Studies on Wound Healing: Effects of Calcium D-Pantothenate on the Migration, Proliferation and Protein Synthesis of Human Dermal Fibroblasts in Culture

Bernd J. Weimann and Danielle Hermann

F. Hoffmann-La Roche Ltd, Vitamins and Fine Chemicals Divsions, Research and Development, 4070 Basel, Switzerland

Received for publication: July 16, 1998

Abstract: The effect of calcium D-pantothenate on the migration, proliferation and protein synthesis of human dermal fibroblasts from three different donors was investigated. The migration of cells into a wounded area was dose-dependently stimulated by Ca D-pantothenate. The number of cells that migrated across the edge of the wound increased from 32 ± 7 cells/ mm without Ca D-pantothenate to 76 ± 2 cells/ mm with 100 mg/ml Ca D-pantothenate. Moreover, the mean migration distance per cell increased from 0.23 ± 0.05 mm to 0.33 ± 0.02 mm. The mean migration speed was calculated to be 10.5 mm/hour without and 15 mm/hour with Ca D-pantothenate. Cell proliferation was also dose-dependently stimulated. The final cell densities were 1.2 to 1.6-fold higher in cultures containing 100 mg/ml Ca D-pantothenate. The protein synthesis was modulated, since two unidentified proteins were more strongly expressed in pantothenate supplemented cultures.

In conclusion, Ca D-pantothenate accelerates the wound healing process by increasing the number of migrating cells, their distance and hence their speed. In addition, cell division is increased and the protein synthesis changed. These results suggest that higher quantities of pantothenate are locally required to enhance wound healing.

Key words: Calcium D-pantothenate, wound healing, human dermal fibroblasts, migration, proliferation

Introduction

The human skin is probably the organ which is most often injured. Disturbance of the normal arrangement of skin by wounding causes coordinated cellular and biochemical processes leading to wound healing. Generally, three distinct phases of epidermal wound healing are observed: 1. inflammation, migration and proliferation, 2. repair including formation of substances like glycosaminoglycans and collagen and 3. the remodelling phase [1–10]. Immediately after injury and clotting of the extra-vasated blood, inflammatory cells accumulate within the wounded area.

Wound fluids conditioned by these cells were shown to contain mitogenic and angiogenic activities [11–14]. After 18–24 hours epidermal cells migrate from the surrounding wound margins into the wounded area. Distal to the wound margins an increase in epidermal mitotic activity is observed resulting in sufficient cell divisions to fill and approximately restore the original structure. In the repair phase, new connective tissue fibers are formed and deposited. The tensile strength increases continuously as the small collagen fibrils aggregate into large collagen fibers. The mitotic activity of fibroblasts ceases with the beginning of collagen formation. The wounds are then closed by wound contraction due to modified fibroblasts, the myofibroblasts [15–17]. Finally, a re-epithelialization of the wound is observed.

Pantothenic acid is a vitamin of the B group, which is widely found in plants and animals. As active principle it is part of coenzyme A and an acyl carrier protein, a constituent of fatty acid synthetase [18]. Apart from being involved in many other biochemical processes, it is an essential cofactor in the synthesis of fatty acids and for the co-translational and post-translational acetylation of proteins, either at the amino terminal end or within the protein at the ε-amino group of lysine [19, 20]. This N-terminal acetylation protects proteins from degradation. Recent reports suggest that some of the pantothenate dependent modifications are important for the assembly of multisubunit protein complexes of membranes and for the transport of glycoproteins through the cisternae of the Golgi apparatus [21–23].

In standardized wound healing models in animals (rats, rabbits and guinea pigs), closure of skin wounds was shown to be accelerated by orally administered pantothenic acid or by panthenol topically applied as ointment [24–27]. Pantothenic acid also increased skin strength and fibroblastic content of scar tissue [28]. It is therefore possible that migration, proliferation and maturation (protein synthesis) are affected by pantothenate, which could augment wound repair by stimulating these processes. The results reported here strongly support this hypothesis.

Materials and Methods

Calcium D-pantothenate was from F. Hoffmann-La Roche Ltd, Basel, Switzerland. Cell culture media, trypsin/EDTA solution, and foetal bovine serum (FBS) were obtained from GIBCO, Paisley, Scotland.

Cell cultures: Human dermal fibroblasts (HF) in culture were obtained by courtesy from Dr. H. J. Müller and Mr. Wenzel, Kinderspital Basel, Switzerland. Different isolates of human dermal fibroblasts were grown from biopsies taken from the forearm of hospitalized children. Experiments were done with cells ranging between the 5th and 10th passages. They were cultured in RPMI-1640 medium supplemented with 10% FBS, 2 mM glutamine, 1% non-essential amino acids, 10 mM Hepes, pH 7.3, 100 U/ml penicillin, and 100 µg/ml streptomycin. For the migration and proliferation experiments, a pantothenate-free RPMI-1640 medium was used to which Ca D-pantothenate was added where indicated. FBS was extensively dialyzed against Hank's balanced salt solution (HBSS).

Cell migration: The effects of Ca D-pantothenate on the migration of cells into the wound was studied using the method described by Bürk [29]. Two to 3×10^4 cells were seeded into 35 mm Petri dishes (Falcon 3001 F, Becton Dickinson, Oxnard, CA, USA) in 2.5 ml of medium. After confluent cell layers had formed, cells were kept for 24 hours in Ca pantothenate-free medium containing 2.5% FBS. Using a razor blade, wounds of an approximate size of 2×1 cm were made by scraping cells off the dish. Medium with detached cells was removed by aspiration and wounded cell layers were washed once with 3 ml of HBSS. Then, 2.5 ml of D-pantothenate-free medium, 2.5% FBS containing either 12.5, 25, 50 or 100 µg/ml (2.6×10^{-6}–2.1×10^{-5} M) Ca D-pantothenate, respectively, were added and dishes were incubated for 22 hours at 37 °C in a humidified 5% CO_2/95% air atmosphere. Control cultures were kept without Ca D-pantothenate. Cells were fixed and stained with 1.5 ml of Leishman's eosin-methylene blue (1.5 g/l methanol, Merck AG, Darmstadt, Germany) for 10 min at room temperature and then diluted with 3.5 ml of water. After 45 min, dishes were rinsed thoroughly in tap water and dried. The number of cells crossing the starting line was counted using a computer-assisted morphometric system (IBM AT03) with a graphic tablet combined with a light microscope connected to a video system. The system was driven by the DIASYS 2 software packet (H. Meier, DataLab, Thörigen, Switzerland). Cells invading the wound from the starting line were counted at a total length of 9 mm per plate in five different areas of 1.8 mm. In addition, the distance of migration from the starting line was determined. The results are given as means of four plates per point.

Cell proliferation: Cells were seeded at densities of 5×10^3 HF/ml per well into multiwell tissue culture plates (24 well tissue culture cluster 3524, Costar, Cambridge, MA, USA) and grown with Ca D-pantothenate at different concentrations as indicated in Figure 4. Controls were done without Ca D-pantothenate. Cell numbers were measured after washing the cells three times with 1 ml of HBSS followed by incubation with 0.5 ml trypsin/EDTA solution

for 15 min at 37 °C. The detached cells were fixed in 0.5 ml of 1% formaldehyde, 0.145 M NaCl, 3.4 mM EDTA, dispersed by vigorous pipetting and counted using a Coulter counter (Coulter Electronics, Ltd., Harpenden, England).

Protein synthesis: Approximately 5×10^4 cells/ml per well were cultured in medium containing 10% FBS without or with 100 µg/ml Ca D-pantothenate for 24 hours. They were then incubated for 24 hours in methionine-free medium containing 1% FBS and 50 µCi/ml of [^{35}S]methionine (spec. act. 1400 Ci/ mmol, The Radioactive Centre, Amersham, England) without or with 100 µg/ml Ca D-pantothenate. Cell supernatants were centrifuged for 10 min at 12 000 × g and forced through 0.45 µm filters. Electrophoresis of proteins was carried out using 15% SDS polyacrylamide gels [30]. Radioactivity of single bands in the gels was scanned using a phosphorImager 400B (Molecular Dynamics).

Results

Morphology: Cell layers of confluent human dermal fibroblasts grown in presence of Ca D-pantothenate showed the typical elongated, spindle-shaped form. They were orientated in parallel arrays. Cells in the early logarithmic growth phase were morphologically very similar, irrespective of the concentration of Ca D-pantothenate in the medium. In absence of Ca D-pantothenate, however, the morphology of some cells changed in densely populated cultures. A substantial proportion of these cells had a larger surface area and seemed to form a single-layered cell sheet. These cells did not grow in a predominant direction (Fig. 1).

Cell migration: Dermal fibroblasts at the edge of the wound separated from the confluent cell layers and migrated as individual cells rather than in clusters in the direction of the microgrooves (Fig. 2). When increasing quantities of Ca D-pantothenate were added to the culture medium of wounded cell layers, the number of cells migrating across the edge of the wound increased within 22 hours from 32 ± 7.1 cells/mm without Ca D-pantothenate to 76 ± 2.4 cells/mm ($\bar{x}$ ± SEM, n = 4) in the presence of 100 µg/ml of Ca D-pantothenate (Fig. 3A). Furthermore, not only the number of migrating cells but also the mean migration distance per cell increased from 0.23 ± 0.05 mm to 0.33 ± 0.02 mm ($\bar{x}$ ± SEM, n = 4) (Fig. 3B). From these data, the mean migration speed was calculated to be 10.5 µm/hour without and 15 µm/hour with Ca D-pantothenate.

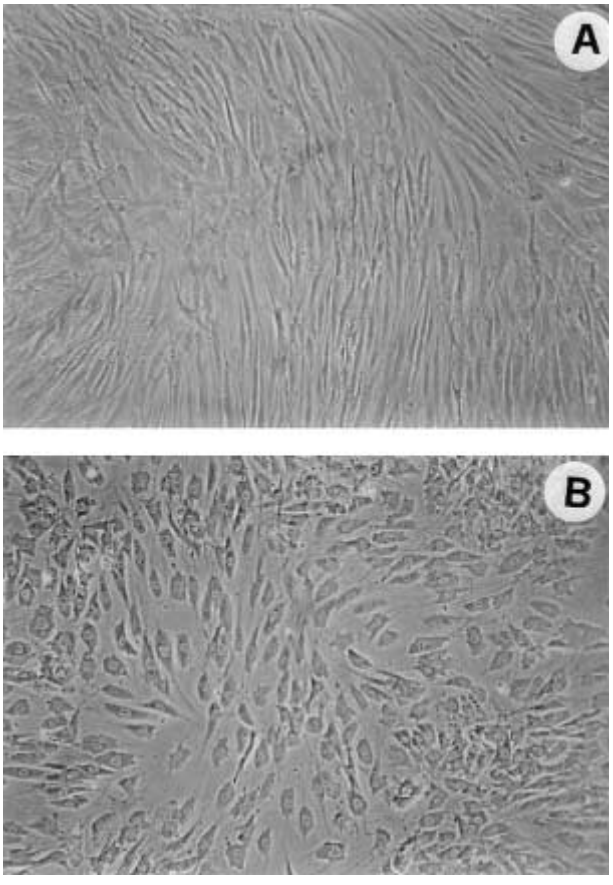

Figure 1: Micrographs of human dermal fibroblasts in culture. Cells were grown in A: normal RPMI-1640 medium, and B: in pantothenate free medium. Magnification × 75.

Cell proliferation: In order to demonstrate the influence of Ca D-pantothenate on the rate of proliferation, dermal fibroblasts were grown without or with Ca D-pantothenate at concentrations between 12.5–100 µg/ml. Proliferation was measured by cell counting and is shown in Figure 4. Growth of the fibroblasts was dose-dependently stimulated. All cultures formed confluent layers by day 8. Increasing cell numbers at confluency were observed in cultures supplemented with increasing concentrations of Ca D-pantothenate. The effect was observed with cells from all donors, although to a variable extent. In cultures of two isolates, the final cell numbers were 1.6-fold higher in the presence of Ca D-pantothenate, while only a 1.2-fold increase was found with the other two isolates. This effect became evident during the later growth, whereas in the early logarithmic growth phase cell numbers were similar in cultures with or without Ca D-pantothenate. Similar results were obtained with zinc D-pantothenate.

Protein synthesis: Fibroblasts from three different donors were treated without or with 100 µg/ ml Ca D-pantothen-

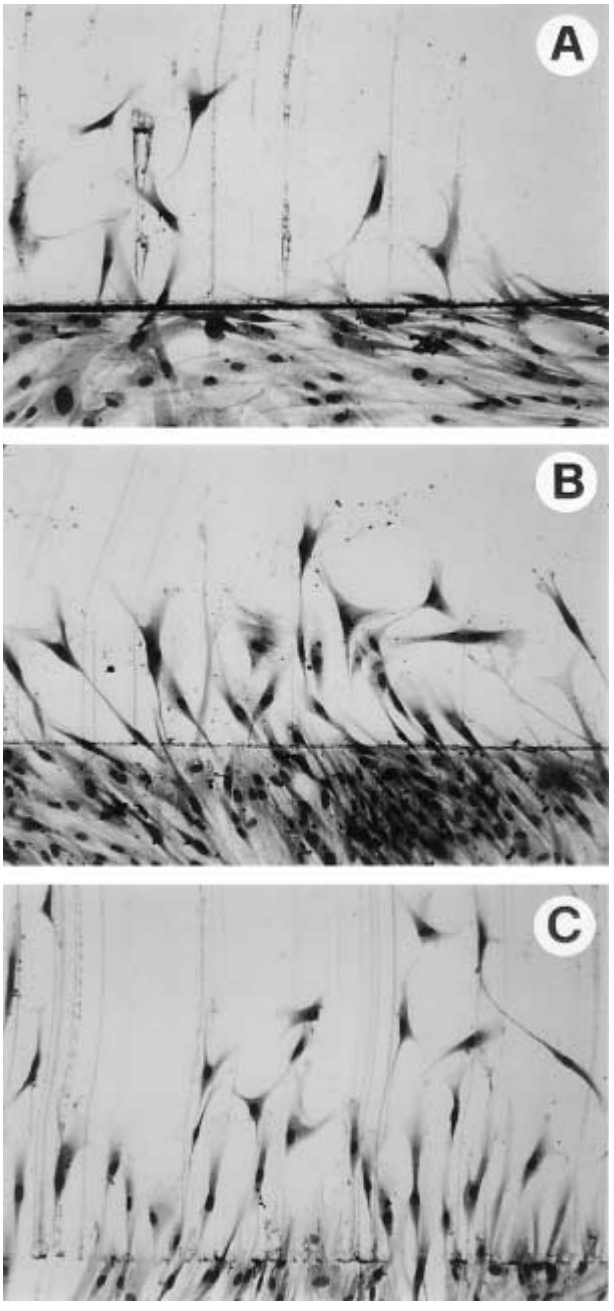

Figure 2: Effect of calcium D-pantothenate on the migration of human dermal fibroblasts. Wounded cell layers were incubated in medium containing calcium D-pantothenate (µg/ml), A:0 (control); B:25; C:100. Magnification × 130.

ate and metabolically labelled with [^{35}S]methionine. Secreted proteins were analyzed by SDS-PAGE and radioactivity was scanned using a phosphorimager. All protein bands observed in cultures treated with Ca D-pantothenate were also seen in untreated controls. However, increased amounts of proteins with estimated molecular weights of about 12 000 (43 233 ± 7737 cpm vs. 10 384 ± 5314 cpm; $\bar{x}$ ± SEM, n = 3) and 46 000 (61 349 ± 6214 vs. 50 083 ± 3742 cpm) were found in culture media of treated cells as compared to untreated controls. The intensity of the band with a molecular weight of 43 000 did not change (134 508 ± 20 532 cpm vs. 130 670 ± 19 019 cpm) and served as internal control. The nature of the two proteins has not yet been identified.

Discussion

In the absence of Ca D-pantothenate, morphological changes were observed in some dermal fibroblasts suggesting the importance of the vitamin in maintaining the structural integrity of the cells. Futhermore, we have shown that Ca D-pantothenate stimulated both the migration of cells and their growth. In addition, the quantities of some of the secreted proteins were increased in cultures supplemented with Ca D-pantothenate.

Migration of cells into a wound is one of the initial events in the wound repair process. It is influenced by a number of factors including platelet-derived growth factor (PDGF). Apart from being a major mitogen in serum for fibroblasts and smooth muscle cells [31], PDGF very potently stimulates the migration of dermal fibroblasts. In the migration experiments a serum concentration of 2.5% corresponding to about 25 pg PDGF/ml medium [32] was used. As shown, Ca D-pantothenate augmented the motility of dermal fibroblasts.

After wounding confluent layers of human dermal fibroblasts, cells did not move in clusters, but rather migrated as single cells in the direction of the microgrooves in the plastic dish. This phenomenon was observed earlier by Weiss and termed contact guidance [1]. Regarding cell migration in the presence of Ca D-pantothenate, the results merit discussion in two points: the increased number of cells observed in the wounded area and the increased mean migration distance and hence the mean speed. After wounding *in vivo,* a large quantity of cells must rapidly migrate from the surrounding tissue into the wound in order to form a provisional closure. Therefore, a stimulation of the initial migration seems to be of importance to accelerate wound healing. As shown here, pantothenate exerted a stimulatory effect on the initial phase of migratory cells. Increasing concentrations of pantothenate caused increasing numbers of cells to migrate. In addition, the mean distance and hence the speed of migration also depended on the concentration of Ca D-pantothenate in the medium. In the absence of Ca D-pantothenate cells migrated with an average speed of about 10.5 µm/hour. The average speed was accelerated to 15 µm/hour with Ca

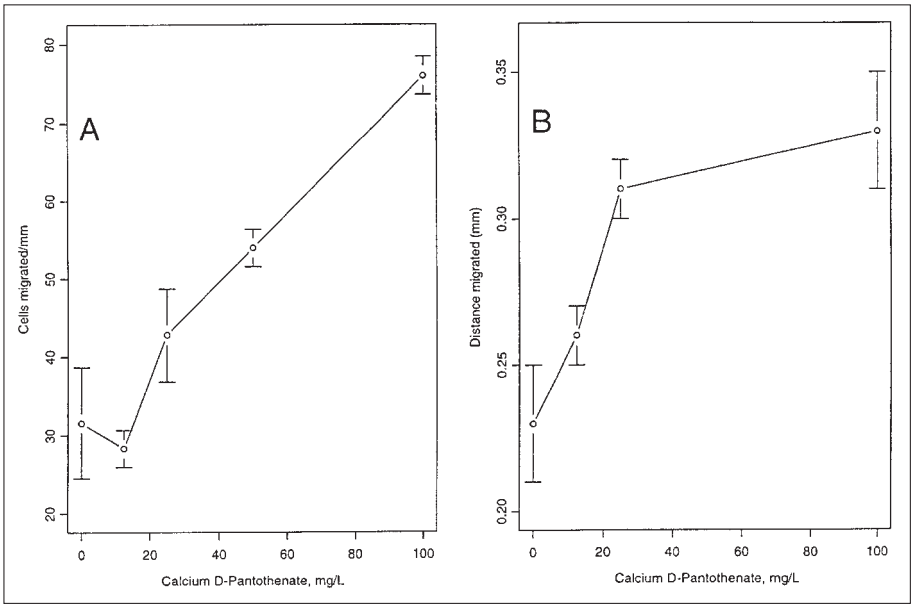

Figure 3: Migration of human dermal fibroblasts into the wound. Confluent cell layers were wounded and exposed to calcium D-pantothenate as indicated. A: Number of cells crossing the edge of the wound. B: Distance of migration. $\bar{X} \pm$ SEM, n = 4.

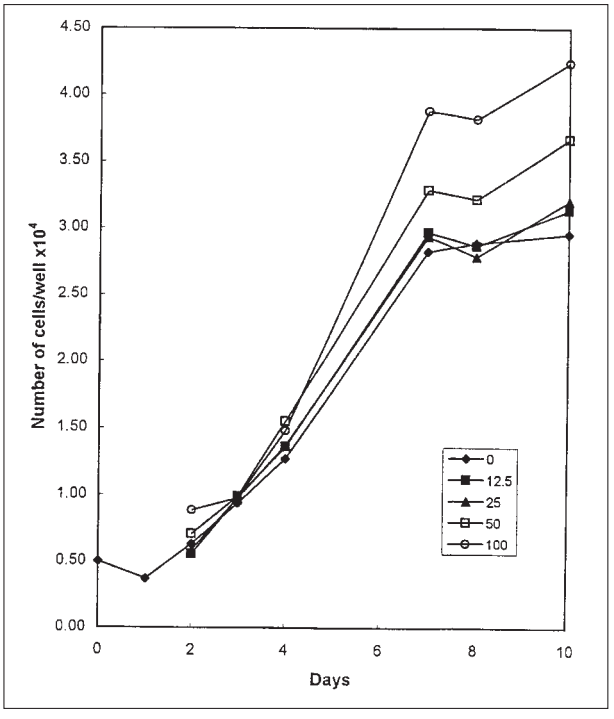

Figure 4: Proliferation of human dermal fibroblasts in culture. Cells were grown in presence of calcium D-pantothenate at the indicated concentrations (µg/ml). Control experiments were done in parallel without calcium D-pantothenate.

D-pantothenate reflecting a substantial effect of the vitamin on cell motility. Generally, the average speed of migrating cells depends on environmental factors such as moisture. As pointed out by Winter [33], different types of cells from various species migrated about 5–10 µm/hour in dry wounds and about 12–21 µm/hour in wet wounds. For example, cells of injured pig skin migrated at a speed of about 21 µm/hour in moist wounds, whereas a speed of only 7 µm/hour was observed in dry wounds [33]. Other factors such as interleukin-2 and transforming growth factor-β (TGF-β) derived from activated T cells were also shown to stimulate migration of cultured rat epitenon cells participating in wound repair of tendons [14]. The average speed doubled from 14 µm/hour in control cultures to 28 µm/hour in the presence of activated T cells, interleukin-2 or TGF-β.

Pantothenic acid is a component of coenzyme A (CoA) which is present in every tissue with a characteristic cellular distribution between cytosol and mitochondria (for reviews see 34, 35). The cellular content of CoA is maintained in a narrow range, but it changes in some pathological conditions. For example, pantothenate is decreased in all tissues of diabetic rats [36]. The turnover of CoA in damaged skin may be relatively high so that the requirement for pantothenic acid may increase in wound healing, an interpretation consistent with the results. The question arises as to whether the normal dietary supply is sufficient and acts rapidly enough to optimize the many cellular processes involved in wound healing. It is likely that additional quantities of pantothenate are locally needed. Cells in the undisturbed epidermis are in a condensed, spheroid form. When they start to migrate, they become elongated and spindle-shaped. This implies continuous membrane alterations for cell adhesion and cell detachment on the wound surface and hence changes in metabolic patterns, e. g. energy production and consumption.

Dietary pantothenate is absorbed in the intestine and co-transported with sodium, using the sodium electrochemical gradient which is maintained by the Na^+, K^+-ATPase on the basolateral membrane (for ref. see [34]). After intestinal absorption, pantothenate is rapidly taken up by blood cells and tissues. To achieve local concentrations comparable to those found effective *in vitro,* one could either consume higher amounts of pantothenate or apply it locally in form of an ointment. By this treatment, drying of the wound would be prevented which is important since cell migration and mitotic activity are reduced in dry wounds.

The question whether cell replication might in part account for the number of cells that was found beyond the starting line was investigated. The number of migrating cells increased gradually with time. When replication (DNA synthesis) was inhibited by γ-irradiation or hydroxyurea, migration of cells was unaffected. These results are in agreement with previous observations which showed that DNA synthesis was first observed 20–24 hours after the beginning of cell migration [37–40]. Small wounds can be fully repaired by migrating cells alone [37, 38]. In addition, colchicine or vinblastine sulfate blocking mitotic activity have no influence on epidermal cell migration at 24 hours after wounding [3]. It is, therefore, reasonable to assume that in our experiments the majority of cells migrated into the wound and that only a minor part of cells originated from cell proliferation.

A dose-dependent stimulation of growth of human dermal fibroblasts was observed in presence of Ca D-pantothenate. Similar results have been reported by others [41]. The final cell densities in confluent cultures grown with 100 µg Ca D-pantothenate/ml medium were 1.2–1.6 times higher than those observed in control cultures. In the absence of Ca D-pantothenate some cells changed their morphology from a spindle-shaped elongated form to a more rounded appearance. These cells tended to form single-layered sheets and seemed to have lost the tendency to grow in multilayers, usually observed with fibroblasts grown under optimal growth conditions. The *in vitro* results are in agreement with results obtained in rabbits, where the fibroblastic content of the scar was higher in pantothenate supplemented animals [28].

The patterns of secreted proteins were very similar in cultures grown with and without pantothenate. Induction of new protein species by pantothenate was not observed, whereas the protein synthesis was modulated. Two proteins with apparent molecular weights of 12 000 and 46 000 were more strongly expressed in the presence of pantothenate. The ratios were 4.0 and 1.2, respectively. This pattern was observed with cells from three different donors. The two proteins remain to be identified. The expression of the other secreted proteins remained unchanged.

In conclusion, calcium D-pantothenate accelerates the cell migration and proliferation and modulates the protein synthesis of cultured human dermal fibroblasts. The data are in agreement with results obtained with pantothenate on the healing of artificial wounds *in vivo* showing an accelerated repair process and a significantly increased fibroblastic content 10–15 days after operation [24–28]. The main processes needed to rapidly close a wound are enhanced by pantothenate, namely migration of cells from the surrounding area into the injured region and proliferation to fill the gap. These observations are consistent with the interpretation that pantothenate has an important therapeutic role in the regeneration of wounded tissue *in vivo.* They also support the conclusion that pantothenate as a nutritional factor plays a critical role in wound healing.

Acknowledgement

We are grateful to Dr. H. Kuhn and B. Wessner for introducing us to the morphometric methods. We thank Drs. B. Glatthaar, W. Schalch and J. Schwager for critically reading the manuscript.

References

1. Weiss, P. (1959) The biological foundations of wound repair. Harvey Lect. 55, 13–42.
2. Ross, P., Everett, N. B. and Tyler, R. (1970) Wound healing and collagen formation. VI. The origin of the wound fibroblast studied in parabiosis. J. Cell. Biol. 44, 645–654.
3. Krawczyk, W. S. (1971) A pattern of epidermal cell migration during wound healing. J. Cell. Biol. 49, 247–263.
4. Marks, R. and Nishikawa, T. (1973) Active epidermal movement in human skin in vitro. Br. J. Dermatol. *88,* 245–248.
5. Marks, R. (1981) The healing and the nonhealing of wounds and ulcers of the skin. In: Tissue repair and regeneration (Glynn, L. E. ed.) vol. 3, pp. 309–342, Elsevier, Amsterdam.
6. Harris, A. K., Stopak, D. and Wild, P. (1981) Fibroblast traction as a mechanism for collagen morphogenesis. Nature 290, 249–251.
7. Barrandon, Y. and Green, H. (1987) Cell migration is essential for sustained growth of keratinocyte colonies: The roles of transforming growth factor-α and epidermal growth factor. Cell 50, 1131–1137.
8. Wokalek, H. (1988) Cellular events in wound healing. CRC Crit. Rev. Biocompatibility 4, 209–246.
9. Clark, R. A. F. (ed.) (1996) The molecular and cellular biology of wound repair. Plenum, New York.
10. Martin, P. (1997) Wound healing – Aiming for perfect skin regeneration. Science 276, 75–81.
11. Leibovich, S. J., Polverini, P. J., Shepard, H. M., Wiseman, D. M., Shively, V. and Nuseir, N. (1987) Macrophage-induced

angiogenesis is mediated by tumour necrosis factor-α. Nature 329, 630–632.
12. Rappolee, D. A., Mark, D., Banda, M. J. and Werb, Z. (1988) Wound macrophages express TGF-α and other growth factors in vivo: Analysis by mRNA phenotyping. Science 241, 708–712.
13. Kovacs, E. J. (1991) Fibrogenic cytokines: the role of immune mediators in the development of scar. Immunol. Today 12, 17–23.
14. Wojciak, B. and Crossan, J. F. (1994) The effects of T cells and their products on in vitro healing of epitenon cell microwounds. Immunology 83, 93–98.
15. Majno, G., Gabbiani, G., Hirschel, B. J., Ryan, G. B. and Statkov, P. R. (1971) Contraction of granulation tissue in vitro: Similarity to smooth muscle. Science 173, 548–550.
16. Ryan, G. B., Cliff, W. J., Gabbiani, G., Irlé, C., Montandon, D., Statkov, P. R. and Majno, G. (1974) Myofibroblasts in human granulation tissue. Hum. Pathol. 5, 55–67.
17. Majno, G. (1979) The story of myofibroblasts. Am. J. Surg. Pathol. 3, 535–542.
18. Wakil, S. J., Stoops, J. K. and Joshi, V. C. (1983) Fatty acid synthesis and its regulation. Ann. Rev. Biochem. 52, 537–579.
19. Persson, B., Flinta, C., von Heinje, G. and Jornvall, H. (1985) Structures of N-terminally acetylated proteins. Eur. J. Biochem. 152, 523–527.
20. Driessen, H. P. C., de Jong, W. W., Tesser, G. I. and Bloemendal, H. (1985) The mechanism of N-terminal acetylation of proteins. CRC Crit. Rev. Biochem. 18, 281–306.
21. Sefton, B. M. and Buss, J. E. (1987) The covalent modification of eukaryotic proteins with lipid. J. Cell. Biol. 104, 1449–1453.
22. Brambl, R. and Plesofsky-Vig, N. (1986) Pantothenate is required in *Neurospora crassa* for assembly of subunit peptides of cytochrome c oxidase and ATPase/ATP synthase. Proc. Natl. Acad. Sci. (USA) 83, 3644–3648.
23. Glick, B. S. and Rothman, J. E. (1987) Possible role for fatty acyl-coenzyme A in intracellular protein transport. Nature 326, 309–312.
24. Casadio, S., Mantegani, A., Coppi, G. and Pala, G. (1967) On the healing properties of esters of D-panthenol with terpene acids, with particular reference to D-pantothenyl trifarnesylacetate. Arzneim. Forsch. 17, 1122–1125.
25. Weiser, H. and Erlemann, G. (1988) Acceleration of superficial wound healing by panthenol zinc oxide. Cosmetics Toiletries 103, 79–84.
26. Niedermeier, S. (1987) Animal experiment studies on the problem of treating corneal lesions. Klin. Monatsbl. Augenheilkd. 190, 28–29.
27. Hosemann, W., Wigand, M. E., Gode, U., Langer, F. and Dunker, I. (1991) Normal wound healing of the paranasal sinuses: clinical and experimental investigations. Eur. Arch. Otorhinolaryngol. 248, 390–394.
28. Aprahamian, M., Dentinger, A., Stock-Damgé, C., Kouassi, J. C. and Grenier, J. F. (1985) Effects of supplemental pantothenic acid on wound healing: experimental study in rabbits. Am. J. Clin. Nutr. 41, 578–589.
29. Bürk, R. R. (1973) A factor from a transformed cell line that affects cell migration. Proc. Natl. Acad. Sci. (USA) 70, 369–372.
30. Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.
31. Ross, R., Glomset, J., Kariya, B. and Harker, L. (1974) A platelet dependent serum factor that stimulates the proliferation of arterial smooth muscle cells in vitro. Proc. Natl. Acad. Sci. (USA) 71, 1207–1210.
32. Bowen-Pope, D. F., Harts, C. E. and Seifert, R. A. (1989) Sera and conditioned media contain different isoforms of platelet-derived growth factor (PDGF) which bind to different classes of PDGF receptor. J. Biol. Chem. 264, 2502–2508.
33. Winter, G. D. (1964) Movement of epidermal cells over the wound surface. Adv. Biol. Skin. 5, 113–127.
34. Tahiliani, A. G. and Beinlich, C. J. (1991) Pantothenic acid in health and disease. Vit. Hormon. 46, 165–228.
35. Robishaw, J. D. and Neely, J. R. (1985) Coenzyme A metabolism. Am. J. Physiol. 248, E1–E9.
36. Reddi, A. S., Jyothirmayi, G. N., DeAngelis, B., Frank, O. and Baker, H. (1993) Tissue concentrations of water-soluble vitamins in normal and diabetic rats. Int. J. Vit. Nutr. Res. 63, 140–144.
37. Lipton, A., Klinger, I., Paul, D. and Holey, R. W. (1971) Migration of mouse 3T3 fibroblasts in response to a serum factor. Proc. Natl. Acad. Sci. (USA) 68, 2799–2801.
38. Sholley, M. M., Gimbrone, M. A. and Cotran, R. S. (1977) Cellular migration and replication in endothelial regeneration: a study using irradiated endothelial cultures. Lab. Invest. 36, 18–25.
39. Thorgeirsson, G. and Robertson, A. L. (1978) Platelet factors and the human vascular wall. II. Such factors are not required for endothelial cell proliferation and migration. Atherosclerosis 31, 231–238.
40. Majack, R. A. and Clowes, A. W. (1984) Inhibition of vascular smooth muscle cell migration by heparin-like glycosaminoglycans. J. Cell. Physiol. 118, 253–256.
41. Lacroix, B., Didier, E. and Grenier, J. F. (1988) Effects of pantothenic acid on fibroblastic cell cultures. Res. Exp. Med. 188, 391–396.

Bernd J. Weimann

F. Hoffmann-La Roche Ltd
Vitamins and Fine Chemicals Divisions
Research and Development
Building 93/344, 4070 Basel, Switzerland