MEDICAL EVALUATION OF NUTRITIONAL STATUS

IX. THE RELIABILITY OF VISUAL THRESHOLD DURING DARK ADAPTATION AS A MEASURE OF VITAMIN A DEFICIENCY IN A POPULATION GROUP OF LOW INCOME

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Serial dark adaptation measurements of adults of low income are described in the present report. The measurements represented one aspect of a larger inquiry into the utility of visual threshold measurements in the appraisal of subclinical vitamin A deficiency. The dark adaptation studies referred to were part of the Cooperative Nutrition Study, the purpose and scope of which have been discussed elsewhere (23). The measurements now reported were made to ascertain: 1, The effect upon visual threshold of vitamin A therapy administered to approximately one-half of the subjects; and 2, the extent of variations in threshold at specified dark times which were not due to therapy or to instrumental factors. The threshold variations to be described are errors of measurement at different levels of experience with the adaptation test and temporal variations of the individual’s nearly dark adapted threshold during a six-week period. Quantitative information dealing with such variations is scant. With reference to the adaptometer (14), the instrument used, errors of observation and day to day variation have been commented upon in three reports (15, 27, 22). Errors of single measurements of inexperienced subjects were described in an earlier report from the Cooperative Nutrition Study (17).

1 This paper is the ninth of a series from a cooperative investigation by the Cornell University Medical College, Department of Public Health and Preventive Medicine and Department of Pediatrics; Milbank Memorial Fund; the New York City Department of Health; and the United States Public Health Service, Division of Public Health Methods, National Institute of Health.

The cooperating agencies have been assisted in carrying out this investigation by the Work Projects Administration for the City of New York, Official Project No. 65-1-97-21 W.P. 24, “Medical Evaluation of Nutritional Status.”

2 The time (minutes) elapsed in the dark following light adaptation.
The Sample. Sixty men and twenty-three women were the subjects selected for study. The age range of the group was 20-65 years. The only criteria of selection were willingness to participate in the study and employment as a project worker certified to receive public assistance. The group represented a low-income level. Expenditures for food were necessarily small and the daily provision of vitamin A in the diet was accordingly believed to be below recommended allowances for adults (31). Subsequent gross and biomicroscopic examination of the eye revealed that signs of vitamin A deficiency, either in the form of thickening and opacity or marked translucency of the conjunctiva were present in 72 per cent of the subjects who participated throughout this study. A report of the biomicroscopic examinations has appeared elsewhere (24).

Apparatus. Three adaptometers were used. The operating current of each instrument, controlled by a rheostat and voltage regulator assembly, was adjusted to provide a preadaptation brightness of 1,004 millilamberts. Calibrations of the preadapting lights were made with the Macbeth illuminometer immediately before the initial tests. The National Bureau of Standards checked the manufacturer's calibrations of wedges, "neutral" filters, and chromatic filters for two instruments. The corresponding parts of the third instrument were calibrated by the manufacturer only. Results with this instrument were comparable to those of the other instruments when the same subjects were examined.

The adaptometers were operated in a suitably constructed dark room. The preadapting light and the test flash were viewed 7 degrees nasally, using the right eye. The area of the retinal field during preadaptation for 3 minutes was approximately 35 degrees. During dark adaptation the test field occupied a retinal area 3 degrees in diameter. The duration of the test flash was .2 second. The violet filter (Corning 511) was used during threshold determinations to transmit wave lengths below 460 millimicrons. Brightness of threshold was expressed in the logarithm of micromicrolamberts (log μμπ).
United States Public Health Service. Each of the technicians had tested approximately 300 subjects in the Cooperative Nutrition Study before the present series of tests was made. All tests of one subject were made by the same technician with one adaptometer.

Threshold was defined as the least brightness perceptible to the subject. The subject's oral response to this critical brightness was "yes" (seen). To slightly less brightness than this critical value the oral response was "no" (not seen). The critical brightness was found by means of a sequence of test flashes in which brightness was increased following the "no" response, and decreased following the "yes" response until alternating responses were obtained at relatively narrow limits of brightness.

Measurements of threshold were made within each of twelve approximately 1-minute intervals equally spaced during dark adaptation. These observation intervals were centered at the following dark times: 0.5, 3.5, 6.5, 9.5, 12.5, 15.5, 18.5, 21.5, 24.5, 27.5, 30.5, and 33.5 minutes. To obtain the threshold within a given observation interval, the technician began the flash sequence about 30 seconds or more before the earlier limit of the interval so as to bring the subject near the stage of alternating "no" and "yes" responses to small changes in brightness before the interval was reached. The threshold was then finally determined at or near the midpoint of the interval and the actual observation-time was recorded to the nearest 5 seconds. A multiple correlation analysis of threshold, dark time, and number of flashes in a large number of flash sequences showed no relation between the threshold obtained and the number of flashes employed.

Duplicate dark adaptation tests were made in the following manner: the first test was completed in about 40 minutes (3 minutes light adaptation and 33-34 minutes dark adaptation) and the subject rested for 20 minutes in office illumination. The subject was then light adapted again and a second dark adaptation test was made by the same technician. In the second test, threshold in each of the twelve observation intervals was measured exactly as in the first test. The record of the first test was collected from the dark room by a clerk before the second test was begun. In all observation intervals the actual observation-time at which the threshold was determined was very nearly the same in the first and second tests of any duplicate pair. A study of the differences between actual observation-times in the first and second tests respectively, at
each interval, showed that 1, the mean difference in time was zero, 2, the standard deviation of the difference was less than ±.25 minute. Because of the close correspondence in actual observation-time, thresholds in the first and second tests of a duplicate pair, within any observation interval, were regarded as directly comparable.

Schedule of Examination and Therapy. During the study period of six weeks duplicate tests of all subjects were scheduled at each of four examination periods. Preliminary to the arrangement of the examination schedule the subjects were classified according to age (less than 40 years of age, 40 years and older) and sex. The schedule was then set up so that the several technicians tested approximately equal numbers of subjects drawn at random from the subjects in each age-sex group.

Ten work days, or two calendar weeks, were required by each technician to complete duplicate tests of the subjects assigned. These two weeks constituted an examination period. The order of testing the subjects in the first period was maintained with minor exceptions at each of the three later periods. Thus the interval between repeated tests of the same subject was regularly two weeks and at all examination periods a given subject was tested at the same time of day.

The entire group of eighty-three subjects was tested in duplicate at the first examination period. For a number of reasons all of these subjects could not be retested at each of the three examination periods which followed. For example, certain subjects were transferred to another work-relief project. Others discontinued tests because of lack of interest or inability to be released from office work at the necessary hours. Fifty subjects were examined in duplicate at each of four examination periods and thirty completed duplicate tests at less than four periods.

Subjects who were to receive vitamin A therapy were designated when the examination schedule was arranged by selecting by a random procedure approximately one-half of the cases from each of the subgroups resulting when the subjects were classified according to age, sex, and technician. Of the fifty subjects who were examined at four periods, thirty received vitamin A therapy and twenty served as controls. The case record of treatment for the thirty subjects in the therapy group showed that the supplement was taken 90 per cent or more of the days during the study by twenty-one of these subjects. Seven others in this group of thirty had therapy 80-89 per cent of the study period and the
remaining two subjects had therapy 76 and 78 per cent respectively of the total days under observation.

Therapy was begun immediately after the initial duplicate tests in examination period I. Each subject in the therapy group was given daily one Afaxin capsule containing 10,000 International units of vitamin A. The supplements of vitamin A had the effect of increasing the dietary supply of this vitamin to a level of intake which was well above the recommended daily allowance for adults. On week days the capsules were dispensed by a clerk in the laboratory and swallowed in his presence. Capsules to be taken at home on Saturdays and Sundays when the laboratory was closed were delivered to the subjects on Friday afternoons. A report on the use of the week-end supply was required Monday mornings.

**Results**

*Individual Trends of Threshold in the Therapy and in the Control Groups.* Measurements at 33.5 minutes in the dark were selected for a comparison of change in threshold in the therapy and control groups respectively, during the six weeks. The selection of a threshold late in adaptation was made because: 1, immediate errors of measurement, which will be discussed in a later section, were smaller than at earlier dark times; 2, the nearly dark adapted threshold approximates a cumulative measure of the secondary phase of adaptation (10); and 3, such thresholds have been found to be more responsive than earlier ones to changes in the dietary supply of vitamin A (3, 26, 44, 15).

The trend of threshold at 33.5 minutes was measured for each of the fifty subjects, who were tested in duplicate at each of four examination periods. An individual linear regression of the threshold at this dark time on days elapsed during the study was used for this purpose. The individual regression coefficient, or the slope of threshold, was taken as a measure of the consistent day to day change in threshold \((\log \mu d per day)\). Each coefficient was derived on the eight observations of the subject obtained in duplicate tests at four examination periods.
If supplements of vitamin A had the effect during the study period of lowering threshold at 33.5 minutes, one would expect negative individual slopes to occur more frequently in the therapy group than in the control group. The summary of individual slopes in Table 1 provides no evidence of such a difference in the present study. Negative slopes were found with about equal frequency in both treatment groups. For example, for the nine subjects in the

Table 1. Individual slope of threshold at 33.5 minutes (dark time) during six weeks for subjects in the therapy and in the control groups who had significant trends, and the number of cases in each group for whom the individual slope did not differ significantly from zero.

<table>
<thead>
<tr>
<th>Item</th>
<th>Therapy Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Subject Having Significant Slope of Threshold at 33.5 Minutes During Six Weeks</td>
<td>1 D60 .016</td>
<td>1 D15 .007</td>
</tr>
<tr>
<td>Subject Having Significant Slope of Threshold at 33.5 Minutes Not Different from Zero</td>
<td>1 D60 .016</td>
<td>1 D15 .007</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Subjects Having Significant Slope of Threshold at 33.5 Minutes</td>
<td>21</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>20</td>
</tr>
</tbody>
</table>

1 b = $\frac{S_{xy}}{S_{xx}}$, where x represents deviations from the mean observation day (X); y, deviations from the mean threshold at 33.5 minutes (Y) during six weeks; and the summations (S) extend over 8 points.  
2 p. < .05 for Student's "t" obtained by dividing the individual standard error of estimate by the standard error of the individual slope.  
3 p. > .05 by the same criterion.
therapy group who had slopes for threshold differing significantly from zero, four had negative values, indicating a fall in threshold during the study and five had positive slopes, corresponding to a rise in threshold. Similarly in the control group, eight subjects had significant slopes of which half were negative and half positive.

Continued observations on many of the subjects indicated that the similarity of consistent changes in threshold in the therapy and control groups was probably not a result of the moderate level at which therapy was administered, nor due to the short period. After the close of the present study twenty-one subjects of the therapy group and nineteen control subjects were retested from time to time for several months. Seventy-five per cent of these subjects were under observation for at least six months and the remainder for three, four, or five months. The amount of therapy was increased from 10,000 International units of vitamin A daily to 100,000 International units per day. Despite the more extended period and higher level of therapy the changes in threshold at 33.5 minutes were essentially alike in therapy and control groups and they resembled the pattern described in Table 1.

The conclusion appears to be justified, therefore, that any benefits received by subjects of the therapy group through the supplements of vitamin A were not reflected in their visual thresholds late in adaptation. With respect to change in threshold at 33.5 minutes the therapy and control subjects can be regarded as a homogeneous group, in which during six weeks significant rise in threshold level occurred in approximately 18 per cent of the cases and in the same period significant fall in a nearly equal proportion, 16 per cent. Furthermore, during the six weeks thirty-three subjects, or 66 per cent of the total group, were characterized by variations of threshold at 33.5 minutes which were in no part accounted for by a linear trend.

**Age Differences.** In the group of fifty subjects, whose individual slopes are summarized in Table 1, the mean threshold at 33.5 min-
For subjects less than 40 years of age was lower than the mean for subjects 40 years of age or older. In Table 2, mean threshold at 33.5 minutes is shown for subjects in these broad age groups. The variability of threshold in each age group is also given in this table. Mean threshold in the younger age group was 2.698 log $\mu l$ and in the older group 3.010 log $\mu l$. The difference, .312 log $\mu l$, was much larger than could be explained by expected variation due to sampling alone.

The variability between different subjects in the older age group was greater. However, the coefficients of variation in the two age groups were approximately the same.

**Variation of Visual Threshold.** The two dark adaptation curves resulting from duplicate tests of the same subject were not identical. Similarly, serial adaptation curves for a single individual obtained by periodic testing at two-week intervals differed considerably from

Table 2. Mean and standard deviation of threshold at 33.5 minutes during dark adaptation for subjects who were less than 40 years of age and for subjects 40 years of age or older.

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>Number of Cases</th>
<th>Number of Observations</th>
<th>Mean $^2$ Log $\mu l$</th>
<th>Standard Deviation $^3$ for Different Subjects Log $\mu l$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects Less Than 40 Years</td>
<td>31</td>
<td>248</td>
<td>2.698 ± .150</td>
<td>± .250</td>
</tr>
<tr>
<td>Subjects 40 Years or Older</td>
<td>19</td>
<td>152</td>
<td>3.010 ± .318</td>
<td>± .318</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>50</strong></td>
<td><strong>400</strong></td>
<td><strong>2.817</strong></td>
<td><strong>± .281</strong></td>
</tr>
</tbody>
</table>

1. Each of three technicians tested approximately one-third of the subjects in each age group.
2. $\overline{Y}/N$, where $Y$ is the threshold (33.5 minutes) in any test at any period in each age group, and $N$ is the number of observations in the age group.
3. $\sqrt{\sum y^2/(n-Kk)}$, where $y$ is the deviation of the mean of duplicate thresholds (33.5 minutes) of any subject in a technician-period grouping from the mean threshold in that grouping; $y^2$ is summed ($\sum$) within each technician-period grouping; $y^2$ is summed ($\sum$) over all technician-period groupings; $n$ is the number of subjects; $K$, the number of examination periods; and $k$, the number of technicians. The standard deviation between different subjects was computed within technician groups since thresholds measured by different technicians are not directly comparable (18, 22). The subjects were grouped according to examination period to control also the individual variation of threshold between periods.
4. This variability of threshold between different subjects is slightly larger than that given in Table 6, item 2, as it includes the error of measurement whereas the variability in Table 6 does not. The value in Table 6 is a better estimate of the variability between different subjects.
one another and indicated temporal changes in the threshold of the individual. An attempt was made to measure each of these types of variation in order to answer the questions: 1, How reliable is a single threshold observation at a given dark time as an index of the individual's threshold at that time in the particular test, and 2, how reliably will a threshold observation in a single test indicate the characteristic threshold of the individual during a six-week period, at the dark time specified? Reliability in the first question depends, of course, upon the immediate error of measurement. The differences between thresholds at each dark time in the duplicate tests provided a means of estimating immediate error. The reliability referred to in the second question is limited by error and also by the amount of temporal deviation of threshold of the individual from his characteristic (mean) level during the limited period. Temporal variation of the individual's threshold at 33.5 minutes was estimated from observations in repeated tests of the same subject at successive examination periods.

I. **Immediate error of measurement throughout adaptation and the effects of experience:** The immediate error described here was derived on differences for all subjects tested in the same period. At each period differences were computed at each of twelve observation intervals (dark times) by subtracting the measurement in the second test of a pair of duplicates from that in the first test. At successive examination periods the differences at a given dark time reflected the response of subjects as their experience with the test increased. In general, subjects tested in period I were inexperienced, subjects in period II had experienced not more than two tests, those in period III not more than four tests, and those in period IV not more than six tests. In the course of repeated testing of the same individuals the technicians may also have become increasingly aware of the characteristic behavior of particular subjects. Significant change in the technician's understanding of the test procedure and facility in conducting the test probably did not occur, inasmuch
Fig. 1. Cumulative frequency distributions of differences between duplicate measurements of threshold during dark adaptation at each of five dark times for eighty-three inexperienced subjects in examination period I and sixty-nine of the same subjects when experienced in period IV.

as each of the technicians had previously tested several hundred subjects.

Typical distributions of differences between duplicate measure-
ments are illustrated in Figure 1 for eighty-three inexperienced subjects tested in period I and for sixty-nine of the same subjects, tested in period IV. In the latter period 72 per cent of the sixty-nine cases had been tested previously six times and the remainder four times. The curve in each panel of this figure was obtained by arranging the differences in a cumulative frequency distribution. Cumulative frequency at each interval of difference was then expressed in per cent of the total frequency and plotted against the scale of differences, which increased from negative values through zero to positive values of the differences. Distributions are shown at five of the twelve observation intervals during adaptation. At other intervals and also at periods II and III the distributions were similar in type to those illustrated here.

Several general observations can be made on the basis of Figure 1. 1. Positive and negative differences occurred with about equal frequency and the differences showed a tendency to center about zero. 2. In the first examination period the total range of the distribution of differences declined as adaptation proceeded from 0.5 to 33.5 minutes in the dark. 3. After the subjects had become experienced in the fourth examination period a similar but less marked decline in the range was found. 4. At each dark time before 33.5 minutes the total range of the distribution for experienced subjects in period IV was smaller than that for inexperienced subjects in period I.

The mean difference between duplicate measurements of threshold and the standard deviation of this difference for different subjects are shown in Table 3 at each examination period according to time in the dark. The subjects are those whose differences were illustrated in Figure 1. It will be seen in this table that the mean differences for inexperienced subjects (period I), at the beginning and also late in adaptation were significantly different from zero. After the first examination period the mean difference at all dark times showed only insignificant fluctuations from zero. The mean differences showed, therefore, that the mean error of measurement
### Difference Between Duplicate Measurements of Threshold

<table>
<thead>
<tr>
<th>Examination Period</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Cases</td>
<td>75</td>
<td>75</td>
<td>66</td>
<td>69</td>
</tr>
<tr>
<td>Mean Log (\mu L)</td>
<td>+.014</td>
<td>+.070</td>
<td>+.063</td>
<td>+.033</td>
</tr>
<tr>
<td>Standard Deviation Log (\mu L)</td>
<td>±.172</td>
<td>±.435</td>
<td>±.501</td>
<td>±.297</td>
</tr>
<tr>
<td>Number of Cases</td>
<td>66</td>
<td>66</td>
<td>66</td>
<td>69</td>
</tr>
<tr>
<td>Mean Log (\mu L)</td>
<td>+.016</td>
<td>+.054</td>
<td>+.090</td>
<td>+.025</td>
</tr>
<tr>
<td>Standard Deviation Log (\mu L)</td>
<td>±.126</td>
<td>±.430</td>
<td>±.504</td>
<td>±.176</td>
</tr>
<tr>
<td>Number of Cases</td>
<td>69</td>
<td>69</td>
<td>69</td>
<td>69</td>
</tr>
<tr>
<td>Mean Log (\mu L)</td>
<td>+.009</td>
<td>+.051</td>
<td>+.011</td>
<td>+.002</td>
</tr>
<tr>
<td>Standard Deviation Log (\mu L)</td>
<td>±.135</td>
<td>±.331</td>
<td>±.382</td>
<td>±.156</td>
</tr>
</tbody>
</table>

**Table 3.** The mean difference between duplicate measurements of threshold during dark adaptation, and the standard deviation of the difference at each of four examination periods, according to dark time.

The variability of the error of measurement for experienced and inexperienced subjects is shown in Table 4 according to examination period and dark time. Each value in Table 4 is the standard deviation of the error of measurement derived from the corresponding standard deviation of the differences between duplicate measurements in Table 3. It will be seen that regardless of experience the variability of the error of measurement is largest at 9.5 minutes dark time and smaller after 21.5 minutes than at any other time. The effect of increased experience upon error is clearly illustrated in the smaller variability of error in period IV as compared with period I at each dark time before 24.5 minutes. At 24.5 minutes and later in adaptation, error was unaffected by the extent of experience.
### Table 4. Variability of the error of measurement of threshold during dark adaptation of any subject at each of the four examination periods according to dark time.

<table>
<thead>
<tr>
<th>Dark Time (Minutes)</th>
<th>I Inexperienced Subjects</th>
<th>II Subjects Tested Previously not More Than Twice</th>
<th>III Subjects Tested Previously not More Than Four Times</th>
<th>IV Subjects Tested Previously not More Than Six Times</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Log $\mu \mu l$</td>
<td>Log $\mu \mu l$</td>
<td>Log $\mu \mu l$</td>
<td>Log $\mu \mu l$</td>
</tr>
<tr>
<td>0.5</td>
<td>±.188</td>
<td>±.122</td>
<td>±.089</td>
<td>±.095</td>
</tr>
<tr>
<td>3.5</td>
<td>±.196</td>
<td>±.141</td>
<td>±.217</td>
<td>±.100</td>
</tr>
<tr>
<td>6.5</td>
<td>±.346</td>
<td>±.308</td>
<td>±.304</td>
<td>±.234</td>
</tr>
<tr>
<td>9.5</td>
<td>±.399</td>
<td>±.354</td>
<td>±.356</td>
<td>±.270</td>
</tr>
<tr>
<td>12.5</td>
<td>±.223</td>
<td>±.243</td>
<td>±.210</td>
<td>±.176</td>
</tr>
<tr>
<td>15.5</td>
<td>±.161</td>
<td>±.201</td>
<td>±.153</td>
<td>±.115</td>
</tr>
<tr>
<td>18.5</td>
<td>±.153</td>
<td>±.170</td>
<td>±.124</td>
<td>±.110</td>
</tr>
<tr>
<td>21.5</td>
<td>±.146</td>
<td>±.129</td>
<td>±.125</td>
<td>±.103</td>
</tr>
<tr>
<td>24.5</td>
<td>±.098</td>
<td>±.076</td>
<td>±.111</td>
<td>±.086</td>
</tr>
<tr>
<td>27.5</td>
<td>±.086</td>
<td>±.080</td>
<td>±.088</td>
<td>±.068</td>
</tr>
<tr>
<td>30.5</td>
<td>±.062</td>
<td>±.079</td>
<td>±.086</td>
<td>±.071</td>
</tr>
<tr>
<td>33.5</td>
<td>±.064</td>
<td>±.077</td>
<td>±.081</td>
<td>±.071</td>
</tr>
</tbody>
</table>

1 Standard deviation of the difference between duplicate measurements of threshold divided by \( \sqrt{2} \).

Table 4. Variability of the error of measurement of threshold during dark adaptation of any subject at each of the four examination periods according to dark time.

It may be assumed that twice the value of the standard deviation of the error of measurement will include approximately 95 per cent of the errors liable to occur in connection with single measurements of any subject. These limits (±2$\sigma$) define in a uniform way the error component of unreliability of single measurements of threshold at different dark times and following different degrees of experience. For example, the observed threshold of an inexperienced subject (period I) at 6.5 minutes has an error of measurement which will probably not exceed ±.692 log $\mu \mu l$ more than five times out of one hundred. Similarly, for inexperienced subjects at 15.5 minutes the odds are the same that the error of measurement will not exceed ±.322 log $\mu \mu l$. For measurements of experienced subjects,

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* The form of the distributions shown in Figure 1 appear to justify this assumption with the possible exception of the distribution at 9.5 minutes.

* Assuming that the variance of error for other samples of the same size will not differ greatly from that of the present sample.
tested previously not more than six times, the corresponding limits of error at the same dark times are ±.468, ±.230 log µµl, respectively.

The only evident learning effect in the present study was an increase in the immediate reliability of single observations before 24.5 minutes. Experience did not alter the level of the individual's threshold consistently in one direction. As indicated by the findings presented in Table I linear trend of threshold at 33.5 minutes was absent in the majority of cases and the significant trends were as frequently in the direction of elevated as of lowered thresholds. A graphical analysis at 30.5 and 27.5 minutes gave similar results. Before 27.5 minutes the same tendencies prevailed. It will be seen in Table 5 and the corresponding Figure 2 that mean thresholds in periods I and IV did not differ significantly at any dark time. Thus after having taken six tests (period IV), the mean threshold of the

| Time in the Dark (Minutes) | Number of Cases | Number of Observations | Mean Threshold
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Period I Log µµl</td>
</tr>
<tr>
<td>.5</td>
<td>53</td>
<td>106</td>
<td>6.525</td>
</tr>
<tr>
<td>3.5</td>
<td>53</td>
<td>106</td>
<td>5.827</td>
</tr>
<tr>
<td>6.5</td>
<td>53</td>
<td>106</td>
<td>5.271</td>
</tr>
<tr>
<td>9.5</td>
<td>53</td>
<td>106</td>
<td>4.383</td>
</tr>
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<td>12.5</td>
<td>53</td>
<td>106</td>
<td>3.766</td>
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<td>15.5</td>
<td>53</td>
<td>106</td>
<td>3.433</td>
</tr>
<tr>
<td>18.5</td>
<td>53</td>
<td>106</td>
<td>3.191</td>
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<td>53</td>
<td>106</td>
<td>3.021</td>
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<td>24.5</td>
<td>53</td>
<td>106</td>
<td>2.886</td>
</tr>
<tr>
<td>27.5</td>
<td>53</td>
<td>106</td>
<td>2.809(^{2})</td>
</tr>
<tr>
<td>30.5</td>
<td>53</td>
<td>106</td>
<td>2.773</td>
</tr>
<tr>
<td>33.5</td>
<td>52</td>
<td>104</td>
<td>2.761</td>
</tr>
</tbody>
</table>

1 Mean threshold in the first and in the second test for subjects tested in the period at the dark time specified.
2 \(n = 104\) observations for 52 subjects.
3 \(n = 102\) observations for 51 subjects.
fifty-three subjects was the same at each dark time as in period I, when they were without previous experience.

2. Temporal variability of threshold and immediate error of

Table 6. Variability of threshold at 33.5 minutes during dark adaptation for fifty subjects tested in duplicate at each of four examination periods, according to source of variation.

<table>
<thead>
<tr>
<th>Source of Variability</th>
<th>Standard Deviation of Threshold (( \bar{\sigma} )) Log ( \mu \mu l )</th>
<th>Proportion of Variance (( \bar{\sigma}^2 )) Per Cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Total, ( \bar{\sigma}_{ijk} ) (400 Observations).</td>
<td>±.316</td>
<td>100.0</td>
</tr>
<tr>
<td>2. Different Subjects, ( \bar{\sigma}_y ) (50 Individuals).</td>
<td>±.253</td>
<td>64.0</td>
</tr>
<tr>
<td>3. Temporal Changes of the Individual’s Threshold during Six Weeks, ( \bar{\sigma}_t ) (Four Examination Periods).</td>
<td>±.173</td>
<td>30.0</td>
</tr>
<tr>
<td>4. Immediate Error of Measurement, ( \bar{\sigma}_e ) (200 Pairs of Duplicate Observations).</td>
<td>±.077</td>
<td>6.0</td>
</tr>
<tr>
<td>5. Immediate Error of Measurement and Temporal Changes (( \sqrt{\bar{\sigma}_t^2 + \bar{\sigma}_e^2} )).</td>
<td>±.189</td>
<td>36.0</td>
</tr>
</tbody>
</table>
measurement at 33.5 minutes: The variability of 400 measurements of threshold at 33.5 minutes is shown in Table 6. The measurements were duplicate observations on fifty subjects tested at each of four examination periods. The total variability, ±3.16 log µµ, was a composite of variations arising from error, and temporal deviations from mean of the individual's threshold during the study.

The standard deviations (σ) shown in Table 6 were obtained in an analysis in which the assumptions and procedures were as follows: Any threshold (Y) was assumed to be the sum of (i) an error component, ε, (ii) a temporal fluctuation, t, and (iii) the individual’s characteristic threshold value, y. According to this view, Y_{ijk} = ε_{ijk} + t_{ij} + y_i, where i = the individual, j = examination period, and k = a single observation in any period. It was assumed also that: ε_{ijk}, t_{ij}, and y_i are mutually uncorrelated; Eε_{ijk} = Eε_{ijk} = 0 for all i, j, k, and E y_i = m for all i, where the symbol E represents the expected or mean value of the quantity immediately following it. As a rough approximation, it was assumed furthermore that σ^2_{ijk} = σ^2_ε for all i and j, and σ^2_{tij} = σ^2_ε for all i, j, and k. The symbol σ^2_ε will be used to represent the variance of y_i.

The total sum of squares is given by ∑_i=1^n ∑_j=1^p ∑_k=1^q (Y_{ijk} - Y_{ij.})^2, where Y is the general mean of all observations, n = the number of individuals, p = the number of periods, and q = the number of repeated tests of a subject in a given period. This sum of squares is the sum of three parts:

(a) ∑_i=1^n ∑_j=1^p ∑_k=1^q (Y_{ijk} - Y_{ij.})^2, in which Y_{ij.} is the sum of any pair of duplicate observations;

(b) q ∑_i=1^n ∑_j=1^p (Y_{ij.} - Y_{i..})^2, in which Y_{i..} is the mean for any individual of all observations at all periods; and finally

(c) pq ∑_{i=1}^n (Y_{i..} - Y)^2.

The expected values for the sums of squares indicated under a, b, c are:

\( (i) E \sum_{i=1}^n \sum_{j=1}^p \sum_{k=1}^q (Y_{ijk} - Y_{ij.})^2 = np(q - r) \sigma^2_\varepsilon \)

\( (ii) E q \sum_{i=1}^n \sum_{j=1}^p (Y_{ij.} - Y_{i..})^2 = nq(p - r) \sigma^2_\varepsilon + nq^2(p - r) \sigma^2_t \)

\( (iii) Epq \sum_{i=1}^n (Y_{i..} - Y)^2 = (n - r) \sigma^2_\varepsilon + q(n - r) \sigma^2_\varepsilon + pq(n - r) \sigma^2_y \)

These expressions show that: the expected value of ∑_{i=1}^n ∑_{j=1}^p ∑_{k=1}^q (Y_{ijk} - Y_{ij.})^2 is a function of the variance of error (σ^2_ε), the expected value of q ∑_{i=1}^n ∑_{j=1}^p (Y_{ij.} - Y_{i..})^2 is a function of the variance of error (σ^2_ε) and of temporal fluctuations (σ^2_t), and the expected value of pq ∑_{i=1}^n (Y_{i..} - Y)^2 is a function of σ^2_ε, σ^2_t, and the variance of the characteristic thresholds of the different subjects (σ^2_y).
and differences in the characteristic thresholds of different sub-
jects. Sixty-four per cent of the total variance represented differ-
ences between the subjects. This variability, $\pm .253 \log \mu \mu l$, reflects
age effects upon visual function, one aspect of which was noted here,
and, presumably, differences with respect to many other factors af-
flecting threshold, which are relatively stable over a period of time,
for example: intelligence and educational level (51); individual
functional patterns of the sympathico-adrenal system (30); indi-
vidual efficiency in the utilization of any of the nutrients essential
to retinal and cerebral metabolism, relative to the individual’s char-
acteristic supply of these nutrients; and pupil size in the light and
in the dark (35).

Temporal deviations of the individual’s threshold and error ac-
counted for 36 per cent of the total variance and of this proportion
the greater part, 30 per cent, arose from temporal variability. The
individual temporal variability, $\pm .173 \log \mu \mu l$, was significantly
greater than the immediate error of measurement, $\pm .077 \log \mu \mu l$.

Hence an unbiased estimate for $\sigma^2_\varepsilon$ is given by $\frac{\sum \sum \sum (Y_{ijk} - \bar{Y}_{ij})^2}{npq(q-1)}$; an unbiased
estimate of $\sigma^2_\tau$ is given by $\frac{\sum \sum (Y_{ij} - \bar{Y}_j)^2}{npq(q-1)}$; and an un-
biased estimate of $\sigma^2_\gamma$ is given by $\frac{\sum\sum (Y_{ij} - \bar{Y}_j)^2}{n-1}$.

The values in Table 6 of $\sigma_\gamma$ (item 2), $\sigma_\varepsilon$ (item 3) and $\sigma_\tau$ (item 4) are square roots of
weighted mean variances ($\bar{\sigma}^2_\gamma$, $\bar{\sigma}^2_\varepsilon$, $\bar{\sigma}^2_\tau$) for the three technician-groupings.

The variances of $\sigma^2_\gamma$, $\sigma^2_\varepsilon$, and $\sigma^2_\tau$ were computed initially within technician-groupings
since different technicians do not obtain exactly the same threshold in testing the same
subject, other conditions being equal. Accordingly, the variance, $\sigma^2_\varepsilon$, if computed without
regard to technicians, would probably be somewhat larger than the value given in Table 6,
and unless the technicians were equally consistent in measurement procedure over a period
of time $\sigma^2_\varepsilon$ would also be affected. In the case of $\sigma^2_\varepsilon$, data of the present study indicated
that this quantity at 33.5 minutes was the same for all technicians.

The analysis within technician-groupings showed that the proportions of $\sigma^2_{Yijk}$ con-
tributed by $\sigma^2_\varepsilon$, $\sigma^2_\tau$, and $\sigma^2_\gamma$, respectively, varied somewhat in the 3 technician-groupings,
but in all such groupings $\sigma^2_\gamma$ accounted for the largest proportion of the variability, $\sigma^2_\varepsilon$ was next in importance, and $\sigma^2_\varepsilon$ was the smallest component, the order of importance
for $\gamma$, $\varepsilon$, and $\tau$ shown in Table 6. Under these circumstances the use of weighted mean
variances for the different technicians was considered justified.
The greater variability of serial measurements of the individual, as compared with duplicate measurements, might be expected, since in duplicate tests within approximately one and a half hours the physiological state of the subject is relatively stable, whereas in serial testing the measurements may vary as a result of temporary fluctuation of factors affecting threshold. The latter fluctuations may be due to differences in certain aspects of test conditions at different examination periods, such as unequal ventilation of the dark room or variations in the amount of bright sunlight to which the subject is exposed on different examination days. They may also include, for example, variations in the concentration of vitamin A in the blood arising from dietary changes or for other reasons (49, 50, 33) and transitory changes in level of blood sugar (29).

In practice, any threshold measurement of the individual in a particular test reflects immediate error and the factors of temporal variation jointly. The standard deviation for the combined variability due to both sources was ±.189 log \( \mu \mu l \). This result indicates that threshold of any subject in a particular test deviates very frequently from the individual’s characteristic mean level of threshold during a limited period (six weeks) to the extent of ±.189 log \( \mu \mu l \), higher or lower. Since temporal variations as well as immediate error appeared to be normally distributed it is likely that the limits ±.189 log \( \mu \mu l \) would account for approximately 68 per cent of the deviations from characteristic individual level. If no more than 5 per cent of the deviations are disregarded, ±.378 log \( \mu \mu l \) would appear to be required.

From the above considerations it follows that in the population group represented here a single threshold measurement at 33.5 minutes is commonly unreliable as an index of the individual’s characteristic mean level within a limited period to the extent of ±.189 log \( \mu \mu l \) and, less frequently, ±.378 log \( \mu \mu l \).

The unreliability of single threshold measurements, ±.378 log \( \mu \mu l \), can be used to indicate roughly the amount of change in
threshold of the individual from one examination period to another
which is to be expected in view of temporal variation and error
of measurement and, therefore, need not be regarded as evidence
of a significant shift in the characteristic threshold level of the indi-
vidual. In single tests two weeks apart a change in threshold at 33.5
minutes, which is less than $\pm 0.523 \log \mu\mu l$ is of doubtful signifi-
cance and only changes as great as or exceeding $\pm 0.688 \log \mu\mu l$ can
be considered highly significant.

**Comment**

1. Serial measurements of dark adaptation failed to indicate that
subjects who received vitamin A therapy were benefited. Assuming
that visual threshold, measured at 33.5 minutes in the dark, is
closely related to the intake of vitamin A, this result might be ex-
pected if the body reserves of vitamin A were already adequate.
The latter presumption of adequate reserves of this vitamin does
not appear to be justified, in as much as eye examinations in the
gross and also by biomicroscope showed conjunctival lesions in 72
per cent of the eighty-three subjects in the present study and regres-
sion of these lesions in cases receiving vitamin A therapy was re-
ported (24). It seems highly probable, therefore, that the group
concerned here contained a fairly large number of cases of avita-
minosis A of some degree. The latter view receives some support
also from the fact that the subjects were drawn from an unmistak-
ably underprivileged group in which average annual family income
was approximately $750. At this income level the food expenditure
of 50 per cent of the families concerned might be expected to be
insufficient to secure nutritionally adequate diets (47). Great econ-
omy in expenditures combined with scientific planning of the diets
would alter this expectation. In the group studied it is doubtful that
more than a few individuals possessed requisite training and ex-

$^e \pm t_{0.05} s \bar{d}$, where $t_{0.05} = 1.960$ and $s \bar{d} = \sqrt{2 \left(\sigma_1^2 + \sigma_2^2\right)}$.

$^f \pm t_{0.01} s \bar{d}$; $t_{0.01} = 2.576$. 
The similarity of threshold changes at 33.5 minutes in the therapy and control groups suggests a lack of relationship between threshold measurements at this dark time and vitamin A in the diet. This finding is at variance with the evidence from a number of depletion experiments with human subjects in which a correlation between threshold and intake of vitamin A was shown by the rise of threshold, which accompanied a greatly reduced supply of this nutrient in the diet (20, 6, 44, 15, 16). Blanchard and Harper also found, early in adaptation, an increase in "adaptation time" among students whose intake of vitamin A was reduced to a low level for 42-45 days (4). Furthermore, highly probable cases of vitamin A deficiency, diagnosed on the basis of low vitamin A content of the serum as well as relatively high threshold late in adaptation, have shown marked improvement in adaptation under vitamin A therapy (26). Similar cases of high threshold late in adaptation due to vitamin A deficiency and responding to vitamin A therapy, were reported recently by Rajagopal (36), Yudkin (50), and Campbell (8). On the other hand the lack of evidence of relationship between threshold as measured and intake of vitamin A in the present study is in line with the results of experiments in which attempts to produce nightblindness in human subjects by diets low in vitamin A were unsuccessful (39, 19, 45, 11). In view of the latter reports and results in the present study, it appears that the correlation of threshold and intake of vitamin A is less direct than indicated by the findings in the first group of experimental and clinical investigations cited here.

Studies of the correlations between threshold measurements and vitamin A in the blood and in the diet also have not revealed a uniformly high degree of relationship among the three variables. Three groups of investigators (41, 2, 9) found no correlation between threshold and vitamin A in the blood, but the intake of
vitamin A affected the concentration of vitamin A in the blood. The biophotometer was used to measure threshold early in adaptation. The instrument, customary procedure and results obtained have been critically reviewed elsewhere (26, 32, 38). Similar results were obtained by Yarbrough and Dann (48) for the nearly dark adapted threshold measured with the adaptometer and the concentration of vitamin A in plasma determined from non-fasting blood samples of 154 subjects in a southern rural mill town. The correlation between the threshold and vitamin A in plasma was +.131 and not significant. Josephs, Baber, and Conn (21) in a study of 147 persons, mostly children, found an increase in mean “recognition time” (after about 4 minutes of dark adaptation) associated with a mean decrease in the amount of vitamin A in the serum when subgroupings of subjects at progressively poorer dietary and socio-economic levels were compared. When paired values of “recognition time” and vitamin A in plasma for different individuals were correlated little relationship between the variables was found. Bodansky, Lewis, and Haig (5, 25) observed a significant mean difference in the plasma vitamin A concentration of forty-six infants, 3 weeks to 6 months of age, receiving 1,200-1,500 units of vitamin A daily and forty-seven infants of the same age on the same diet but supplemented with 17,000 units of vitamin A daily. A corresponding difference in mean thresholds late in adaptation was not present. When vitamin A in the diet was reduced to 335 units daily, a decrease in plasma concentration of vitamin A followed without the appearance of abnormal adaptation. The latter appeared, however, when vitamin A was entirely withheld from the diet and the plasma vitamin A concentrations fell below 45 units of 100 cc. of plasma (seven cases). Administration of small amounts of vitamin A (150 units daily for thirty days) returned the threshold to normal but were insufficient to increase the plasma concentrations to normal level. The results showed that the concentration of vitamin A in plasma in infants is
more closely related to vitamin A deficiency than threshold late in adaptation. Lindquist (26) found that clinical patients (fifty adults less than 40 years of age) with low concentrations of vitamin A in serum (less than 100 I.U. per 100 cm.) had a higher mean threshold (late in adaptation) than similar subjects (175 cases) whose vitamin A content of serum was 100 I.U. per cm. or more. For subjects more than 40 years of age and with the serum concentration of vitamin A less than 100 I.U. per 100 cm. (thirty-seven cases) a significant correlation (.29) between threshold and vitamin A concentration of serum was observed, whereas none was found when the vitamin A content of serum was 100 I.U. per cm. or greater. In a study of 214 healthy persons and thirty-one patients, Saksela (37) reported normal adaptation in 85.2 per cent of the subjects whose vitamin A content of the serum was 226 I.U. per cc. or greater, as compared with 18 per cent in the group in which the vitamin A of serum was 120 I.U. per cc. or less. Twenty-six individuals observed by Pett and Le Page (34) showed a relationship regarded as significant between “regeneration time” (early in adaptation) and the vitamin A concentration of plasma.

2. The conflicting appraisals of the degree of relationship between threshold measurements, intake of vitamin A and the vitamin A content of the blood, raise the question whether, frequently, the actual relationships may not be obscured either by inherent inaccuracies of the procedures employed or by lack of control over factors and circumstances which greatly increase the variability of the observations. It may be asked in the present study, for example, whether a preadaptation in the neighborhood of 1,004 millilamberts provided a searching appraisal of individual capacity with respect to dark adaptation. In studies of nutritional nightblindness in rats, a prolonged preadaptation at relatively high brightness appeared to be an important factor in the experimental production of impaired dark adaptation (13). Dark adaptation following preadaptation at relatively low brightness may be well within the range of successful
performance for even moderately impaired capacity whereas recovery from more intense light exposure may tend to differentiate more clearly, excellent, average, and poor capacity. It should be noted also that the test, as given, examined but a small field of the retina.

3. The age difference noted was similar to those described elsewhere. In a study employing the adaptometer a slight tendency of the nearly dark adapted threshold to be distributed toward higher values than were common in younger age groups was evident among persons 40 to 65 years of age (15). Basu and De, using an instrument similar to the adaptometer, reported thresholds after 15-30 minutes dark adaptation in a group of 341 school children which were on the average lower than those obtained for 161 adult employees (1). Higher and more variable threshold of the nearly dark adapted eye in persons over 40 years of age was found in examinations with the Gullstrand photometer (26). With another apparatus and procedure, "light minimum" of persons over 35 years of age was found to be higher than that of younger subjects (12). A diminished power of visual distinction among older persons has also been observed (7). Wittkower and Rodger (51) and also Stewart (43) reported a correlation of adaptation ability with age. Some part of this correlation is apparently due to a decrease in the size of the pupil in the dark in older persons (35).

4. The variations of threshold measurements described in the present paper differ in two respects from those reported heretofore for the adaptometer or a similar instrument. In the first place, the errors of single measurements are larger in size than those reported either for adults at a nonrelief level of income or for practiced laboratory subjects tested by the same or a closely similar procedure (44, 15, 46, 27). For the types of subjects mentioned the "generally expected" error at one sitting is .10 log units. Yudkin gives the figure, .10 log unit, for variation of the individual's threshold within a few days (50). In the present study .10 log unit (0 ± .05 log μμd)
accounted for only about 30 per cent of the errors of single measurements late in adaptation. The limits including about 95 per cent of the errors were $\pm .151 \log \mu \mu l$ or an interval of $.30 \log \mu \mu l$ for the nearly dark adapted eye. Second, the error of single measurements was not constant throughout adaptation. Measurements were least reliable in the neighborhood of 6.5-12.5 minutes and had the smallest error at 30.5-33.5 minutes dark time. In connection with the measurement of the limit of the “cone” threshold and the “transition” time, Jung and Greenberg have also reported larger observational errors early in adaptation than for the nearly dark adapted eye (22).

Inasmuch as the fluctuation in error during adaptation decreased significantly as the subjects became more experienced and the technician better acquainted with individual subjects, it is clear that a definite learning factor was operative during the first 20-25 minutes of adaptation. The pattern of error indicates that “poor” subjects are commonly met in routine field testing. This appraisal of error in routine testing is consistent with the comments of McDonald and Adler with regard to allowances which must be made in the interpretation of threshold measurements of clinical and ward patients (28).

5. A criterion of vitamin A deficiency, employing measurement of the threshold of the nearly dark adapted eye has recently been proposed (42), and commented upon (48, 50). The procedure of measurement was similar to the one used here. According to this criterion a decrease in threshold of the dark adapted eye of at least $.3 \log \text{unit}$ accompanying vitamin A therapy or eventual lowering by this amount when therapy is continued at least two weeks may be regarded as evidence that the threshold is “vitamin A labile.” In the population group represented here, a $.3 \log \text{unit}$ change in threshold of the nearly dark adapted eye might occur more frequently than 5 times out of 100 without therapy, due simply to temporal and error variations of the individual’s threshold. A just
significant difference between thresholds at two examinations, two weeks apart, was considerably larger than .3 log units, \textit{i.e.,} ±.523 log units and the highly significant change was more than twice as great, ±.688 log units. The difference .5 log unit was previously suggested by Steffens, Bair, and Sheard (40).

6. The large variability of temporal change and error for threshold measured at 33.5 minutes, ±.189 log \( \mu \mu \) (36 per cent of total variance) suggests that distinctions between individuals with respect to characteristic thresholds can not be made with certainty in this and similar population groups when factors associated with temporal variation, particularly, are uncontrolled. For, if different subjects are tested in one examination period, the differences between them will be due to a considerable extent to the fact that they are in different phases or stations of individual temporal change from characteristic level in a limited period. Jung and Greenberg reported a similar expectation for individuals in a more privileged group, \textit{i.e.,} medical and graduate university students (22). Furthermore because of temporal and error variations, different individuals tested in one period can not be ranked accurately in respect to characteristic threshold, relative to narrow standards of normal response such as have been employed in many population surveys. A critical review of the latter studies up to 1938 is given by Lindquist (26).

The relatively large temporal and error variations of threshold at 33.5 minutes, together with the fact that threshold level late in adaptation in a particular test is not a good indication of how the individual will respond to vitamin A (16) or other therapy administered subsequently, lead to the conclusion that the incidence of nightblindness in a population group can not be accurately determined by means of a cross-sectional survey of thresholds late in adaptation. Since error increases rapidly at earlier dark times and there is no reason to expect smaller temporal variations before 33.5 minutes, the cross-section survey gives results which are probably undependable throughout adaptation.
If significant regression of the nearly dark adapted threshold in response to vitamin A therapy is used as a criterion of nightblindness due to vitamin A deficiency, individuals must be observed serially over a period of time, a procedure unsuited for practical reasons to the study of a large number of individuals. From the latter and previous considerations it appears that threshold measurements may be useful and practicable for the diagnosis of nightblindness due to vitamin A deficiency in laboratory or clinical or very small scale field investigations but undependable or impractical in studies of a large population group.

**Summary**

The dark adaptation of fifty under-privileged adults was measured periodically, at intervals of two weeks, during a period of six weeks. Supplements of 10,000 International units of vitamin A were given daily to one-half of the group during the study.

1. The adaptation of individuals receiving therapy was not improved.

2. Experience in taking the adaptation test decreased the error of threshold measurements made before 24.5 minutes in the dark but did not affect error at this dark time or others late in adaptation (27.5, 30.5, 33.5 minutes). Throughout adaptation mean threshold level for the group of subjects was unaltered by experience.

3. Regardless of the amount of experience threshold measurements before 20 minutes in the dark were subject to larger observational errors than those made after 20 minutes of adaptation. At 33.5 minutes the error of measurement was $0 \pm 0.077 \log \mu\mu l$. Approximately 95 per cent of the errors were within the limits $\pm 0.151 \log \mu\mu l$, or an interval $0.302 \log \mu\mu l$ in width.

4. Due to error of measurement and individual temporal variation, the threshold of the nearly dark adapted eye (33.5 minutes) deviated in any particular test from the individual's characteristic (mean) threshold during a six-week period. The standard devia-
tion of these variations was $\pm 0.189 \log \mu l$, which represented 36 per cent of the total variance of threshold at 33.5 minutes.

5. The variability of thresholds of different individuals at 33.5 minutes was $\pm 0.253 \log \mu l$, or 64 per cent of the total variance.

6. In semi-monthly examinations in the population group studied smaller changes in the individual’s threshold at 33.5 minutes than $\pm 0.523 \log \mu l$ are not significant evidence of change in the individual’s characteristic threshold. A change as large or greater than $\pm 0.688 \log \mu l$ would appear to be highly significant as such differences are expected in no more than 1 per cent of fluctuations due to temporal variation and error of measurement.

7. If nightblindness due to vitamin A deficiency is to be determined by means of threshold measurements, serial study of the individual is necessary during a period of administered intake of vitamin A, as (a) the temporal and error variations of the individual’s threshold are large, (b) the threshold level in a particular test does not indicate the probable response to therapy, and (c) threshold level is affected by many other factors as well as vitamin A.

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