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Percutaneous Uptake of Zinc in Rabbit Skin[1] (37927)

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It has been suggested that zinc plays an important role in the metabolism of skin epidermis and hair, especially during keratogenesis (1–4). The presence of sulfhydryl groups and their association with zinc in (—SH) areas of keratinization has been demonstrated (5, 6). It has also been reported that zinc accumulates primarily in the epidermis and hair regardless of the method of administration, i.e., dietary, systemic or topical application (7). Information on zinc metabolism and its localization in skin is based primarily on biochemical and *in vitro* studies. With the exception of a few studies, radioautographic and histochemical localization of zinc in the skin and the hair follicle has not received the attention it merits. Therefore, the present study was designed to demonstrate, *in situ,* localization of zinc and sulfhydryl (-SH) groups in rabbit skin after topical application of various zinc compounds.

Materials and Methods. Three adult rabbits weighing approximately 2 kg each were used in this study. The animals were immobilized, the skin on the back was shaved and four circular areas, 1 in. in diameter, were outlined on either side of the spinal column. Zinc oxide, zinc omadine, zinc sulfate, and zinc undecylenate, each with a specific activity of 131 μCi/mole of ^{65}Zn were used for topical application.

These compounds were prepared in our laboratories[2] and were chosen for application due to differences in their oil/water solubility coefficient, molecular weight, and pH. These properties are assumed to affect the rate of penetration of zinc compounds into the skin. Each application consisted of 2.5 mg zinc compound containing 5 μCi ^{65}Zn. A 1:1 mixture of glycerin and propylene glycol was used as vehicle.

Animals I and II received one application on all four skin areas left of the spine, while the four skin areas on the right of the spine were given two applications. The second application was made 24 hr after the first dose.

Animal I and animal II were sacrificed 6 hr and 24 hr, respectively, after the second application. Animal III served as the control and was treated with the four corresponding nonradioactive zinc compounds. The treated skin areas were excised and fixed in cold alcoholic formalin. Estimation of total ^{65}Zn retained in the whole skin block was done in a well-type automatic

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[2] ^{65}Zinc oxide was prepared by adding sodium hydroxide to a solution of ^{65}zinc chloride (International Chemical and Nuclear Corporation, Irvine, CA) and drying the resulting precipitate at 125°C.

^{65}Zinc omadine was precipitated from an aqueous solution of sodium omadine (Vick Chemical Company, Mount Vernon, NY) by ^{65}zinc chloride.

^{65}Zinc undecylenate was obtained by adding ^{65}zinc oxide to an ethanolic solution of undecylic acid. The residue was filtered off, washed with ethanol, and dried at 115°C.

^{65}Zinc sulfate was prepared by adding ^{65}zinc hydroxide to dilute sulfuric acid and crystallization from the supernatant with acetone.

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Gamma Counter (Nuclear Chicago Model 4222 with a 3-in. sodium iodide crystal). Subsequently, the skin blocks were routinely processed and embedded in paraffin for histological study. Serial sections, 6 μm in thickness, were coated with fresh NTB_2 nuclear track emulsion (Eastman Kodak Co., Rochester, NY) diluted 1:1 with distilled water. The coated slides were stored in boxes at 4°C. After an exposure time of 3 weeks, tissue sections were developed in Kodak D-17 and fixed in hypo. After several rinses in distilled water, some sections were stained with hematoxylin and eosin and mounted in permount, while the remaining sections were dehydrated, cleared, and mounted in permount without staining.

Blank slides coated with emulsion, developed and fixed in a similar fashion were used as controls for checking the background fog.

Zinc was localized histochemically by the diphenylthiocarbazone method of Mager, McNary, and Lionetti (8). The alkaline tetrazolium method of Barrnett and Seligman (9) was used to localize sulfhydryl (—SH) and disulfide (—SS) groups. 0.1 *M* *n*-ethyl maleimide in 0.1 *M* phosphate buffer was used at pH 7.4 for blocking the sulfhydryl (—SH) groups.

Results. Table I shows radiocounts of ^{65}Zn in the excised areas of whole skin as obtained from a well-type automatic Gamma Counter. It can be noticed that the counts at different dose levels and time schedules are erratic for various zinc compounds except zinc oxide for which the results are consistent.

Radioautographic counts of emulsion grains were, however, consistent but no significant quantitative differences were observed in the amount of ^{65}zinc absorbed from the four compounds used in this study, at any given time. Further, optimal activity for all compounds used was observed at 6 hr after each application. ^{65}Zn counts were markedly reduced at 24 hr after application of first dose and also 24 hr after application of second dose in the same area of the skin.

Radioautographic evaluation showed high concentration of ^{65}Zn in the cortical and cuticular zones of the hair shaft, concentrations being optimal in the keratogenous zone (Fig. 1). Localization of ^{65}Zn was, however, relatively poor in the hair shaft of skin 24 hr after the last application of the zinc compounds.

Localization of ^{65}Zn was very low in the epidermis, which in rabbit is only two to three cell layers deep. On the contrary, ^{65}Zn accumulation was heavy in the subdermal muscle layer (Fig. 2). Only trace amounts of ^{65}Zn were present in the dermis

TABLE I. ^{65}Zn Retention on Excised Skin Blocks (cpm × 10^3).[a]

Compound	Total applied dose of Zn compound	Time after last application: 6 hr Applied	6 hr Retained	6 hr %	24 hr Applied	24 hr Retained	24 hr %
Single dose							
Zinc oxide	2.5 mg	784	161	20.6	784	185	23.6
Zinc omadine	2.5 mg	835	34	4.1	835	242	29.0
Zinc sulfate	2.5 mg	763	496	65.0	763	145	19.0
Zinc undecylenate	2.5 mg	966	360	37.3	966	219	22.7
Double dose							
Zinc oxide	5.0 mg	1478	314	21.2	1478	369	25.0
Zinc omadine	5.0 mg	1670	120	7.2	1670	138	8.3
Zinc sulfate	5.0 mg	1526	50	3.3	1526	181	11.9
Zinc undecylenate	5.0 mg	1931	106	5.5	1931	155	8.0

[a] Results corrected for background and counted simultaneously with an aliquot of the applied sample. Second dose was always applied 24 hr after the first application.

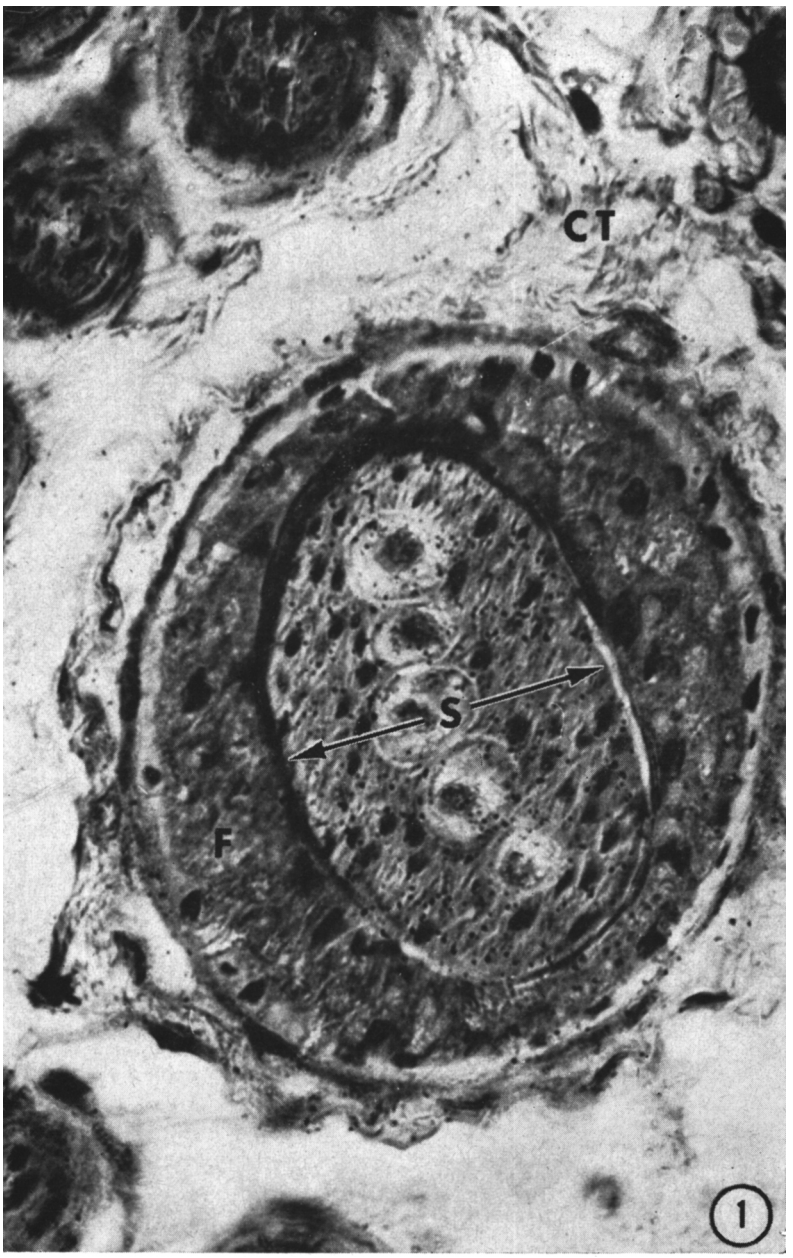

FIG. 1. Zinc undecylenate. Two applications of total 10 μCi ^{65}Zn. Animal killed 6 hr after second application. ^{65}Zn activity is optimum in the hair shaft (S) with some activity present in the hair follicle (F) (transverse section) and in the surrounding connective tissue (CT). Hematoxylin and eosin counterstain ($\times$625).

and in blood vessels of both dermis and hypodermis.

Sections stained in alkaline tetrazolium for sulfhydryl (—SH) and disulfide (—SS) groups demonstrated a positive reaction in sites where ^{65}Zn was observed in radioautographs. The optimal staining intensity was seen in the cortex of the hair shaft and in the keratogenous matrix of the hair papilla. Reaction for sulfhydryl (—SH)

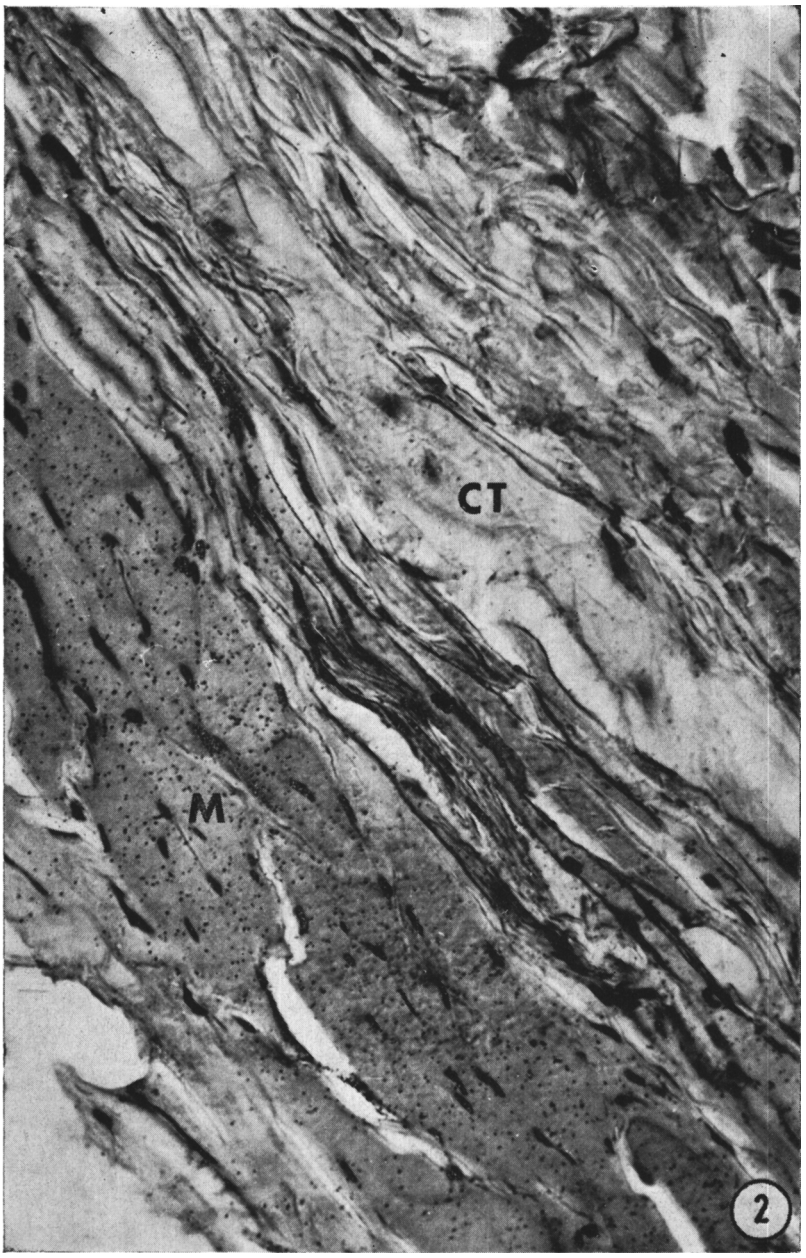

FIG. 2. Zinc undecylenate. Two applications of total 10 μCi ^{65}Zn. Animal killed 6 hr after second application. Notice heavy localization of ^{65}Zn in the subdermal muscle (M) in comparison to that in the adjoining dermal connective tissue (CT). Hematoxylin and eosin counterstain (×625).

and disulfide (—SS) groups was markedly positive in the epidermis (Fig. 3).

Zinc localized by the diphenylthiocarbazone reaction exhibited a pattern similar to that of ^{65}Zn seen in the radioautographs and sulfhydryl (—SH) and disulfide (—SS) groups.

Discussion. No significant differences were found in the amount and location of ^{65}Zn in skin treated with four different zinc com-

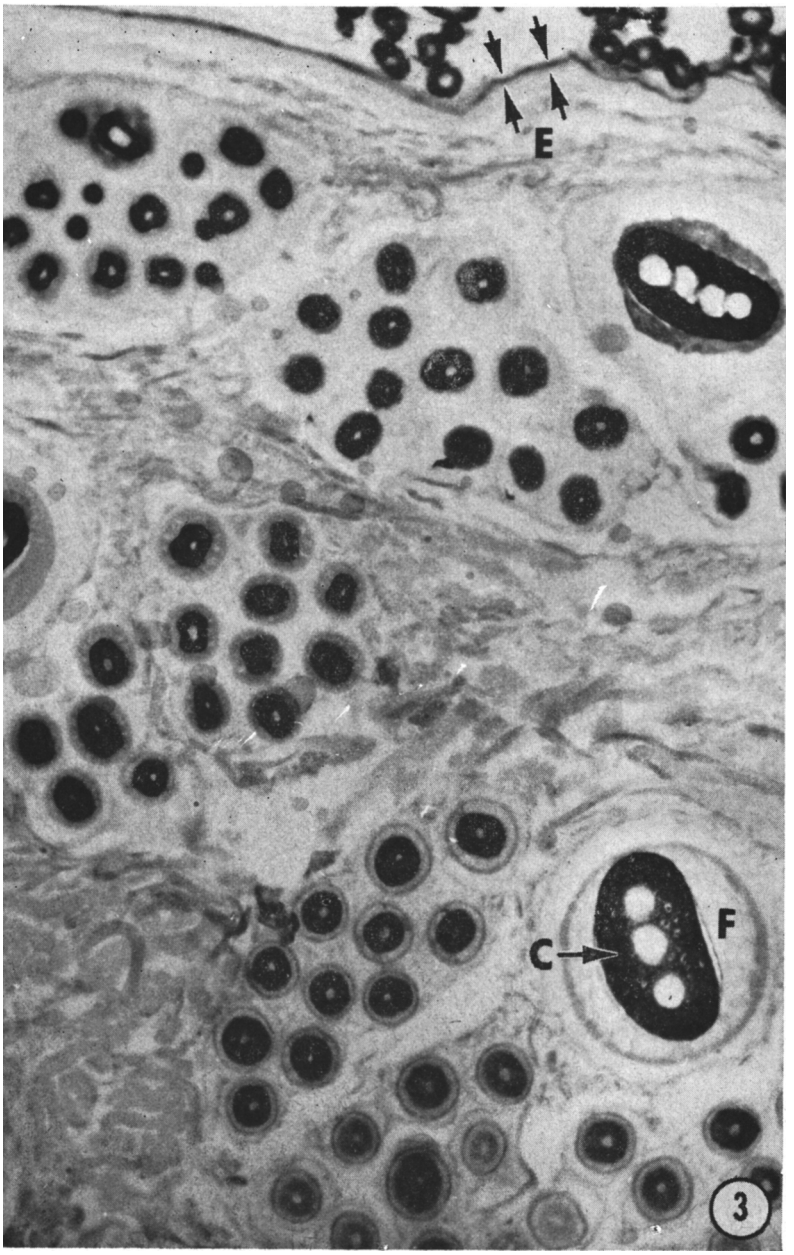

FIG. 3. Alkaline tetrazolium reaction for sulfur amino acids is optimal in the cortex (C) of the hair shaft (transverse section) and is present to a lesser degree in the skin epidermis (E). (F) Hair follicle. (×110 approximately).

pounds. Since these zinc compounds markedly differ in their oil/water solubility, pH, and molecular weight, an exclusive transepidermal absorption of ^{65}Zn would be expected to show different rates of absorption and concentration. However, the heavy localization of ^{65}Zn from four different compounds observed primarily in the hair shaft and hair follicles suggests that the major mode of ^{65}Zn uptake in skin is by diffusion through the hair follicles (10). This further emphasizes that chemical dif-

ferences in the compounds may not play a very important role in absorption of ^{65}Zn.

It is noticed that in skin, zinc is primarily concentrated in the cortex of the hair shaft, especially in the keratogenous zone (1, 2). These sites also demonstrate a high concentration of sulfur amino acids (2). It may thus be speculated that zinc diffusing to the base of the follicles is bound to sulfhydryl groups, thereby setting a favorable gradient for its absorption and utilization. The presence of a rich capillary bed in this area may also allow for systemic absorption and clearance of zinc.

It was observed in this study that ^{65}Zn almost completely disappears from skin 24 hr after the first and second application though its concentration is high at 6 hr. This may be explained by stating that 2.5 mg zinc containing 5 μCi of ^{65}Zn administered in a single dose is large enough to saturate all available binding sites in the skin during the first 6 hr after application. And that excess and unbound ^{65}Zn from the first dose as also from the second dose may pass directly into systemic circulation in 24 hr. Further, the low concentrations of radiozinc, especially in the hair shaft, 24 hr after application, may be explained by a high rate of exchange with the total available body zinc, in the keratogenous portion of the hair. This zone of the hair papilla has a rich vascular bed that should allow for a greater turnover rate of ^{65}Zn.

Radiocounts of ^{65}Zn made in the excised whole skin blocks are erratic and not reconcilable with the radioautographic results. It is suggested that randomness of the radiocounts is due to the unabsorbed ^{65}Zn compounds that remain adhered to the treated skin blocks. It is our opinion that radioautographic techniques prove more reliable in such studies since the unabsorbed compounds are removed during the preparation of the slides and only the tissue bound radioactivity is realized.

Summary. The percutaneous uptake of zinc in rabbits was studied radiochemically and radioautographically using single- and double-dose applications of four different zinc compounds. All compounds were absorbed equally, regardless of their chemical composition. ^{65}Zn was optimally located in the keratogenous zone of the hair shaft and in the subcutaneous muscle layer. Histochemical demonstration of zinc and sulfur amino acids was also optimal in the same sites.

There was a depletion of ^{65}Zn in the skin radioautographs of animals sacrificed 24 hr after application of a first and a second dose. A rationale for these results is discussed.

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