THE GROWING HAIR ROOTS OF THE HUMAN SCALP AND MORPHOLOGIC CHANGES THEREIN FOLLOWING AMETHOPTERIN THERAPY*

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The hair roots of man, as in most animals, cyclically pass through periods when hair is actively produced (anagen) and periods of total mitotic quiescence (telogen). In many animals all the hairs of the pelage synchronously pass from one phase of the cycle to the other. In man, however, the growth pattern is a mosaic one wherein the state of activity of any one hair root is completely independent of that of neighboring hairs (1, 2, 3). Hence all stages of the growth cycle are simultaneously found in the pelage of man.

The growing hair root of the human scalp, producing approximately 0.35 mm. of hair shaft daily (4, 5), is a tissue of high metabolic and mitotic activity. Hair loss as a sequela to toxicity induced by drugs or disease is evidence that the hair root is sensitive to metabolic or mitotic interferences.

Folic acid antagonists used chemotherapeutically, such as aminopterin and amethopterin, have certain undesirable side effects. Among the most serious of these phenomena is depression of hematopoiesis, reflected by a decrease of cellular elements of the circulating blood. A less serious phenomenon is interference with the growth of hair on the scalp, the result of which is marked loss of hair. The mechanism of this hair loss is unknown.

The present report describes a technic for examining hair roots. Microscopic changes in the hair root following amethopterin (methotrexate) therapy are described.

THE METHOD AND MATERIAL

Human hair was pulled from the parieto-occipital scalp by means of a surgical needle-holding forceps, the jaws of which had been covered on the grasping surfaces with one to several layers of cellulose tape to insure a tight closure and prevent slipping of hair shafts during the pulling procedure. Approximately fifty hairs were simultaneously grasped close to the surface of the scalp and extracted with a quick, forceful pull. The proximal ends of the extracted hairs were cut off and dropped into a 5.5 cm. x 1.5 cm. glass petri dish, the bottom of which was covered with water. A grid pattern of 16 divisions had previously been scored on the underside of the petri dish. The root ends of at least 100 hairs were thus obtained. Aggregates of hairs in the water were dispersed by means of a dissecting needle. In order to minimize movement of the hair roots on the grid, two 24 mm. × 40 mm. cover glasses were floated on the surface at right angles to each other and the excess water removed with an eye dropper.

The hair roots were examined with a dissecting microscope under 20× and 45× magnification, using transmitted light subdued by covering the sub-stage mirror with a sheet of lens tissue.

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Hair roots were obtained from staff physicians, laboratory personnel, and patients hospitalized for neoplastic diseases. Data obtained from microscopic examination of the hair roots included the number of roots present, the number in anagen phase of the growth cycle, the number in telogen phase, and the number in catagen (involutional phase between anagen and telogen). The hair roots of patients receiving amethopterin therapeutically were examined before administration of the drug and at intervals thereafter to detect morphologic changes which might occur.

RESULTS

Differential counting of types of hair roots. Roots of hairs pulled from the human scalp may be identified as either anagen (growing) or telogen (resting) when viewed microscopically with transmitted light. An actively *growing* (anagen) root was characterized by a darkly appearing keratogenous zone, immediately distal to the hair bulb; melanin pigment usually was seen within the matrix of the bulb; the internal root sheath and external root sheath were either present and intact, partially present, or absent (Figures 1, 2, 3, 4). A *resting* (telogen) root had no keratogenous zone, and it usually had no melanin pigment. Although the resting root had no internal or external root sheath, its club-shaped keratinized tip was surrounded by an epithelial sac (Figures 7, 8). Hair roots in transition from anagen to telogen (catagen) could be identified by the fact that they possessed both internal and an external root sheaths, as well as a keratinizing bulb (Figures 5, 6).

These criteria permitted differential counting of the three types of hair roots described, and the number of anagen hairs was expressed as a percentage of the total number of roots present. The remaining hair roots comprised preponderantly telogen hairs, since catagen hairs were rather uncommonly seen.

Reliability of the method. To determine the limits of accuracy of a single differential counting of hair roots repeated examinations were performed on nine patients hospitalized for neoplastic diseases at a time when they were receiving no chemotherapy for their disease and no other therapy which is known or suspected to affect hair growth. In each patient one hundred or more scalp hair roots were extracted and examined at intervals of one week or less. In each patient the percentage of growing hairs found on the first examination and that found on the second examination made up a pair for comparison; similarly the percentage found on the second examination and that of the third examination comprised another pair; the third in turn was compared with the fourth, etc. The percentages of each pair were averaged and the error of either percentage expressed as $\pm$ the percentage points which either deviated from this average (mean). In twenty-five such paired determinations the deviation from the mean ranged from 0 to $\pm$ 4.5 percentage points; the average deviation and median deviation were $\pm$ 1.5 and $\pm$ 1.0 percentage points respectively.

Range of proportion of growing hairs in scalps of normals. Examination of the roots of scalp hairs from sixteen healthy staff physicians and laboratory workers who had no discernible diseases of the scalp was performed. This group consisted of 11 males and 5 females; fifteen were white, one was Negro; their ages ranged from 18 to 38 years. The proportion of growing hairs ranged from 63%–96%

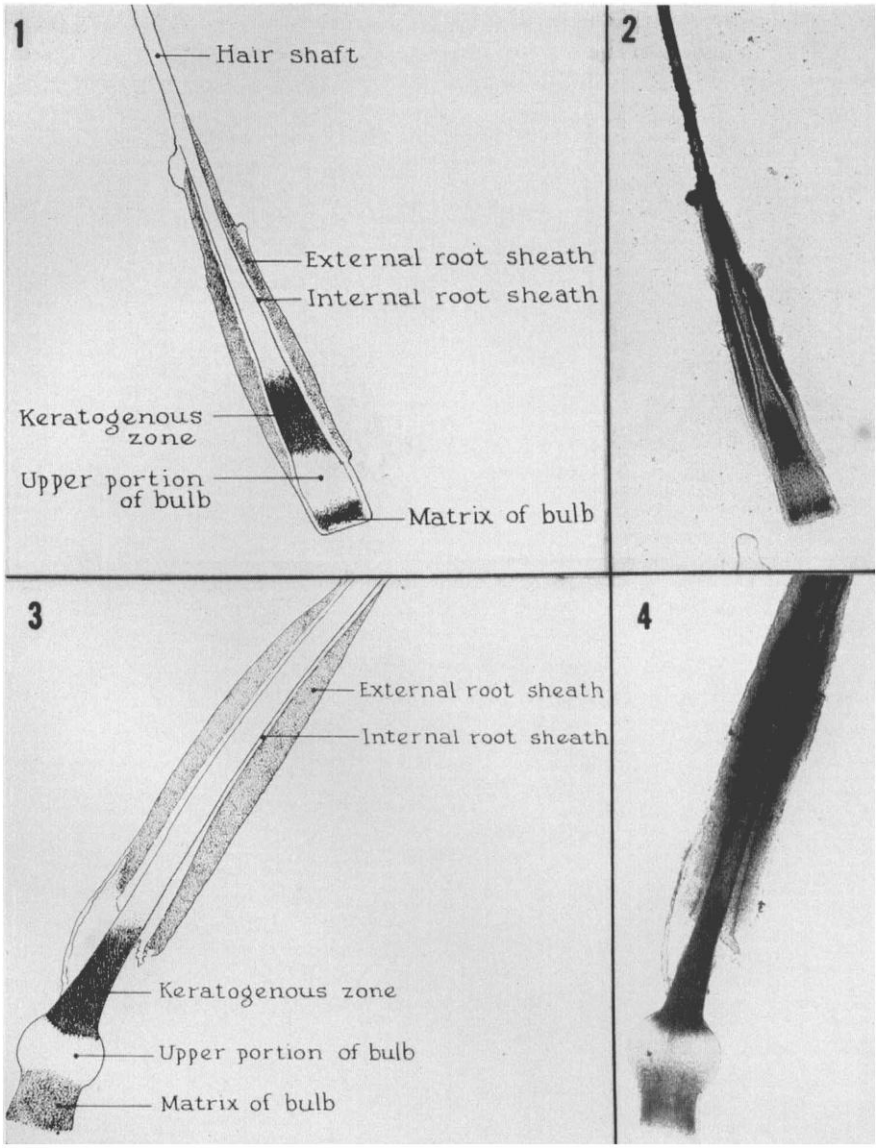

FIG. 1 (sketch) and FIG. 2 (photograph). Normal anagen (growing) root of scalp hair. ×17.

FIG. 3 (sketch) and FIG. 4 (photograph). Normal anagen root of scalp hair without bulbar portions of external and internal sheaths. ×41.

(Table 1). No correlation of age or sex to proportion of growing hairs could be made in this group.

Range of proportion of growing hairs in scalps of patients with neoplastic diseases. The roots of scalp hairs from twenty-four white patients with neoplastic diseases were examined. The ages of these patients ranged from 4 years to 89 years. Nine

were female, fifteen were male. Ten had a diagnosis of acute leukemia, 3 had a diagnosis of melanoma; there were 2 cases each of Hodgkin's disease, carcinoma of breast, and carcinoma of the stomach; there was one case each of carcinoma of the prostate, lung, and skin, and one case each of chronic leukemia and chorio-

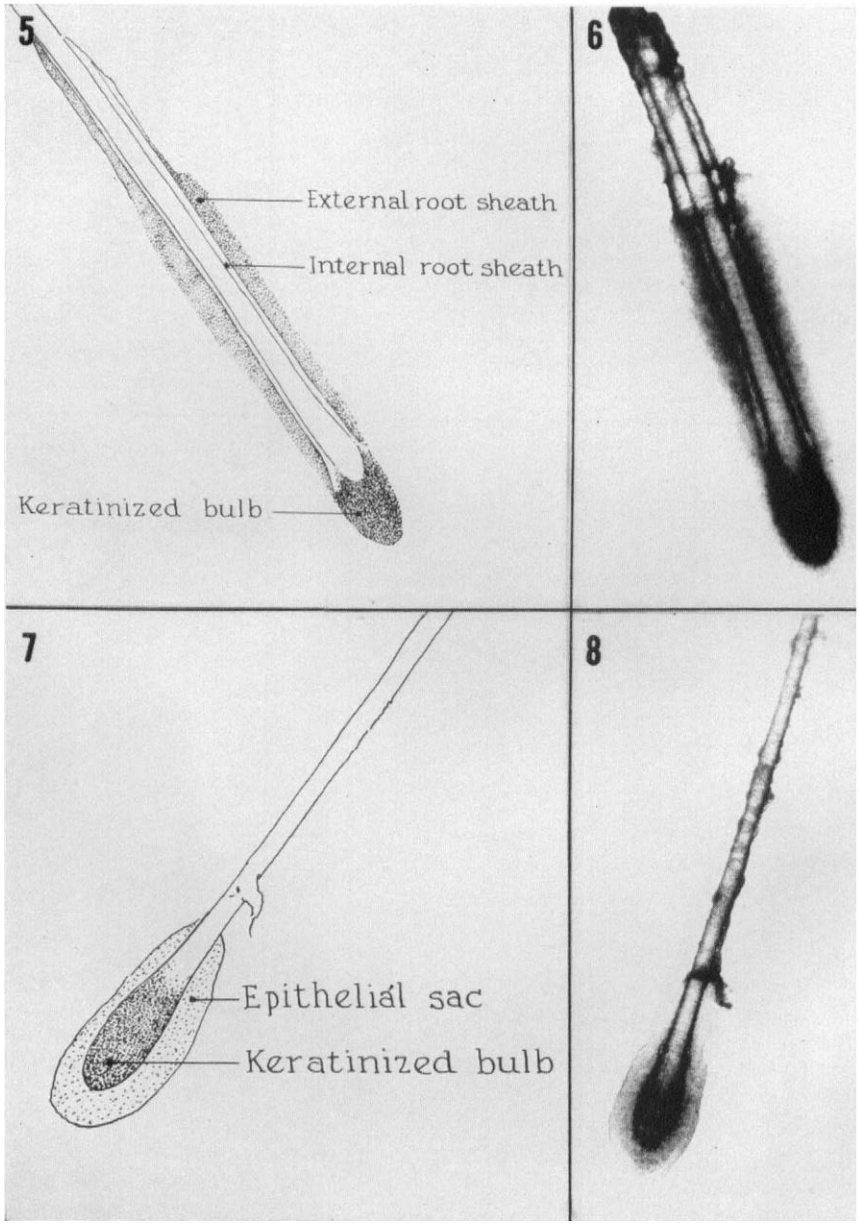

FIG. 5 (sketch) and FIG. 6 (photograph). Normal catagen (involuting) root of scalp hair. ×45.

FIG. 7 (sketch) and FIG. 8 (photograph). Normal telogen (resting) root of scalp hair. ×43.

carcinoma. The proportion of growing hairs found ranged from 24% to 98%; the median and mean were 92% and 77% respectively. In this group of patients no correlation of proportion of growing hairs with sex or diagnosis could be made. The range of the proportions of growing hairs was, however, greater than that found in normals.

Changes in proportion of growing hairs over periods of time in patients with neoplastic diseases. Repeated examinations of the roots of scalp hairs were done on eleven patients with neoplastic diseases during periods when these patients received no therapy which is known or suspected to affect hair growth. The patients were followed from 3 to 14 weeks; examinations of their hair roots were generally done once weekly. Comparison of the proportion of growing hairs found on the last examination with that of the first examination revealed that in one case the proportion of hairs remained unchanged; in four cases the proportion increased, the largest increase being 9%; in six cases the proportion diminished, the greatest decrease being 9% (Table 2).

Changes in the hair root following amethopterin therapy. All changes due to amethopterin were confined to anagen hairs and were the result of reversible atrophy of the hair bulb. These effects were observed in 10 patients who were given amethopterin intravenously in single doses varying from 2.0 mgm./kg.

TABLE I

Proportion of growing (Anagen) hairs in the scalp of normal individuals

Ages	# Females	# Males	% Growing Hairs		
			Range	Median	Mean
18–26	2	5	82–96	86	87
28–38	3	6	63–91	86	82
Total 18–38	5	11	63–96	86	84

TABLE 2

Changes in proportion of growing (Anagen) hairs in the scalp of patients with neoplastic disease

Patient	# of Examinations	Interval (days)	% of growing hairs		
			Initial	Final	Change
1	4	36	92	90	−2
2	7	28	77	68	−9
3	9	26	87	84	−3
4	4	24	93	99	+6
5	2	28	43	52	+9
6	2	26	24	32	+8
7	5	33	84	93	+9
8	7	44	94	89	−5
9	9	98	96	96	0
10	13	103	81	77	−4
11	4	20	85	77	−8

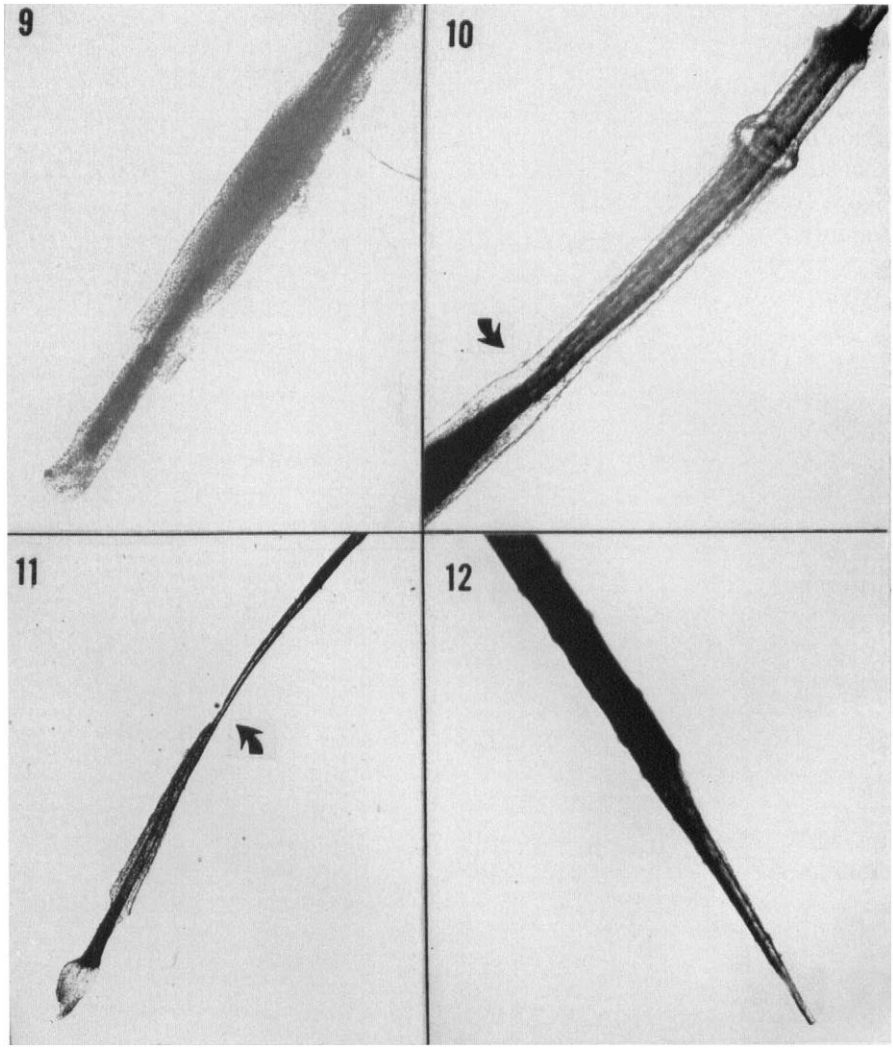

FIG. 9. Atrophy of bulb of scalp hair five days following amethopterin therapy. ×27.

FIG. 10. Constriction in shaft of scalp hair immediately above keratogenous zone eight days following amethopterin therapy. ×70.

FIG. 11. Constriction in distal portion of shaft of scalp hair fourteen days following amethopterin therapy. ×27.

FIG. 12. Hair broken at site of severe constriction in shaft induced by amethopterin. ×80.

body weight to 15.0 mgm./kg. body weight, and in five patients who were given the drug orally or intravenously in repeated daily doses which varied from 2.5 mgm./day to 30 mgm./day. The ages of the patients ranged from 2 years to 67 years.

Examinations of hair roots were performed before and after the institution of amethopterin therapy. Since changes were not expected to occur prior to one

week after therapy was begun, post-treatment examinations were not performed prior to this time in most cases. Changes were detected however in anagen hair roots of one patient five days following intravenous administration of the drug in a single dose of 4 mgm./kg. body weight. Changes were confined to the matrix portion of the bulb, the diameter of which was diminished (Figure 9).

Constriction of the hair shaft immediately distal to the keratogenous zone (Figure 10) was observed in three patients on the eighth, ninth, and tenth days after intravenous administration of amethopterin in single doses of 5 mgm., 8 mgm., and 15 mgm./kg. body weight respectively.

In all patients who received amethopterin intravenously in single doses, marked constrictions in the shafts of scalp hairs were seen two weeks following the administration of the drug (Figure 11). Many hairs were broken off by the extraction procedure at a point of severe constriction and had a characteristic appearance (Figure 12). Similar constrictions were observed to occur in the hairs of patients who were given amethopterin orally or intravenously in repeated daily doses and appeared two weeks or more after administration of the first dose.

Hairs which were broken off at the site of constriction were arbitrarily used as a criterion for severe effect of the drug on hair growth. The number of such hairs was established for each patient by differential examination of one hundred or more extracted hairs; the number of anagen hairs which did not break off at a constricted site was similarly established. The remaining hairs were telogen hairs and were morphologically normal. Such differential examinations were done repeatedly on five patients during periods of two weeks or more. The greatest proportion of broken hairs was found in a patient who received a large single intravenous dose of amethopterin (10 mgm./kg/body weight); on the 16th day after administration of the drug all hairs, excepting those with telogen roots, were found to be broken off at a site of severe constriction.

DISCUSSION

The morphologic changes in the roots and shafts of scalp hairs described above appeared in the absence of, or prior to, clinically evident spontaneous loss of hair. The changes varied in severity, the most marked occurring in a patient receiving one of the higher doses of amethopterin. Although the effect of amethopterin on reproduction of the cells of the hair bulb was severe the bulb rapidly recovered to continue producing a morphologically normal hair. Progressive atrophy of the hair bulb, as may be seen following ionizing radiation (6), did not occur. Hair loss resulting from amethopterin therefore occurs by breaking off of the hair at a site of constriction in the hair shaft, and not by falling out at the roots.

The presence and magnitude of these changes associated with amethopterin therapy might be utilized to differentiate between toxicity due to disease and toxicity due to drug, particularly in patients with leukemia where difficulties in making this differentiation are frequently encountered.

Growth-inhibiting effects of metabolic antagonists other than amethopterin

may also be detectable by morphologic changes induced in the hair root and may serve to further evaluate the anti-mitotic action of such compounds in man.

SUMMARY

1. Roots of manually extracted scalp hairs were examined microscopically and the proportions of growing (anagen) hairs to resting (telogen) hairs determined.

2. Most of the hairs in the scalps of normal adults were found to be in the growing stage.

3. The range in the proportions of growing hairs was found to be greater in patients with neoplastic diseases than in healthy individuals.

4. Anagen hair bulbs were found to become atrophic in response to amethopterin therapy and temporarily produced a hair shaft markedly diminished in diameter. The bulb recovered promptly following cessation of therapy, and again produced a hair shaft of normal diameter. This resulted in a focal constriction of the hair shaft.

5. Loss of hair following amethopterin is due to breaking of hair shafts at a constricted site, and is not due to hairs falling out at the roots.

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