Ninhydrin positive bands were the same or not, we stained them with Ninhydrin after we marked the position of the fluorescent bands. In all commercial goods, the nearest fluorescent band to start line was in the same position of the Ninhydrin positive band. The other fluorescent bands were not always coincident with the Ninhydrin positive fraction.

After comparing the exact position of these bands exposed by two different methods above mentioned with each other, it was found to be most **To study whether the fluorescent bands and the Ninhydrin positive band were the same or not, we stained them with Ninhydrin after we marked the position of the fluorescent bands. In all commercial goods, the nearest fluorescent band to start line was in the same position of the Ninhydrin positive band. The other fluorescent bands were not always coincident with the Ninhydrin positive fraction.
Fig. 1-A
Pherograms of Penicillin G
Irradiated with UV 2536 A
Y—whitish yellow green
fluorescence
P—pink fluorescence
1—Meiji Seika Co. Ltd.
2—Sankyo Co. Ltd.
3—Takeda Yakuhin Co. Ltd.
4—Nihon Kayaku Co. Ltd.

Fig. 1-B same above
5—Banyu Seiyaku Co. Ltd.
6—Fujisawa Yakuhin
7—Kaken Yakukako Co. Ltd.

Table 1. $R_{Lp}$ of Fluorescent Fractions

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>number of fractions</th>
<th>$R_{Lp}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>0.24 0.17 0.07</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.14 0.06</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>0.29 0.18 0.10</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>0.16 0.10</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>0.11 0.07</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>0.19 0.10</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>0.16 0.09 0.06</td>
</tr>
</tbody>
</table>

Table 2. $R_{Lp}$ of Ninhydrin Positive Fractions

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>number of fractions</th>
<th>$R_{Lp}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>1.01 0.64 0.44 0.24 0.06</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>1.02 0.50 0.43 0.29 0.22 0.15 0.06 0</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>1.04 0.71 0.57 0.48 0.42</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>1.02 0.76 0.68 0.53 0.42</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>1.01 1.01 0.54</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>1.02 0.80 0.70 0.50</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>1.01 0.56</td>
</tr>
</tbody>
</table>
probable that the fluorescent band of L-Lysine under the same condition was estimated. A serious of the mobility ratio thus obtained, which is called $R_{\text{LyS}}$, was shown in Table 1 and 2.

From the results of this experiment, it was concluded that the commercial penicillin G, although there are some differences with each brand, could be regarded as the mixture of two or three fluorescent substances and of from five to nine Ninhydrin positive substances. The mobility ratio of fluorescent bands to L-Lysin, $R_{\text{LyS}}$, was found to be of comparatively small value ranging 0.06–0.29. $R_{\text{LyS}}$ values of Ninhydrin positive bands, on the other hand, were distributed in the wide range from 0–1.04. Among
those Ninhydrin positive substances, three of which showed the marked coloring with the $R_{f_{st}}$ of 0.06–0.10, 0.24–0.29 and 1.01–1.04, and it was the case with every sample. The antibiotic effect of each fraction has to be investigated in future.

**Summary**

Seven different bands of commercial penicillin G were analyzed by means of the high-potential paperelectrophoresis. We thought that these results would become important for the study of penicillin. We knew that in commercial penicillin, Meiji Seika, Sankyo, Takeda Yakuhin, Nihon Kayaku, Banyu Seiyaku, Fujisawa Yakuhin, Kaken Yakukako, they had similar fractions by the high-potential electrophoresis (horizontal type), and the pherogram of Penicillin G could be separated into several fractions, although there were slight differences in number with each band.

With ultraviolet rays two or three fluorescent bands were detected, and by staining with Ninhydrin, from five to nine fractions could be recognized. The significance of the findings was discussed.

**Literature**