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STUDIES OF COMMON BALDNESS IN THE STUMPTAILED MACAQUE

III. *DNA Synthesis in Regrowing Hair*

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Abstract. Bald and nonbald scalp areas of 2 adult stumptailed macaques were plucked at different intervals and injected with 3H-Thymidine for a study of DNA synthesis in regrowing hairs. Partial regrowth occurred in both bald and nonbald regions, with a delayed onset in the former. DNA synthesis occurred only in those plucked telogen hair follicles with well-developed secondary germs; statistical analysis of the quantitative data showed that it was significantly lower in regrowing hairs in the bald scalp. The delayed onset of plucked hairs in this region of the scalp correlates with the lesser DNA synthesis in the hair follicles of the bald scalp.

An earlier paper (11) reported the development of common baldness in the stumptailed macaque. Baldness in these animals is characterized not by a decrease in the number of follicles but by the transformation of hair follicles from terminal to vellus type. In the present work, we studied the uptake of 3H-Thymidine in hair follicle cells in bald and nonbald scalp regions before and after plucking. Since in the regrowing hairs of the guinea pig DNA is synthesized mainly in the matrix cells below the so-called critical level (2, 1), we focused our attention on this part of the follicle. We also compared the activity of transformed vellus hairs (10) with that of terminal hairs in the "hairy" regions of the scalp.

MATERIAL AND METHODS

The hairs from restricted areas of the bald foreheads and parietal-occipital regions of 2 adult male stumptailed macaques (8 to 10 years old) were thoroughly plucked. This procedure was repeated 25, 14, 7 and 3 days before biopsy specimens were excised (Fig. 1). One hundred μc of 3H-Thymidine were injected intradermally one hour before excision, each area receiving approximately 10 μc. Two nonplucked control areas in both bald and

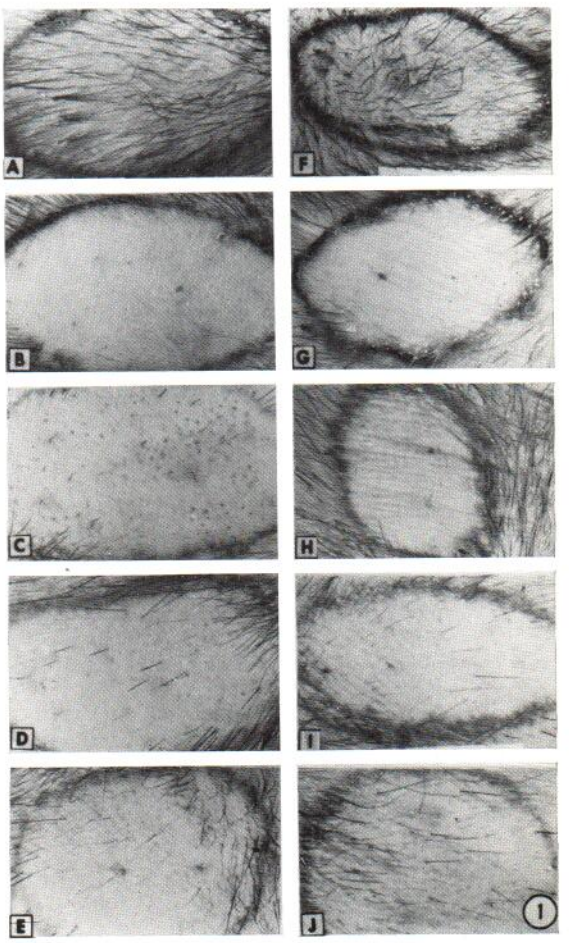

Fig. 1. (*A*) *Nonbald* scalp before plucking; (*B*) nonbald scalp 3 days after plucking; (*C*) 7 days after plucking; (*D*) 14 days after plucking; (*E*) 25 days after plucking; (*F*) *Bald* scalp before plucking; (*G*) bald scalp 3 days after plucking; (*H*) 7 days after plucking; (*I*) 14 days after plucking; (*J*) 25 days after plucking.

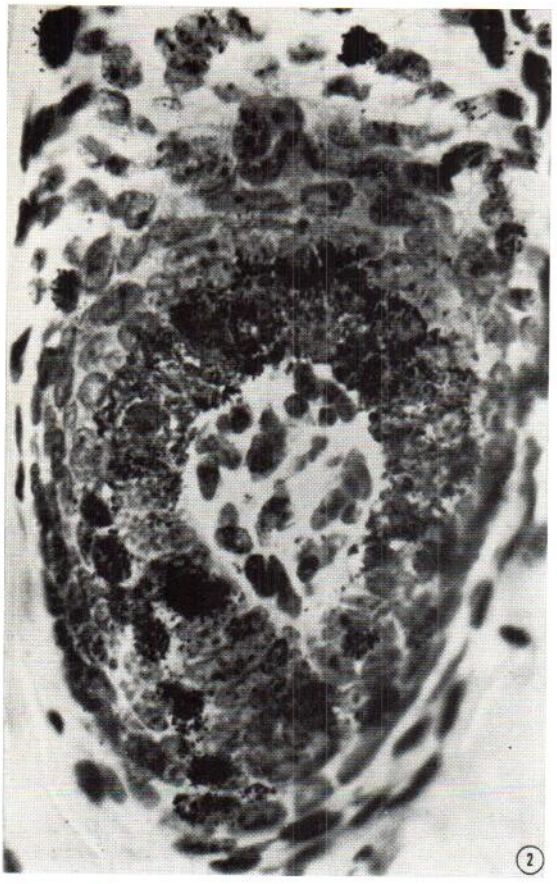

Fig. 2. Nonbald scalp, normal anagen. Labelled cells below the critical level. In the upper part, many cells are filled with pigment.

nonbald regions were also injected. Each specimen was fixed in Bouin's fluid and embedded in paraffin. Serial longitudinal sections, 5 μ thick, were coated with NTB 2 Kodak emulsion and developed after 21 days of exposure. Harris hematoxylin and eosin were used as routine counterstain.

For quantitative evaluation of the data, we investigated the actual number of labelled cells per 100 germ cells, using the "t" method for paired comparisons between nonbald and bald hair follicles.

RESULTS

Nonbald scalp (Fig. 1 A)

After 3 days the plucked area was completely free of hairs (Fig. 1 *B*); after 7, 14, and 25 days, the number of regrown hairs was conspicuously lower than that previously seen in normally haired areas (Fig. 1 *C*, *D*, *E*).

The uptake of 3H-Thymidine by the matrix cells of anagen (growing) follicles registered mostly below the critical level (Fig. 2), and no labelling occurred in telogen (quiescent) follicles, Three days after plucking, the walls of empty follicles were filled with labelled cells and resembled short, thick, irregularly shaped epithelial columns (Fig. 3). Seven, 14, and 25 days after plucking, DNA synthesis occurred only in the germ cells near the base of plucked telogen follicles (Figs. 4, 5 and 6). The matrix cells of plucked anagen hairs, showing a transverse fracture of the hair shaft below the keratotic zone, were weakly labelled (with a few granules) compared with those in regrowing hairs or in normally growing anagen (Fig. 7). Some were not labelled at all. Twenty-five days after plucking, the regrown hair follicles had recovered their normal shape and size.

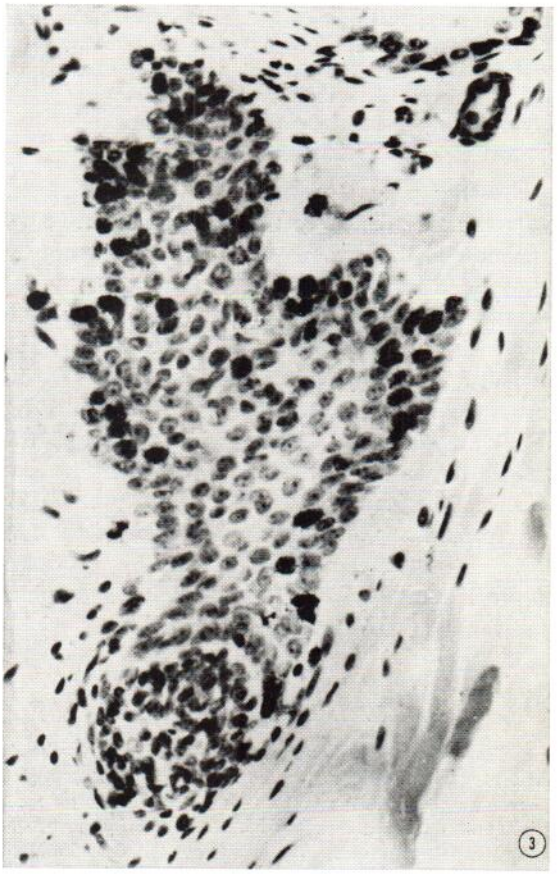

Fig. 3. Nonbald scalp; 3 days after plucking, the hair follicles are filled with DNA-synthesizing cells.

The number of labelled cells per 100 germ cells or cells in the wall of follicles was significantly higher in the nonbald area than in the bald area. As shown in Table I, there was no significant difference only 7 days after plucking.

Bald scalp (Fig. 1 F)

Seven days after plucking only a few, lanugo-like hairs had regrown on the bald scalp surface (Fig. 1 *H*); the number of hairs remained less than that in "hairy" regions after 14 and 25 days (Fig. 1 *I, J*). Cells synthesizing DNA were observed in the matrix of vellus hairs below the critical level (Fig. 8). No 3H-Thymidine uptake occurred in telogen hairs.

Regrowing plucked hairs in the bald scalp differed somewhat from those in the nonbald scalp. Three and 7 days after plucking, cells synthesizing DNA were few (Figs. 9 and 10); whereas 14 and 25 days after plucking, labelled cells occurred in those plucked telogen hairs having a well-developed secondary germ (Figs. 11 and 12). Twenty-five days after plucking there were fully developed vellus hairs.

The number of labelled cells per 100 germ cells was "significantly" lower than that in the nonbald area although no significance was obtained 7 days after plucking.

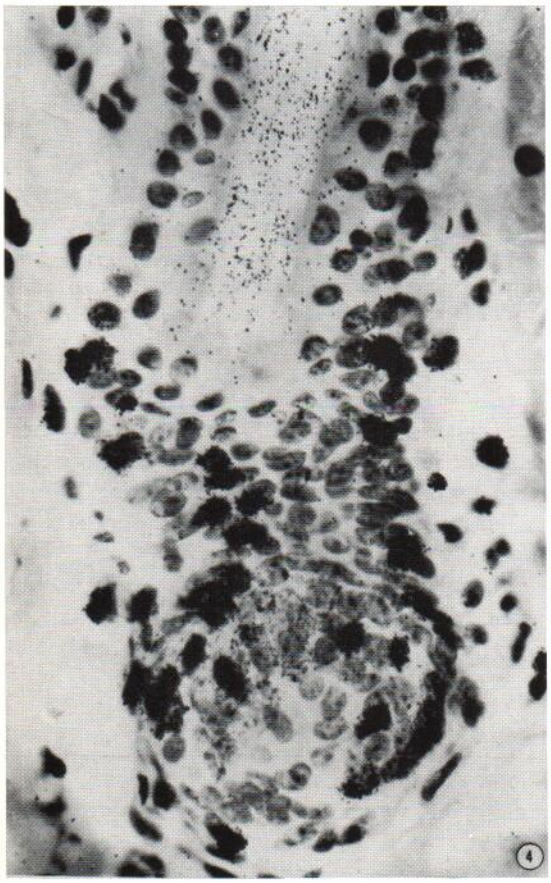

Fig. 4. Nonbald scalp, 7 days after plucking; telogen hairs with secondary germs attached with a burst of DNA-synthesizing cells.

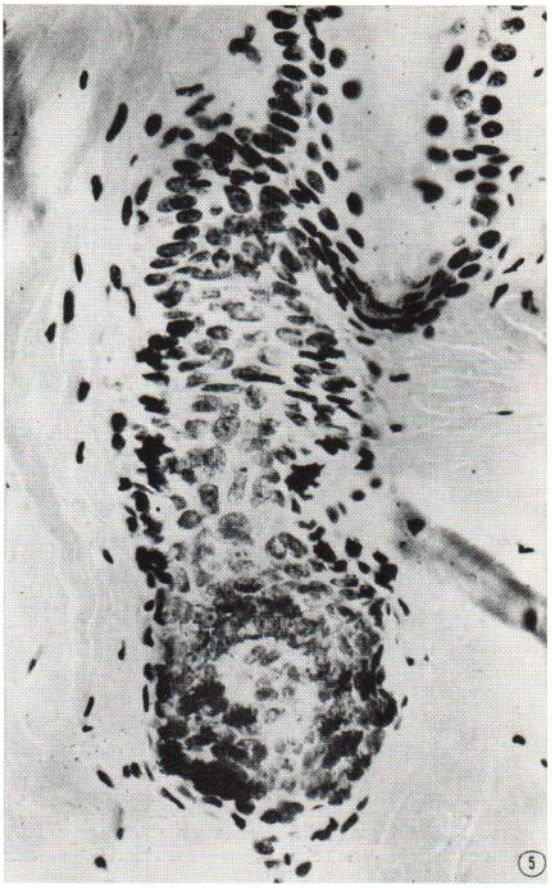

Fig. 5. Nonbald scalp, 14 days after plucking; telogen with a more developed secondary germ.

DISCUSSION

Antimitotic drugs and X-rays have been and still are widely used to study phenomena related to the regrowth of hair (8, 12, 13). However, we prefer the plucking method since it obviates the environmental modifications that result from the use of

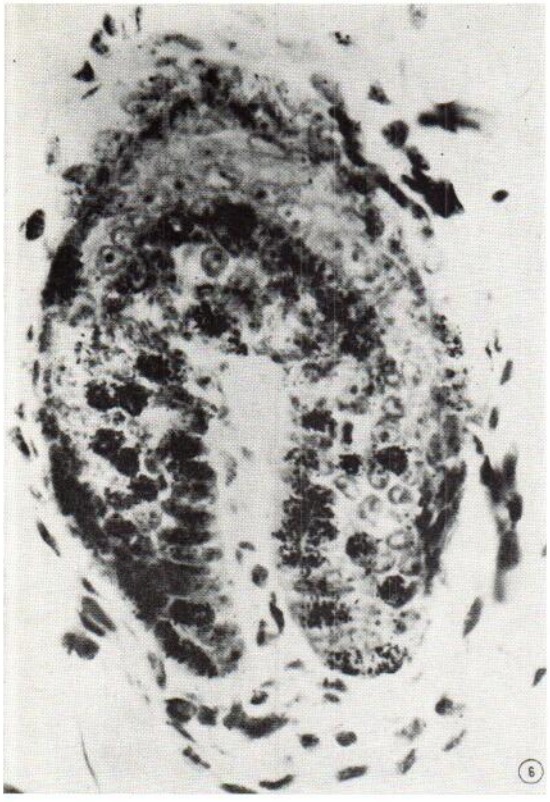

Fig. 6. Nonbald scalp, 25 days after plucking; a fully developed hair follicle, showing the matrix cells labelled with 3H-Thymidine.

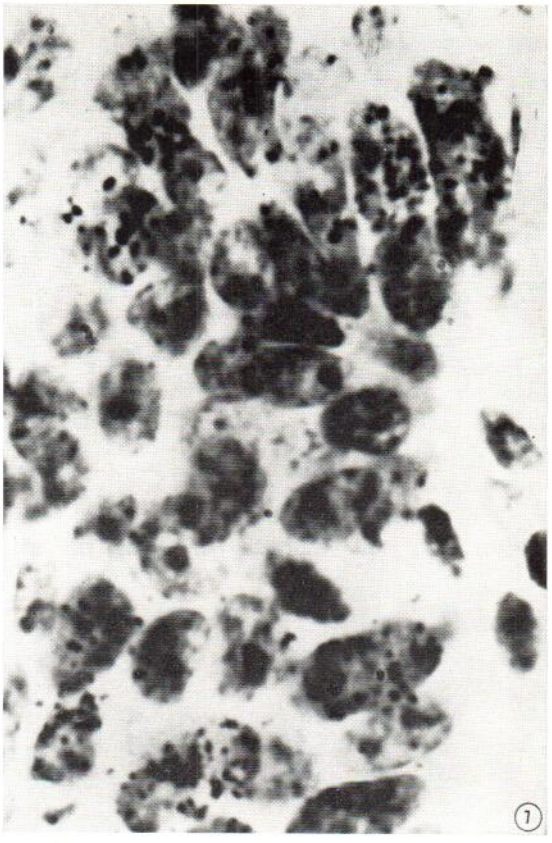

Fig. 7. A fully grown anagen, 14 days after plucking. This highpower picture shows the cells of the matrix, thinly and randomly labelled.

drugs and X-rays. Furthermore, plucking has advantages that outweigh its sometimes traumatic effects which are mostly brief and negligible. Plucking has the extra advantage of starting all hairs in the plucked region at the same growing stage and of effecting the transformation from telogen (quiescent) to anagen (growing) hairs (3, 4, 5, 7). Ebling & Johnson (6) demonstrated in rats that plucking hairs at the onset of the resting phase increased mitotic activity and that the results of plucking club hairs after mitotic activity has begun in the hair matrix were negative. These data are important when one is trying to obtain a homogeneous population of regrowing hairs in animals with a random hair cycle—men, monkeys, and guinea pigs.

Our results in the stumptailed macaque scalp agree with the above findings: hairs do not regrow synchronously after plucking, and only

Table I

Days	0	3	7	14	25
Nonbald	26.4^a	23.0^a	24.8^a	36.8^a	23.6^a
t	1.9822	2.7113	0.5844	1.7134	1.4813
Significance	$0.05 > p > 0.02$	$0.02 > p > 0.01$	n. s.	$0.1 > p > 0.05$	$0.1 > p > 0.05$
Bald	16.6^a	8^a	22^a	26.2^a	15.4^a

[a] Number of labelled cells per 100 germ cells or cells in the wall of follicles.

some of the previously visible hairs begin to grow 25 days after plucking. This likewise confirms our belief that the possibility of inducing a burst of mitotic activity in the matrix by plucking is related to the stage of the hair cycle. In the scalp of stumptailed macaque, where the hair population is not homogeneous, only the plucked hairs in the resting phase regrow. Plucked anagen are easily distinguished from plucked telogen because they are more deeply embedded in the dermis and, when plucked, leave behind a veritable "streamer" of perifollicular connective tissue.

The few hairs reappearing on the surface after plucking demonstrate that only those follicles in the resting phase start to regrow. Data on 3H-Thymidine uptake substantiate this finding. In all areas studied, plucked telogen hairs showed the

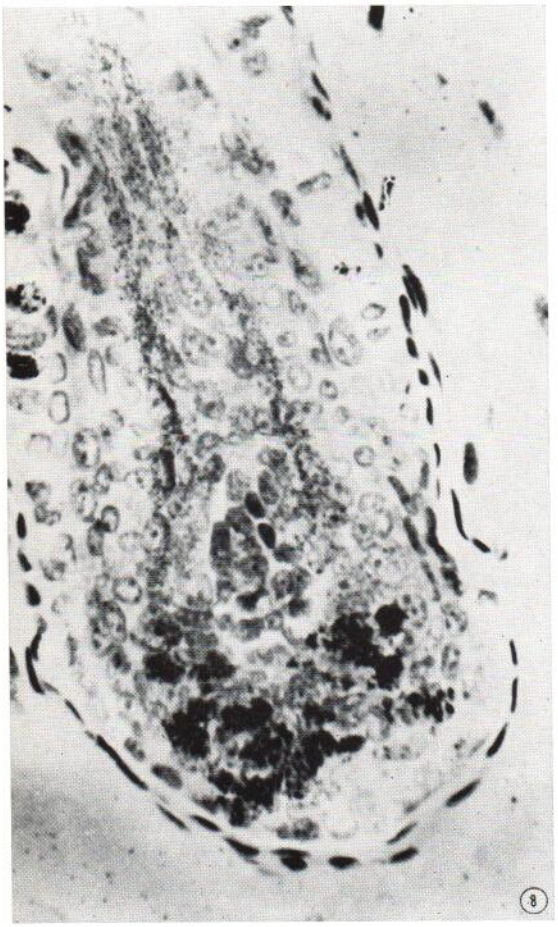

Fig. 8. Bald scalp, transformed vellus hair. Labelling of the matrix cells occurs as in normally growing anagen.

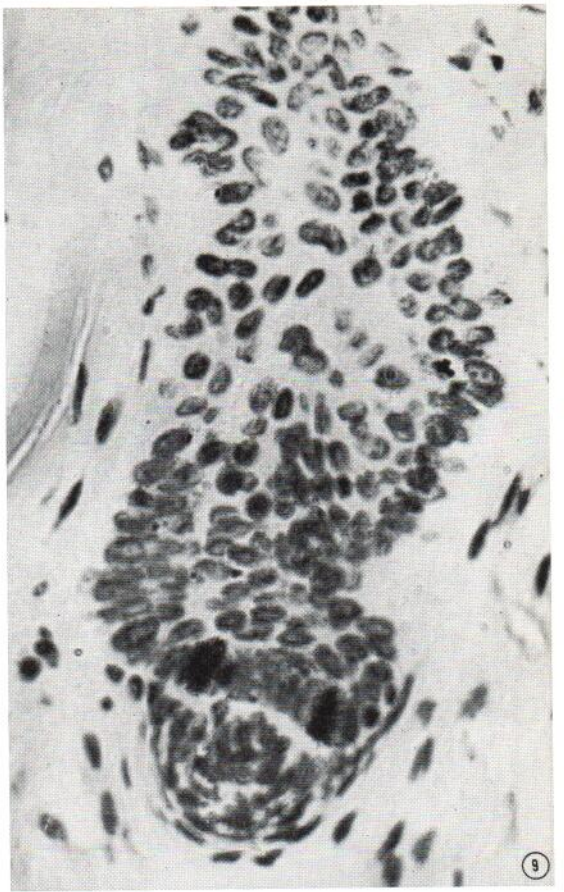

Fig. 9. Bald scalp, 3 days after plucking. The hair follicle contains few DNA-synthesizing cells (cf. Fig. 3).

secondary germ filled with 3H-Thymidine labelled cells. On the other hand, only a few matrix cells of fully grown anagen hairs were labelled after plucking; moreover, regardless of the area in which they were found, the number of silver granules in the nuclei was limited. Moffat & Pelc (9) attributed the blocking of DNA synthesis (especially shortly after plucking) to an inhibition of phosphorylating enzymes or of DNA-polymerase. The present study demonstrates again that only telogen hairs are able to regrow after plucking; (it is not possible to elicit an effective burst of mitotic activity in the matrix cells of plucked anagen). By contrast, in the unplucked bald and non-bald scalp, both the growing anagen and the transformed vellus hairs show mitotic activity in the matrix cells, whereas no labelled cells are seen in the resting follicles.

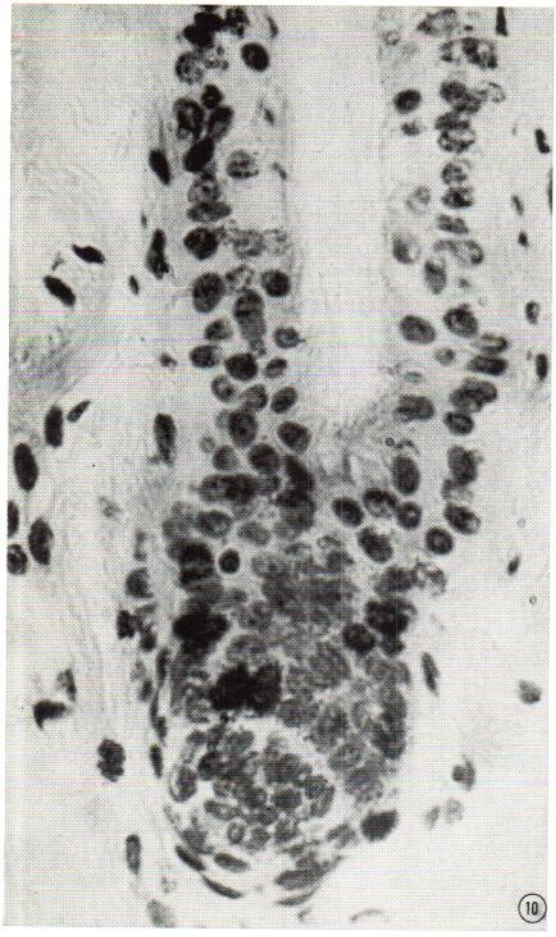

Fig. 10. Bald scalp, 7 days after plucking. Only a few cells are in DNA synthesis (cf. Fig. 4).

Hair regrew faster in the nonbald than in the bald scalp (Fig. 1 *C–H*). We speculate that this may be related to a lesser DNA synthesis in the hair follicles of the bald scalp (Figs. 9 and 10) and may be conditioned by its peculiar environmental situation.

Quantitative data (Table I) confirm that DNA synthesis occurs in a significantly higher number of germ cells in hair follicles of the nonbald area, both in normal condition (day 0) and after plucking (day 3). The significance is minor on day 14 and 25 and is lacking on day 7.

The conclusions from this study can be summarized as follows:

1. Only some of the plucked hairs are able to regrow.

2. Regrowth seems to be faster in nonbald areas.

3. In nonplucked hair follicles, DNA synthesis occurs in the matrix cells of both anagen and transformed vellus hairs but not in resting hairs. A significantly lower number of labelled cells was noticed in hair follicles of bald areas than in those of nonbald areas.

4. Only those follicles in the resting phase giving rise to the secondary germ are labelled with 3H-Thymidine after plucking. Anagen hairs after plucking are labelled either not at all or only weakly; therefore, we surmise that they stop growing and probably start involutionary changes.

5. Minor DNA synthesis occurs in the regrowing hair follicles of bald areas after plucking.

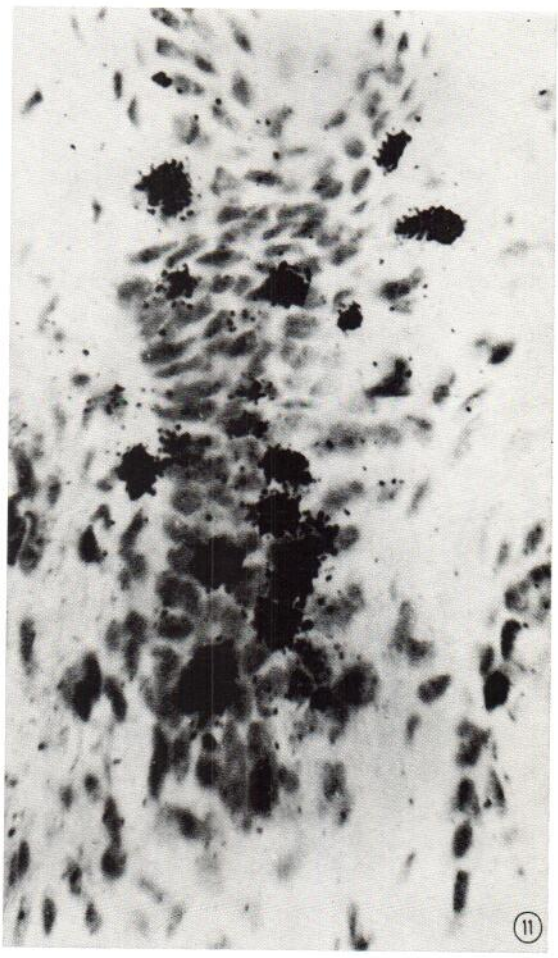

Fig. 11. Bald scalp, 14 days after plucking; a telogen hair giving rise to a secondary germ with many DNA-synthesizing cells.

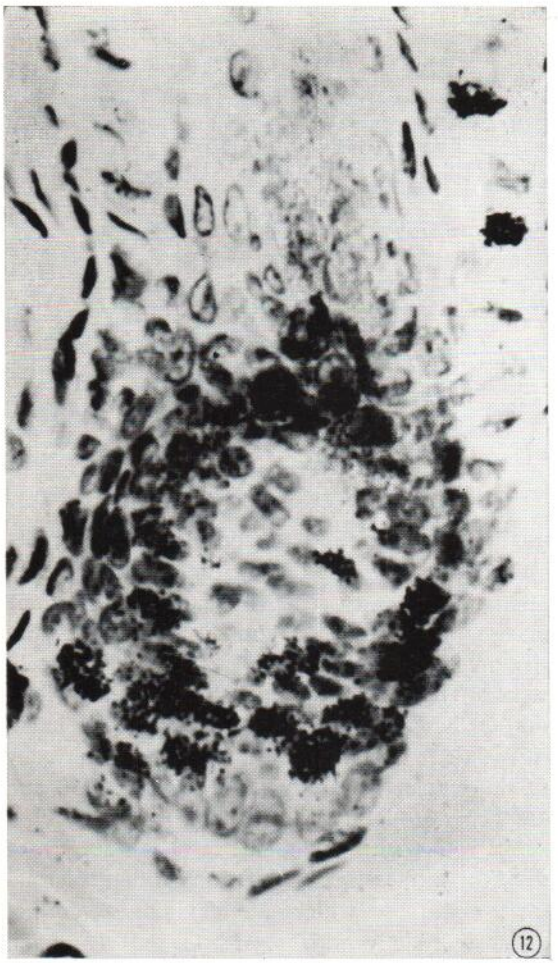

Fig. 12. Bald scalp, 25 days after plucking; a fully regrown transformed vellus hair with DNA synthesis in the matrix cells comparable to that of Fig. 8.

Therefore, we can reasonably postulate a correlation between the delayed onset of regrowing hairs in bald areas and this minor DNA synthesis activity.

ACKNOWLEDGEMENTS

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