METHODS FOR MEASURING FINGERNAIL GROWTH RATES IN NUTRITIONAL STUDIES 1, 2

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INTRODUCTION

Information on nutritional status may be obtained from physical findings, biochemical analysis, or the composition of diets. The most conclusive evidence of a deficiency is, however, usually considered to be the alleviation of a nutritional deficiency syndrome by dietary supplementation. In nutrition surveys the curative test has been used only infrequently because most of the signs of mild nutritional deficiencies are so poorly defined that quantitative measurements cannot be made of the small changes that occur in short-term dietary studies. Long-term studies, besides being expensive, are difficult to interpret because other pertinent factors, such as variation in the basal diet, activity, season, and illness, are likely to change the conditions during the experiment.

One of the signs most frequently associated with nutritional deficiency is decrease in growth rate. This index of nutrition is, however, usually limited in its application to young, growing animals, and, in the case of the larger, slow-growing species, such as man, to long-term studies. Although growth

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of the whole body is a slow process, certain tissues, such as hair, nails and epithelium, grow at relatively rapid rates in all animals, large and small, young and old. Of these continuously growing tissues, the nails are probably best adapted to measurement, because they are readily accessible and rigid structures.

These considerations suggest that a change in the growth rate of nails induced by supplementation of the diet might provide a measure of certain nutritional deficiency states and that this measure would be applicable to mature, as well as growing, animals. Evidence in the literature suggests that the state of nutrition frequently has an effect on fingernails, but very few quantitative studies have been made of the problem because suitable methods for measuring nail growth have not been available. This paper presents a review of previous work and describes a new method for measuring fingernail growth that is suitable for short-term studies.

The medical literature contains many references to qualitative changes in fingernails thought to be associated with nutrition. These changes include transverse and longitudinal ridging, brittleness, and the "spoon nails" (koilonychia) associated with anemia. Nutrients that have been implicated are: vitamins, iron, calcium, zinc, fluorine, protein and unsaturated fats.

Although nail tissue is composed primarily of keratin, the fact that this tissue is formed by mitosis and cornification of epithelial cells indicates that the growth of nails involves much more than the synthesis of protein. Nail tissue has been shown to contain cholesterol (Hotta and Takazi, '37) and various metals (Goldblum et al., '53). Sinclair ('48) has stated that deficiencies of at least 11 nutrients probably cause alterations in the epidermis in mammals. The wide variety of nutrients that may have a role in the growth of nails presents many possible applications for nail growth studies in the field of nutrition. The numerous observations of nail anomalies associated with endocrine disorders and disease, recently reviewed by Rönchese ('51) suggest further that
quantitative nail growth studies might be of value in following the course of such metabolic derangements. As with other clinical and nutritional measures, however, the likelihood that various factors may affect the response necessitates careful control and interpretation of all studies (Bean, '50).

Quantitative studies of fingernail growth over periods of a month or more have been made by several workers. In 1930, Voit reported a long-term study on the weight of fingernail and toenail clippings of three subjects. He related the weight of the clippings to age, body growth, and hair growth, to different fingers, hands, months, and years, and to the frequency of cutting, surface area, and moisture content of the nails. Halban and Spitzer ('29) cited studies of earlier workers dating back to research by Robert Boyle in 1684. Halban and Spitzer showed that, during pregnancy, fingernail growth rates were one-fourth to one-third higher than normal. These workers, and others (Bau Kien-Tsing, '38; Wigand, '37) usually measured growth by the distance a stain on the nail advanced from the cuticle. Clark and Buxton ('38) improved this procedure by making measurements from the lunula with the aid of an illuminated magnifying glass. They showed that the nails on the longer digits grew more rapidly, but that all responded similarly to seasonal and other variations. Using the thumbnails only for further studies, they showed that growth was essentially the same for left and right thumbs, for male and female, and for ages 10 to 23 years, but that it was greater in the summer and for nail-biters, and that there was wide variation between individuals.

Using the same technique, Gilchrist and Buxton ('39) studied the relation of fingernail growth to nutritional status of East London elementary school children. They found a highly significant difference in fingernail growth rates between children rated as having "subnormal" nutrition, and those rated as "normal" or as "excellent," but no significant difference between the "normal" and "excellent" groups. They recognized the subjective errors and probable over-
lapping of their clinical ratings, but felt that the low average economic level of the "subnormal" group gave added support to the clinical assessment of that group. It is pertinent that the English school medical inspections were based on "general physical appearance" (Dunstan, '38), rather than on clinical signs associated with specific nutrient deficiencies. Since the clinical ratings may have been influenced by factors that had little effect on fingernail growth, it is not surprising that Gilchrist and Buxton found relatively small differences in the nail growth rates of their three clinically-rated groups.

Petersen ('33) has stated that meteorological conditions, particularly barometric pressure, and the period of the menstrual cycle affect the rate of fingernail growth. Further studies are needed, however, because his observations were limited to one subject, and the negative growth rates (retraction of the nail) recorded on some days throw some doubt on the accuracy of the method used (not stated) for his measurements.

Basler ('37) noted wide variation in nail growth from hour to hour. Using a special "biomicrometer," he measured the increase in space between two brass strips, one cemented to the nail, the other cemented to the skin and overlapping the nail. The slower growth rate of nails at night observed by Basler may be caused by reduced blood pressure since Riddle ('08) has shown that the growth of feather barbules is affected by diurnal variations in blood pressure, as well as by nutrition.

Bean ('53) has recently reported a 10-year study of the growth of his left thumb nail. His method of measuring the time required for a mark on the nail to grow from the cuticle to the point of separation of the nail from its bed was not sensitive because it gave only average growth rates over periods of approximately 120 days. His measurements showed a slight but consistent decline in average growth rate with age and a retardation of growth during an attack of mumps, but they did not reveal any relationships of nail growth to season, geographic location, occupation, or other factors. Bean showed that weights of fingernail clippings are subject to
large errors because of wearing away of the nail. He also cited early observations on retarded nail growth in paralysis and immobilization. In 1871, Weir Mitchell had observed that nail growth was retarded in paralyzed hands and that growth was resumed prior to return of motor power. Head and Sherren in 1908 had observed a similar retardation of nail growth when the hand was immobilized in a splint or cast, but massage stimulated the growth.

EXPERIMENTAL PROCEDURE

Thickness or weight measurements were not considered to be satisfactory for short-term measurements of nail growth because it takes about 5 months for changes in thickness to grow out to where they can be measured (Voit, '30), and because the terminal portion of the nail may wear away rapidly (Bean, '53). Increase in the length of nails involves measuring the distance between a mark on the nail and a reference point on the finger. Since nails grow at the rate of about 0.1 mm per day, this distance is so small in short term experiments that magnification is necessary to obtain accurate readings. Measurements with a microscope are difficult because of movement of the finger (Basler, '37). It was felt that photographs would be more suitable than direct measurements on live subjects because photographs permit optical enlargement of the distance to be measured. Photographs also allow rapid collection of the data with more thorough examination of the permanent records.

The greatest problem in measuring fingernail growth is the choice of a reference point for the measurements. The cuticle, used by early workers, is inaccurate for short-term studies because it can easily be pressed back. The same applies to the point of separation of the nail from its bed. The lunula frequently is not visible on all fingers and does not appear as a sharp line when viewed through the nail with a magnifying glass. It does not show clearly on ordinary photographs, and it is said to be affected by pathologic
changes that affect the nails or nail bed (Anonymous, '34).
In the studies reported here, three reference points have been
tested, the phalanx bone, the skin near the nail, and the lunula.

Comparison of x-ray and skin-photographic methods

The skin between the nail and the terminal finger joint
offers a possible reference point that has not previously been
investigated. The reference point could be either a mark
made on the skin, or the small skin wrinkles in this area.
Since nutrition survey subjects might object to semipermanent
skin markings, a study was made to determine whether
the skin wrinkles would provide suitable reference points.

For an "absolute" standard reference point in these studies,
it was decided to use the bone rather than any portion
of the soft tissues. The root of the nail is closely attached
to the terminal phalanx by means of dense connective tissue.

To obtain growth records, the nails were marked with a
material opaque to roentgen rays (a deep scratch in the nail
was filled with bismuth amalgam) and an x-ray photograph
was made. At the end of a 41-day growth period, another
x-ray photograph was made under identical conditions. The
two photographs of the bone were then exactly superimposed,
and the distance the scratch-mark had advanced was measured
with the aid of a microscope ocular micrometer scale and a
magnifying glass. On the same days the x-ray photographs
were taken, ordinary photographs (slightly larger than life-size)
were also taken. These were read in a similar manner
by superimposing the images of the skin wrinkles and measuring
the distance the mark on the nail had advanced. In all cases, 5 individual readings were made and the averages
were converted to microns per day, using factors obtained
by photographing a metal caliper rule under identical conditions.

Nail growth data were obtained by the x-ray and skin-
photographic methods described above from 6 subjects, rang-
ing in age from 6 to 52 years, using the index, middle, and
ring fingers of each hand. To investigate the effects of dif-
ferent fingers, hands, and subjects, as well as the two methods
used, a factorial analysis (Cochran and Cox, '50) was applied
to the 72 items of data. The summary of the analysis of
variance data, presented in table 1, shows that there was
no significant difference between the two methods, though

| TABLE 1 |
| Analysis of variance comparison of x-ray and skin-photographic methods for measuring fingernail growth |

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>DEGREES OF FREEDOM</th>
<th>MEAN SQUARE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method</td>
<td>1</td>
<td>15.68 (^1)</td>
</tr>
<tr>
<td>Fingers</td>
<td>2</td>
<td>153.45 (^1)</td>
</tr>
<tr>
<td>Subjects</td>
<td>5</td>
<td>1,797.39 (^1)</td>
</tr>
<tr>
<td>Hands</td>
<td>1</td>
<td>132.31 (^1)</td>
</tr>
<tr>
<td>Error</td>
<td>37</td>
<td>13.33</td>
</tr>
</tbody>
</table>

\(^1\) Not significant at the 5% level.
\(^2\) Significant at the 1% level.

there were significant differences in the nail growth rates
between fingers, between right and left hands and very large
differences between subjects.

The overall averages indicated a difference of one micron
between the two methods. According to the sensitivity of
this statistical analysis, the odds were better than 9 to 1
that a difference of three or more microns would have been
found to be significant. It is concluded from this analysis
that there is virtually no bias or inaccuracy in the photo
graphic method, using the x-ray method as an absolute reference for measuring growth.

Reproducibility of the skin-photographic method

Having established that the skin-photographic method gave a reliable measure of nail growth as compared with the x-ray method, when used with a 41-day growth period, tests were made to determine the reproducibility of the skin-photographic method when adapted to short-term (one week) studies. In short-term studies the small distance that the scratch-mark image advanced was increased, to facilitate measurements, by taking enlarged photographs (6.4 ×) of a single finger. Duplicate photographs, two at the beginning and two at the end of the growth period, were taken to provide a measure of variation caused by positioning the finger. The dispersion of nail growth measurements obtained from different combinations of these photographs gave a measure of the reproducibility of the method.

Photographs were made of the nails of the left middle fingers of 5 subjects, ranging in age from 7 to 35 years, under the following conditions. The camera was prefocused on a three-fourths inch square opening in a firmly mounted piece of sheet metal, and the finger was gently pressed upward against this frame. The illumination was standardized by clamping two 375-watt reflector-type photoflood bulbs 8 inches from the frame. The exposure (two seconds at f:22) was controlled by an automatic timer switch connected to the lights. Two photographs were taken at the beginning of the growth period, and two photographs were made at the end of one week. Replicate growth measurements were thus obtained, one from each pair of photographs. Each growth measurement was based on 5 individual readings of the photographs, as in the previous experiment. Different skin wrinkles were used to superimpose the photographs for each reading.

The standard deviation of the growth measurements, calculated from the analysis of variance, was 6.88 μ, and the coefficient of variation was 6.3%.
FINGERNAIL GROWTH RATE METHODS

Lunula-photographic method

Since the skin-photographic method did not have the desired degree of precision when used with short growth periods, tests were made to determine whether the lunula could be used as the reference point for nail growth measurements. Photographic conditions were selected to obtain maximum contrast of the lunula. It was illuminated with light of a wavelength absorbed by hemoglobin and oxyhemoglobin, and high contrast film and developer were used. The thumbnail was studied because the lunula of the thumbnail is usually more distinct than that of the other fingernails. Details of the technique follow.

The thumbnail was lightly scratched near the lunula with a sharp scalpel blade. The scratch was filled with red pigment (glass-marking crayon), and the excess was wiped off. A few drops of glycerol were applied to the nail, and the nail was very lightly pressed upward against a microscope slide that was firmly mounted. The glycerol served as an optical mounting medium to prevent reflections from the surface of the nail. Care was taken to avoid pressing hard against the glass because blood would be forced out of the nail-bed capillaries and the lunula would have less contrast. The camera was clamped vertically over the microscope slide and prefocused on a marked area of the slide. The fingernail was illuminated with two General Electric H 100 SP 4 mercury vapor spotlights, one on each side mounted at about a 45° angle. A special transformer and socket (admedium) are required for each bulb, and the bulbs should be turned on 5 minutes before photographs are made. Corning glass filters no. 351 and no. 978 were placed in the camera optical system to isolate the 546 μm mercury line. A Bausch and Lomb, Type II photomicrographic camera with 48 mm lens and extension bellows was used to obtain negatives enlarged about 6 times. Eastman Kodak Contrast Process Panchro-

Corning now manufactures a single filter (no. 4-102) to isolate the 546 μm mercury line.
matic sheet film and D-8 developer were used. One-second exposure at f:22 with over-development (4 minutes instead of two minutes tray development) gave negatives of the desired density and adequate contrast. Tank development (5 minutes) has also given satisfactory results with D-8 developer.

One week later the nail was again photographed under exactly the same conditions (lens distance, bellows extension, etc.). The two developed negatives were placed against a bright light source (Keystone Overhead Projector) and the two images of the lunula were carefully superimposed. The distance between the two positions of the scratch mark was measured with a microscope ocular micrometer scale containing 140 divisions in 8.4 mm and a 7.5 × magnifying glass (Bausch and Lomb linen tester no. 81-34-46). The negatives were separated, then reread 4 more times. The average of the 5 readings was converted to microns of growth per day by using appropriate factors derived from a photograph of an accurate (caliper) scale taken under the same conditions.

Photographs were made of the left thumbnails of 9 subjects (5 male, 4 female), ranging in age from 10 to 60 years. As in the test of reproducibility of the skin-photographic method, duplicate pictures were taken at the beginning and at the end of the growth period, and the dispersion of the growth measurements was used to measure the reproducibility of the method. The standard deviation of the growth measurements, calculated from the analysis of variance, was 3.23 μ, and the coefficient of variation was 3.1%.

_Lunula-transparency method_

Preliminary tests revealed that it was somewhat easier to align a photographic negative with a positive transparency than with another negative. Tests were made, therefore, to determine whether the use of positive transparencies would increase the precision of the measurements. For this study,
the initial films used in the lunula photographic study were printed on Eastman Kodak Panatomic X film. These transparencies were read, as before (5 readings each) against the final negatives to obtain mean growth measurements for each of the 9 subjects. The standard deviation of these growth measurements, calculated from the analysis of variance, was 2.19 \( \mu \), and the coefficient of variation was 2.2%.

DISCUSSION

The relative precision of the short-term skin-photographic, lunula-photographic, and lunula-transparency methods is inversely proportional to the ratio of their variances, 47.3, 10.4 and 4.8, respectively. Thus, the lunula-transparency method is 2.2 times as precise as the lunula-photographic method and 9.9 times as precise as the skin-photographic method. The lower precision of the skin-photographic method is attributed to distortion of the skin by contact with the focusing frame. In the lunula methods, the increase in precision and ease of reading of transparencies probably justifies the extra work required to print them. Since the original photographs are taken in exactly the same manner, whether or not positive transparencies are used, this choice can be made after the experiment is completed.

As carried out in this laboratory (5 individual readings from each pair of films), the precision of the lunula-transparency method, when applied to an individual, would be sufficient to show a difference in growth rate greater than 5 \( \mu \) per day to be significant at the 5% level. That is, with an average fingernail growth rate of approximately 100 \( \mu \) per day, application of the method should reveal a difference greater than about 5% in mean growth rate.

Although the lunula may change over a period of years, or with disease (Anonymous, '34), it appears to be a relatively stable reference point for short-term studies. Slight variations in the edge of the lunula would have little effect on the readings because the negatives are placed in register.
by superimposing the two images of the entire lunula, rather than by measuring from a specific point on the edge of the lunula. The greater precision of the lunula methods makes them preferable to the skin-photographic method for short-term studies. With subjects whose lunula is indistinct, however, the skin-photographic method could be applied, preferably with a growth period longer than one week to increase precision. The equipment required for the lunula-photographic method can be adapted easily to the skin-photographic method.

These methods for measuring nail growth rates in short-term studies provide a means for testing the effects of dietary supplementation, or other treatment, on nail growth rates, with each subject serving as his own control. A consistent increase in the fingernail growth rate of a subject following dietary supplementation with a nutrient would suggest that the previous nutritional status of the subject had been less than optimal with respect to that nutrient, provided, of course, that other factors affecting nail growth had remained unchanged. Since the control of extraneous variation between successive measurement periods becomes increasingly difficult as the length of the experimental period is increased, methods for measuring nail growth over short time periods are to be preferred.

Animal studies (in which a tattoo mark could be used as the reference point) are needed, as well as controlled studies with humans, to answer questions concerning the sensitivity of nail growth responses to various nutrients, drugs, metabolic disturbances, disease, and environmental factors before the place of nail growth tests in medicine, physiology, and nutrition surveys can be evaluated. Fingernail growth measurements offer an objective measure of anabolic processes that is readily obtainable without discomfort to the patient. Since the original observations are recorded permanently in photographic form, they may be checked at any time. The methods described here require little equipment and skill beyond those needed for ordinary photographic procedures.
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SUMMARY

Evidence in the literature has shown that fingernail formation is probably influenced by the state of nutrition, endocrine factors, disease, and environmental factors. It is suggested that quantitative measurements of fingernail growth response following dietary supplementation might be useful in assessing the nutritional status of individuals. A method for measuring nail growth rates over short time periods is presented which was found to be capable of detecting differences in fingernail growth rates of approximately 5%. The method is based on measuring the distance a scratch-mark on the thumbnail advances with respect to the lunula during one week. Enlarged photographs are used to record the positions of the mark and increase the precision of the measurements. The method gives a permanent, objective measure of anabolic processes without discomfort to the subject.

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LITERATURE CITED


