Basic studies were carried out on 3', 4'-dideoxykanamycin B (DKB), a new antibiotic derived from kanamycin B. DKB proved to be highly effective against *Klebsiella pneumoniae*, *Salmonella*, and *Pseudomonas aeruginosa*. DKB showed similar MICs against *P. aeruginosa* to those of gentamicin (GM). Serum level of DKB reached a peak (38.0 mcg/ml on average) 30 minutes following a single intramuscular injection of 20 mg/kg. Of the injected DKB 63.3% was recovered from the urine within 24 hours. The fact that high levels of DKB were detected in both serum and urine might suggest an excellent therapeutic effects in the treatment of systemic infections as well as urinary infections. The biliary excretion rate of DKB was only 1.22%.

3', 4'-Dideoxykanamycin B (DKB) is a new antibiotic derived from kanamycin B, and has a structure shown in Fig. 1.

The present paper is concerned with the results of basic studies on this new antibiotic, including the *in vitro* antibacterial activity, absorption and excretion in animals.

**Materials and Methods**

1. **Antibiotics tested:** Antibiotics used were DKB (Meiji Seika Kaisha Ltd.), carbenicillin (Fujisawa Pharm. Co., Ltd.), gentamicin (Shionogi Pharm. Co., Ltd.), polymyxin B (Taito Pfizer Co., Ltd.), kanamycin (Meiji Seika Kaisha Ltd.), thiopenicol (Eisai Co., Ltd.) and colistin (Banyu Seiyaku Co., Ltd.).

2. **Bacterial strains:** Standard strains stored in our laboratory and *P. aeruginosa* clinically isolated in our hospital.

3. **Animals:** Male and female rats of the Sprague-Dawley strain, each weighing 150~270 g.

4. **Susceptibility test:** *In vitro* antibacterial activity of antibiotics was determined by the agar dilution method. One loopful of an overnight culture was streaked on heart infusion agar containing graded concentrations of any of the test antibiotics. The minimal inhibitory concentration (MIC) was determined after incubation at 37°C for 20 hours.

5. **Assay method:** Petri dishes containing 10 ml of sodium citrate agar (1% agar, 1% Na-citrate, 0.5% polypeptone, and 0.3% meat extract), with about 10⁸ spores per ml of *Bacillus subtilis* ATCC 6633, were used for the assay.

6. **Stability in tissue homogenates:** Three rats were killed by cervical dislocation. The lungs, liver and kidneys were removed and washed with saline. These organs were homogenized in a Waring blender, and the homogenates were diluted with *KREBS-RINGER'S* phosphate (pH 7.2) to make 30% solutions. One ml of DKB solution (200 mcg/ml) was added to equal ml of any of the three homogenates, and incubated at 37°C for 60~120 minutes. The enzymatic reaction was terminated by the addition of 2 ml of 99% ethanol and the antibacterial activity still residing in

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7. Protein binding of DKB: The degree of serum protein-binding was measured by centrifugal ultra-filtration method using Visking tubing (size: 8/32). Serum used was of human origin ("CONSERA": Nissui Pharm. Co. Ltd.). A 4.5 ml portion of the serum was added to 0.5 ml of 0.1 M phosphate buffer (pH 7.4) containing DKB at 500 mcg/ml, and incubated at 37°C for 1 hour. The mixture was then introduced into Visking tubing, and centrifuged at 1,000×g for 30 minutes. The DKB activity present in the protein-free ultra-filtrate was assayed by the disc method. As a control, the DKB solutions without serum were treated similarly. Unbound DKB was calculated from the ratio of the concentration of the ultra-filtrate to the total concentration in serum.

8. Serum concentration and urinary excretion: A single dose of 20 mg/kg of DKB was injected intramuscularly to animals. Blood samples were withdrawn from the heart 0.5, 1, 2 and 4 hours after administration. The urine was collected at regular intervals over a period of 24 hours after administration. The concentrations in the serum and urine were determined by the disc method.

9. Identification of active substances recovered in urine: DKB was injected intramuscularly to rats at a single dose of 20 mg/kg. The urine samples were collected over a period of 24 hours, and examined by thin-layer chromatography. A solvent system of n-BuOH-ETOH-CHCl₃-17% NH₄OH (4:5:2:5 in volume) was used. The adsorbent was Eastman Chromagram Sheet No. 6065 (Cellulose) and No. 6061 (Silica gel). For bioautography, dried sheets were placed on agar plates previously seeded with 0.2% of a spore suspension (2×10⁸ spores/ml) of B. subtilis ATCC-6633.

10. Biliary excretion: Animals were anesthetized with sodium thiopental, and the bile duct was cannulated with a polyethylene tube by standard laboratory procedure. DKB was administered intramuscularly at a single dose of 20 mg/kg. The bile was collected for 6 hours.

11. Tissue distribution: Nine rats were injected intramuscularly with 20 mg/kg of DKB, and divided into three animal groups. Animals of each group were killed 0.5, 1 and 2 hours after administration by cervical dislocation. Three other rats were given 50 mg/kg and killed 30 minutes afterward by the same method. The lungs, liver and kidneys were removed and washed with saline. Each organ was then homogenized with twice the volume of 99% ethylalcohol in a Waring blender. The homogenates were centrifuged at 10,000 × g for 10 minutes, and the supernatant fluids were used for bioassay.

Results

1. Antibacterial Activity

The antibacterial activity of DKB and other antibiotics are summarized in Table 1.

As this table shows, DKB...
proved to be a broad-spectrum antibiotic highly active against *K. pneumoniae*, *Shigella*, *Salmonella* and *P. aeruginosa*. Moreover, DKB was found to be as active as GM against *P. aeruginosa*.

2. Distribution of Activity against 40 Strains of Freshly Isolated *P. aeruginosa*.

The fact that DKB is highly active against *P. aeruginosa* prompted us to investigate the distribution of its activity against freshly isolated strains of this organism. As Table 2 shows, DKB at concentrations ranging from 0.39 to 12.5 mcg/ml, inhibited all the strains tested. In addition, as many as 39 of the 40 strains were suppressed at concentrations as low as 6.25 mcg/ml or below. DKB was thus found to be the most effective agent among the antibiotics tested.

3. Serum and Urine Concentrations

When a single dose of 20 mg/kg of DKB was injected intramuscularly, a mean peak serum concentration of 38.0 mcg/ml was attained 30 minutes after administration. The serum concentration decreased to about half after 1 hour, and almost undetectable by disc method after 4 hours (Table 3).

The urinary recovery was concurrently studied (Table 4). The recoveries in 0~24-hour urine samples were 69.7%, 56.8% and 63.4%, and averaged 63.3%. The maximum urinary con-
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Table 5. Excretion in bile 0~6 hour after 20 mg/kg intramuscular injection of DKB

| Exp. No. | 0~6 hour |  |  
|----------|----------|---|---|
|          | mcg/ml   | mg | %  |
| 1        | 5.8      | 0.046 | 0.92 |
| 2        | 8.3      | 0.079 | 1.52 |
| Mean     | 7.1      | 0.063 | 1.22 |

Table 6. Tissue distribution

<table>
<thead>
<tr>
<th>Dose</th>
<th>Tissue</th>
<th>30 min.</th>
<th>60 min.</th>
<th>120 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mg/kg</td>
<td>Lung</td>
<td>N. D.*</td>
<td>N. D.</td>
<td>N. D.</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>28.2</td>
<td>4</td>
<td>N. D.</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>4.1</td>
<td>(36.4)</td>
<td>(15.0)</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>Lung</td>
<td>12.4</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>50.6</td>
<td>(89.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>4.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7. Protein binding of DKB

Concentration of serum: 90%
Concentration of antibiotic: 50 mcg/ml

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>% Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp. 1</td>
</tr>
<tr>
<td>DKB</td>
<td>39.0</td>
</tr>
<tr>
<td>CB-PC</td>
<td>40.3</td>
</tr>
</tbody>
</table>

Concentration of 1,037 mcg/ml was found in 0~6-hour sample.

4. Biliary Excretion

The biliary excretion of DKB is shown in Table 5. When a single intramuscular dose of 20 mg/kg was given, the recovery in the 0~6-hour bile sample was only 1.22% with an average concentration of 7.1 mcg/ml.

5. Tissue Distribution

After a single intramuscular dose of 20 mg/kg, DKB was found at the concentration of 28.2 mcg/ml in the kidneys, 4 mcg/ml in the liver and not detected in the lungs after 30 minutes. When 50 mg/kg were given, the concentration 30 minutes after administration was 50.6 mcg/g for the kidneys, 12.4 mcg/g for the lungs, and 4.5 mcg/g for the liver (Table 6).

6. Serum Protein Binding

As shown in Table 7, the binding of DKB to human serum protein is low in comparison with that of carbenicillin (CB-PC).

7. Identification of an Active Substance Recovered in Urine

The urine sample was examined by thin-layer chromatography and subsequent bioautography. The results are shown in Fig. 2. Under the test conditions, only a single active substance was
recovered, the Rf value of which was found to be identical to that of the parent DKB. Accordingly, the active substance recovered in the urine is considered to be DKB itself.

8. Stability of DKB in Tissue Homogenates

The stability of DKB in tissue homogenates is shown in Fig. 3. DKB lost its activity in tissue homogenates of rats. The loss of activity was most remarkable in liver homogenate, in which only 15% of the initial potency was recovered after incubation at 37°C for 120 minutes. The activity of DKB was also lost in kidney and lung homogenates but the loss was lower than in liver homogenate.

Discussion

Kanamycin has long been extensively used as a broad-spectrum antibiotic\(^2\).\(^4\). DKB, a new kanamycin derivative, is more active than CB-PC and as potent as GM against \textit{P. aeruginosa in vitro}. The present study showed that all the tested strains of \textit{P. aeruginosa} were sensitive to DKB. It suggested that this new antibiotic is likely to be equal or superior to any of the currently available antibiotics effective against infections caused by \textit{P. aeruginosa}. The high serum level of 38 mcg/ml after a single intramuscular dose of 20 mg/kg and its low MIC against \textit{P. aeruginosa} indicate the excellent therapeutic effectiveness of this antibiotic for systemic infections caused by this organism. The fact that levels of DKB in the 0~6 hour urine samples exceeded 1,000 mcg/ml suggests its usefulness against urinary infections.

Biliary concentrations, however, were not necessarily high (7.1 mcg/ml at the highest) after the same intramuscular dose, which indicates the need for further investigation.

As to its tissue distribution, difference in doses caused different findings: when an intramuscular dose of 50 mg/kg was given, DKB was detected in the kidneys, lungs and liver, meanwhile, when 20 mg/kg were given, the tissue concentrations were low except in the kidneys, and were considerably lower than those of penicillins and cephalosporins. One of the explanations for this low tissue distribution must be the low recovery rates in the tissue homogenates. However, it is unknown whether this is due to inactivation or possible binding to homogenate components.

Acknowledgement

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Reference

1) Text book of DKB. Meiji Seika Kaisha Ltd. 1972