Progressive Epithelial Dysplasia In Mouse Skin Irradiated With 10 MeV Protons

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DISCLAIMER

It has been previously reported[1] that within twenty days following bombardment of mice with 10 MeV protons (as well as with 20 MeV deuterons and 40 MeV alpha particles) that atypical epithelial hyperplasia developed without underlying recognizable vascular or collagen alterations as predisposing factors. The source of these monoenergetic accelerator-produced heavy ionizing particles was the 60-inch cyclotron of the Brookhaven National Laboratory. The technique employed utilized a variable-thickness filter in the beam in order to deliver multiple Bragg peaks in depth in the path of the charged particles in the tissue being irradiated. In this way a cylinder of skin was bombarded with essentially uniform ionization limited to a depth of 1-2 mm. In some instances the epidermal lesions resulting from an exposure of 2000 to 5000 rad resembled the type of lesion considered in the skin of man to be carcinoma in situ. The eventual fate of such lesions then constituted a question of importance in the possible relationship of atypical hyperplasia in the pathogenesis of carcinoma in situ and of invasive carcinoma in skin. It is with this problem that the currently reported study is concerned.

The next natural step in this study appeared to be one in which mice of the same strain and age should be irradiated as in the previous procedure at similar doses and allowed to live for longer periods of time, with arbitrarily selected time periods of sacrifice up to 6 months. It was not known at the onset of these experiments as to whether the lesions noted up to 20 days would remain the same for months, progress to frank carcinoma or conceivably regress completely. It was also not known as to what the eventual outcome of established atypical hyperplasia would be if, instead of one

exposure to ionizing particle bombardment, another similar irradiation was administered at a later date. Would it be necessary in a possible transition from atypical epithelial hyperplasia to carcinoma to require further irradiations to develop invasive neoplastic cells or would a single exposure followed by sufficient lapse of time result in such an effect? Perhaps also a wider variety of doses might be advantageous. All of these considerations seemed important and formed the basis for planning this series of experiments.

Methods

The method for irradiation of the mice with the 60-inch cyclotron of the Brookhaven National Laboratory as well as the dosimetric considerations have been described previously.[2,3,4] In principle, essentially uniform distribution of ionization throughout the irradiated tissue was achieved by placing a disc filter in the proton beam. About 300 BNL mice, 6 to 8 weeks old, and of both sexes were used in the experiments. The dosages selected were 600 rad alone: 600 rad plus an additional 600 rad 30 minutes later: and 1200 rad alone. The higher doses of 2000 and 5000 rad used in the previous study for determination of acute effects were not employed this time.

Results

Examples of the atypical epithelial hyperplasia obtained in previous acute effect experiments are shown in Figures 1 and 2. In the former, 19 days after irradiation with 2000 rad protons the dyskeratosis was quite marked and again in the latter with 2000 rad deuterons at a similar post-exposure period the hyperplasia was extensive, even involving hair follicles.

In each, however, the basement membrane remained intact and no degenerative changes were found in the blood vessels or collagen of the dermis. In the present series of experiments 600 rad protons were chosen for the first irradiated group because this amount of irradiation was also followed at 20 days by distinct epithelial hyperplasia although not nearly as marked as with the doses of Figures 1 and 2. It was felt that it would be of interest to determine what a lower dose of this amount would be followed by in skin reaction at periods up to 6 months. In a second group of mice 30 minutes after 600 rad proton exposure another similar dose was administered and the sacrifice periods were the same. In the third group 1200 rad proton dose was given and mice were sacrificed up to 6 months. At this time of sacrifice only animals exposed to 2000 rad alpha particles have been studied and similar doses with protons and deuterons will be reported later.

In Figure 3 is shown the effect of the 600 rad dose of protons 6 months after exposure. The hyperplasia is distinct and definite hydropic degeneration is noted in most of the cells adjacent to the basement membrane. In those mice receiving 1200 rad (600 plus 600 rad) protons at two months after exposure (Figure 4A and B) the degree of hyperplasia is greater but again the cells do not appear anaplastic and have not invaded the basement membrane. At 4 months post-irradiation with a similar dosage plan as noted in Figure 5, there is more irregularity in cell shape and suggestion of coalescent destruction of groups of adjacent cells. With both 600 rad proton dose alone and with a single 1200 rad proton dose at 6 months post-exposure as seen in Figures 6A, B and C; and Figure 7A and B there is

hyperplasia, dysplasia and complete degeneration of many of the epithelial cells with intra-epithelial cyst formation and hemorrhage. In Figure 7 the destruction of basement membrane is quite obvious as is disruption of dermal collagen fibers. No obliterative changes are found in blood vessels. In the final Figure, number 8, a higher dosage was used, namely 2000 rad alpha particles, 6 months post-irradiation. Again the hyperplasia, dysplasia, focal basement membrane interruption and localized areas of collagen destruction are noted. Again it is apparent that as yet the epithelial cells are not neoplastic and in spite of total destruction of basement membrane in some areas there is no invasion into the dermis.

Summary

Up to 6 months after proton irradiation at 600 and 1200 rad epithelial hyperplasia persisted in the exposed mouse skin. Hydropic degeneration of many epithelial cells occurred with intra-epithelial cyst formation with hemorrhage. Focal areas of basement membrane degenerated. Interruption of and fragmentation of underlying collagen fibers was prominent. Of interest is the paramount observation that with this degree of cellular alteration and with complete breakdown of basement membrane the epithelial cells did not invade into the dermis. This suggests that the altered epithelial cells must actually be definitely neoplastic for true invasion to occur and that a single exposure at these doses did not alter the cells sufficiently to render them anaplastic. It is possible however that larger single doses with subsequent time interval elapse (months) might provoke the formation of neoplastic cells. This and the effect of repeated small doses and with longer periods after irradiation before sacrifice of the animal are now being investigated.

LEGENDS

Figure 1 Atypical epithelial hyperplasia with dyskeratosis in mouse skin at 19 days following 2000 rad protons. 150 X.

Figure 2 Atypical epithelial hyperplasia involving hair follicles in mouse skin at 20 days following 2000 rad deuterons. 150 X.

Figure 3 Epithelial hyperplasia with early cellular regressive alterations in mouse skin at 6 months following 600 rad protons. 100 X.

Figure 4 Atypical epithelial hyperplasia in mouse skin at 2 months following 1200 rad (600 + 600) protons. A X 100. B X 250.

Figure 5 Epithelial hyperplasia with cellular degenerative changes in mouse skin 4 months after 1200 rad (600 + 600) protons. X 250.

Figure 6 Marked callular and basement membrane degeneration in hyperplastic mouse skin with focal hemorrhage at 6 months following 600 rad protons. A X 100. B X 250. C X 400.

Figure 7 Epitheliâl dysplasia with intra-epithelial cystic degeneration in mouse skin at 6 months following 1200 rad protons. A X 100. B X 250.

Figure 8 Marked atypical epithelial hyperplasia with degeneration of dermal collagen at 6 months following 2000 rad alpha particles. 150 X.

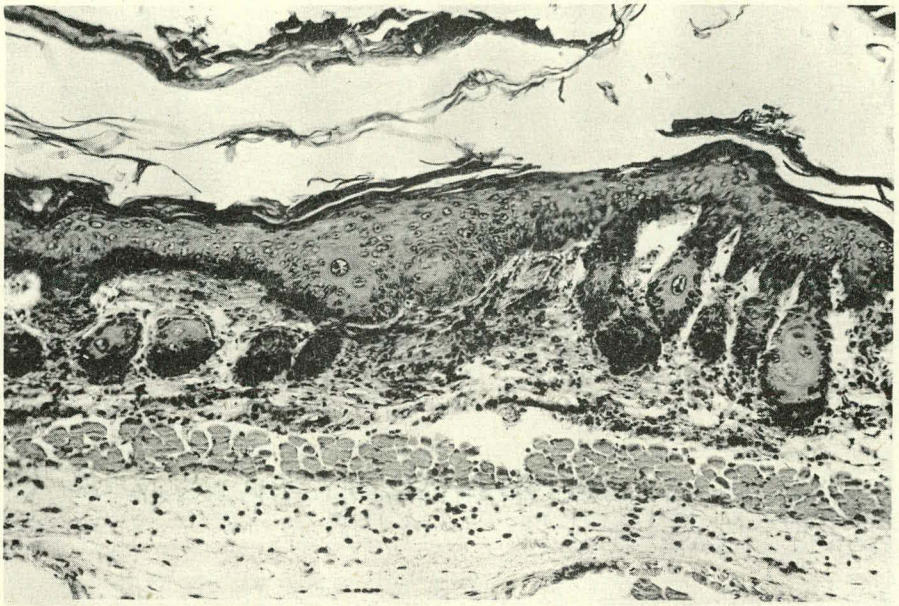

FIGURE 1

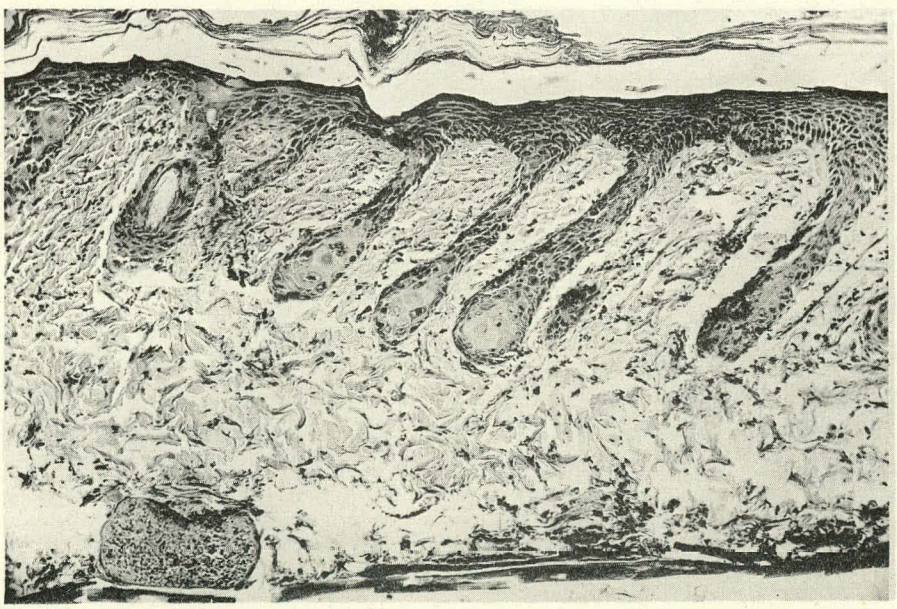

FIGURE 2

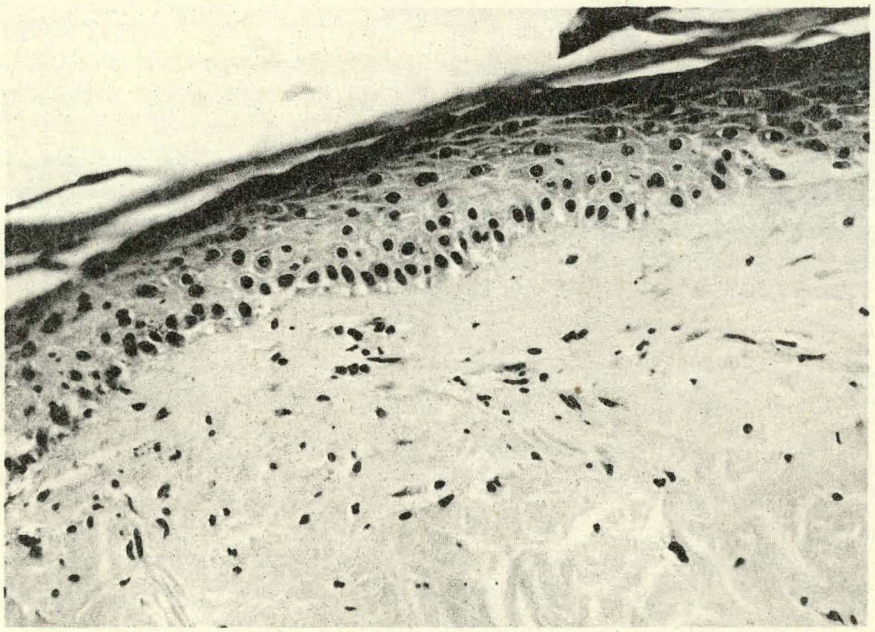

FIGURE 3

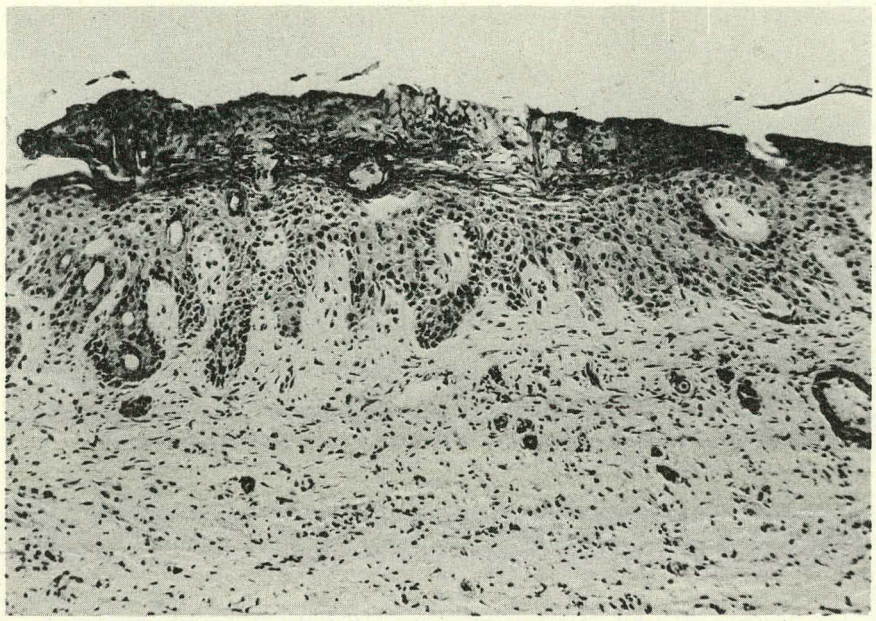

FIGURE 4 a.

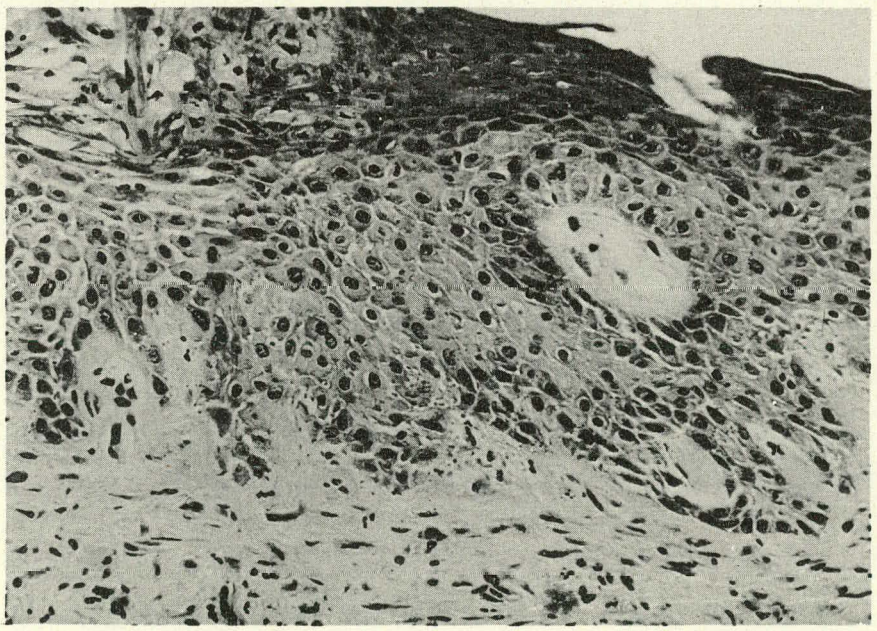

FIGURE 4 b.

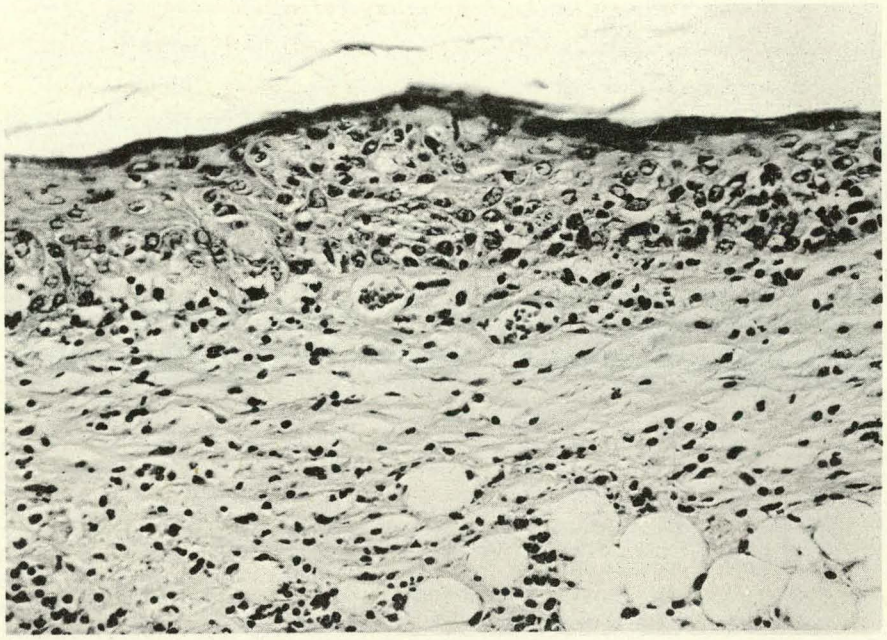

FIGURE 5

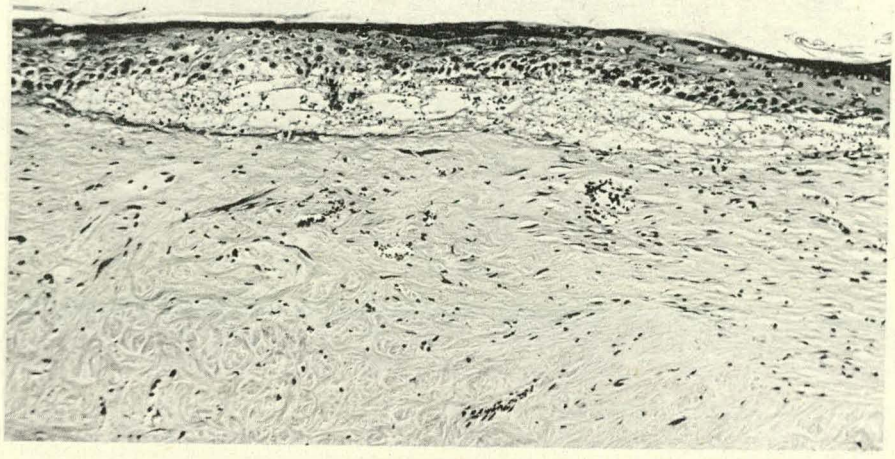

FIGURE 6 a.

FIGURE 6 b.

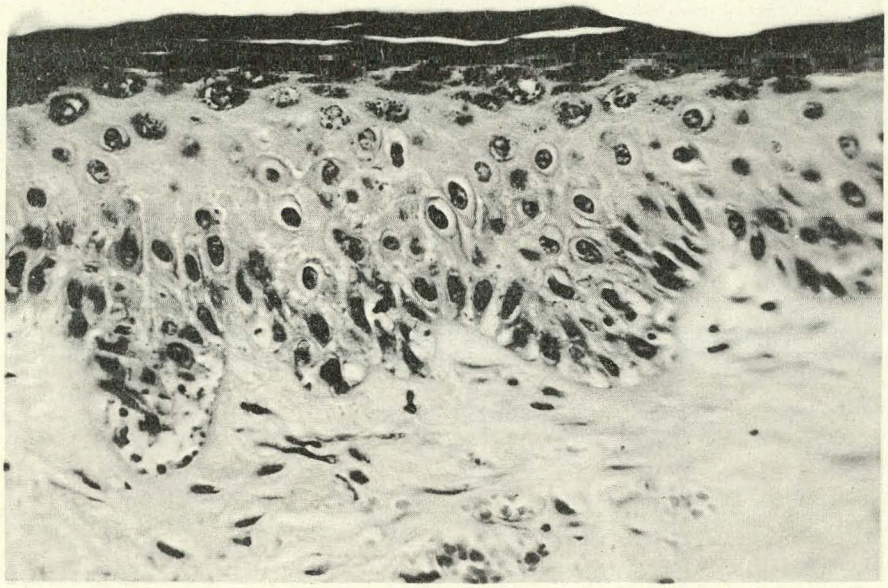

FIGURE 6 c.

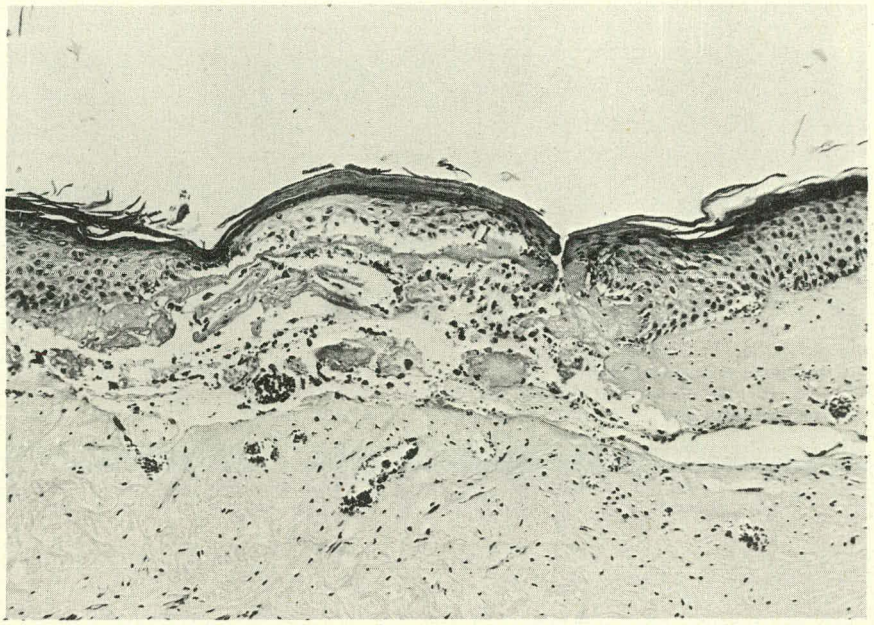

FIGURE 7 a.

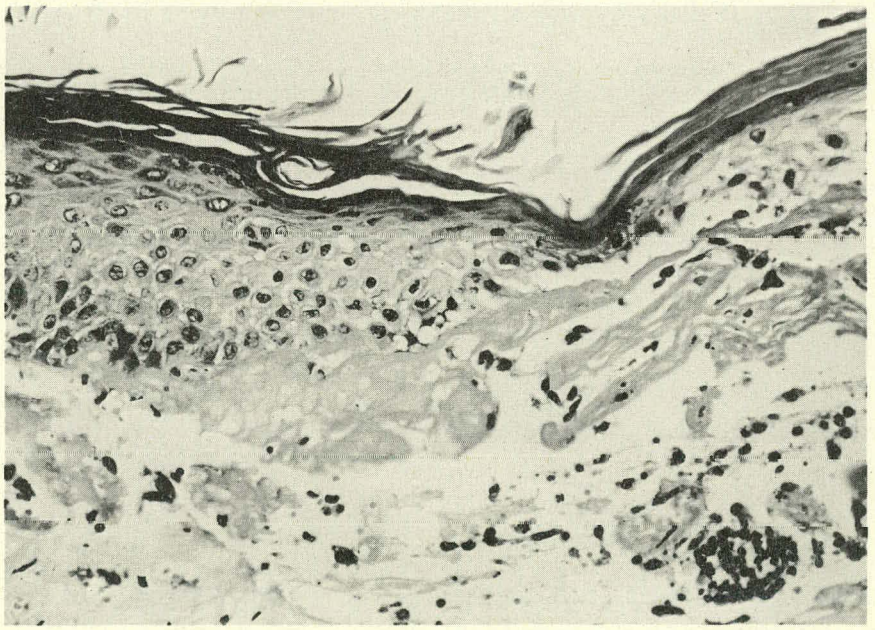

FIGURE 7 b.

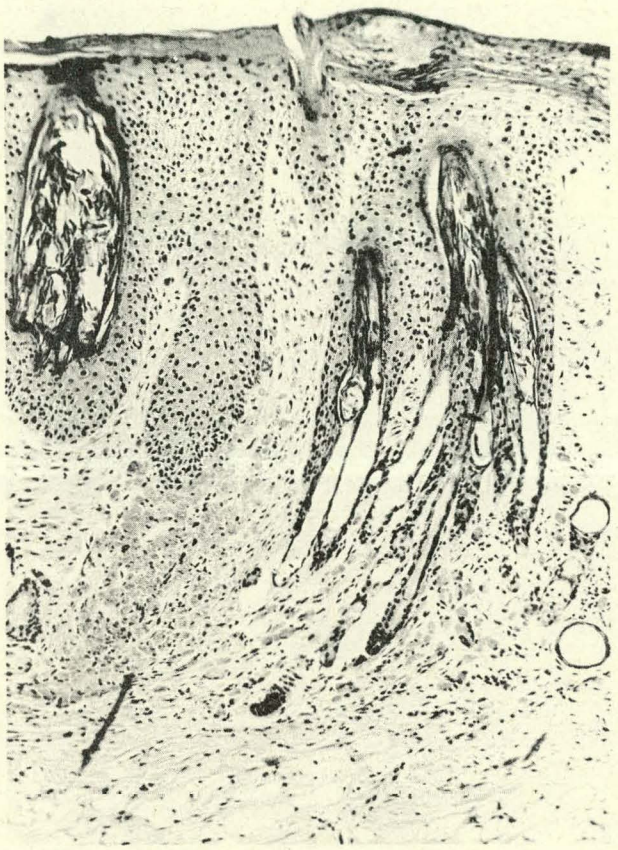

FIGURE 8

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