Variations of Hair Follicle Size and Distribution in Different Body Sites

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For the evaluation and quantification of follicular penetration processes, the knowledge of variations of hair follicle parameters in different body sites is basic. Characteristics of follicle sizes and potential follicular reservoir were determined in cyanoacrylate skin surface biopsies, taken from seven different skin areas (lateral forehead, back, thorax, upper arm, forearm, thigh, and calf region). The highest hair follicle density and percentage of follicular orifices on the skin surface and infundibular surface were found on the forehead, whereas the highest average size of the follicular orifices was measured in the calf region. The highest infundibular volume and therefore a potential follicular reservoir was calculated for the forehead and for the calf region, although the calf region showed the lowest hair follicle density. The calculated follicular volume of these two skin areas was as high as the estimated reservoir of the stratum corneum. The lowest values for every other parameter were found on the forearm. The present investigation clearly contradicts former hypothesis that the amount of appendages of the total skin surface represents not more than 0.1%. Every body region disposes its own hair follicle characteristics, which, in the future, should lead us to a differential evaluation of skin penetration processes and a completely different understanding of penetration of topically applied drugs and cosmetics.

J Invest Dermatol 122:14–19, 2004

The knowledge of permeation and penetration processes is a prerequisite for the development and optimization of drugs and cosmetics. In the past, percutaneous absorption was described as a diffusion though the lipid domains of the stratum corneum. It was presumed that skin appendages, which mean hair follicles and sweat glands, play a subordinate role in absorption processes. The amount of appendages of the total skin surface was estimated to represent up to 0.1% (Schaefer and Redelmeier, 1996). Nevertheless, previous studies show higher absorption rates in skin areas with higher follicle density (Feldmann and Maibach, 1967; Maibach *et al*, 1971; Hueber *et al*, 1994a,b; Tenjarla *et al*, 1999). On the other hand, hair follicle size and density and the amount of the absorbed drug have never been correlated. The variation in the thickness of the stratum corneum in different body areas was considered to be the main reason for the varying absorption rates. Feldmann and Maibach (1967) and Maibach *et al* (1971) found regional variations of percutaneous absorption in different skin areas. They assumed that the density and size of hair follicles might be the reason for their findings. More recent studies more strongly suggest that skin appendages play an important role in permeation and penetration processes of topically applied substances. Tenjarla *et al* (1999) and Hueber *et al* (1994a,b) found significant differences in percutaneous absorption of appendage-free scarred skin and normal skin. Turner and Guy (1998) found a significant iontophoretic drug delivery across the skin via follicular structures. Essa *et al* (2002) performed an *in vitro* Franz cell experiment for iontophoretic drug delivery. A new technique involving a stratum corneum/epidermis sandwich method was used for blocking the follicular orifice. A five times lower absorption rate was found when the potential shunt routes were blocked.

Moreover, Hueber *et al* (1994a,b) found a follicular reservoir of radiolabeled triamcinolone acetonide in human skin. Lademann *et al* (1999, 2001) found an amount of topically applied titanium dioxide microparticles, located in the hair follicles of the forearm. They showed that some follicles were open, whereas others were closed for the penetration process.

Hair follicle density has mainly been measured for terminal hair follicles on the scalp. Blume *et al* (1991) determined the vellus hair follicle density on the forehead, cheek, chest, and back by phototrichogram. Seago and Ebling (1995) measured hair follicle density on the upper arm and thigh using a classical trichogram. Pagnoni *et al* (1994) used the cyanoacrylate technique for the measurement of hair follicle density in different regions of the face.

Hair follicles are the most important appendages in terms of surface area and skin depth. An exact knowledge of hair follicle densities, size of follicular orifices, follicular volume, and follicular surface is necessary for the understanding of follicular penetration processes.

Therefore, our aim was to quantify characteristics of hair follicle sizes. This includes the measurement of the following parameters: density, size of follicular orifice, amount of orifice of the skin surface, hair shaft diameter, and volume and surface of the infundibula in different regions of the body by using noninvasive cyanoacrylate skin

surface biopsies and light microscopy. The volume of the follicle infundibula may represent the potential follicular reservoir for topically applied substances.

Results

Hair follicle density was measured on seven different body sites in six healthy volunteers. The samples were taken from corresponding areas in the different body areas of the volunteers. Figure 3 gives the average density with standard deviation for every test area. The average density was highest on the forehead (292 follicles/cm^2), significantly higher than in all other regions ($p = 0001$). The skin regions on the back showed 29, on the thorax 22, on the upper arm 32, on the forearm 18, on the thigh 17, and on the calf region 14 follicles per cm^2 on average. The back and upper arm showed no significant differences in hair follicle density ($p > 0.05$).

Significant intersite variations of the diameters of the follicular orifices could be found ($p = 0001$). The thigh and calf showed no significant difference in diameters ($p > 0.05$). The diameters showed great variations in every body site. High standard deviations were found, especially on the forehead and back. (These areas belong to the seborrheic regions of the body, where small vellus hair follicles are found together with large sebaceous follicles.) Comparing the mean values of diameters of the hair follicle orifices, the smallest diameters were found on the forehead with 66 μm and on the forearm with 78 μm. The calf region showed the largest diameter of the follicular orifices (Fig 4).

The percentage of follicular orifices on the skin surface is given in Table I for the seven different test regions. The amount was calculated by adding all circle areas of the follicle orifices (calculated with the formula for circle areas: $A = \pi(d/2)^2$) in 1 cm^2 of the different test regions.

Although the forehead shows the smallest diameter, it also shows, owing to the elevated hair follicle density, the highest percentage of follicular orifices on the skin surface. Significant intersite variations of the percentage of the follicular orifices on the skin surface were found ($p = 0001$). The thigh and calf showed no significant difference ($p > 0.05$).

Additionally, the hair shaft diameter was determined. Figure 5 gives the average hair shaft diameters with standard deviation for the seven measured body sites. The hairs on the thigh and calf region were significantly thicker compared to the other five regions ($p = 0.01$). Lateral forehead, back, thorax, upper arm, and forearm showed no significant differences in hair shaft diameters ($p > 0.05$). The thigh showed hair shaft diameters of 29 μm, and the calf region 42 μm.

The volume of the follicular infundibula was measured by dividing the casts on the surface biopsy in truncated cones, calculating each volume, and adding all volumes within 1 cm^2 and subtracting the hair shaft volumes. The results of these measurements are given in Fig 6 related to 1-cm^2 skin surface. The forehead (0.19 mm^3/cm^2) and the calf regions (0.18 mm^3/cm^2) showed the highest volume with no significant difference ($p > 0.05$), although the hair follicle density is around 20 times higher on the forehead. The forearm shows the lowest volume at 0.01 mm^3/cm^2.

The potential surface for the penetration of topically applied drugs and cosmetics has been estimated as the skin surface, disregarding the fact that substances penetrate into skin appendages. The potential penetration surface of the hair follicles was calculated by measuring the curved surface of the follicular casts on the cyanoacrylate biopsy. Significant intersite variations of the surface area were found ($p = 0001$). The largest surface was found on the forehead with 13.7 mm^2 on average or 13.7% of the skin surface. The smallest surface was found on the forearm (0.95 mm^2), in accordance with the small follicles and the low follicle density (Fig 7).

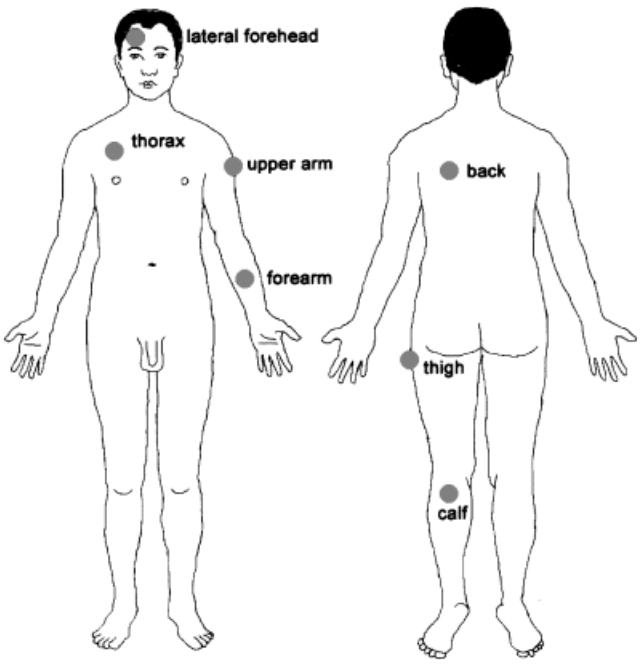

Figure 1
Localization of the seven test regions.

Discussion

The knowledge of hair follicle density and size is important for the understanding and calculation of follicular penetration and permeation processes.

Only a few studies have been performed for the determination of the vellus hair follicle density on the human body. Blume *et al* (1991) determined the hair follicle density on the forehead, cheek, chest, and back. An average density of 423 follicles per cm^2 was found on the forehead and a mean density of 92 follicles on the back. Pagnoni *et al* (1994) found a density of 455 hair follicles on the lateral forehead and the highest density on the nasal wing with 1220 follicles per cm^2. These results show higher values, compared to our findings for the forehead (292 follicles/cm^2) and the back (29 follicles/cm^2). High standard variations as an expression of intraindividual variations occurred in every study. Even within the forehead region the hair follicle density can extremely differ to a high extent.

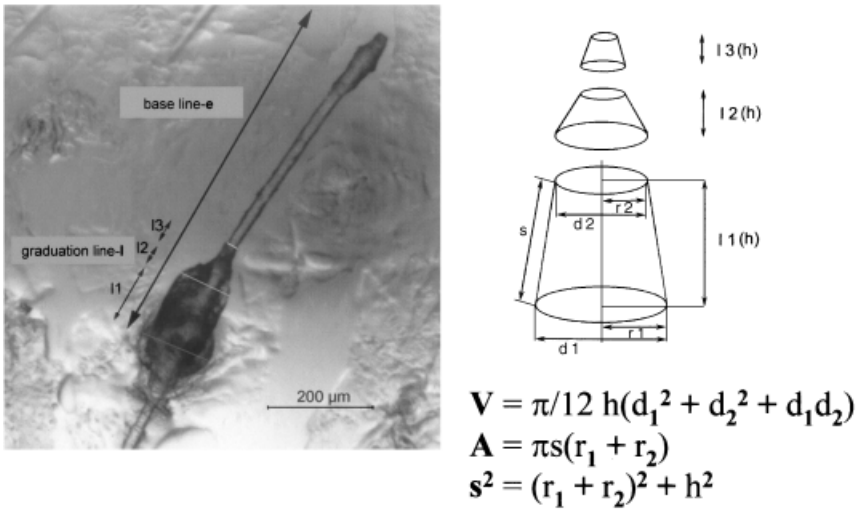

Figure 2
Cyanoacrylate skin surface biopsy with infundibular cast and vellus hair.

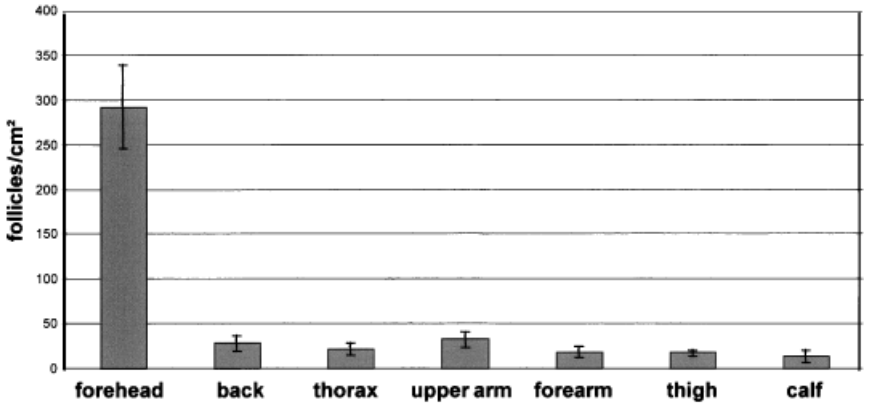

Figure 3
Hair follicle density on seven body sites.

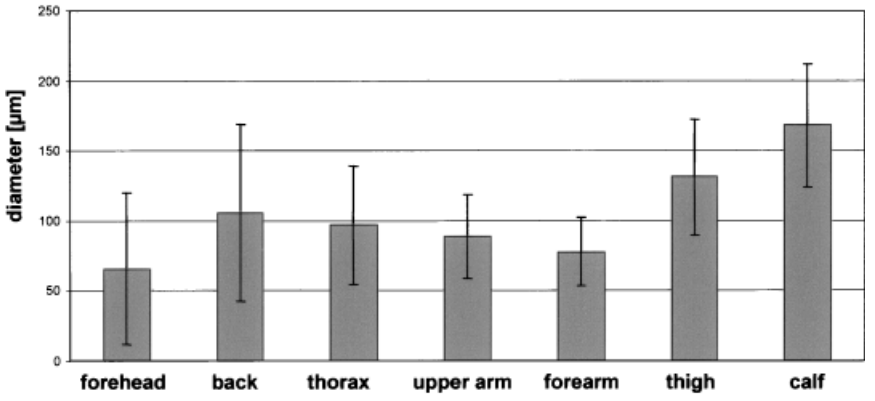

Figure 4
Diameter of hair follicle orifices on seven body sites.

Table I. Percentage mean (± SD) of follicular orifices on the skin surface in seven body sides

Skin area						
Forehead	**Back**	**Thorax**	**Upper arm**	**Forearm**	**Thigh**	**Calf region**
1.28 (± 0.24)	0.33 (± 0.15)	0.19 (± 0.08)	0.21 (± 0.09)	0.09 (± 0.04)	0.23 (± 0.12)	0.35 (± 0.25)

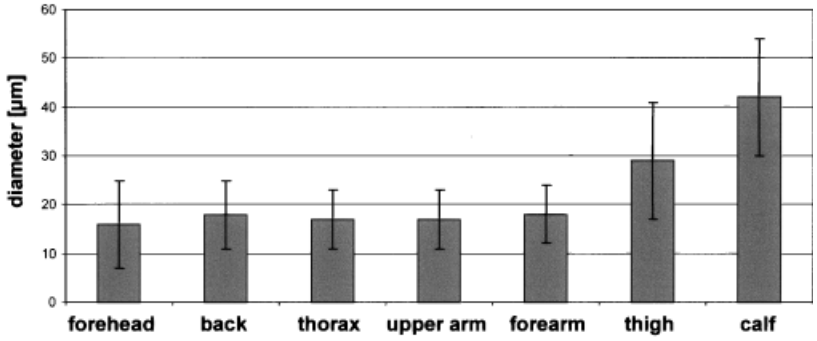

Figure 5
Hair shaft diameter on seven body sites.

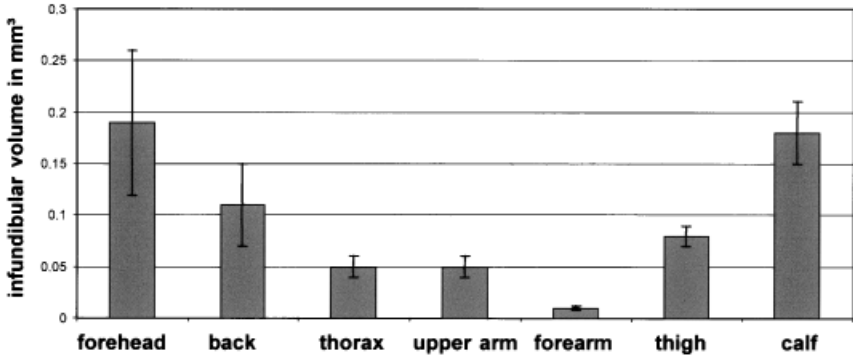

Figure 6
Volume of the follicular infundibula per square centimeter skin on seven body sites.

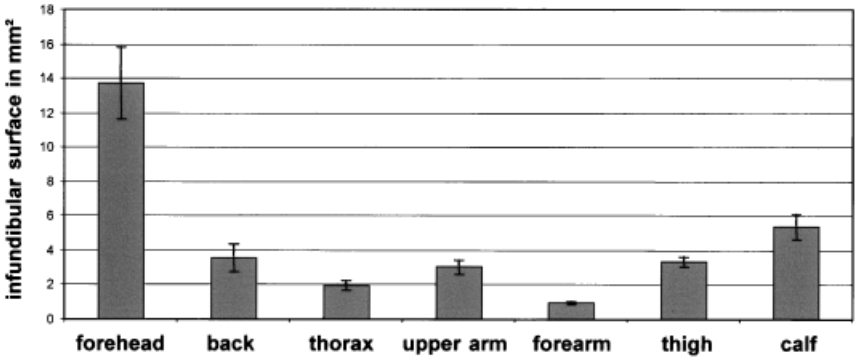

Figure 7
Surface of the follicular infundibula per square centimeter of skin on seven body sites.

Seago and Ebling (1995) measured the hair follicle density on the upper arm and thigh using a classical trichogram. They found a mean density of 18 follicles on the upper arm and 17 follicles in the skin area on the thigh, which correspond to our findings.

The mean hair follicle density depends on the skin area, because hair follicles are built in the early fetal period. After birth, the body proportions change and the hair follicles move apart according to the growth of body and skin. Because of the relatively lower growth of the head compared to the extremities, hair follicles are much more numerous on the scalp and in the face than on arms and legs (Pagnoni *et al*, 1994; Seago and Ebling, 1995). Table II gives an overview of vellus hair follicle density found in literature compared to the presented results.

In spite of the fact that the size of hair follicles shows great intra- and interindividual differences, hair shaft diameters showed relatively low variations. The thicker hair in the androgen-dependent areas on the thigh and calf, which were thicker than 30 µm, can be regarded as intermediate follicles. This means that these follicles are in a transitional stage between vellus and terminal hair (Whiting, 2000).

Our findings show that the assumption of an appendage account of not more than 0.1% of the total skin surface (Schaefer and Redelmeier, 1996) is valid for the forearm. The value of 0.1% corresponds well to our findings on the inner side of the forearm, which is most commonly used as an investigational area for skin penetration experiments. Skin areas with a higher follicle density, such as the

Table II. Review of vellus hair follicle density (per cm^2) on different body sites

	Author				
Body site	**Own results**	**Pagnoni *et al* (1994)**	**Blume *et al* (1991)**	**Seago and Ebling (1995)**	**Scott *et al* (1991)**
Lateral forehead	292	455	414♀	—	—
			432♂		
Tip of the nose	—	1112	—	—	—
Nasal wing	—	1220	—	—	—
Preauricular region	—	499	—	—	—
Back	29	—	93♀	—	—
			90♂		
Thorax	22	—	—	—	—
Abdomen	—	—	—	—	6
Upper arm	32	—	—	17–19	—
Forearm	18	—	—	—	—
Thigh	17	—	—	14–20	—
Calf	14	—	—	—	—

forehead, or with larger follicle orifice, such as the calf region, showed much higher values. A higher transfollicular absorption in these areas can be assumed.

The measurement of a potential follicular reservoir for topically applied substances showed extreme differences between the investigated body sites. The volume of the infundibula on the forehead was 0.19 mm^3. This is five times less than the volume of the stratum corneum, assuming that the stratum corneum shows a thickness of 10 μm with a volume of 1 mm^3 per cm^2 skin surface. The determination of the reservoir function of the stratum corneum is part of recent studies. Lademann *et al* (1999, 2000) found that a topically applied substance is found mainly in the upper 20% of the stratum corneum. This means that we have an approximately comparable reservoir volume in the stratum corneum and in the follicles of the forehead, assuming that all follicles are open for the penetration process (Lademann *et al*, 2001). In contrast to the forehead, the forearm shows a volume of 0.01 mm^3, which is 100 times less than the volume of the stratum corneum. It can be estimated that the hair follicles in this skin area play a minor reservoir function role.

The enlargement of the penetration surface through the follicular epithelium was measured by calculating the surface of the infundibular cast on the cyanoacrylate biopsy. The infundibular surface proved to be 13.7% on the forehead and only 1% on the forearm; thus only relatively low values for the enlargement of the potential penetration surface could be demonstrated.

A significantly higher amount of drug absorption in skin areas with high follicle densities or large follicles cannot be explained only by an enlargement of the penetration surface through the follicular epithelium. The reason for the better permeation through the hair follicles can likely be found in the ultrastructure of the follicular epithelium and in the special environment of the follicular infundibulum. The hair follicle epithelium shows an epidermal differentiation in the infundibulum. The epithelium of the uppermost parts shows no difference from the interfollicular epidermis; in the lower parts of the infundibular epithelium corneocytes are smaller and appears crumbly. This part of the follicular epithelium can be seen as an incomplete barrier for topically applied substances (Pinkus *et al*, 1981; Braun-Falko *et al*, 1996).

This study shows a body-region-dependent hair follicle characteristics concerning follicular size and follicular distribution. Differential evaluation of skin penetration and absorption experiments and the development of new standards for the testing of topically applied drugs and cosmetics on different skin areas are mandatory. By knowing the differences of hair follicle size and density, we suggest that skin absorption experiments be performed on human skin not only on the inner forearm but also in skin areas with other follicular properties, for example, forehead or calf.

Materials and Methods

The study was performed on six healthy volunteers (3 women, 3 men) aged 27–41 y with normal body mass indices (21–24). None of the volunteers suffered from any kind of skin disease, hormonal dysregulation, or adipositas. Cyanoacrylate surface biopsies were taken from each volunteer from seven different regions of the body (lateral forehead, back, thorax, upper arm, forearm, thigh, and calf region, just below the popliteal space), on the same day under the same conditions, which means same room temperature and humidity. Figure 1 shows the localization of the seven test regions. Hair follicle parameters were measured by light microscopy in combination with digital images. None of the volunteers showed terminal hair growth in the test regions. All volunteers gave written informed consent and the protocol was approved by the institutional review board. The study was conducted according to the declaration of the Helsinki principles.

Cyanoacrylate is a nontoxic, nonadherent, optically clear adhesive that polymerizes and bonds rapidly in the presence of small amounts of water and pressure (Marks and Dawber, 1971). A drop cyanoacrylate (UHU, GmbH, Brühl, Germany) is placed on the untreated skin and

covered with a glass slide under light pressure. After polymerization, which occurs in 1 min, the glass slide can be removed. A thin sheet of horny cells, hair shafts, and casts of the follicular infundibula are ripped off with the cyanoacrylate (Holmes *et al*, 1972; Plewig and Kligman, 1975; Mills and Kligman, 1983).

The surface biopsies were investigated using microscopy (Olympus, BX60M system microscope) in combination with digital image analysis and a special software program (analySIS, Soft Imaging System GmbH SIS, Münster, Germany). The follicular casts were counted in a marked area of 1 cm^2. The diameters of the follicle orifice and of the hair shaft were measured directly. The percentage of orifices of the skin surface can easily be determined by adding all calculated circle area of the follicular orifices in the labeled biopsy area.

For the measurement of the surface and volume of the infundibula, a special three-dimensional image, EFI (extended focal image), was performed. With the module extended focal image of the software program analySIS (Soft Imaging System GmbH SIS) microscope images can be taken in different focuses and can than be calculated to a three-dimensional picture. With the same software program length, height and diameter of the infundibular casts can be measured. Every infundibular cast in the marked square centimeter was divided into truncated cones. Figure 1 explains the measurement of the infundibular cast. It shows a cyanoacrylate skin surface biopsy from the lateral forehead. The volume (V) of each truncated cone was calculated with the formula

$$V_n = \pi/12 h_n (d_1^2 + d_2^2 + d_1 d_2), \quad (1)$$

where h is the height of the truncated cone and d is the diameter of the covers.

The whole infundibular volume per square centimeter was calculated by adding all single volumes and subtracting the hair shaft volumes. The hair shaft volume (V_{hs}) was calculated by the formula

$$V_{hsn} = \pi/4 d^2 h_n, \quad (2)$$

where h is the height of the whole infundibulum and d is the hair shaft diameter.

The surface of the follicular infundibula was measured by calculating the curved surface (A) of the truncated cones by the formulas

$$A_n = \pi s (d_1/2 + d_2/2) \quad (3)$$

$$s^2 = (d_1/2 - d_2/2)^2 + h_n, \quad (4)$$

where h is the height of the truncated cone and d is the diameter of the covers. The follicular penetration surface was calculated by adding all curved surfaces within the marked area (Fig 2).

For statistical analysis, we utilized the Mann-Whitney *U* test for the comparison of two variables and Kruskal-Wallis's *h* test for the comparison of more than two variables and SPSS software (SPSS, Chicago, IL). Data are expressed as means $\pm$ SD with $p = 0.05$ considered significant, $p = 0.01$ considered very significant, and $p = 0.001$ considered highly significant.

DOI: 10.1046/j.0022-202X.2003.22110.x

Manuscript received March 13, 2003; revised June 15, 2003; accepted for publication September 9, 2003

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