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REPORTS

LONG-TERM MAINTENANCE OF PSORIATIC HUMAN SKIN ON CONGENITALLY ATHYMIC (NUDE) MICE

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Transplantation of involved psoriatic and nonpsoriatic human skin onto congenitally athymic (nude) mice has been performed successfully. Although biopsies at selected intervals demonstrate that the excess glycogen deposition normally seen in psoriasis is no longer consistently present, the psoriatic grafts did retain the usual characteristic histologic differences throughout the life of the animal, up to 11 weeks. This grafting procedure potentially represents a useful method whereby the study of psoriasis can be made in a nonhuman, living system.

Further insight into the pathogenesis of psoriasis as well as the development of new treatment programs, topical or systemic, has been hampered by the lack of an animal model for psoriasis. This paper describes a potenital model in which human psoriatic skin is maintained on the congenitally athymic (nude) mouse.

Nude (nu/nu) mice are unique in that they are congenitally athymic and thus are unable to mount thymus-dependent immune responses, e.g., cell-mediated rejection of a foreign graft, as well as the antibody production for most antigens. Nude is a mutant allele of the nu locus of the VII linkage group, is recessive, and is being put onto inbred backgrounds by repeated backcrossing [1]. Under non-germ-free states, nude mice develop at a near normal rate for approximately 3 to 4 months, then characteristically they develop wasting which manifests as rapidly progressive weight loss and death [2,3]. Surgical procedures, anesthesia, etc., seem to hasten the onset of this wasting phenomenon [4].

Recently there have been two reports of the long-term maintenance of normal human skin transplants on the nude mouse [5, 6]. On the basis of these observations, we conducted a pilot project to determine whether similar maintenance could be accomplished with transplanted psoriatic skin. A more definitive project was then carried out which standardized the variable of thickness of grafts, i.e., amount of dermis included. Common to both studies after transplantation were the reduction of some of the features of psoriasis and the persistence of the typical psoriasiform acanthosis for the life span of the animal, up to at least 11 weeks post grafting.

MATERIALS AND METHODS

Initially each of 13 nude mice on a BALB/c background had an 8-mm punch biopsy of involved psoriatic skin (10) and noninvolved psoriatic skin (3) from 1 of 3 living psoriatic donors transplanted onto a graft bed. Prior to grafting, varying amounts of the dermis were trimmed so that the thickness of the human graft approximated the thickness of the mouse skin. Grafts were secured in the graft bed utilizing the casting technique of Billingham [7].

Following the initial study, 11 particularly healthy-appearing nude mice, also on a BALB/c background, 40 to 50 days of age, were used as recipients for donor grafts of both noninvolved psoriatic skin (NIPS) and involved psoriatic skin (IPS) from another 3 patients with psoriasis. All mice were housed away from other animals, maintained on autoclaved Purina 5010C feed and acidified-chlorinated water, and bedding that was sterilized and changed weekly. These procedures were implemented to keep the nude mice relatively pathogen free in an attempt to lengthen their expected survival.

Informed consent was obtained from the psoriatic subjects, and IPS and NIPS donor specimens 20 mm wide, 36 mm long, and 0.4 mm thick were taken aseptically under local anesthesia with a Castroveijo electrokeratome as described by Voorhees et al [8]. To prevent ripping of the edges during removal, a sterile template 20 × 36 mm was placed on the skin and a very superficial cut with a scalpel made along each edge, approximately 0.5–0.7 mm in depth. The specimen was placed in a sterile Petri dish with Minimal Essential Medium for transport to the lab, donor graft wounds were

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covered with sterile Vaseline-impregnated gauze and wrapped with a Kling dressing.

The skin specimens were placed on a sterile cork board, stretched and pinned in place, and 4 round, 1-cm grafts were taken from each specimen with a sharp, 1-cm sterile punch. The remaining donor scraps were fixed in 95% ethyl alcohol and histologic sections prepared to serve as a control of the thickness of the graft as well as to determine the degree of acanthosis and parakeratosis. Figure 1 is representative of these donor NIPS and IPS specimens. Periodic acid-Schiff (PAS) stains with amylase hydrolysis were also made to determine the amount of glycogen in both the initial IPS and NIPS specimens.

In these experiments we used a new grafting procedure which is much faster than conventional casting techniques of Billingham [7] and induces less wasting in the nude mice. This technique, which we have reported in detail elsewhere [4], is described here briefly (Fig. 2). Mice were anesthetized with intraperitoneal sodium pentobarbital dosage according to weight-dose scale [9]. Recipient graft beds, slightly larger than the donor graft, were then prepared, right thorax for the IPS and left thorax for the NIPS. Grafts on each animal were from the same donor. Donor grafts were secured in place with Permabond 102 cement (Pearl Chemical Company, Tokyo) and covered with a Band-Aid trimmed to size. Band-Aids were removed at day 7, and 2- to 3-mm sliver biopsies (Fig. 2*D*) were taken at 2, 4, and 6 weeks post grafting while the animal was under pentobarbital anesthesia. These biopsies were fixed in 95% ethyl alcohol, and PAS as well as H & E histologic sections were prepared.

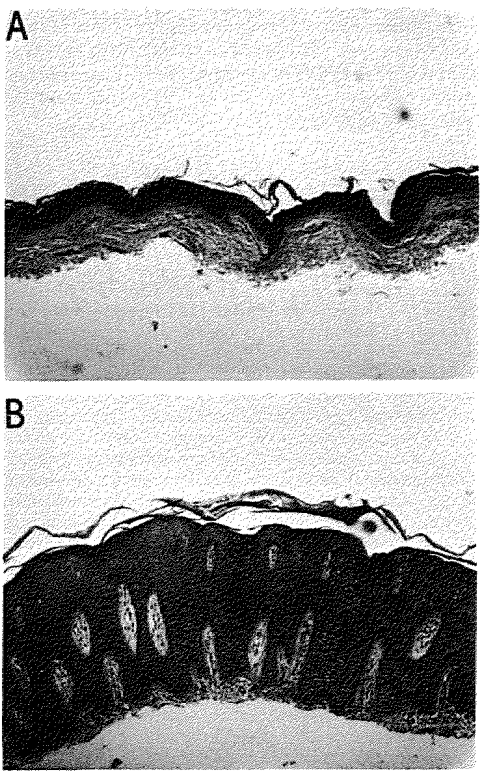

FIG. 1. *A:* NIPS-Noninvolved psoriatic skin. *B:* IPS-Involved psoriatic skin. Both 0.4 mm thick, prepared from scraps of donor specimen at the time of grafting.

RESULTS

Initial studies. Five of the 13 initial grafts were technical failures which were felt to be due to either an excessively thick graft with subsequent inadequate perfusion, or slipping of the graft from the graft bed during casting, both resulting in necrosis of the graft. Four out of 13 mice developed the severe wasting peculiar to nude mice and died shortly after the grafts were placed; thus, no interpretation was possible in these animals. The remaining 4 mice had successful grafts. One such graft with evident psoriatic acanthosis 11 weeks after transplant (the longest surviving animal) is shown microscopically (Fig. 3). Superficial biopsies [10] of these successful grafts showed some parakeratosis, but very few "halo nuclei" peculiar to scale of psoriasis [11], suggesting that this technique would not be useful in determining whether the transplanted skin still exhibited typical psoriasis. Further, it was apparent that we could not distinguish between the noninvolved psoriatic grafts and the involved psoriatic grafts on a macroscopic basis.

This initial study was encouraging enough to justify a second, more definitive study, using 11 more animals and the methods described above. All results reported hereafter are from this second experiment.

Animal and graft survival. Seven of 11 animals survived at least 6 weeks post grafting; 1 died at day 1, 2 died at approximately 4 weeks, and 1 was sacrificed at 5 weeks after grafting. The 10 animals surviving at least 4 weeks after grafting all had successful grafts, both grossly and microscopically. Human skin which appeared "normal" macroscopically was present in all 20 grafts placed; an example is illustrated in Fig. 2*D*.

The changes which occurred in the grafts as they healed in place were: (1) thin crust formation (7/20) and what appeared to be a "ghost" of stratum corneum; (2) on occasion (1/20) a necrotic crust formed. Both types of crust sloughed by day 14, and normal-appearing human skin was seen. The necrotic crust rarely (1/20) occurred with the 0.4-mm shave biopsy of donor skin, but was very common in our initial study, where the dermis was trimmed manually. By day 14, the grafts appeared grossly as they were to remain for the rest of the animal's life.

Gross appearance. No gross difference between IPS and NIPS grafts could be detected at any time after grafting. There was never evidence of graft rejection, i.e., shriveling and necrosis, after a graft was healed in place.

Histology. Sliver biopsies from NIPS and IPS grafts were obtained every 2 weeks. Animal #6 at 4 weeks (NIPS) and animals #6 and #11 at 6 weeks

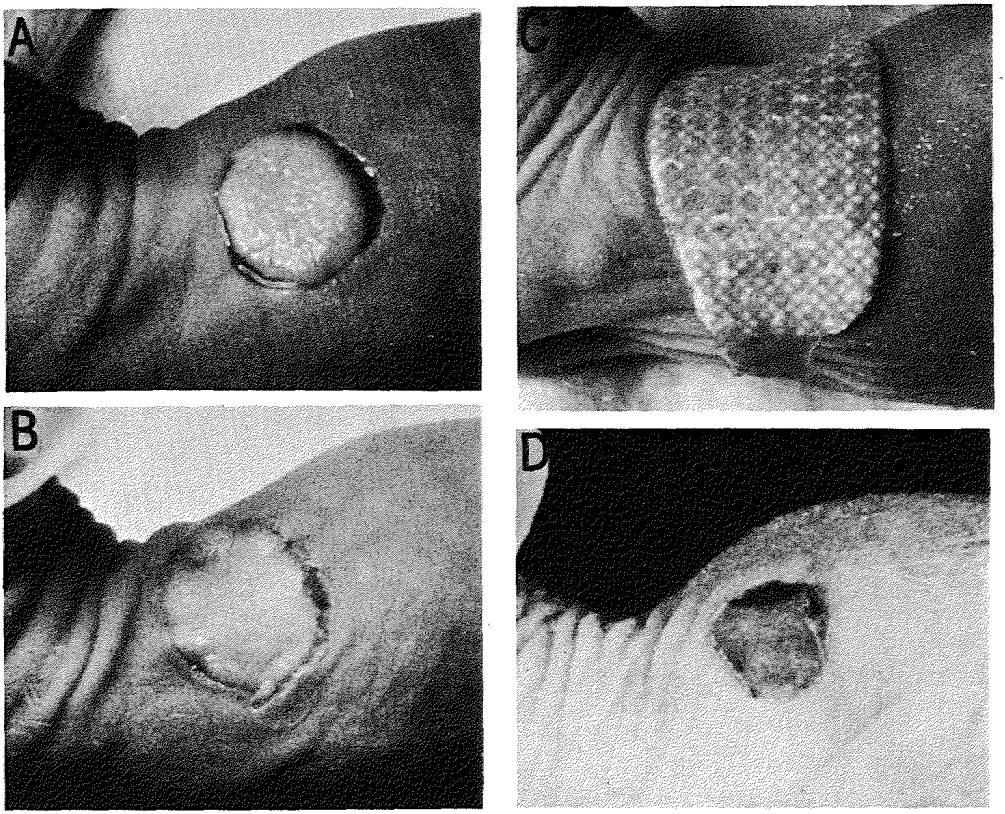

FIG. 2. *A:* NIPS donor graft in place. *B:* MIPS graft secured in place with cyanoacrylate cement. *C:* Band-Aid in place to protect graft. *D:* IPS graft site at 4 weeks immediately after removal of sliver biopsy.

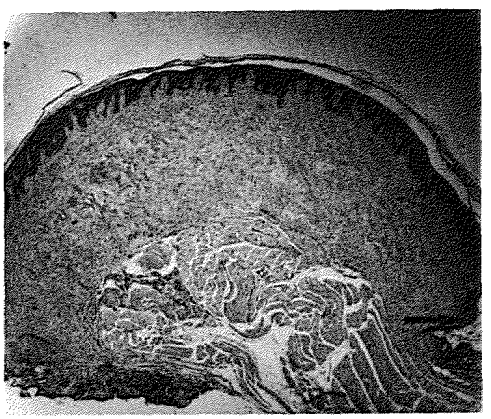

FIG. 3. Cross-section IPS graft at 11 weeks at 25×; *arrow* points to mouse skin.

(both IPS and NIPS) had no remaining human skin due to overaggressive biopsies at an earlier date. Data in the Table demonstrate the findings in a summary form.

In the psoriasis grafts, all animals had typical psoriasiform acanthosis of the epidermis at some time during the sequential biopsying. Such a series from IPS is seen in Figure 4, which represents both the typical (Figs. 4*A* and 4*B*) and the atypical (Fig. 4*C*) acanthosis seen. Figure 5 is the NIPS and is representative of the histology seen.

Figure 6 shows the junction of IPS to mouse epidermis to be quite distinct. The human epidermis is many cell layers thick, while the mouse epidermis is only 2 to 3 cell layers thick. The granular layer of the nude mouse is different from the human in that the granules in the mouse epidermis appear to not be as distinctly intracellular as in humans (Fig. 7).

PAS stains of IPS at the time of grafting revealed excess glycogen accumulation which was amylase hydrolyzable in all specimens. The NIPS skin showed no PAS granules. Figure 8 demonstrates the finding of glycogen in both an IPS and NIPS biopsy. Attempts to grade the amount of glycogen present in the biopsies by the PAS stain on a scale of 0–4 did not result in substantial differences between the involved and uninvolved groups (see Table).

Biopsies taken early (2 weeks) showed an in-

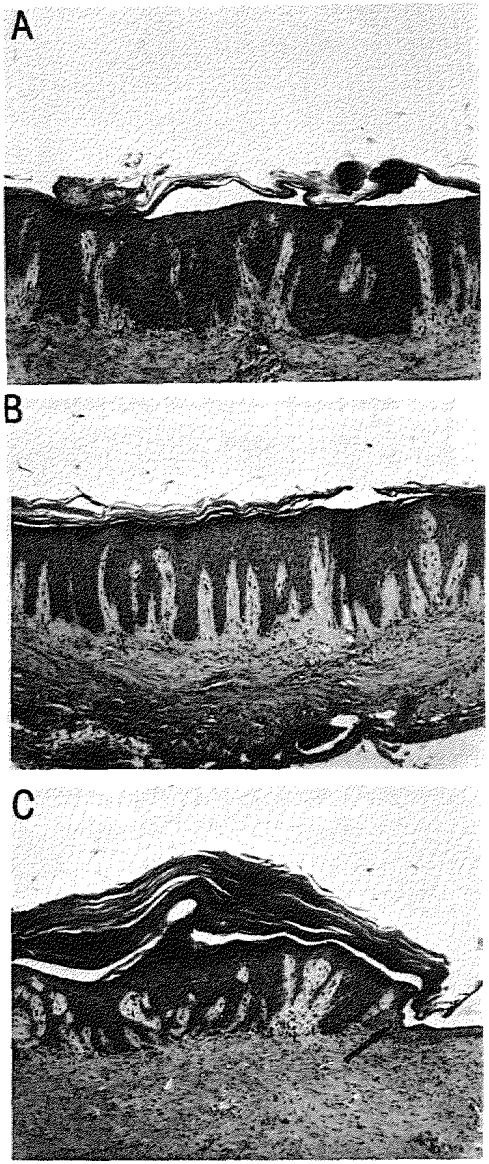

FIG. 4. Sliver biopsies from 3 different IPS grafts demonstrating the variation in acanthosis at 68×. *A:* IPS at 4 weeks, animal #1. *B:* IPS at 6 weeks, animal #2. *C:* IPS at 2 weeks, animal #7; *arrow* points to mouse-human junction.

creased number of fibroblasts in some specimens, a phenomenon which decreased with time.

DISCUSSION

Not all of the biopsies from IPS grafts revealed the acanthosis typical of that seen in psoriasis. Other investigators have noted that within any given plaque of psoriasis there may be areas without the typical histologic changes of psoriasis [12,13]. This, plus the problem of improper orientation during embedding, may explain the occasional failure to demonstrate the typical pso-

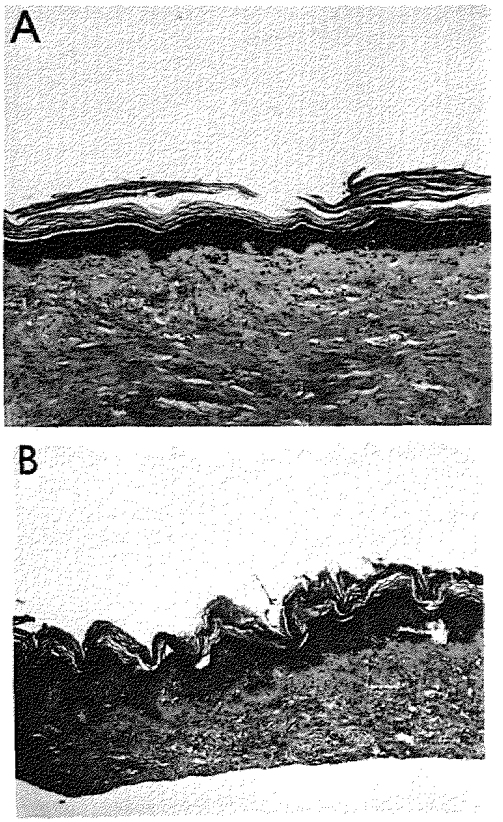

FIG. 5. Sliver biopsy from 2 different NIPS grafts demonstrating variations seen histologically at 74×. *A:* NIPS at 4 weeks, animal #1. *B.* NIPS at 2 weeks, animal #7.

FIG. 6. IPS graft at 4 weeks; *arrow* points to junction of mouse and human skin (× 250).

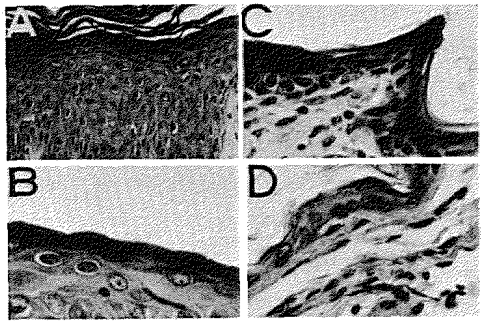

FIG. 7. *A:* IPS at 2 weeks, animal #1 (× 350). *B:* IPS at 2 weeks, animal #1 (× 770). Note granules of upper epidermis. *C:* Mouse skin of animal #1 (× 350). *D:* Mouse skin of animal #1 (× 770). Note difference in granules of upper epidermis.

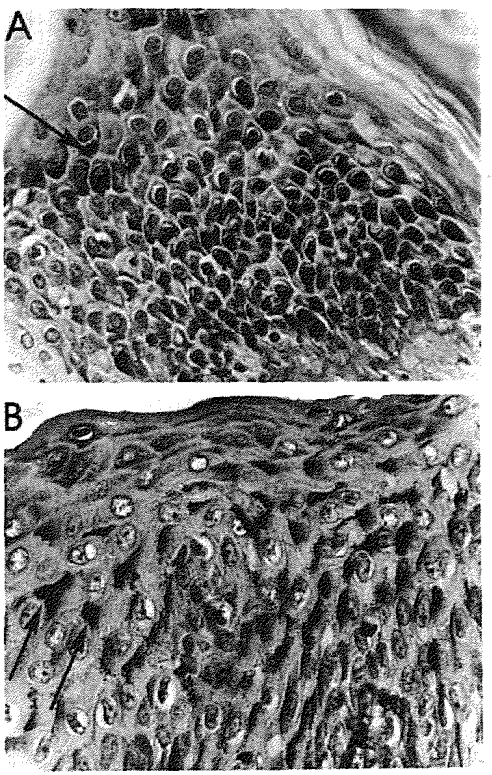

FIG. 8. *A:* NIPS. *B:* IPS. Both at 4 weeks post graft, 460×. PAS stain; *arrows* point to glycogen deposits.

riatic acanthosis in some of our biopsies. It should be emphasized that all of the animals had typical psoriatic acanthosis in at least 2 of the IPS biopsies taken over the 6-week period.

Since inflammation can result in parakeratosis [14], the presence of parakeratosis in 9 of 23 of the NIPS biopsies may be attributed to the inflammation of the healing process of the graft as well as inflammation secondary to healing from previous biopsy sites. The presence of parakeratosis in 12 of 24 IPS biopsies is not markedly different from the presence of parakeratosis in the NIPS group. Not recorded, but paralleling the disapperance of parakeratosis, was the reappearance of the granular layer.

If glycogen persisted in the epidermal cells of IPS grafts it might indicate a decrease in intracellular cyclic AMP, a finding which in some laboratories has been seen in psoriasis [15]. The fairly good relationship between parakeratosis and the presence of excess glycogen in 30 out of 46 versus no relation in the remaining 16 out of 46 suggests that these parameters are related. This led to the conclusion that even though there is psoriatic acanthosis in the IPS biopsies, the excess glycogen accumulation normally seen in psoriasis probably reverts to a more normal state, i.e., decreased to normal levels. Perhaps necessary host factors are not present in the nude which are needed to maintain all of the typical characteristic features of psoriasis. Evidence to support this is the fact that the nude in the non-germ-free state goes into wasting at about 3 to 4 months of age [3], and a general state of nutritive deficiency develops which could be a factor in not keeping the IPS grafts "fully psoriatic."

The presence of glycogen, usually with accompanying parakeratosis, demonstrates that some of our NIPS grafts appear to take on some psoriatic changes without the acanthosis. Reasons for this are unclear unless they are also related to the inflammatory response associated with healing. The sliver biopsies may have induced unintentional inflammation even though attempts were made to biopsy away from previous sites. Each previous biopsy site appeared to be well healed prior to new biopsies.

The unlikely possibility that in this study mouse epidermis built on a grafted human epidermal skeleton must be addressed. This hypothesis would suggest that mouse epidermal cells flow in from the edge and replace the human epidermal cells one by one until all that is left is mouse epidermal cells on

TABLE. *Histology of grafts*

Biopsy time	Psoriasiform acanthosis		Parakeratosis		Glycogen	
	I	NI	I	NI	I	NI
Initial	11/11	0/11	11/11	0/11	11/11	0/11
2 Weeks	8/10	0/10	5/10	5/10	8/10	10/10
4 Weeks	8/8	0/7	4/8	2/7	7/8	5/7
6 Weeks	4/6	0/6	3/6	2/6	3/6	4/6

I = involved (IPS)
NI = noninvolved (NIPS)
Numerator = number of grafts showing effect
Denominator = number of grafts showing human skin histologically

a human skeleton. The points of evidence against this are:

a. The granular layer of mouse epidermis is different from that of human skin (see Fig. 7).

b. Gross appearance of the graft complete with Langer's lines appear totally human (see Fig. 3).

c. Distinct histologic differences can be seen at the margins where human and mouse skin meet (see Fig. 6). This has been noted previously [5, 6].

d. Implantation of a thymus gland causes human xenografts on nude mice to be rejected [16]. This should not be possible unless the skin remained human.

The presence and significance of antihuman antibodies in nude mice grafted with human skin, of the type reported in nudes carrying human tumors [1], are uncertain. One of us (D.M.) has attempted to demonstrate these antibodies unsuccessfully (unpublished results).

REFERENCES

1. Wortis HA: Immunological studies of nude mice, Contemporary Topics in Immunology, vol B. Edited by MD Cooper, NL Warner. New York, Plenum, 1974, pp 243–263
2. Cantrell JC, Jutila JW: Bacteriologic studies on wasting disease induced by neonatal thymectomy. J Immunol 104:79–85, 1969
3. Flanagen SP: Nude, a new hairless gene with pleiotropic effects in the mouse. Gen Res 8:295–301, 1966
4. Manning DD, Krueger GG: Use of cyanoacrylate cement in skin grafting congenitally athymic (nude) mice. Transplantation (in press) 1974
5. Reed ND, Manning DD: Long term maintenance of normal human skin on congenitally athymic (nude) mice. Proc Soc Exp Biol Med 143:350–353, 1973
6. Rygaard J: Skin grafts in nude mice. 3. Fate of grafts from man and other taxonomic classes. Acta Pathol Microbiol Scand 82:105–112, 1974
7. Billingham RE: Chapter 1, Transplantation of Tissues and Cells. Edited by RE Billingham, WK Silvers. Philadelphia, Wistor Institute, 1961, pp 1–17
8. Marks R, Dawber RPR: In situ microbiology of the stratum corneum. Arch Dermatol 105:216–221, 1972
9. Goldschmidt H, Thew MA: Exfoliative cytology of psoriasis and other common dermatoses. Arch Dermatol 106:476–483, 1972
10. Voorhees JJ, Duell EA, Bass LJ, Powell JA, Harrell ER: Decreased cyclic AMP in the epidermis of lesions of psoriasis. Arch Dermatol 105:695–701, 1972
11. Pilgrim HI, DeOme KB: Intraperitoneal pentobarbital anesthesia in mice. Exp Med Surg 13:401–403, 1955
12. Cox AJ, Watson W: Histological evidence of the nature of psoriasis, Psoriasis, Proceedings of the International Symposium. Edited by E Farber, AJ Cox. Palo Alto, Stanford University Press, 1971, pp 151–159
13. Braun-Falco O: Dynamics of growth and regression in psoriatic lesions: alterations in the skin from normal into a psoriatic lesion, and during regression of a psoriatic lesion. Psoriasis, Proceedings of the International Symposium. Edited by E. Farber, AJ Cox, Palo Alto, Stanford University Press, 1971, pp 215–237
14. Graham JH, Johnson WC, Helwig EB: Dermal Pathology. Hagerstown, Harper and Row, p 972
15. Voorhees JJ, Duell EA, Stawiski M, Harrell ER: Cyclic nucleotide metabolism in normal and proliferating epidermis, Advances in Cyclic Nucleotide Research. Edited by P Greengard, GA Robinson. New York, Raven, 1974, pp 117–162
16. Manning DD, Reed ND, Shaffer CF: Maintenance of skin xenografts of widely divergent phylogenetic origin on congenitally athymic (nude) mice. J Exp Med 138:488–494, 1973