A STUDY OF THE URINARY EXCRETION OF VITAMIN B₆
BY A COLORIMETRIC METHOD

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Chemical methods for the determination of biological substances, in contrast with animal assays, offer a considerable saving in time, and the analytical data are usually more precise and reproducible. For these reasons, a study of the vitamin B₆-indophenol color reaction has been in progress in these laboratories for some time (1). In the course of this work a procedure has been devised whereby solutions containing a minimum of 2 micrograms of the vitamin per cc. can be analyzed with precision (2). The general applicability of this method, the nature of interfering substances, and the specificity of the test are under investigation. Details of this work will be presented elsewhere.

Method

It has been found that this method may be used in a very simple form to measure the urinary excretion of vitamin B₆ administered orally or parenterally. This application of the method depends upon the analysis of samples of urine obtained before and after the administration of the vitamin. Both analyses are necessary because interfering substances present in the preadministrative or control sample give rise to a small concentration of colored reaction products. This concentration, measured in the Evelyn colorimeter with a No. 660 filter to reduce the influence of extraneous colors, is subtracted from the concentration found in the postadministrative sample. The difference is the vitamin B₆ output. This is based on the assumption, however, that the interfering substances are the same in both samples of urine, which seems likely under the conditions employed.
In general, the conditions used are such as might be used clinically in a saturation test of vitamin B₆ deficiency. A fasting subject was given water to flush the urinary tract. The urine was discarded. After 1 hour, the control sample was taken and the bladder was emptied. At this time additional water was given to provide an adequate diuresis and the vitamin was injected intravenously or given orally. Postadministrative samples were then taken at measured intervals of time. Under these conditions, interfering substances should be essentially the same in both samples, although the concentration might be slightly lower in the postadministrative samples. This would tend to
give low, rather than high results. Urine samples taken from test subjects were analyzed both by the chemical method and by the curative assay with vitamin B₆-deficient rats (3) and the results were found to be in excellent agreement. Furthermore,

![Diagram](http://example.com/diagram.png)

**Fig. 2.** Rate of excretion following the intravenous administration of vitamin B₆. Curve 1, Dog 31, dose 25 mg., 1 hour output 17.2 per cent; Curve 2, Dog 97, dose 25 mg., 1 hour output 16.8 per cent; Curve 3, Dog 97, dose 50 mg., 1 hour output 15.0 per cent; Curve 4, Dog 31, dose 50 mg., 1 hour output 20.8 per cent; Curve 5, Dog 97, dose 100 mg., 1 hour output 14.4 per cent; Curve 6, Dog 97, 100 mg., 1 hour output 16.5 per cent; Curve 7, Dog 31, dose 250 mg., 1 hour output 20.8 per cent; Curve 8, Dog 97, dose 250 mg., 1 hour output 18.0 per cent; Curve 9 (urine by catheter), Dog 66, dose 500 mg., 1 hour output 20.8 per cent; Curve 10, Dog 97, dose 500 mg., 1 hour output 20.5 per cent.

100 per cent recovery was obtained upon the addition of vitamin B₆ to samples of both dog and human urine. Nevertheless, in order to eliminate further the possibility of variations in urine samples, both the control and postadministrative samples of urine were diluted equally until the control urine gave a blank, or
very faint color reaction. The concentration of the postadministra-
tive sample was then reckoned directly from a calibration
curve, or by difference as the case required. This dilution reduces
the sensitivity of the test, and may be a handicap if doses lower
than 50 mg. are required in the development of a saturation test
in man.

Experiments in Dogs—Six dogs, weighing 7 to 15 kilos and
maintained on a diet of Purina Dog Chow, were used. Control

| Table I |

Vitamin B₆ Output in Human Subjects after Intravenous and Oral
Administration

<table>
<thead>
<tr>
<th>Subject</th>
<th>After intravenous administration of 50 mg. vitamin B₆</th>
<th>Total output 4 hrs. after oral administration of 100 mg. vitamin B₆</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 min.</td>
<td>30 min.</td>
</tr>
<tr>
<td>1</td>
<td>3.3</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>3.1</td>
<td>0.7</td>
</tr>
<tr>
<td>3</td>
<td>3.9</td>
<td>1.2</td>
</tr>
<tr>
<td>4</td>
<td>3.6</td>
<td>2.2</td>
</tr>
<tr>
<td>5</td>
<td>2.4</td>
<td>0.9</td>
</tr>
<tr>
<td>6</td>
<td>2.9</td>
<td>0.7</td>
</tr>
<tr>
<td>7</td>
<td>3.7</td>
<td>0.3</td>
</tr>
<tr>
<td>8</td>
<td>4.3</td>
<td>0.3</td>
</tr>
<tr>
<td>9</td>
<td>3.1</td>
<td>0.5</td>
</tr>
<tr>
<td>10</td>
<td>5.2</td>
<td>0.7</td>
</tr>
<tr>
<td>11</td>
<td>4.5</td>
<td>0.3</td>
</tr>
<tr>
<td>12</td>
<td>3.1</td>
<td>0.0</td>
</tr>
<tr>
<td>13</td>
<td>3.5</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Average .................................. 8.7±1.6 7.6±1.1
Maximum deviation .......................... 3.1 3.1

samples of urine and postadministrative samples were taken
either by catheterization or through a bladder fistula at the time
intervals indicated in Figs. 1 and 2. A point-line method of
plotting was used for clarity in presenting these data.

Fig. 1 shows the urinary output of fasting dogs following the
oral administration of the vitamin. Maximum excretion oc-
curred within 1 hour, indicating rapid absorption from the gastro-
intestinal tract. In fed dogs (recoveries shown in the legend
under Fig. 1) maximum excretion was delayed 2 to 3 hours. In all experiments 20 per cent of the vitamin (±1, maximum deviation 3.2 per cent) was recovered within 6 hours.

After the intravenous injection of doses of the vitamin varying from 25 to 500 mg. per dog, the maximum output was attained within 30 minutes (Fig. 2). Within 1 hour, 18 per cent of the vitamin was excreted, regardless of the weight of the animal. This approximates the 6 hour output following oral administration.

*Experiments in Man*—A group of thirteen non-fasting, apparently healthy, adult male subjects weighing 60 to 85 kilos were given 50 mg. of the vitamin intravenously. Postadministrative samples were then taken at the intervals shown in Table I. The results obtained are more variable than those found in our study with dogs. This may be due to the non-fasting condition of the subjects.

After the oral administration of 100 mg. of the vitamin to a group of eleven non-fasting male subjects, an average recovery of 7.6 per cent of the vitamin was obtained at the end of 4 hours.

**DISCUSSION**

It has been reported (2) that 50 to 70 per cent of test doses of vitamin $B_6$ was recovered in normal rat urine regardless of the mode of administration of the vitamin. In fasting dogs, urinary excretion of the vitamin is essentially complete within 6 hours after oral administration, but there was only 20 per cent recovery. An almost equal recovery (18 per cent) was obtained in dogs within 1 hour after intravenous administration of the vitamin. These results were remarkably constant, regardless of the weights of the animals and the doses (25 to 500 mg.) administered. In non-fasting human subjects only 8.7 per cent of a 50 mg. intravenous dose of the vitamin was recovered within 1 hour, although excretion appeared to be practically complete by this time.

In earlier experiments (2) it was observed that the fraction of the vitamin excreted in the urine of deficient rats was smaller than that excreted by normal animals. However, the amounts of the vitamin given (100 micrograms per rat, or approximately 2 mg. per kilo) were too small to permit a quantitative interpretation of the results obtained. Nevertheless, it was believed that
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at this same dose level (2 mg. per kilo) or even smaller doses, the urinary output of the vitamin could be measured quantitatively in larger animals. This is amply substantiated by the present work. The fraction of the vitamin excreted following doses of 2 to 3 mg. per kilo has been measured in the dog, and the output following less than 1 mg. per kilo has been quantitatively measured in human subjects.

SUMMARY

1. A colorimetric method based on the indophenol reaction has been used to study the excretion of test doses of vitamin B₆ in the normal dog and man.

2. The short interval between oral administration of the vitamin and its appearance in the urine indicates that it is rapidly absorbed from the gastrointestinal tract and readily excreted.

3. After the intravenous administration of the vitamin in doses ranging from 25 to 500 mg., 18 per cent of the vitamin was recovered from the urine of dogs within 1 hour. After oral administration, these dogs excreted 20 per cent of the vitamin within 6 hours.

4. In a group of apparently healthy human subjects, an average of 8.7 per cent of a 50 mg. intravenous dose of the vitamin was recovered within 1 hour, while 7.6 per cent of a 100 mg. oral dose was recovered within 4 hours.

BIBLIOGRAPHY
