The urinary excretion of thiamine has been used for some time as an empirical indication of thiamine nutrition. The tacit assumption is made that when the dietary intake of the vitamin is at or below the human "requirement" the urinary excretion falls below a certain value, but as long as the dietary intake exceeds the requirement the urinary excretion remains above this critical level (10).

This assumption was originally made without a clear understanding of the range of the variation in excretion values under controlled dietary conditions. In most cases the controlled studies were made over very short periods, so it is likely that many of the subjects had not attained equilibrium in their urinary excretion of thiamine. Furthermore, in many cases, the methods used for the determination of thiamine in the urine are open to some question (12).

Very few reports have utilized statistical methods, even where the data were sufficient to warrant such use. A notable example of this is the series of papers by the group at the Mayo Clinic (9, 18). Although they collected urine samples from subjects who were maintained on constant thiamine intakes for periods as long as 7 months, only a single average value for the urinary thiamine is given for each period. Elsom, Reinhold, Nicholson, and Chornock (4) have given the standard deviations for the urinary thiamine excretions of nine women who consumed a constant amount of thiamine for indefinite periods. They and Wertz and Mitchell (17) are the only investigators who seem to have given this problem any considera-
tion, but their data are so scanty that a true picture of the variation in urinary thiamine excretion is not possible. Their results, however, do bring out the great variation in the urinary excretion of thiamine by "normal" adults.

The present paper presents a statistical evaluation both of thiamine and of pyramin excretions by normal young men of comparable body size who were living under the same conditions of diet and activity. These experiments have been carried on in this Laboratory from 1943 to date. They have involved the prolonged maintenance of the subjects on controlled thiamine intakes from zero up to 16 mg. per day. A survey of the general results of one of these experiments has already been published (8).

**EXPERIMENTAL**

In one experiment, groups of two, four, and four men were given 1.81, 1.01, and 0.61 mg. of thiamine per day, respectively, for a period of 24 weeks. In another experiment, two groups of six subjects each received 1.00 and 2.00 mg. of thiamine, respectively, per day for a period of 34 weeks, after a control period of a month on the lower level of thiamine intake. Following this experiment the intake levels of the two groups were reversed. A period of 1 month on a partially controlled diet intervened between the two parts of the latter experiment. In each of these experiments, all men received the same basic diet. All meals throughout the experiment were served as weighed portions from the diet kitchen. The differences in the thiamine intake were adjusted by means of thiamine pills and placebos. The thiamine content of the diet averaged close to 0.5 mg. per day. The day to day variation of the dietary intake of thiamine from the given mean values was of the order, $\sigma = \pm 0.06$ to 0.08 mg. per day. This was determined by actual analyses of the diets served. Finally, the excretion values at higher levels of intake, i.e. 5, 11, and 16 mg. of thiamine per day, were obtained from other subjects on somewhat less strictly controlled thiamine intakes.

**Analytical Methods**

In all of these experiments, except those in which 5, 11, and 16 mg. of thiamine were used, diet samples were collected daily. An exact duplicate of all the food served to the subjects was saved for analysis. This was analyzed for thiamine by the method of Hennessy and Cerecedo (6).

All of the values reported in this paper for urinary thiamine excretion represent 24 hour samples which were collected in bottles containing 5 ml. of toluene and 5 ml. of glacial acetic acid as preservatives. Thiamine was determined in these samples by our modification of the thiochrome procedure (12).
The pyramin content of the urine was determined by a modification of the yeast fermentation method of Schultz, Atkin, and Frey (15). According to the original description of the method, the pyramin value is represented by the sulfite blank. The standard used in these determinations was 2-methyl-4-amino-5-ethoxymethylpyrimidine hydrochloride.

Statistics

Many of the statistical methods used in the analysis of our results are not available to most biochemists or physiologists. To clarify our methods and to avoid confusion as to the definition of terms, we include a brief summary of the statistical items used in this report. For a more complete discussion and for the mathematical derivation of the formulas, see Snedecor (16).

The variability of the thiamine and pyramin excretion data has been submitted to the analysis of variance (16). In the discussion of variability, the variances $\sigma^2_{id}, \sigma^2_i, \sigma^2_d, \sigma^2_{wit},$ and $\sigma^2_{wd}$ are related to the mean squares, $V_{id}, V_i, V_d, V_{wd},$ and $V_{wi},$ as indicated in the following equations:

\begin{align}
V_{wi} &= \sigma^2_{wi} = \sigma^2_{id} + \sigma^2_i = \frac{\sum y^2 - \sum I^2/k}{nk - n} \\
V_{wd} &= \sigma^2_{id} + \sigma^2_i = \frac{\sum y^2 - \Sigma D^2/n}{nk - k} \\
V_i &= \sigma^2_i + k\sigma^2_d = \frac{\Sigma I^2/k - T^2/nk}{n - 1} \\
V_d &= \sigma^2_i + n\sigma^2_d = \frac{\Sigma D^2/n - T^2/nk}{k - 1} \\
V_{id} &= \sigma^2_{id} = \frac{\sum y^2 - \Sigma D^2/n - \Sigma I^2/k + T^3/nk}{nk - k - n + 1}
\end{align}

where, in a given table of data showing the excretion values, $y,$ for $n$ individuals on $k$ days, the sums of the excretion values for each of the individuals over this period of days are $I_1, I_2, I_3, \ldots, I_n,$ respectively; the daily totals summing all individual values for each day are $D_1, D_2, D_3, \ldots, D_k,$ respectively, and $T$ is the grand total of all $nk$ values of $y.$

These terms are measures of:
- $V_{wi} = \sigma^2_{wi},$ the within individual (intraindividual) variation. This is a pooled ("statistically averaged") value for day to day variations for each of the individuals within the group and represents the average day to day variations of each individual from his own mean.
- $V_{wd} = \sigma^2_{wd},$ the within day (interindividual) variation. This is a pooled value for the $k$ days of the deviation of individual values on a given day from the mean group value on that day.
$V_i$, the deviation of the $n$ individual mean values from the grand mean for the total period.

$V_d$, the deviation of the $k$ daily mean values from the grand mean.

$V_{id} = \sigma_{id}^2$, the interaction term or purely random "error." This can be represented graphically as the degree to which the individual values from day to day fail to move in a parallel fashion.

$\sigma_i$, that part of the interindividual variation (not attributable to random error) which is consistent for the individual from day to day.

$\sigma_d$, that part of the day to day "general up and down movement" of the group values which is over and above that expected from purely random "error."

### TABLE I

**Analysis of Variances of 24 Hour Urinary Excretion of Pyramin by Normal Young Men on Different Levels of Thiamine Intake**

The men were maintained at each level of intake for periods of 2 or more months (see Table III for the exact time). The urinary excretion values are expressed as micrograms of 2-methyl-4-amino-5-ethoxymethylpyrimidine hydrochloride per day. The thiamine intake is expressed as mg. per day. The definition of $\sigma$ is given in the text. The pooled values are the weighted averages for each column of figures.

<table>
<thead>
<tr>
<th>Thiamine intake</th>
<th>24 hr. urinary pyramin excretion</th>
<th>$c_{id}$</th>
<th>$c_{id}$</th>
<th>$c_{id}$</th>
<th>$c_{id}$</th>
<th>$c_{id}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.61</td>
<td>125.5</td>
<td>21.0</td>
<td>26.3</td>
<td>18.9</td>
<td>8.9</td>
<td>18.2</td>
</tr>
<tr>
<td>1.00</td>
<td>165.4</td>
<td>19.0</td>
<td>29.3</td>
<td>18.2</td>
<td>5.3</td>
<td>22.9</td>
</tr>
<tr>
<td>1.01</td>
<td>171.5</td>
<td>22.9</td>
<td>30.0</td>
<td>22.0</td>
<td>6.5</td>
<td>20.5</td>
</tr>
<tr>
<td>1.81</td>
<td>267.4</td>
<td>27.7</td>
<td>40.1</td>
<td>29.4</td>
<td>0</td>
<td>27.4</td>
</tr>
<tr>
<td>2.00</td>
<td>232.6</td>
<td>23.1</td>
<td>28.9</td>
<td>21.5</td>
<td>8.4</td>
<td>19.4</td>
</tr>
<tr>
<td>Pooled values</td>
<td>21.58</td>
<td>29.65</td>
<td>20.45</td>
<td>6.90</td>
<td>21.48</td>
<td></td>
</tr>
</tbody>
</table>

**Excretion of Pyramin**—The values of the different variances (Table I) are quite constant for pyramin, being homogeneously distributed throughout the data from all of the experimental groups on all levels of thiamine intake and pyramin excretion.

The pyramin values from all of our pooled experimental data have been used in calculating the following variations in micrograms per day:

$$\sigma_{wi} = 30.46; \sigma_{wd} = 22.61; \sigma_{id} = 20.81$$

In each case there are 500 to 600 degrees of freedom. By calculation from the above values, $\sigma_i = 8.84$ and $\sigma_d = 22.24$. In the urinary pyramin excretion data, $\sigma_i$, the individual variation corrected for random "error," is very small, in some cases almost insignificant. Considering the careful
control of the dietary intake of thiamine, the day to day variation as represented by $\sigma_{\text{net}}$ is surprisingly large. This may be partly due to the fact that, while the diet is carefully controlled with respect to thiamine, it was not possible to control the intake of the pyrimidines which may be related to thiamine or its decomposition products. Direct analysis of the diets for pyrimin has been found to be valueless because of the tendency of compounds that are inactive in the fermentation method to become “active” on feeding (3).

**Excretion of Thiamine**—The variance values in the thiamine excretion data are not so uniformly distributed as they were in the pyrimin data. The variation in the thiamine excretion depends to a large extent upon the magnitude of the mean with which it is associated. This has been noted to a certain extent by other workers (4) who have preferred to express deviations in thiamine excretion in terms of percentages of the mean. This strong dependence of variance upon the mean can readily be seen in Table II, A. Where the variance is so intimately related to the mean, it is proper to convert the excretion values to logarithms before proceeding with the analysis of variance, and then express the final figures as percentages of the mean ((16) p. 448). This helps somewhat, but, as may be seen in Table II, B, even according to this method the variances are not homogeneously distributed throughout the various intake levels. The logarithmic treatment of the thiamine excretion data overcompensates for the trend seen in the ordinary analysis of variance (Table II, A). The proper method of analysis would involve some intermediary statistical procedure which has not yet been developed. Both of the presently available methods.

<table>
<thead>
<tr>
<th>Thiamine intake</th>
<th>Excretion mean</th>
<th>$\sigma_{\text{ad}}$</th>
<th>$\sigma_{\text{at}}$</th>
<th>$\sigma_{\text{id}}$</th>
<th>$\sigma_{\text{it}}$</th>
<th>$\sigma_{\text{d}}$</th>
<th>$\sigma_{\text{ad}}$</th>
<th>$\sigma_{\text{at}}$</th>
<th>$\sigma_{\text{id}}$</th>
<th>$\sigma_{\text{it}}$</th>
<th>$\sigma_{\text{d}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg. per day</td>
<td>$\gamma$ per day</td>
<td>$\gamma$ per day</td>
<td>$\gamma$ per day</td>
<td>$\gamma$ per day</td>
<td>$\gamma$ per day</td>
<td>$\gamma$ per day</td>
<td>$\gamma$ per day</td>
<td>$\gamma$ per day</td>
<td>$\gamma$ per day</td>
<td>$\gamma$ per day</td>
<td>$\gamma$ per day</td>
</tr>
<tr>
<td>0.61</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>6</td>
<td>184</td>
<td>182</td>
<td>104</td>
<td>115</td>
</tr>
<tr>
<td>1.01</td>
<td>26</td>
<td>16</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>9</td>
<td>1</td>
<td>125</td>
<td>95</td>
<td>25</td>
<td>118</td>
</tr>
<tr>
<td>1.00</td>
<td>65</td>
<td>25</td>
<td>23</td>
<td>16</td>
<td>16</td>
<td>20</td>
<td>17</td>
<td>41</td>
<td>96</td>
<td>7</td>
<td>40</td>
</tr>
<tr>
<td>1.81</td>
<td>195</td>
<td>36</td>
<td>29</td>
<td>27</td>
<td>27</td>
<td>22</td>
<td>13</td>
<td>22</td>
<td>18</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>2.00</td>
<td>224</td>
<td>106</td>
<td>81</td>
<td>57</td>
<td>57</td>
<td>90</td>
<td>58</td>
<td>52</td>
<td>26</td>
<td>16</td>
<td>42</td>
</tr>
</tbody>
</table>

**Table II**

*Analysis of Variance of 24 Hour Urinary Thiamine Excretion Data of Normal Young Men As Calculated by Two Different Methods (See Table III for Details)*

$A$, values calculated from actual excretion data expressed in terms of micrograms of thiamine per day, and $B$, values calculated from the logarithms of the excretion data, expressed in terms of per cent of the mean thiamine excretion.
for the analysis of variance, when applied to the thiamine data, yield results
which are markedly different and are inconsistent among themselves
(Table II). This does not permit large scale pooling of values or even
proper comparison of samples, and it makes strict analysis of variance
almost impossible with the thiamine data. It has been found possible to
obtain reasonable results for the significance of the changes in thiamine
excretion following a drastic change in physical activity or composition of
diet (the thiamine intake maintained at the previous constant level) by
treating the urinary thiamine values on an individual basis. The values
for each individual, under these circumstances, must be compared with
his own normal values. In this way \( \sigma_i \), one of the large variables, can be
eliminated from the calculations.

The very large values found for \( \sigma_i \) constitute one of the more striking
phenomena of the thiamine excretion data. The thiamine excretion level
is highly characteristic of the individual as well as the intake. A group of
men on identical and carefully controlled intakes of thiamine will show
very different but individually consistent levels of thiamine excretion.
Some of the men may consistently excrete twice as much thiamine as others.
At the same time, however, the pyramin excretion level of these same
individuals will be much less variable. There are no visible outward signs
to indicate a reason for this difference in thiamine excretion (13).

The intraclass (or intragroup) correlation, \( r = (\sigma_i^2)/(\sigma_i^2 + \sigma_{id}^2) \), is a measure
of the consistency with which the various individuals in a group maintain
their relative positions within the group ((16) p. 243). The values for
each of the groups are given in Table III for both thiamine and pyramin.
In general, the thiamine excretions show highly significant values of \( r \)
close to 0.7 (0.4 to 0.9), while those for pyramin are about 0.1 (0 to 0.2)
and statistically non-significant. In the second of the two experiments
previously described, the reversal of intake levels of the two groups of men
afforded an opportunity to check the consistency with which the individuals
of a group hold their relative positions in that group when the intake levels
are reversed. It was found that, when the individuals were ranked with
respect to their position in the group both before and after the change in
intake, the rank correlations ((16) p. 164) obtained were +0.89 and +0.99,
as compared with the intragroup correlation values ((16) p. 243) of +0.85
and +0.97, respectively, found for these groups during the control period
of 6 to 8 months. This effect is very important, and has been discussed in
greater detail elsewhere (13).

All of the experimental groups do not show as high correlation of thiamine
intake and excretion values as the above. The lower values for the intra-
group correlations obtained at some of the thiamine intake levels are
possibly attributable to the facts that (1) at low levels of thiamine intake,
where excretion values and the attendant variabilities all approach zero, the possibility of distinguishing individuals decreases, and (2), since the levels of thiamine excretion are highly dependent upon the individuals in a group, the intraclass correlations obtained from small groups would be expected to vary considerably.

Time Required for Stabilization—One factor that must be kept in mind in attempts to obtain thiamine and pyramin excretion values characteristic of a given intake of thiamine is the period of time required for an individual to come into metabolic equilibrium with the new level of intake. Fig. 1

### Table III

**Summary of Urinary Excretion of Thiamine and Pyramin by Normal Young Men Maintained on Different Levels of Thiamine Intake for Periods of 2 or More Months**

The excretion values are given only for the collections made after the men were stabilized at their intake levels. The excretion of thiamine is given in micrograms of thiamine hydrochloride per 24 hours, whereas that of pyramin is in micrograms of 2-methyl-4-amino-5-ethoxymethylpyrimidine hydrochloride. The definitions of the variances and correlation terms are given in the text. The groups with an asterisk are those whose thiamine intakes were reversed after the 8 months experiment.

<table>
<thead>
<tr>
<th>Daily thiamine intake (mg)</th>
<th>No. of subjects</th>
<th>Months on intake</th>
<th>Average thiamine excretion</th>
<th>Average pyramin excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>$\sigma_{od}$</td>
</tr>
<tr>
<td>0.61</td>
<td>0.07</td>
<td>4</td>
<td>6</td>
<td>4.6</td>
</tr>
<tr>
<td>1.00</td>
<td>0.08</td>
<td>6</td>
<td>8</td>
<td>65.4</td>
</tr>
<tr>
<td>1.00</td>
<td>0.08</td>
<td>6*</td>
<td>3</td>
<td>57.2</td>
</tr>
<tr>
<td>1.01</td>
<td>0.07</td>
<td>4</td>
<td>6</td>
<td>26.2</td>
</tr>
<tr>
<td>1.81</td>
<td>0.07</td>
<td>2</td>
<td>6</td>
<td>195.4</td>
</tr>
<tr>
<td>2.00</td>
<td>0.08</td>
<td>6</td>
<td>8</td>
<td>224.0</td>
</tr>
<tr>
<td>2.00</td>
<td>0.08</td>
<td>6*</td>
<td>3</td>
<td>249.3</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>2</td>
<td>6</td>
<td>971</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>2</td>
<td>8</td>
<td>1875</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>1</td>
<td>2</td>
<td>1430</td>
</tr>
</tbody>
</table>

shows the rate of change of thiamine and pyramin excretion levels found in a group of six men when they were changed from a constant intake level of 1.00 mg. of thiamine per day to a constant intake level of 2.00 mg. of thiamine per day. The group subsequently remained on this higher level of intake for a period of about 8 months. The plateau values shown represent the average thiamine and pyramin excretion values obtained over approximately the last 6 months of this period. The ordinates have been arranged so that the two plateau values coincide exactly in Fig. 1.

The thiamine excretion values show a sharp and immediate rise, which,
however, does not reach the levels characteristic of the new intake during the first 30 days. The pyramin values rise more slowly than the thiamine in the first few days. This may possibly account for previous reports that pyramin excretion values are insensitive to changes of, or are entirely independent of, thiamine intake. The smooth least squares curve passing through the data in Fig. 1 is

Micrograms pyramin = 67.2 - 67.2e^{-0.7t}

where $e$ is the base of the natural system of logarithms, 2.718, and $t$ is the time in days. In this formula $t_1 = 10$ days; that is, this curve traverses half of the remaining distance to the plateau level each 10 days. Multiplication of the values found in the above equation by 2.36 gives the corresponding thiamine values observed in this instance. We have not studied rates of change following a decrease of thiamine intake, nor those encountered at higher or lower levels of thiamine intake. In the normal intake region studied, a group of individuals must be maintained on a carefully controlled thiamine intake for a period of at least 6 weeks before their excretion values may be considered to be truly characteristic of that level of intake.
Because of the great variation in the thiamine excretion data, there is a possibility that certain values may equal or exceed the plateau value before the time indicated in Fig. 1.

Relation between Intake and Excretion—In comparing thiamine and pyramin it must be remembered that the molecular weight of the thiamine standard is almost twice that of the pyramin standard. Thus, on a molar basis, a rise of 60 \( \gamma \) of pyramin is equivalent to a rise of 100 \( \gamma \) of thiamine.

When the average excretion values for both thiamine and pyramin are plotted against the mg. of thiamine ingested per day, the curves in Fig. 2 are obtained. The ever increasing variability in the thiamine data makes it difficult to determine the exact nature of the curve relating these points. There is little to be gained, however, in assuming that it is other than a straight line, especially for excretion values at intakes in excess of 1.0 mg. of thiamine per day. Since each individual shows his own characteristic slope, a great variability must be associated with any value given to the slope of this straight line. Fitting a least squares line to the thiamine excretion data which go to make up the values found in Table II gives the equation

\[
\text{Micrograms thiamine excreted} = -118 + 173 \times \text{mg. thiamine intake}
\]

This indicates that at intakes below about 0.7 mg. of thiamine per day the urinary thiamine excretion would be zero for most individuals. It must be remembered that the level of urinary thiamine excretion is highly characteristic of the individual; consequently it is rather difficult to make useful generalizations from this.

The literature is in disagreement concerning the thiamine excretions on the lower thiamine intakes. This may be attributed to one or more of the following: (1) individual differences of subjects, (2) inadequate time for stabilization of urinary excretion, (3) difficulties in exactly controlling dietary intakes at these lower levels, (4) differences in analytical methods used in the determination of thiamine, and (5) differences in the physical characteristics, activity, and living conditions of the subjects. The method used for the determination of thiamine is especially important when the concentration of this vitamin in the urine is very low. Mickelsen, Condiff, and Keys (12) have discussed the problems involved under these circumstances.

The relation of the stabilized pyramin excretion values to thiamine intakes (Fig. 2) is an exponential curve which can be represented by the equation

\[
\text{Micrograms pyramin} = 400 - 330e^{-0.314A}
\]

where \( A = \) daily thiamine intake in mg. The curve approaches a plateau value of 400 \( \gamma \) of pyramin per day for very high intakes of thiamine. The
significance of the change in the slope of the pyramin excretion curve with increasing thiamine intakes is difficult to evaluate at this time. Under certain conditions, the pyramin excretion may, temporarily at least, greatly exceed 400 γ. These special conditions will be discussed in subsequent publications.

**Excretions of Thiamine and Pyramin As Indicators of Thiamine Intake**

It would be highly desirable to evaluate the relative merits of thiamine and pyramin excretions as indices of thiamine intake. At present there are no statistical methods which allow the thiamine data to be used in a strict analysis of variance. Several attempts at comparison of these two measures, however, have been tried.

In the first of these methods, the thiamine and pyramin excretion values have been plotted against the thiamine intake. At daily thiamine intakes of 2 mg. or less per day two substantially straight lines result, whose slopes on an equimolar basis are not significantly different. The correlation between the corresponding intake and excretion values should indicate how directly the excretion measure is related to the thiamine intake. The measure with the higher correlation coefficient can be taken to be the more directly related to the thiamine intake.

![Graph showing excretion of thiamine and pyramin with different thiamine intakes](https://example.com/thiamine-pyramin-excretion-graph.png)
With the mean excretion values of twenty-two individuals who have been maintained on levels of 0.6, 1.0, 1.8, and 2.0 mg. of thiamine per day (Table III), the correlation coefficient \((r_{1})\) relating thiamine excretion to intake, was +0.88, and the one relating pyramin excretion to thiamine intake, \(r_{2}\), was +0.93. The pyramin excretion data show the higher correlation coefficient, but with twenty-two cases the difference between the two is non-significant. The difference is still found to be non-significant when all of the original data are used.

Another approach involves the use of Hotelling's \(t\) test, which is specifically designed to show which of two variables is best suited for the prediction of a third \((1)\). When the calculations for this test are made, it is again found that pyramin excretion is better, but not significantly so, than thiamine for the prediction of thiamine intake.

Other methods of comparison have also been studied. In one of these, the variations in the excretion values at each intake level have been expressed in terms of thiamine intake by means of the slopes relating the excretion of thiamine and pyramin (expressed in equimolar terms) to thiamine intake. This procedure indicates that the relative superiority of the one excretion test over the other is dependent upon the intake level, but again exact comparisons are difficult.

Below a thiamine intake of about 0.7 mg. per day, the thiamine excretion approaches zero. Statistically these values are very constant, but experimentally not very useful. At these same levels of intake the pyramin excretion values are easily measurable. At thiamine intake levels close to 1.0 mg. of thiamine per day both thiamine and pyramin excretion values are equally reliable as indicators of thiamine intake. At thiamine intakes below 1.0 mg. of thiamine per day the thiamine excretion values are statistically less variable than the pyramin. At thiamine intakes above 1.0 mg. per day the pyramin excretion values are less variable and thus more reliable than the thiamine values.

One of the great drawbacks in the use of thiamine is that the excretion level is highly characteristic of the individual. This property is apparent at the intake levels ordinarily encountered in the normal American dietary, which makes it difficult to compare the data of one group with that from another. Except at intake levels between 0.7 and 1.0 mg. of thiamine per day, the pyramin excretion is slightly more sensitive to intake than is thiamine excretion. Consequently, at intake levels below 1 mg. of thiamine but above 0.7 mg. per day, it is better to use the thiamine excretion as an indicator of the intake. From 1 mg. up to about 5 mg. it is better, however, to use the pyramin excretion. Unless the intake level is known, it is rather difficult to determine which of the two indices is the better. Under such conditions and as a means of improving the accuracy of the intake predic-
tion, it is advisable to determine both excretion compounds. A multiple regression equation ((16) p. 340) or discriminant function (2) may be set up which in turn can be converted to

\[
\text{Mg. thiamine intake} = -0.65 + 0.0086 \text{pyramin} + 0.0027 \text{thiamine}
\]

**TABLE IV**

*Original Urinary Thiamine and Pyramin Excretion Data of Individual Subjects Maintained on Constant Thiamine Intake for 8 Months*

The first six men received 2 mg. of thiamine per day throughout this period, while the other six received 1 mg. \( B_1 \) represents the daily thiamine excretions in micrograms and \( P \), the daily pyramin excretion in micrograms of 2-methyl-4-amino-5-ethoxymethylpyrimidine hydrochloride. Where thiamine and pyramin are the urinary values for these compounds expressed as indicated above. The constants in this equation have been calculated from the data in Table IV. In this equation pyramin is weighted twice as heavily as thiamine, which again indicates that it is a slightly more reliable indicator of thiamine intake (2). It must be emphasized that all

<table>
<thead>
<tr>
<th>Subject</th>
<th>Time of urinary collection</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>December</td>
<td>January</td>
</tr>
<tr>
<td>D</td>
<td>276 294 350</td>
<td>433 398</td>
</tr>
<tr>
<td></td>
<td>230 282 240</td>
<td>228 227</td>
</tr>
<tr>
<td>C</td>
<td>82  87  88</td>
<td>123 140</td>
</tr>
<tr>
<td></td>
<td>275 290 235</td>
<td>236 216</td>
</tr>
<tr>
<td>B</td>
<td>231 141 155</td>
<td>238 336</td>
</tr>
<tr>
<td></td>
<td>266 228 195</td>
<td>220 243</td>
</tr>
<tr>
<td>W</td>
<td>128 191 109</td>
<td>167 180</td>
</tr>
<tr>
<td></td>
<td>238 249 219</td>
<td>203 268</td>
</tr>
<tr>
<td>H</td>
<td>148 122 138</td>
<td>225 100</td>
</tr>
<tr>
<td></td>
<td>231 306 246</td>
<td>280 272</td>
</tr>
<tr>
<td>Pe</td>
<td>114 128 101</td>
<td>183 200</td>
</tr>
<tr>
<td></td>
<td>226 278 222</td>
<td>234 296</td>
</tr>
<tr>
<td>A</td>
<td>117 75 79</td>
<td>105 119</td>
</tr>
<tr>
<td></td>
<td>230 211 190</td>
<td>202 219</td>
</tr>
<tr>
<td>Pa</td>
<td>36  21  39</td>
<td>46  45</td>
</tr>
<tr>
<td></td>
<td>158 214 162</td>
<td>170 186</td>
</tr>
<tr>
<td>R</td>
<td>62  52  60</td>
<td>69  97</td>
</tr>
<tr>
<td></td>
<td>165 193 181</td>
<td>157 179</td>
</tr>
<tr>
<td>E</td>
<td>60  48  53</td>
<td>67  77</td>
</tr>
<tr>
<td></td>
<td>173 220 153</td>
<td>152 192</td>
</tr>
<tr>
<td>M</td>
<td>85  83  86</td>
<td>122 90</td>
</tr>
<tr>
<td></td>
<td>183 243 210</td>
<td>245 205</td>
</tr>
<tr>
<td>S</td>
<td>55  46  49</td>
<td>62  76</td>
</tr>
<tr>
<td></td>
<td>173 200 185</td>
<td>188 206</td>
</tr>
</tbody>
</table>

where thiamine and pyramin are the urinary values for these compounds expressed as indicated above. The constants in this equation have been calculated from the data in Table IV. In this equation pyramin is weighted twice as heavily as thiamine, which again indicates that it is a slightly more reliable indicator of thiamine intake (2). It must be emphasized that all
of the equations given in this paper have been secured from our data and are meant to hold only for our set of conditions, and must be rigidly checked before they are used under any other circumstances.

DISCUSSION

In the past, surprisingly little work has been done with pyramin, especially in prolonged experiments. Pollock, Ellenberg, and Dolger (14) were probably the first to make actual analyses of the pyramin content of the urine (called by them "pyrimidine"). They suggested that the urinary thiamine excretion represented only the immediately preceding vitamin intake, whereas the pyramin excretion more faithfully mirrored the body stores of thiamine. Since the urinary pyramin excretion increases after the injection of 100 mg. of thiamine, they postulated that pyramin is derived from thiamine.

Wertz and Mitchell (17) used the pyramin values expressed as thiamine equivalents, plus the actual thiamine values as a measure of the "total" thiamine excretion. Although their experiments were of only a few days duration, there is an indication that at the lower levels of thiamine intake a greater fraction of the intake can be accounted for on the basis of "total" urinary thiamine excretion than at the higher levels of intake. Our work confirms and supplements this. Fig. 3, which is based on our data, shows that with increasing thiamine intakes the excretion of thiamine increases, but tends to reach a plateau at about 13 per cent of the intake. When the pyramin content of the urine is added to this, the total per cent of the intake accounted for in the urine decreases as the intake increases. Even at the lower dietary intakes, a maximum of 42 per cent of the intake was accounted for as the sum of pyramin and thiamine, whereas at levels of intake of 1 mg. only 35 per cent was accounted for. There is no indication as to the fate of the remaining thiamine. Nothing can be said about the stability of pyramin in the body until it is isolated and characterized. The pyrimidine ring in thiamine may be broken down by the body to compounds other than pyramin.

Gorham, Abels, Robins, and Rhoads (5) found a marked increase in the pyramin excretion following the injection of 5 mg. of thiamine, whereas the pyramin excretion was almost unaffected by the injection of 5 mg. of 2-methyl-4-amino-5-methoxyethylpyrimidine (sic.). Their work also brings out the greater variation in the excretion of thiamine, when compared to that of pyramin.

Regardless of whether thiamine or pyramin is used, a comprehensive statistical evaluation of the urinary excretion is necessary before any assessment can be made of this technique as a measure of vitamin requirement. Many workers have come to conclusions on the human vitamin
requirement based only on unappraised urinary excretion data. Only one such example will be mentioned to illustrate a common fallacy. Mason and Williams (9) increased stepwise the thiamine intake from 0.4 up to 2.0 mg. per day. They noticed a "marked" change in the slope of the urinary thiamine excretion when the intake was increased from 0.8 to 1.0 mg. This led them to conclude "that a level of intake of 800 micrograms was just enough for physiologic purposes and that above this level a greater amount was excreted as surplus" (p. 251). Statistical examination of their data fails to show any significant change in the slope of the urinary excretion curve at this point. In fact, the total change in the excretion values at the two intakes is just barely significant ($p = 0.02$), even when advantage is taken of paired variance assumptions.

Various workers have argued strongly for the use of the urinary excretion of thiamine as an index of the nutritional status of the individual. The supporting data consist primarily of a number of isolated short time experiments which were poorly controlled more often than not. In spite of the many analyses of urinary thiamine that have been reported by many workers, practically no one has attempted a statistical analysis to establish the possible significance of the data. The result has been a neglect of some
of the basic facts. Excretion equilibrium at a given thiamine intake level requires many days. We have as yet no proper information as to variations in the rate of attainment of equilibrium. But even when equilibrium has been established at a highly constant thiamine intake, there are, as we have shown, very large consistent individual differences and considerable day to day variations. Marked inter- and intraindividual variations in thiamine excretion associated with a constant thiamine intake have been noted without statistical analysis by Wertz and Mitchell (17). Limitation of analysis to a casual comparison of group averages has obscured the question of the meaning of the individual estimation and has led to unwarranted optimism.

The facts are that one "normal" person may excrete twice or even 3 times as much thiamine as another "normal" person on exactly the same diet. In any one day these differences are apt to be considerably larger than the mean differences. Finally, we may note that when the thiamine intake is changed the rate of response in urinary excretion is such that only half the change is completed in 10 days (Fig. 1). This is true for both thiamine and pyramin excretion. Obviously this fact is of great importance in all experiments relating thiamine excretion to intake and attempts to interpret thiamine "load" or "saturation" tests. Melnick (11) has criticized some of our earlier reports on thiamine excretion associated with low thiamine intakes (7) as being "too low." Our reported excretions, being achieved at the true plateau level, are naturally lower than the values obtained only a few days after the change from a high to a low intake.

In this paper we have discussed only "normal" young men, that is young men with no history, signs, or symptoms of nutritional, digestive, or metabolic peculiarities. We can only believe that the variations in a less rigidly "normal" and homogeneous population must be more than with our subjects.

SUMMARY

1. Thiamine and pyramin excretion values from twenty-two normal young men maintained for prolonged periods on very carefully controlled thiamine intakes of 0.6, 1.0, 1.8, and 2.0 mg. per day have been studied statistically.

2. Thiamine excretion values appear in a general way to be linearly related to the thiamine intake, but are also highly characteristic of the individual. One "normal" individual may excrete twice as much as another "normal" individual on the same thiamine intake. The interindividual, intraindividual, and random variabilities occurring in the thiamine data increase markedly (but not quite in direct proportion) as the excretion values increase. Strict analysis of variance is almost impossible with these data.
3. The relationship between pyramin excretion values and thiamine intake is exponential, with the curve approaching a plateau of about 400 \( \gamma \) of pyramin at very high intake values. In the region of normal intake levels (1 to 2 mg. per day) the relationship is very close to linear. The variabilities are quite homogeneously distributed, a fact which greatly facilitates statistical treatment of the data.

4. Several advantages appear to be secured from the use of pyramin over the thiamine excretion data: (a) greater ease of statistical treatment of the data, with a corresponding increase in certainty of the results; (b) thiamine excretion values are subject to large, consistent, individual differences, while in the pyramin data the individual differences are very small; and (c) at low levels of thiamine intake the excretion of pyramin is still determinable, whereas the excretion of thiamine itself becomes zero.

5. When the thiamine intake is increased from 1 to 2 mg., it requires a period of about 6 weeks for thiamine and pyramin urinary excretion values to come to equilibrium with the new intake level. Half of this change occurs during the first 10 days.

6. The necessity for statistical treatment of excretion data and, further, the necessity for recognizing the nature of the variabilities involved in this data have been pointed out.

Mr. Howard Condiff and Miss Laura Werner made the analyses for thiamine; Mr. Eugene Sunnen and Miss Doris Fredson assisted with the pyramin analyses; Mr. Ersal Kindel built the apparatus for the determination of pyramin; Dr. Howard Alexander helped with the statistical work involved in this paper, for which we are sincerely grateful. A large number of Civilian Public Service men served diligently as subjects for these experiments.

BIBLIOGRAPHY

MICKELSEN, CASTER, AND KEYS

A STATISTICAL EVALUATION OF THE THIAMINE AND PYRAMIN EXCRETIONS OF NORMAL YOUNG MEN ON CONTROLLED INTAKES OF THIAMINE
Olaf Mickelsen, W. O. Caster and Ancel Keys


Access the most updated version of this article at [http://www.jbc.org/content/168/2/415.citation](http://www.jbc.org/content/168/2/415.citation)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/168/2/415.citation.full.html#ref-list-1](http://www.jbc.org/content/168/2/415.citation.full.html#ref-list-1)