occurred which was manifest on the 10th and 12th postirradiation days.

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Penetration and Local Effect of Vitamin A on the Skin of the Guinea Pig.*† (21197)

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Vitamin A penetrates the intact skin of the guinea pig largely by way of the pilosebaceous canals and the sebaceous glands, and the speed of its penetration is dependent upon the vehicle used. Topical applications of massive doses of vit. A over a period of 2 weeks cause an hypertrophy of the epidermis but no appreciable inhibition of keratinization. The growth of hair follicles is never sufficiently affected by the vitamin to be observable either in gross appearance or in histological sections.

Materials and methods. These experiments were carried out in duplicate: in one set crystalline vit. A alcohol was used; in the other, vit. A acetate. Since the results were identical, no attempt will be made to describe or discuss them separately.

In the study of penetration, 10 mg of the crystalline vitamin were dissolved in 2 ml of 95% alcohol, chloroform, oleic acid, linoleic acid or a paste made up of petrolatum, zinc oxide and talcum. After one topical application of vit. A dissolved in each of the above vehicles to the clipped skin of guinea pigs, biopsy punctures of skin were removed from each animal after 10 minutes, one hour, and 2 hours. In some cases, specimens were removed after 4 and 8 hours. The animals were maintained in a dark room to avoid inactivation of the vitamin by light. The skin samples were frozen without fixation. Sections were cut at 10 and 20 μ, mounted in glycerine, and studied under near-ultraviolet light filtered to transmit rays at 3600 Å. The penetration of vit. A can be traced by its brilliant fluorescence. Unlike the fluorescence of natural vit. A which fades quickly under ultraviolet light, that of the synthetic vitamin fades very slowly and is visible for about 20 minutes.

For the study of the effect of vit. A and of the vehicles alone on the skin, an aluminum ring ¼″ wide and ½″ deep was fastened to the clipped backs of guinea pigs with methyl methacrylate and adhesive tape (Fig. 1, 2). The well was left empty overnight to make

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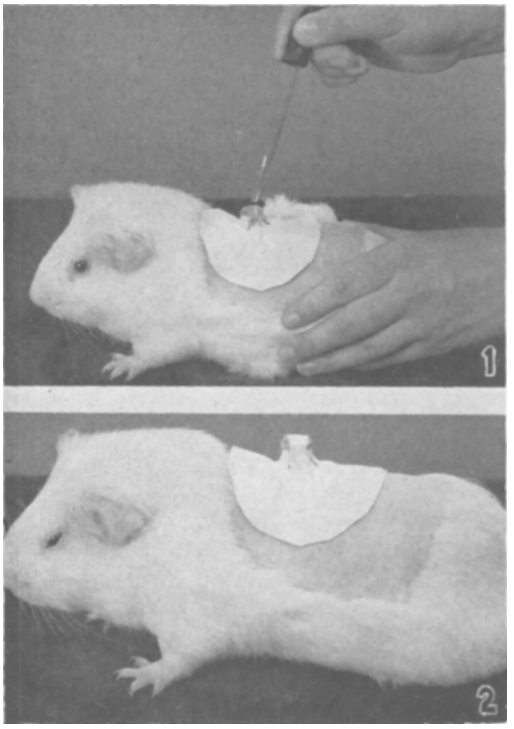

FIG. 1. Open aluminum well has been affixed to the skin with methyl methacrylate and steadied with adhesive tape. The well is ready for use.

FIG. 2. Well filled and closed with a fitted aluminum lid. A strip of adhesive tape over the lid prevents it from falling off.

certain that it had adhered properly. The next day the solutions of vit. A or of the vehicles alone were introduced into the well, which was then covered with a perfectly fitted aluminum lid. No leakage or spillage occurred. In this way the skin is in constant contact with the substances used and no other factors should influence the results. The following substances were used in groups of at least 6 animals: a) one milliliter of linoleic acid containing 5 mg vit. A; b) one milliliter of oleic acid with 5 mg vit. A; c) oleic acid alone; d) linoleic acid alone. Specimens of skin in contact with these agents as well as untreated control specimens from the same animal were removed after 3, 6, and 12 days. In another experiment the skin was treated twice daily with vit. A dissolved in 95% alcohol. At the end of 12 days a total of 20 mg had been applied to the area of skin within the well. Control animals received an equal amount of alcohol alone. Specimens of skin treated with alcoholic vit. A and with alcohol alone were removed 6 and 12 days after the beginning of treatment. All specimens were cut into 2 pieces. One of these was fixed in Helly's fluid, the other in 1% trichloracetic acid in 80% alcohol. Skin fixed in Helly's fluid was imbedded in paraffin and sectioned at 5 μ. For each specimen, alternate sections were stained with 0.05 toluidin blue buffered to pH 5.0(1), and with the periodic acid-Schiff method for glycogen(2). The material fixed in the alcoholic trichloracetic acid was sectioned at 10 μ and the sections were used for the demonstration of sulfhydryl and disulfide groups(3,4).

Observations. Penetration. The normal, untreated skin of guinea pigs shows little autofluorescence. In the epidermis, the Malpighian layer is non-fluorescent, and the keratin layer emits a pale yellow light of very low intensity. The dermis emits a blue-green light of low intensity, but the elastic fibers fluoresce with a strong white light. The hair follicles and sebaceous glands are non-fluorescent. White hairs emit a very pale green light, red or brown hairs fluoresce orange to red, and black hairs are non-fluorescent.

Ten minutes after the application of vit. A dissolved in alcohol, the stratum corneum and the sebum in the pilosebaceous orifices fluoresce with a bright greenish-yellow light. One hour after application, the fluorescence progresses to the sebaceous glands (Fig. 3, 4). After 2 hours the dermis around each sebaceous gland has small clouds of dust-like fluorescent particles. The subcutaneous fat, which is normally non-fluorescent, emits a pale greenish light.

Vit. A dissolved in chloroform penetrates even more rapidly and effectively than when dissolved in alcohol. Ten minutes after application, fluorescence can be seen in the stratum corneum of the epidermis, in the pilosebaceous orifices, and in the sebaceous ducts. After one hour, the stratum spinosum of the epidermis becomes fluorescent as do nearly all of the cells of the sebaceous ducts and glands. In resting hair follicles the fluorescence extends to the club, but in growing ones it does not pass deeper than the sebaceous

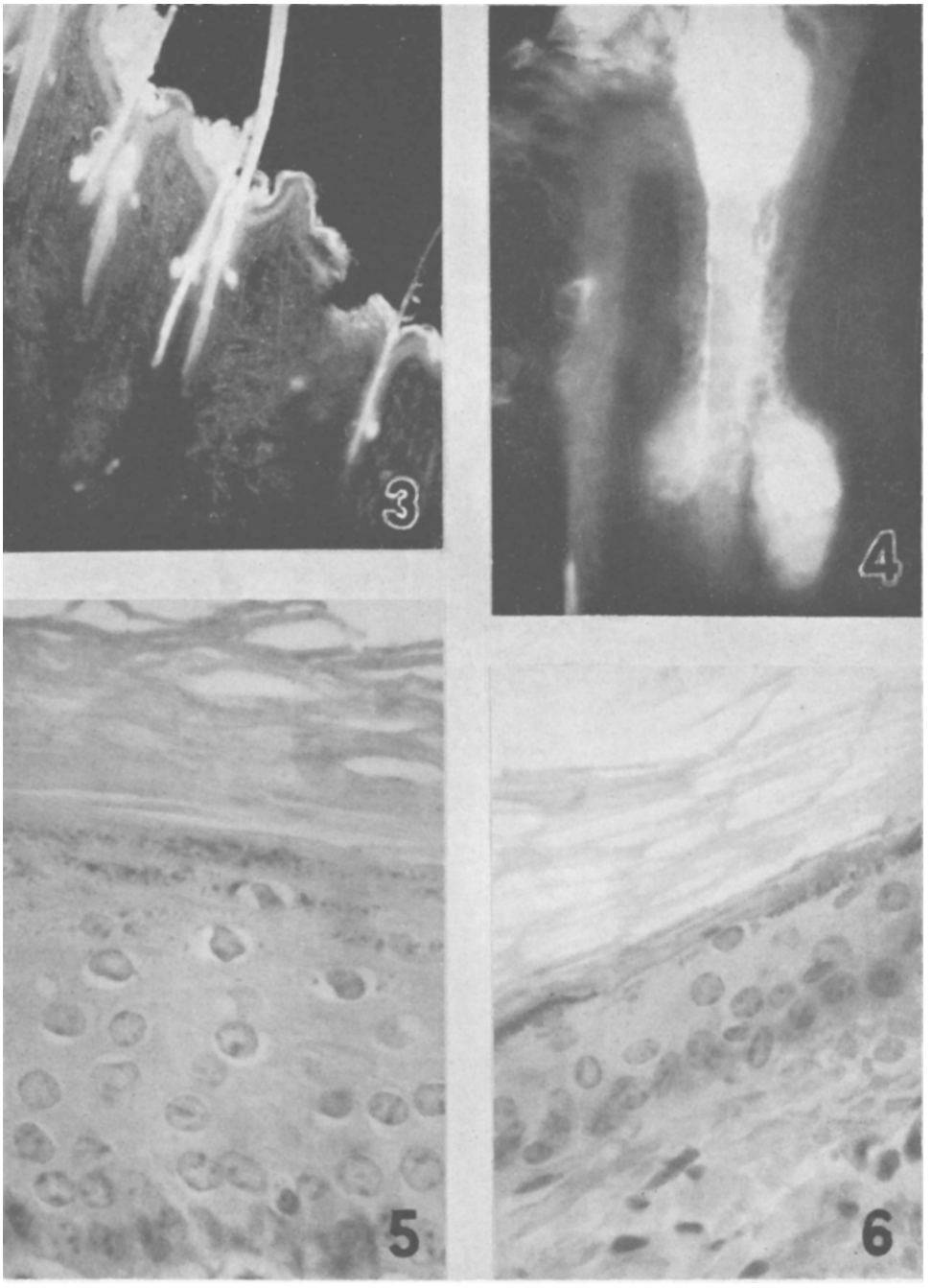

FIG. 3. Section through the skin one hr after application of vit. A dissolved in alcohol, viewed under near-ultraviolet light. Vit. A fluorescence is striking in stratum corneum, pilosebaceous ducts and sebaceous glands. Fluorescence of the hair is not due to vit. A but to a red pigment.

FIG. 4. Vit. A fluorescence in sebaceous glands and ducts one hr after application of vit. A dissolved in chloroform.

FIG. 5. Hypertrophied epidermis after one wk of contact with vit. A dissolved in oleic acid. Observe relatively thick stratum corneum. Stained with hematoxylin and eosin.

FIG. 6. Normal guinea pig epidermis stained with hematoxylin and eosin.

duct. After 2 hours there is some fluorescence in the dermis and in the subcutaneous fat.

Penetration of vit. A in oleic acid is very slow. At the end of 2 hours the vit. A fluorescence can be seen only as far as the sebaceous ducts. After 4 hours the sebaceous glands show a small amount of fluorescence. With linoleic acid, penetration is more rapid. By 2 hours the sebaceous glands show abundant vit. A fluorescence. Linoleic acid itself is slightly fluorescent, but the addition of the vitamin increases its fluorescence considerably. Penetration of vit. A dissolved in the viscous petrolatum paste is very slow. After 2 hours fluorescence was limited to the stratum corneum of the epidermis and to the sebum in the sebaceous ducts. Fluorescence appeared in the sebaceous glands after 4 to 8 hours.

Effect of vit. A on the skin. When in contact with vit. A dissolved in oleic or linoleic acids, the epidermis becomes progressively thicker (Fig. 5) and after 12 days it attains a marked hypertrophy. The stratum granulosum, which is normally one or 2 layers in thickness (Fig. 6), becomes thicker and the individual keratohyalin granules become larger. Many of the cells in the Malpighian layer are enlarged and misshapen, others are shrunken, with pycnotic nuclei, indicating cell death. The stratum corneum becomes somewhat thickened but it does not keep pace with the hypertrophy of the epidermis. Hair follicles and hair growth show no visible effect even after 12 days.

Histochemical preparations of skin kept in contact with vit. A in oleic or linoleic acid reveal a pronounced diminution in cytoplasmic basophilia (ribonucleic acid), particularly in skin treated for 12 days. Many of the cells in the upper part of the stratum spinosum become laden with glycogen. Glycogen is never present in the normal epidermis of the guinea pig. Also, there is a qualitative drop in the amount of sulfhydryl and disulfide content of the epidermis. The distribution of cytoplasmic basophilia, glycogen, and sulfhydryl and disulfide groups in the hair follicles is normal.

Oleic and linoleic acids used alone produce changes which are identical histologically and histochemically with those just described. There is greater damage to the epidermis when oleic acid is used.

After the use of vit. A dissolved in alcohol, the epidermis also becomes hypertrophied. Cell distortion and necrosis, however, is minimal. The histochemical tests show no diminution of cytoplasmic basophilia and sulfhydryl and disulfide groups and no accumulation of glycogen. Skin treated with alcohol alone shows only a slight hypertrophy of the epidermis. Hair follicles and hair growth are not altered by either the alcoholic solution of vit. A or by the alcohol alone.

Discussion. The speed of penetration of vit. A through the intact skin is dependent upon the vehicle used. The vitamin penetrates most rapidly when it is dissolved in alcohol or chloroform. When dissolved in linoleic acid, in oleic acid, and particularly in the viscous petrolatum paste it penetrates more slowly. The avenue of entry of these substances, like that of carcinogens, is by way of the pilosebaceous canal and the sebaceous glands(5-7). What is being measured at first is the penetration of the vehicle with vit. A serving as an indicator. The spreading of vit. A fluorescence to the dermis and the subcutaneous fat is evidence that the vitamin penetrates beyond the sebaceous glands.

Application of large amounts of vit. A dissolved in oleic or linoleic acid to the skin causes an hypertrophy of the epidermis. The hypertrophy is not a specific effect of vit. A, since the oils alone induce similar effects(8). That the vitamin, however, can stimulate epidermal hypertrophy can be seen when it is applied dissolved in alcohol, for alcohol alone produces only mild hypertrophy. The hypertrophy caused by the vitamin dissolved in oils, or by the oils alone, is accompanied by a certain amount of epidermal damage. Damaged epidermal cells contain stored glycogen whereas undamaged ones do not(9,10). The hypertrophy which follows the application of the vitamin dissolved in alcohol is not accompanied by the storage of glycogen or by other visible epidermal damage.

It is agreed that vit. A inhibits or prevents keratinization(11,12). Large amounts of vit. A are said to have a local, non-specific action

on epidermal cells by interfering with the sulfhydryl metabolism of the epidermis(13, 14). Large doses of vit. A profoundly inhibit keratinization in the tissues of chick embryos grown *in vitro*(15). In the guinea pig local contact with oleic or linoleic acid, with or without vit. A, impairs keratinization of the epidermis only partially as seen by the relatively thick stratum corneum in Fig. 5. Keratinization is unaffected by the vitamin dissolved in alcohol. As the epidermis increases in thickness, the stratum granulosum also becomes thicker. This has been interpreted as an indication of impaired keratinization(12), although there is no evidence that increased production of keratohyalin indicates impairment of keratinization.

Treatment of skin for 6 days or longer with vit. A dissolved in oleic or linoleic acids, or with the oils alone brings about a diminution in histochemically demonstrable sulfhydryl groups. Since massive doses of vit. A dissolved in alcohol do not show this, the decrease in sulfhydryl groups, and thus keratinization, seems to be caused by the oil vehicle.

Vitamin A is said to cause hair loss in the mouse, rat, and rabbit(13,14,16). Yet, regardless of the amount of vit. A or of the type of vehicle used in the present experiments, the skin of the guinea pig shows neither impairment of hair growth nor hair loss. Resting hair follicles undergo some cellular hypertrophy similar to that of the epidermis and may cast off their club hairs, but growing follicles remain unaffected for the 12-day duration of the experiment. Lack of penetration cannot account for this, since, as we have seen, vit. A penetrates the skin readily.

Differences among the investigators may be due to a lack of appreciation of the biology of skin. For example, the "atrophy" of hair follicles, referred to in studies of vit. A deficiency, is not atrophy but quiescence of hair follicles. The *"hypoplasia of hair bulbs"* describing the inhibitory action of vit. A and similar unsaturated compounds on hair growth (16) has been illustrated by photomicrography of club hairs which must have come from quiescent hair follicles. Hair follicles have cycles of growth and rest, and the morphology and physiology of the whole skin undergoes corresponding changes(17). The skin responds to injurious agents differently under these different conditions(18,19).

Summary. Vitamin A dissolved in alcohol or chloroform penetrates the intact skin quickly by way of the sebaceous glands. Dissolved in linoleic or oleic acid, or in a viscous petrolatum paste, it penetrates more slowly. Vit. A dissolved in oleic or linoleic acid or in alcohol causes an hypertrophy of the epidermis, but a similar hypertrophy is brought about by the fatty acids alone. Whereas the fatty acids, with or without vit. A, seem to retard keratinization slightly, the vitamin dissolved in alcohol does not. Hair growth was unimpaired even when the skin had received massive doses of vit. A dissolved in alcohol.

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