Zinc Deficiency and Epithelial Wound Repair: an Autoradiographic Study of ^{3}H-Thymidine Incorporation (36415)

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Wound healing is impaired in zinc deficient calves (1) and rats (2). The tensile strength of a healing surgical incision in the integument was lower in zinc deficient rats than in pair-fed zinc supplemented animals (3). In man, zinc sulfate accelerated wound healing after the incision of pilonidal sinuses (4). The beneficial effect of zinc sulfate was also observed in the healing of patients with leg ulcers (5).

Repair of skin wounds has been extensively studied and many investigators believe that epithelialization begins with an increase of proliferative activity in the epidermis at the wound site, followed by migration of newly formed cells over wound surface (6–8).

It is known that zinc deficiency reduces DNA synthesis in rat liver (9, 10). Our recent studies have demonstrated the need for zinc in the incorporation of cystine-^{35}S into skin protein (11) and of ^{14}C-labeled glycine and proline into skin collagen (12). These findings prompted us to examine this deficiency on cells proliferation and differentiation by studying the formation of the epithelium that covers small wounds. Since thymidine is incorporated into the nuclei of epidermal cells when DNA is synthesized (13), its tritiated form was utilized as a specific DNA label, and the results of autoradiographic studies are presented here.

Materials and Methods. Zinc deficient rats and their controls were produced in the same manner as described in a previous study (11). After experimental feeding of 10–14 days, a 1-in linear incision was made on the back of each rat. On days 2, 4, and 6 after wounding, each animal received a single intramuscular injection of tritiated thymidine (50 μCi/100 g body wt), (sp act 20.2 μCi/mmole). One hour later, biopsy specimens were obtained from the wound site, fixed in Orth's fluid and embedded in paraffin by routine histological methods; sections were stained with hematoxylin eosin. Autoradiographs were prepared by dipping section into Kodak NTB-2 nuclear track emulsion and developed several weeks thereafter.

In one experiment using rats not previously wounded, the uptake of tritiated thymidine was measured in skin samples from zinc-deficient animals and their corresponding controls. The hair on the posterior dorsum of each rat was shaved and then each animal received an im injection of thymidine-$C^{3}H_{3}$ (50 μCi/100 g wt). One hour later a section of skin specimen weighing 50–100 mg was removed with scissors. One-half of the amount was immediately homogenized, solubilized with hyamine and its radioactivity was determined in a Packard liquid scintillation spectrometer. An internal standard was used for correction of quenching. The remaining half of skin biopsy specimen was treated in a manner similar to the one described above for autoradiographic study. Results are expressed as percentage of labeling index, *LI*, which is the percentage of cells labeled in the population as follows: $LI = N_s/N_c \times 100$, where N_s is the number of labeled cells at the time of the pulse, and N_c, the number of cells in each cycle.

Results. Unwounded epithelium of both zinc-supplemented and zinc-deficient rats showed normal histological appearance. However, there appeared to be fewer cells in the zinc-deficient animals per unit of histological section. Thymidine-labeling indices for zinc-

TABLE I. Uptake of Thymidine-C^3H_3 by Skin from Zinc Supplemented and Deficient Rats.

Type of diet	No. of rats	Uptake of thymidine-C^3H_3	
		dpm/mg wet weight	Labeling index (%)
Zinc supplemented	6	$607 \pm 57^{a,c}$	17.1 ± 3.4^{b}
Zinc deficient	6	537 ± 13	7.4 ± 0.8

[a] Mean ± SD.
[b] $p < .01$; [c] $p < .05$.

supplemented animals were significantly higher than that for zinc-deficient rats (Table I). When the uptake of radioactive thymidine was measured by counting method, the values obtained from zinc-supplemented rats were also significantly higher than that from zinc-deficient rats. It is noted that the radioactivity measured by counting technique was increased many fold over the values obtained by determining thymidine labeling index. The differences are probably due to the fact that the radioactivity derived from labeled DNA, labeled thymidine and other possible derivatives are all added in a counting spectrometer, whereas the labeled cells are only employed for the determination of labeling index.

Wounding resulted in destruction of a large number of cells at the lesion site. However, two days after wounding, repair was evident; labeled cells, signifying cell proliferation, appeared in the wound site. In histological sections, there were more labeled nuclei in zinc-supplemented tissue (Fig. 1A) than in zinc-deficient tissue (Fig. 1B). The thymidine labeling index for the zinc-supplemented wounded animals was 29.4 ± 1.2% against 26.3 ± 1.1% for zinc-deficient wounded animals (Fig. 2). Four days after wounding, the labeling index for zinc-supplemented animals increased to about 34% whereas it stayed about 25% for zinc-deficient animals. Histological sections revealed that a considerable number of labeling cells were observed in the skin biopsy specimen of zinc-supplemented rats, but not seen in zinc-deficient animals (Figs 1C, 1D). By the sixth day after wounding, the labeling index for zinc-supplemented animals remained about 36%, but the zinc-deficient labeling index also remained around 26%.

Discussion. The epithelium of the skin is a steady state, cell renewal system; cells are continually lost and replaced by newly formed cells, thereby maintaining the cell population structure and function (13). The renewal tissues are characterized by relatively high rates of ^{3}H-thymidine uptake and mitotic activity, with a high cell turnover rate. Dynamic equilibrium of the cell population is maintained by continued cell birth in a population of proliferating cells which gives rise to differentiating and maturing cells, and sequentially into functional end forms (14). Proliferating cells undergo a *proliferative cell cycle* composed of four phases: pre-DNA synthesis (G_1); DNA synthesis (S); post-DNA synthesis (G_2); and mitosis (M) (15, 16). The progression of cells from the proliferation stage to functional end forms follows an orderly temporal and morphological sequence.

The use of high resolution autoradiography combined with specific labeling of DNA with ^{3}H-thymidine permits analysis of certain quantitative histological parameters of cell population kinetics (17). In this study, *pulse labeling* was employed to examine the proliferative activity of epidermal cells in zinc-supplemented and zinc-deficient wounded and unwounded rats by determination of the thymidine labeling index.

The condition of zinc deficiency disturbs DNA synthesis resulting in a change in the proliferative activity of proliferating cells with a concomitant change in cell population kinetics. This is reflected in the differences between the labeling indices of zinc-supplemented and zinc-deficient unwounded rats

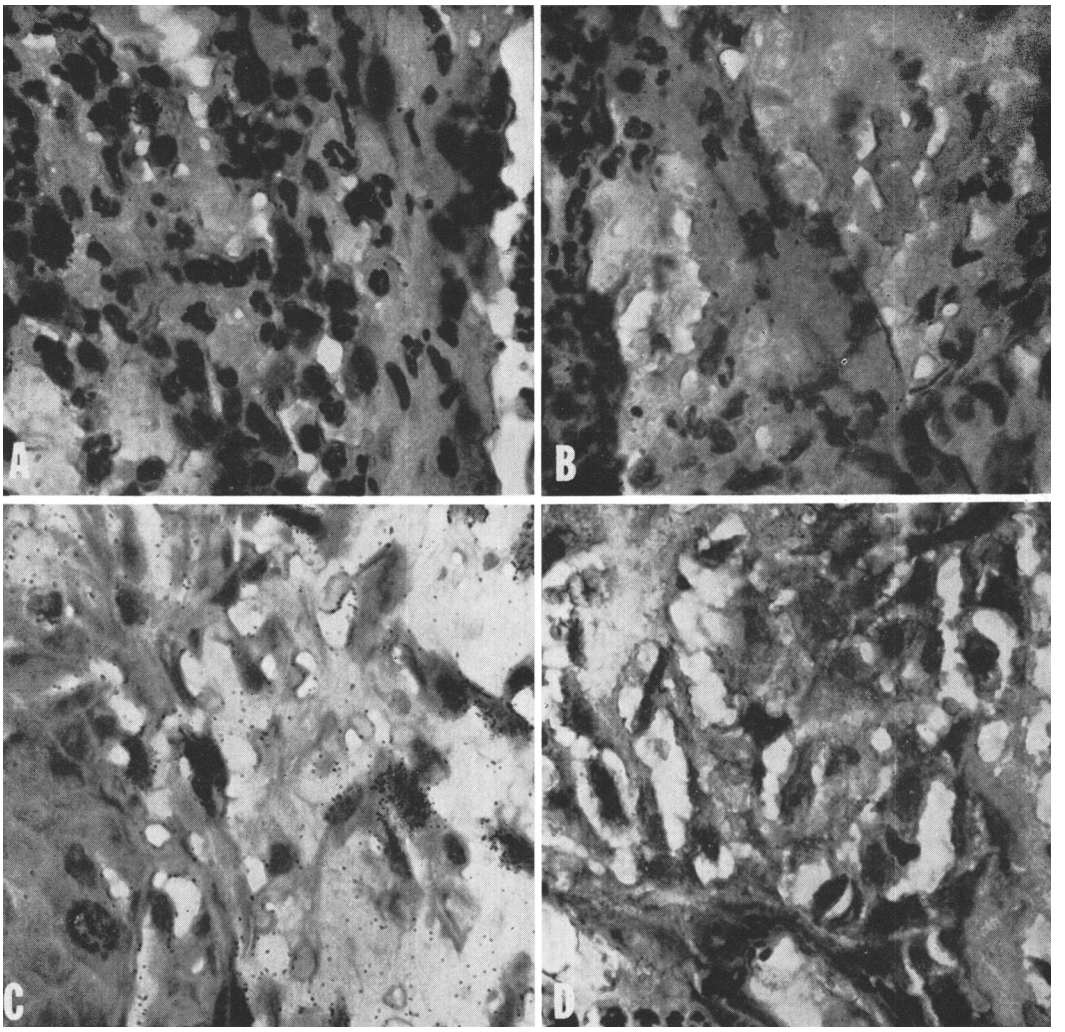

FIG. 1A–D. Autoradiographic analysis of epithelial repair ×780. A and B are sections 2 days after wounding; C and D are sections 4 days after wounding. Animals A and C are zinc-supplemented; B and D zinc-deficient. Proliferative activity of epithelial cells are much more intense in the zinc-supplemented animals, as shown by the number of labeled nuclei. Such activity leads to a quicker and better organized repair of the tissue. (See text)

(Table I). The lower value in zinc-deficient animals (~7.5%) when compared to the value for zinc-supplemented animals (~17%) indicates a lower proliferation ratio of epidermal cells in the former animals.

Wounding places a stress in the normal cell renewal tissue, changing its cell population kinetics from one of steady state to an increasing one; *i.e.*, cell proliferation exceeds that required to maintain tissue homeostasis. Wound repair commences either with the activation of previously dormant cells, or an increase in proliferation compartment. This is reflected by increases in the labeling index, which would mean an enlargement of the growth fraction (18). In both zinc-deficient and zinc-supplemented animals, there was an increase in labeling indices. However, the initial rate of increase was greater in zinc-deficient animals (Fig. 2). But, once the overshoot had ended, the zinc-deficient animals still displayed lower labeling indices dur-

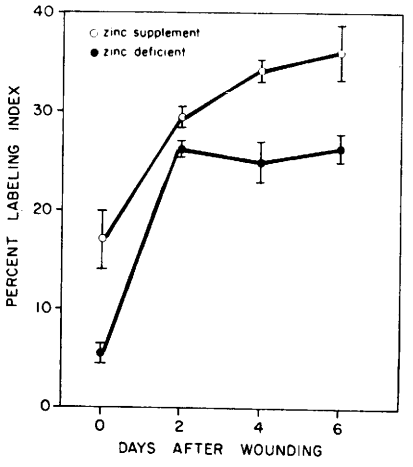

FIG. 2. Percent labeling indices for zinc-supplemented and zinc-deficient rats. Labeling indices for nonwounded animals are shown at day 0, and represents the normal situation. Wounded animals show a much higher labeling index.

ing wound repair.

In conclusion, the experimental results show that a zinc-deficient condition creates a slower rate of epithelial cell renewal but does not lead to destruction of the cell renewal pattern. Therefore, reepithelialization of wounds is much slower in zinc-deficient animals. Further studies are needed to determine the causes of these changes.

Summary. Studies were undertaken to examine the effects of zinc on the repair of the wound in the epithelium. Biopsy specimens obtained from zinc-deficient rats showed a reduced uptake of ^{3}H-thymidine when compared with controls, as shown by liquid scintillation and autoradiography. With wounding, proliferation as indicated by the labeling index increased about twice normal for zinc-supplemented rats, but 3.5 times for zinc-deficient rats. However, despite the increased proliferative rate, repair of wounds in deficient rats was always slower than in zinc-supplemented animals.

1. Miller, W. J., Morton, J. D., Pitts, W. J., and Clifton, C. M., Proc. Soc. Exp. Biol. Med. **118**, 427 (1965).

2. Sandstead, H. H., and Shephard, G. H., Proc. Soc. Exp. Biol. Med. **128**, 687 (1968).

3. Sandstead, H. H., Lanier, V. C., Jr., Shephard, G. H., and Gillespie, D. D., Amer. J. Clin. Nutr. **23**, 514 (1970).

4. Pories, W. J., Henzel, J. H., Rob, C. G., and Strain, W. H., Lancet **2**, 121 (1967).

5. Hausain, S. L., Lancet **1**, 1069 (1969).

6. Loeb, L., J. Med. Res. **41**, 247 (1920).

7. Gillman, T., and Penn, J., Med. Proc. **2**, 93 (1956).

8. Hell, E. A., and Crunickshank, C. N. D., Exp. Cell Res. **31**, 128 (1963).

9. Eujioka, M., and Lieberman, I., J. Biol. Chem. **239**, 1164 (1964).

10. Buchanan, P. J., and Hsu, J. M., Fed. Proc. **27**, 483 (1968).

11. Hsu, J. M., and Anthony, W. L., J. Nutr. **101**, 445 (1971).

12. Hsu, J. M., and Davis, R. L., Fed. Proc. **30**, 643 (1971).

13. Messier, B., and Leblond, C. P., Amer. J. Anat. **106**, 247 (1960).

14. Quastler, H., *in* "Cell Proliferation" (L. F. Lamerton and R. J. M. Fry, eds.), p. 18. Blackwell, Oxford (1963).

15. Howard, A., and Pelc, S. R., Heredity (suppl.) **6**, 261 (1953).

16. Lajtha, L. G., Oliver, R., and Ellis, F., Brit. J. Cancer **8**, 367 (1954).

17. Cleaver, J. E., "Thymidine metabolism and cell kinetics," p. 150. North-Holland, Amsterdam (1967).

18. Mendelsohn, M. L., J. Nat. Cancer Inst. **28**, 1015 (1962).

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