



A rapid tissue technique for the field assessment of protein-calorie malnutrition^{1, 2}

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There is a need for age-independent techniques for the rapid field determination of the incidence and severity of protein-calorie malnutrition (PCM). This is especially true in tropical areas as techniques that are used routinely in well-equipped laboratories may not be adaptable to field conditions of high environmental temperature and humidity and irregular electrical power.

The choice of hair as a tissue for the recognition of protein-calorie malnutrition may at first seem to be a peculiar one, but changes in hair color have been used extensively in the diagnosis of protein-calorie malnutrition. Of the eleven indicators of protein-calorie malnutrition listed by the World Health Organization, four deal with hair (1). During dietary protein deprivation, blood protein levels are maintained at the expense of tissue. It therefore seems reasonable that a test for early protein deprivation should be based on a protein-rich tissue, rather than blood.

For the early recognition of PCM in tropical areas, hair tissue has a number of advantages over other tissues. Hair root specimens can be obtained more easily than most other tissues in non-hospital situations. Obtaining hair samples does not require special equipment, nor a local anesthetic, nor sterilization; and cross-infection problems are avoided. Hair tissue can be transported in hot, humid areas without special precautions for chemical stabilization or refrigeration. The samples can be obtained and examined in ambient temperatures with a low power microscope in laboratories that do not have electricity. Because many tropical hospital laboratories do not have control over

ambient temperatures or humidity and some have no electrical power at all, these factors have to be considered in the selection of practicable techniques for the recognition of PCM. The use of root, rather than shaft, specimens eliminates the 3-week time lag between when protein deficiency affects their formation and when hair reaches a length for practical studies. Root preparations also avoid some of the ethnic effects on hair color and texture.

Method

Obtaining hair root specimens

Hair root samples are pulled from the occipital area, which is chosen because it is the area of most rapid hair growth. Access to the sample area is facilitated by inclining the head. A small group of hairs is lifted firmly from the scalp and stretched taut; pressure should be firm enough so that there is a slight lifting of the scalp in this area (Fig. 1a). Needle-holding forceps are then used to grasp the hairs. The forceps are placed parallel to the scalp, resting snugly against it to secure a sample as close to the scalp as possible. If the grasping surfaces of the forceps are covered with plastic tubing, it will help insure a tight closure and prevent slipping of the hair shafts during epilation. After clamping the

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forceps, it is convenient to grasp the forceps as shown in Fig. 1b and to hold the scalp down firmly with the other hand. Approximately 50 to 100 hairs will probably be grasped and are extracted with a quick, forceful pull. The combination of holding the scalp down and the quick, forceful pull reduces the number of broken hairs considerably. In kwashiorkor, considerably less epilation force is needed due to localized edema.

We have not experienced difficulty in obtaining samples; the mother usually holds the child at the time of sampling and only rarely does a child cry. We find that parents are accustomed to the fact that hair changes occur with malnutrition and it is acceptable to them to examine the hair, particularly when this is done instead of taking blood.

The size of sample necessary is determined by sample variation. When we plotted standard deviation against the size of the samples, there was no advantage in a sample size of more than 15 roots in terms of reducing standard deviation. To get 15 usable roots, however, probably 50 hairs of Caucasian- or Asian-type hair will be needed and 100 hairs of Negroid hair will be required because the follicles of the latter type are different in form. Care should be taken to use slightly different scalp locations on successive samples. The samples are then placed in a paper envelope, such as a stamp-collecting envelope or even an ordinary correspondence envelope. It is convenient to have a rubber policeman or spatula to remove the hair roots from the forceps. Plastic bags are not as convenient in humid areas because the root ends tend to stick to the bag and become distorted when removed. If the root ends do stick to the bag, they should be floated off with water rather than pulled off. It is convenient later to transfer samples to small, wide-mouth, screw-top bottles. In storage trials, we have found that samples taken in humid areas do not change their morphological characteristics over a 4-month period provided they are not wetted. If wetted, the samples have to be examined within several days.

Examination of hair root specimens

Specimens are placed in a shallow Petri dish with just enough water to immerse them fully; this helps to visualize the morphological characteristics. Soaking in water also tends to remove any temporary "set" in the fiber due to storage. (Too much water results in depth-of-field problems with the microscope and permits excessive movement of hairs.) The specimens are examined at a power of between $\times 45$ and $\times 60$ in a dissecting microscope. The specimens are best seen if a top slanting light (45°) is used in conjunction with a standard student-type mirror light for transmitted light. Hairs tend to float on water and the use of forceps is recommended not only to make sure the root is immersed, but also to rotate the bulb and observe conformation of the root and to determine maximum bulb diameter.

Hair roots are first separated by growth phase.

Usually, 85 to 90% of the hair roots will be in a growing phase (anagen), although there is quite a wide range between persons. A shift to the resting phase (telogen) occurs partially in kwashiorkor, and almost totally in marasmus. This is probably more a function of chronicity than protein-calorie density, but it is a useful index of long-term stress. The anagen is characterized by its bell-shaped bulb structure and solid dark pigmentation. External root sheaths are usually present, but may be absent due to epilation trauma (Fig. 2). A telogen hair is shaped like a teardrop and is sometimes referred to as a club hair. It is distinguished from the anagen not only by shape but also by distinct lack of pigment (Fig. 3). An intermediate growth phase called catagen is similar to a telogen in appearance but has external and internal root sheaths. For diagnostic purposes, catagens are included with telogens.

Morphological changes do not occur in roots of telogen hairs inasmuch as they are mitotically and presumably metabolically at rest. Morphological changes are therefore classified only in the growing phase. Anagens are not always ideally formed, and there is some variation between persons. A variety of anagens from different individuals is shown in Fig. 4. The term dysplastic refers to hairs that are broken during epilation and cannot be classified according to growing or resting phase. It is use-

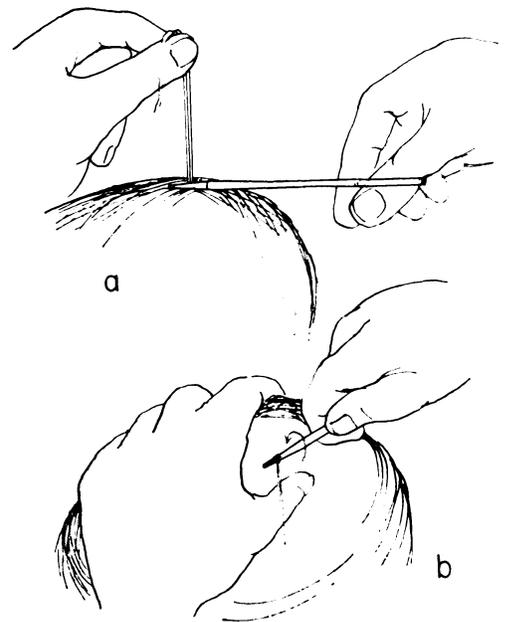


FIG. 1. Procedure used to obtain specimens. Hairs are lifted firmly enough to elevate the scalp slightly and are then grasped close to the scalp with needle-holding forceps. The scalp is held down and the sample taken with a quick, forceful pull.

ful to classify the percent anagen, telogen, and dysplastic hairs.

Classification of morphological changes in the growing phase

Bulb diameter and atrophy. The earliest consistent change in the bulb is a reduction in maximum diameter. Maximum bulb diameter is measured with a micrometer eyepiece. Usually two or three diameters are taken to insure an appropriate measurement. Sheaths are not included in the measurement.

Perhaps a more practical change to classify is the percentage of obvious bulb atrophy. An atrophied bulb is shrunken, often has a shriveled appearance, and shows a definite and considerable decrease in diameter. It is usually less in diameter than that of the shaft itself. Atrophied bulbs tend

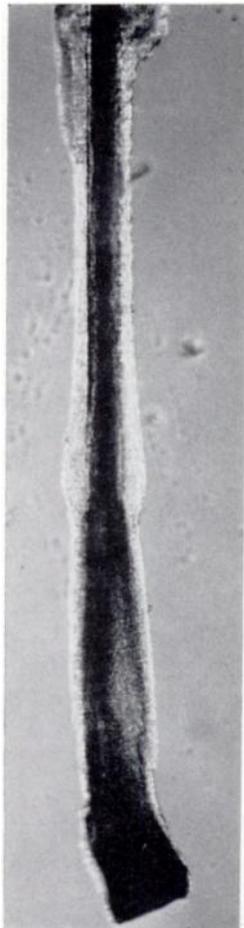


FIG. 2. The hair root in the growing or anagen phase. Note the bell-shaped bulb structure and solid dark pigmentation. Translucent sheaths are usually present.

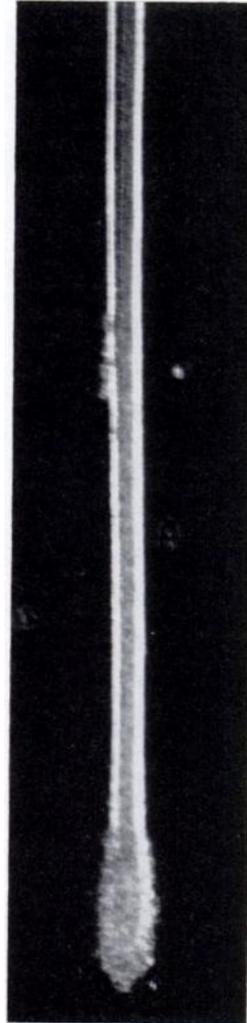


FIG. 3. The hair root in the resting or telogen phase. Note the teardrop shape, lack of pigmentation, and lack of sheath.

to be depigmented and have no sheath present. The shaft is usually depigmented as well. Care must be taken to differentiate between atrophied and twisted bulbs by rotating the specimen as shown in Fig. 5. Figure 6 compares photographically a normal bulb and atrophied bulbs from the same individual after 15 days of protein deprivation. A variety of atrophied bulbs is shown in Fig. 7.

Bulb fraying. Bulb fraying is a fringe-like appearance at the base. The fringed area is frequently dyspigmented and the sheath is rarely intact at the base. Care must be taken to distinguish between fraying and the often slightly irregular outline of an intact normal bulb. Care should also be taken



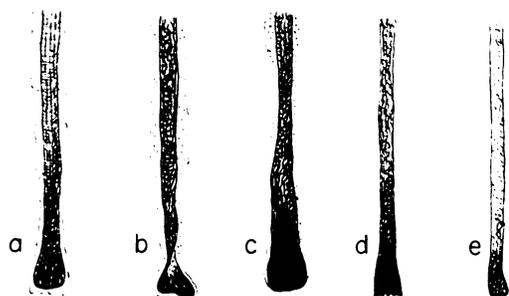


FIG. 4. A variety of anagens from different individuals illustrates the morphological differences found.

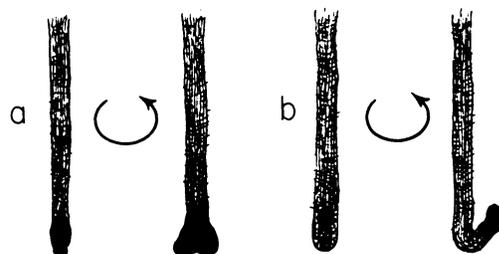


FIG. 5. Care must be taken to distinguish between atrophied and twisted bulbs by rotating the specimen.

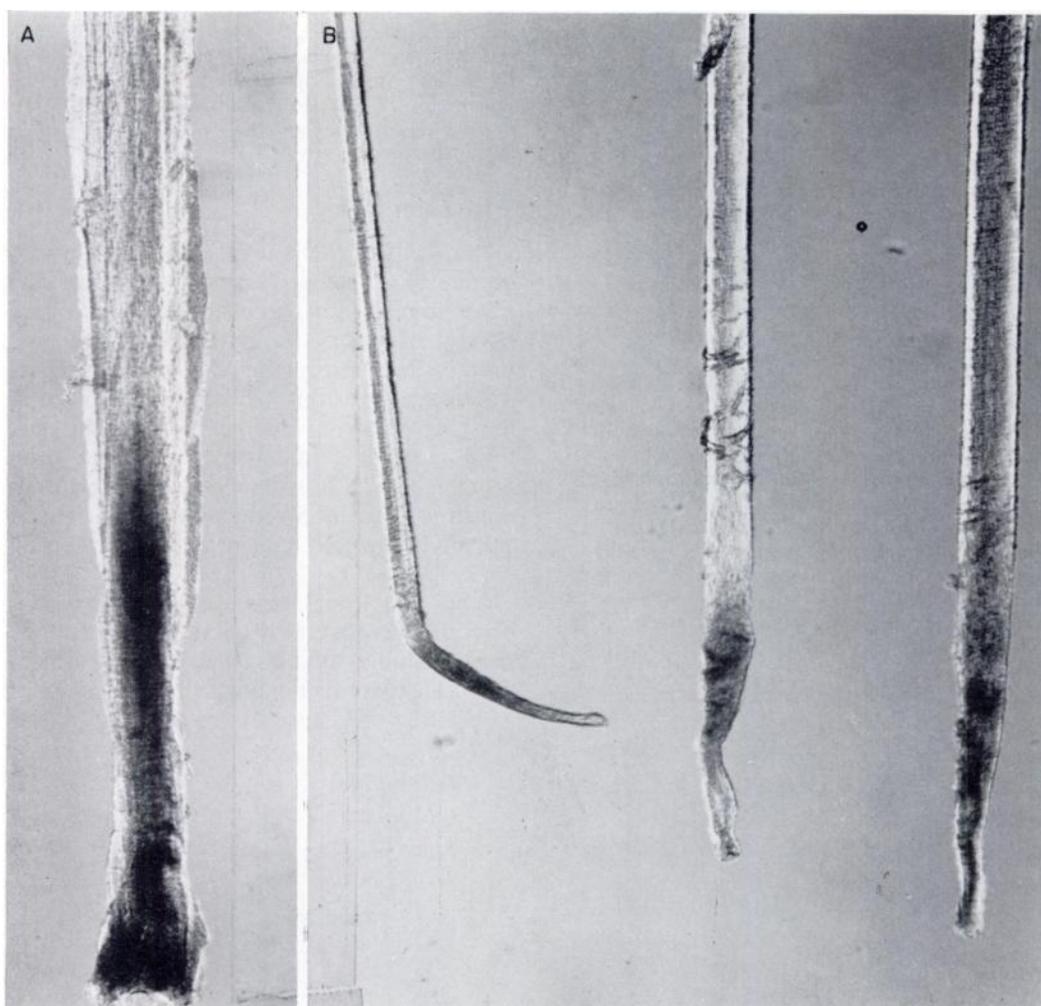


FIG. 6. Comparison of (A) normal and (B) atrophied bulbs in the growth phase. Samples taken from the same individual before and after 15 days protein deprivation. Note the reduction in bulb diameter, the loss of pigmentation, and loss of external and internal sheaths.

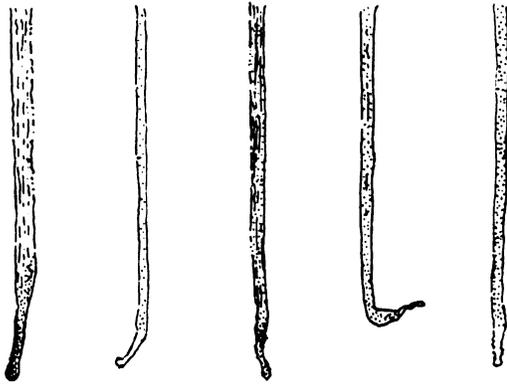


FIG. 7. A variety of atrophied bulbs from different individuals.

to distinguish between bulb fraying and sheath shredding at the base of an otherwise complete bulb.

Bulb dyspigmentation. The normal bulb is darker than the shaft; the pigment is usually evenly distributed. Dyspigmentation is a decrease of coloration that usually precedes atrophy. Bulb dyspigmentation, however, is a relative factor and its assessment is affected by the thickness of the bulb, ethnic differences, and the intensity of the supra-stage light used. Sometimes dyspigmentation appears speckled before the complete depigmentation seen in atrophied bulbs.

Sheath changes. The root and shaft are normally surrounded by a translucent sheath. The presence or absence of a sheath is to some extent affected by epilation, and completely normal bulbs may be found without sheaths. However, atrophied bulbs almost never have sheaths. An increase in the number of bulbs without sheaths usually accompanies atrophy. Sometimes the sheaths are partially present, and this usually takes two forms. The first is sheath fraying (or shredding). The sheath is normally smooth and has a regular outline. Sheath fraying occurs as a shredding that is particularly noticeable along the shaft. The other form of partial sheath disappearance is shortening

or "miniskirting" in which a complete sheath has been broken or peeled up from the bulb base, leaving the bulb bare at the bottom but the sheath complete at the top. Care must be taken to distinguish between a miniskirt and those sheaths that taper off close to the bulb but still continue around the bulb and are therefore intact.

Shaft changes. Slight shaft dyspigmentation is difficult to judge without standardization of lighting as it tends to be highly variable between persons. Marked dyspigmentation of the shaft, however, is easy to classify and usually accompanies bulb dyspigmentation. Shaft narrowing occurs after the bulb has diminished in size. As the child recovers, a normal diameter shaft is produced and a constriction appears as the affected area moves out along the shaft. This constricted area is frequently dyspigmented as well. When an area of the shaft is completely dyspigmented, it is frequently called the flag sign (*signo de la bandera*). Often hair breaks during epilation at the point of severe constriction of the shaft. A summary of the morphological changes that occur with protein deprivation are shown in Fig. 8.

Discussion

The cells of the hair matrix normally proliferate at a rate probably greater than any other tissue, with the possible exception of bone marrow (2), an indication of the high protein synthesizing activity of hair tissue. The majority of this synthesis takes place in the hair bulb (3) and the replacement time for the entire germinative matrix has been estimated to be less than 1 day. During malnutrition there is a reduction in the rate of cell division; also less protein is produced per cell.

The hair root technique described has been used in several types of studies, i.e., 1) clinical studies of marasmus and kwashiorkor, 2) experimental protein deprivation of

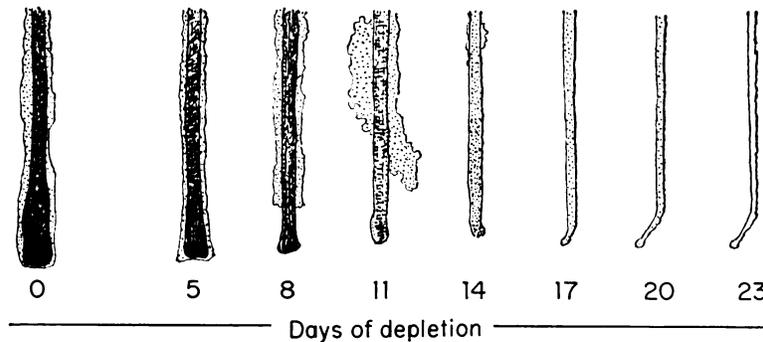


FIG. 8. Summary of the morphological changes that occur with time following protein deprivation.

young adult volunteers, and 3) nutritional status surveys of moderate malnutrition.

Hair root morphology of 13 Andean Indian children suffering from kwashiorkor has been compared with that of 13 healthy children of the same age in the same ethnic group. The bulbs of the healthy children were comparable in size with those of Caucasian children of the same age. In all the samples studied, well-formed and heavily pigmented anagens were present in normal quantity ($66 \pm 6\%$); the bulbs were not atrophied, and the mean diameter was $18 \pm 0.7 \times 10^{-2}$ mm. The remainder of the bulbs were in the telogen phase ($10 \pm 3\%$), with the number of dysplastic hairs being $25 \pm 5\%$ of the total sample; internal and external sheaths were largely intact (100% and $60\% \pm 10\%$, respectively). The hair of children with kwashiorkor exhibited strikingly different physical characteristics. Instead of the usual coarse, lustrous black hair associated with this ethnic group, the hair was fine-textured, dry, and lifeless in appearance. Dysplastic hairs were common and many appeared to have broken at a constriction in the shaft. Examination of the roots revealed many differences from those of normal children, i.e., the number of anagens found was significantly less than normal ($26 \pm 6\%$) ($P < 0.01$), and those that were present were abnormally formed with severe atrophy and shaft constriction immediately distal to the bulb. In most cases there was marked pigment depletion in the anagens, many of which exhibited a speckled appearance. The extent of atrophy is indicated by the mean bulb diameter of $7 \pm 0.4 \times 10^{-2}$ mm, one third of normal values ($P < 0.01$). The number of bulbs in the telogen phase increased significantly to $45 \pm 5\%$. The number of dysplastic hairs was similar to the normal samples ($29 \pm 6\%$). There was a significant loss of both internal and external sheaths present (53 ± 8 and $36 \pm 7\%$, respectively). Hair root samples were also taken during several stages of recovery. The clinical and hair root response to treatment varied greatly with the individual according to the severity of protein deprivation and the relative importance of concurrent gastrointestinal and respiratory infection. From successive sam-

TABLE 1
Suggested standards

1) Maximum mean bulb Diameter, mm $\times 10^{-2}$	= >11 Normal 6-11 Moderate PCM <6 Severe PCM
2) Atrophy, % of anagen	0-25 Normal 26-50 Moderate PCM >50 Severe PCM
3) Anagens, %	>50 Normal 30-50 Moderate PCM <30 Severe PCM
4) Telogens, %	<20 Normal 20-45 Moderate PCM >45 Severe PCM

The above classifications are suggested as tentative standards for preschool children. They have been developed from studies with children with Asian and Negroid hair types.

Changes in bulb fraying and dyspigmentation and sheath alterations have been described but standards have not been suggested due to the difficulty of separating traumatic from nutritional insult.

ples in each child, however, several observations could be made that indicate the sequence of events during protein repletion. The shift to the growth phase appeared to be rapid, because after 6 weeks no sample exceeded 4% telogens. During the first 2 months after admission, a number of atrophied anagens were found and there was still fraying of the bulbs. As the bulbs regained normal size and form, the pigmentation remained speckled in appearance instead of the normal solid dark appearance. After about 3 months of dietary and medical treatment, the bulbs were well-formed and heavily pigmented and the sheaths were complete. The bulbs were not yet normal, however, because twice the normal number of dysplastic hairs was found, and stretched and hooked shafts continued to appear. In several cases hypochromotrichia persisted for 6 to 12 months after discharge from the hospital. We found little relationship between hypochromotrichia and serum albumin levels during either acute or recovery stages (4, 5).

Hair root morphology studies have also been carried out with cases of classical marasmus (6). Although kwashiorkor is the more spectacular form of protein-calorie mal-



nutrition, marasmus is a more significant public health problem in many tropical developing areas in terms of incidence and severity. The relative importance of marasmus is probably grossly underestimated. The most striking change in the hair roots of children with marasmus was the almost complete absence of bulbs in the anagen (growing) phase. In 8 of the 15 cases studied there were no anagens present in the samples. In four of the remaining cases, less than 1% of the bulbs were in the growing phase. The few anagens that were present were abnormally formed, with almost complete atrophy and shaft constriction. The extent of atrophy in the few anagens present is indicated in the mean bulb diameter of $6 \pm 1 \times 10^{-2}$ mm. In all cases there was a marked pigment depletion in the anagens, many of which exhibited complete dyspigmentation. There was a highly significant shift to the telogen (resting) phase ($60 \pm 7\%$). Dysplastic hairs comprised nearly one-half of the total sample (46%) and many appeared to have broken at a constriction in the shaft. In 14 of the 15 cases no complete external or internal sheaths were observed.

In contrast to the kwashiorkor children studied previously, the marasmic children exhibited a striking and highly significant shift to the resting phase of hair growth, as demonstrated both by the almost complete lack of bulbs in the growing phase and by a manyfold increase in the number of bulbs in the resting phase. There was a 50% increase in the number of broken hairs over that found previously in children with kwashiorkor. The finding of no complete sheaths in 14 of the 15 marasmic children is in sharp contrast to the samples from children with kwashiorkor in which more than one-third of the bulbs had complete external sheaths and more than one-half had complete internal sheaths. The results suggest a physiological adaptation to chronic insufficient calorie and protein intake by a complete shift to the resting phase of hair growth. This re-establishment of priorities reduces the amount of nitrogen loss that would otherwise occur if the roots remained in the growing phase. The differences found in the hair root morphology between marasmus and kwashiorkor are

probably due more to comparative differences in chronicity than to specific differences in relative protein-calorie density. Classical marasmus is a severe chronic undernutrition in which the child adapts to the stress by failing to grow. The long-term effect on the hair follicle is that it shifts to the resting phase and conserves nitrogen. In classical kwashiorkor, there has been a period of more normal growth that has been interrupted by an acute condition. Linear growth may continue as the hair follicle adapts to this stress by both atrophy of bulbs already in the growing phase and a partial shift to the resting phase. The fact that defensive physiological adaptations to the calorie and protein stress of marasmus also takes place in hair roots is of interest because hair is a readily accessible tissue (6).

Although the cases reported were limited to classical cases of kwashiorkor and marasmus, studies of malnutrition of hospital admissions leave a great deal to be desired from the standpoint of controlling and isolating the specific effects of malnutrition, because 1) the children are studied on admission and differences in staff and treatment are inevitable with time, 2) differences in the type and amount of concurrent infection and infestation affect both the treatment and recovery rate, and 3) other nutritional deficiencies may complicate the situation further.

To be able better to control conditions of initial nutritional status and clinical conditions, we then carried out studies in the metabolic ward. The subjects were young adult males, aged 24 to 29 years, who were in good physical condition. They were fed a liquid diet complete in all known nutrients. The protein deprivation diet was prepared by the isocaloric substitution of dextro-maltose for egg albumin.

In the first study, eight volunteers were fed the protein deprivation diet for 15 consecutive days. Consistent morphological changes occurred in the hair roots during this period; there was a significant reduction in bulb diameter ($P < 0.05$). The hair roots exhibited severe atrophy and decreased pigmentation. The external root sheaths were consistently absent in atrophied bulbs. Marked atrophy of the bulb was observed in



one-half the total sample in the anagen phase for each subject after protein deprivation. When protein was again added to the diet (7, 8), the changes were reversible to some degree. A second study of six young men confirmed these findings. Consistent and significant hair root morphological changes were evident at 11 days of protein depletion. These included bulb diameter reduction, atrophy, dyspigmentation, and sheath absence but the growth phase of the hair did not change. Urinary nitrogen values reached minimal values by 11 days and serum protein and albumin levels remained normal. (When protein was added to the diet, the hair root changes were partially reversed.) The sequence of root changes commenced with bulb depigmentation occurring as early as bulb diameters began to reduce, but with wide in-person variation. Next, sheath abnormalities occurred when there was substantial root diameter reduction, but prior to atrophy. However, sheath loss is also found in otherwise normal bulbs, due to epilation techniques, limiting the usefulness of this change. Bulb atrophy appeared to be a useful morphological sign because it is uniformly progressive, easy to assess, and shows less person-to-person variation than other measures. During protein repletion, mean bulb diameters tended to return to normal size. The fact that the mean bulb diameter responds promptly to protein deprivation and reverts to normal early in protein feeding suggests that it is a sensitive indicator of body protein status (9).

Bulb diameter is a more sensitive index of protein deprivation than is percentage of atrophy. Reduction in diameter of the well-pigmented bulb occurs before pronounced atrophy. Nevertheless, percentage of gross bulb atrophy can be assessed more rapidly than mean bulb diameter and occurs to such an extent that, in our work with malnourished children, it has been a useful field index. In general, bulb parameters respond to protein deprivation both more quickly and with less in-person variation than either sheath or shaft parameters.

The striking changes in the morphology of the hair root described in children admitted to a hospital with acute kwashiorkor and

marasmus and young men fed diets free of protein showed not only that the morphological changes described are due to protein deprivation, but also that they occur while serum albumin levels are still normal. We then tried to find out whether such morphological changes would occur early enough and regularly enough in preschool children with mild to moderate malnutrition to be of value in community health programs.

The most commonly used criterion for the indication of malnutrition in preschool children is a reduction in the normal weight-for-age relationship. We therefore compared the depression of this ratio with the degree of change from normal hair root morphology in 72 West Indian children of African descent. The sample included positive and negative controls, 15 healthy controls (with normal weight) and 12 clinical cases of protein-calorie malnutrition with a weight-for-age ratio less than 60% of normal. The children in both of the control groups were of the same age and ethnic group and from the same Caribbean island.

The most obvious of several morphological characteristics was the reduction in mean root diameter. The analysis of variance revealed a highly significant ($P < 0.01$) reduction in mean root diameter with reduction in weight-for-age. The samples were then grouped by weight-for-age and the least significant differences method used to ascertain at what weight-for-age class the reduction in mean bulb diameter was significantly different from normal values. There was a significant reduction in mean root diameter ($P < 0.01$) from the normal controls in the 81 to 90% weight-for-age group (10, 11).

The morphological changes occurred early enough and regularly enough to be used to differentiate between normal and moderately malnourished children. Under the conditions of this study, objective measurements of hair root diameter alone could not be used to classify the various levels of weight depression suggested by Jelliffe (1). Subjective classification of the incidence of certain abnormalities (bulb fraying, bulb dyspigmentation, incomplete outer root sheaths) helped to assess the relative severity of protein-calorie

malnutrition. The correlation between reduction in weight-for-age and reduction in mean root diameter was high ($r = 0.96$) (12).

In a second public health evaluation biochemical, hair tissue, and anthropometric methods used for the early recognition of malnutrition were compared simultaneously in 179 preschool children living in the Guatemalan highlands. Decreases in hair root diameter and urinary urea/creatinine ratio were significantly related as early indicators of inadequate protein intake. Increased hair-root atrophy was significantly related to changes in the ratio of nonessential/essential amino acids in sera and also to depressed weight-for-height as later indicators of PCM (13).

The soluble protein and deoxyribonucleic acid (DNA) content of hair roots has been studied in relation to protein stress. The DNA/hair root was found to correlate highly with the protein content. Hair root volume and protein content were directly proportional ($r = 0.9$) (14, 15). The results of these studies suggest that the response of hair root to protein-calorie malnutrition is one of reduction in size and cell number, a phenomenon that has been noted experimentally in other tissues such as intestinal mucosa. The fact that hair root DNA and soluble protein correlate well with cell size is biochemical evidence for something that has already been shown morphologically. It would appear that the morphological measurements are easier to perform in the field.

Comparisons among tests of PCM suffer from the lack of an accepted predictor of malnutrition against which each one can be compared. The interpretation of prevalence of cross-sectional surveys is further complicated by a number of factors. The tests used may show varied responses to different types and severity of nutritional stress. Some reflect nutritional changes more rapidly than others. Also the nutritional insult may be chronic or acute and its timing in relation to the time of survey will influence test response. Many of the tests probably change in a curvilinear rather than a linear fashion due to homeostatic mechanisms. Infection and infestation synergistically affect nutrition status. Interpretation of cross-sectional stud-

ies is therefore complicated. Age-independent measurements are useful because in many areas birth dates have little social significance and cannot be verified by documentation.

The method cannot be regarded as one of quantitative assessment for nutritional status. In this regard it is similar to other methods being used for the assessment of nutritional status. However, the ease and speed of sampling, the simplicity of examination, and possible use of the method under adverse climatic and other physical conditions make it more attractive for lesser developed tropical areas.

It should be emphasized that the method is primarily intended for assessing nutritional status of groups, rather than individuals. As in all such tests, there will be a range of variation both in normal and abnormal sets of individuals. In public health practice, this test could be helpful in locating those segments of the population who are most likely to be malnourished.

Summary

A tissue test is proposed for the field assessment of the incidence and severity of protein-calorie malnutrition. Morphological changes in hair roots are described, classified, and standards suggested. This screening test is particularly applicable to rural tropical areas where high environmental temperatures and humidity and irregular electrical power may preclude the use of more commonly used techniques. The results of the applications of this technique are discussed in connection with clinical cases of classical kwashiorkor and marasmus, in the field assessment of PCM under tropical conditions, and in controlled studies of experimental protein deprivation in the metabolic ward. 

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