quantitative studies, such as those with beta liquid-scintillation measurements, aged ^{3}H-thymidine (as exemplified here by the irradiated ^{3}H-thymidine) could give spurious results. In all such studies, parallel samples must be digested with deoxyribonuclease to rule out a count from unspecific incorporation. With qualitative studies, such as those with autoradiography, the results could be equally misleading. Where just macronuclear labeling is being observed, the use of aged ^{3}H-thymidine might merely decrease the sensitivity of the autoradiographic method. We have used ^{3}H-thymidine with a storage life of 1 year in our autoradiographic experiments and there was only slight change in sensitivity and overall results (*9*). Where cytoplasmic incorporation is being observed, as with many recent studies on cytoplasmic DNA (*10*), the importance of using freshly made ^{3}H-thymidine and digestion controls is stressed.

Although our observations emphasize the possible misleading results which could be obtained with radiochemically impure tritiated thymidine as a tracer, it is equally true that similar erroneous interpretations are possible by the use of other radiochemically impure, labeled compounds. Such misinterpretations are perhaps especially possible in cytological investigations where the results are visualized by autoradiography, and where only a small fraction of the label used is actually incorporated into the cellular structures. Any selectivity of uptake of the impurities by the cell could indeed produce some very misleading results.

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Morphological Changes in Human Scalp Hair Roots during Deprivation of Protein

Abstract. *Human subjects were deprived of protein for 15 days, after which time hair from the scalp of each subject was plucked and examined. Both the bulb and the external root sheath showed morphological changes. This technique may therefore be useful in diagnosing protein-calorie malnutrition.*

Human hair color (*1*) and texture (*2*) change during protein-calorie malnutrition. The cells of the hair matrix normally proliferate at a rate probably greater than any other tissue with the possible exception of bone marrow (*3*), an indication of the high protein-synthesizing activity of hair tissue. The majority of this synthesis is carried on in the hair bulb (*4*). Van Scott (*5*), using histogeometric analysis, estimated replacement time of the entire germinative matrix to be less than 1 day. Fiber growth rate of Merino sheep has been shown to vary with differences in protein quality as well as quantity (*6*). These facts, coupled with the known high mitotic activity of the hair bulb (*7*) and resultant high protein requirement, suggested that hair root activity may be useful in diagnosing protein-calorie malnutrition.

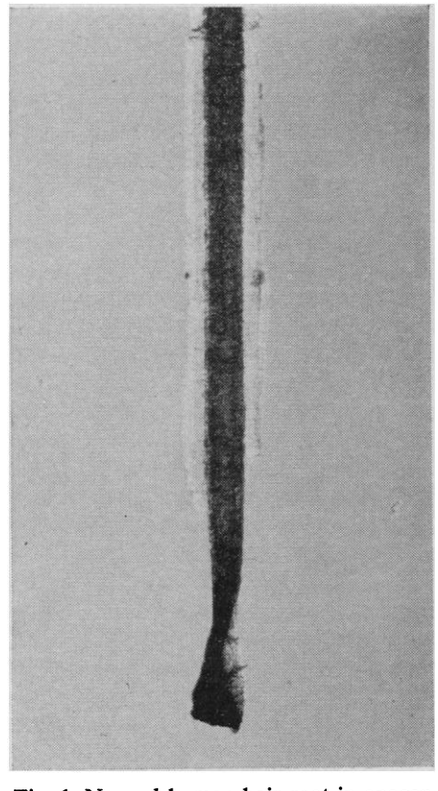

Fig. 1. Normal human hair root in anagen growth phase (× 75).

Eight male subjects in excellent physical condition, aged 24 to 29, were housed in a research ward for more than 3 months. Physical and biochemical examination showed that they remained in good health throughout the study. The subjects were fed a liquid formula diet (*8*) three times daily which provided either 75 g protein per man per day in the form of egg albumen, or no protein. In the latter case Dextri-maltose was substituted isocalorically for the egg albumen. The diet remained at 2800 calories per man per day throughout the study. None of the pathological factors causing telogen effluvium as reported by Kligman (*9*) or Van Scott (*10*) were present, nor were there histories of x-ray therapy or use of cancer therapeutic drugs. At the end of each 15-day experimental period approximately 100 hairs were manually plucked from the occiputal area of each subject with Tygon-coated needle-holding forceps (*11*) and examined in a dissecting microscope (× 45). Care was taken to use slightly different locations on the scalp in successive samples. The fast epilation technique described by Maguire and Kligman (*12*) was used to minimize dysplasia as a function of technique. To avoid bias, both protein-deprived and control subjects were sampled blind at the same time by the same investigator. The morphological changes were observed more clearly with suprastage cross light than with transmitted light. Bulb diameters were measured with an ocular micrometer. In most cases samples were taken during two complete dietary periods and represented a total of 21 samples (11 before, 10 after).

The 15-day periods of protein deprivation were too short to show changes in the ratio of the anagen (active) phase to the telogen (resting) phase. However, consistent morphological changes did occur after the subject was deprived of protein. (i) The hair

bulb exhibited severe atrophy and decreased pigmentation. The atrophied bulbs ranged from 0.025 to 0.075 mm in maximum diameter compared with a range of 0.250 to 0.550 mm before protein deprivation. (This measurement did not include the root sheaths.) The atrophied bulbs manifested distal constriction which extended for a short distance along the shaft. (ii) The external root sheath was consistently absent in atrophied bulbs (Figs. 1 and 2). (iii) The internal root sheath was frequently absent as well.

The marked atrophy of the bulb was observed in one-half of the total sample in the anagen phase for each subject after protein deprivation (range 48 to 65 percent). Care was taken to differentiate between twisted and atrophied bulbs by rotating specimens. Many of the bulbs in the anagen phase which did not atrophy after the subject was deprived of protein had a distinct pinch constriction distal to the bulb. Intrafollicular hair shaft breakage at epilation was considerably more frequent after protein deprivation [before deprivation (mean) $\bar{X}$, 41 percent; after deprivation $\bar{X}$, 77 percent]. Hair samples were taken for chemical analysis 25 days after the root samples in order to obtain hair produced during the deprivation period. Some shaft constriction was noted as a result of the short-term protein deprivation of the subjects but less than that which we have observed with children suffering from acute protein-calorie malnutrition (*13*) or that which has been reported by Van Scott (*11*) with the use of cancer chemotherapeutic drugs.

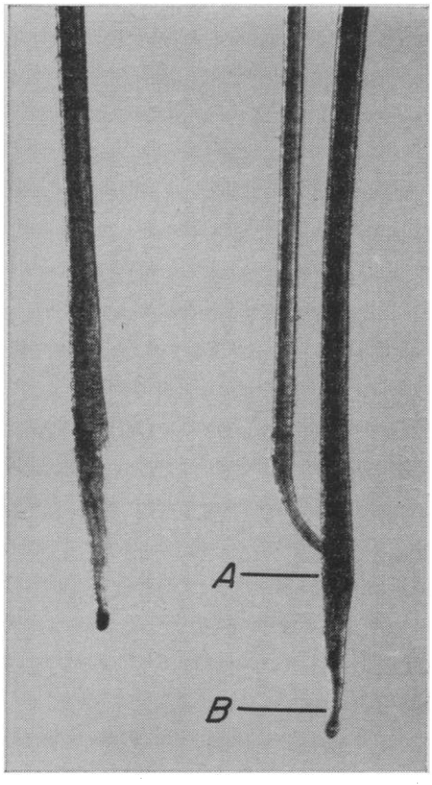

Fig. 2. Hair root of the same subject after 15-day protein deprivation (× 75). (*A*) atrophy of external root sheath; (*B*) marked atrophy and loss of pigment of the bulb.

The morphological changes between the samples taken before and after protein deprivation were consistent in all subjects, although the amount of change varied with the individual. These changes occurred in short-term protein deprivation of normal healthy subjects in the absence of decreases of serum protein, hemoglobin, and hematocrit, and were reversible to some degree when protein was again added to the diet.

Hair root examination may prove to be a useful tool in public health programs as an inexpensive and simple diagnostic aid in protein-calorie malnutrition, particularly in developing areas where more sophisticated equipment is not readily available and where tissue samples must be transported under adverse conditions of temperature and humidity.

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Langerhans Cells: Uptake of Tritiated Thymidine

Abstract. *After irradiation of the skin of the rhesus monkey with ultraviolet light, there is temporary suppression of the proliferating activity of the epidermal cells, followed by some hyperplasia and a striking increase in synthesis of DNA by Langerhans cells. Since Langerhans cells can proliferate, they can no longer be considered to be worn-out melanocytes.*

In spite of the many varied approaches of studies, the function and genealogy of a population of dendritic cells interspersed in the higher level of the epidermis still remain unknown. Discovered by Langerhans (*1*) in 1868 and named after him, these cells were first demonstrated by treating the skin with an acid solution of gold chloride. Since this stain has a particular affinity for nervous tissue, the impregnated cells were considered to be nervous receptors (*2*) derived from cells located in the dermis (*3*). Assuming that they represent terminal stages of previously mature melanocytes or desquamating, effete, pigment cells, investigators have generally included the elusive Langerhans cells with the melanocytes (*4*). No one, however, has conclusively shown activities in Langerhans cells, and evidence for the thesis that they are wornout epidermal melanocyte is circumstantial.

We now show that, after irradiation of the skin with ultraviolet light, Langerhans cells incorporate tritiated thymidine. This fact suggests that Langerhans cells synthesize DNA and that probably they are not effete melanocytes.

Three areas of the chests of three rhesus monkeys were irradiated (*5*) for 3 seconds with a flux of 7×10^5 erg sec^{-1} cm^{-2}; 1, 2, and 3 days later, 1.5 microcuries of tritiated thymidine (^{3}HT; specific activity, 6.7 c/m*M*; New England Nuclear Corp.) was injected intradermally at each site irradiated. For a control, a similar amount of ^{3}HT was injected intradermally in adjacent unirradiated skin of the chest. Biopsy specimens obtained from the irradiated and control areas 1 hour after the injections were fixed in 10-percent formalin and stained by the gold chloride technique of Gairns (*6*). After embedment in paraffin the sections were cut at 6 μ, freed of paraffin, rehydrated, and dipped in NTB_2 Kodak emulsion. The dipped slides were ex-