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Action of Vitamin A on the Skin Following Intracutaneous Injection. (23420)

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Recent clinical reports (Steinberg(1,2), Mullens(3)) indicate that the human wart papilloma and certain types of helomata may be dramatically resolved by injection of vit. A palmitate directly into the lesion. These findings are unexpected since attempts to treat these lesions with vit. A by systemic administration have given equivocal results(4). The experiments described below were therefore undertaken to study the effect of vit. A on normal epidermis when administered by the intracutaneous route.

Methods. A commercial vit. A palmitate solution (Keramin Injection) especially formulated for injection of skin lesions was used throughout. This solution has an activity of 50,000 U.S.P. units per cc in a vehicle consisting in 11% polysorbate 80, U.S.P., 0.25% citrate-phosphate buffer, pH 7, with 0.5% chlorobutanol, 0.4% dl-alpha tocopherol and 0.03% butylated hydroxyanisole as preservatives and antioxidants. A sterile solution containing all ingredients except vit. A was used for control injections.

New Zealand white male rabbits weighing 2-4 kg were used in all experiments. The dorsal thoracic and abdominal area of each rabbit was clipped closely, care being taken to avoid cutting the skin, and the entire surface was washed with 70% ethanol. A volume of 0.03 cc of the solution to be tested was injected intracutaneously by means of a 0.25 cc tuberculin syringe and a sharp, 27 gauge, ½ inch, regular point hypodermic needle. Injections were made only after the bevel of the needle was seen to be just below the surface. The needle was left in the skin for about ½ minute after injection to minimize leakage from the point of puncture. Pairs of injections were made on either side of the middorsal line but never over a bony prominence. When vit. A was injected on the left side, vehicle was injected on the right side at the same level and vice versa. Sites of injection were marked with indelible pencil. The animals were sacrificed always in the late afternoon by concussion 1, 2, 4, 7, 14, 21 and 28 days after injection. In all cases, a strip of skin at the site of injection approximately 40 x 4 mm was excised for histology so as to contain a central injected region and uninjected skin on either side. These latter areas served as additional controls. Tissues were fixed in Bouin's fluid, sectioned through the long axis at 8 μ and stained with Mayer's acid alum hematoxylin and eosin. The thickness of the epithelial layer with the corneum excluded was estimated with an ocular micrometer at 450 X magnification. Measurements were made in the center of each section as well as at points approximately one-tenth of the length in from either edge (uninjected controls). Only areas between the hair follicles were used for evaluation. Mitotic figure counts were made in the regions where epithelial thickness was measured. Two hundred and fifty consecutive nuclei in the basal and spinous* layers were counted for the areas corresponding to vit. A injection, vehicle injection and normal uninjected controls.

* The term spinous layer used in this paper refers to the cells in the germinative layer other than the basal layer even though no "prickle cells" as such can be found in the skin.

Results. On gross examination of the living animals, a mild erythema is seen after vit. A injection which appears in 24 hours and lasts several days. Vehicle injections give rise to a less intense response of somewhat shorter duration.

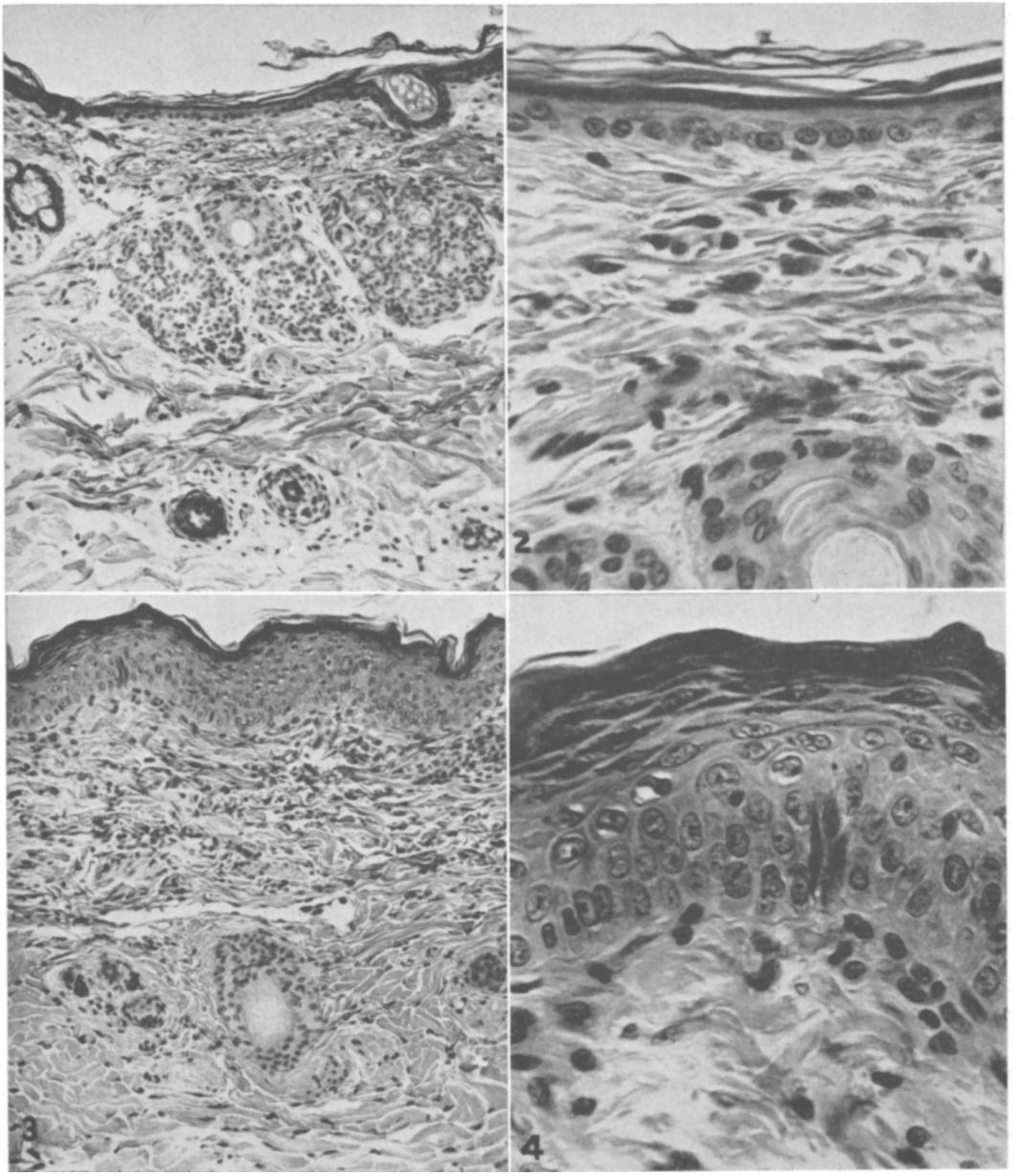

FIG. 1. Section of rabbit skin 7 days after intracut. inj. of vehicle control showing essentially normal skin. H & E × 128.

FIG. 2. Higher magnification of Fig. 1. The epidermis is 1-2 cell layers thick and the corneum diffuse. H & E × 480.

FIG. 3. Section of rabbit skin 7 days after intracut. inj. of aqueous vit. A solution. Epidermis has thickened considerably. H & E × 128.

FIG. 4. Higher magnification of Fig. 3. Nuclei in the basal layer are larger than in controls. Cells in various stages of differentiation are present including several layers of granular cells. Corneum is dense, compact and closely adherent to epidermis. H & E × 480.

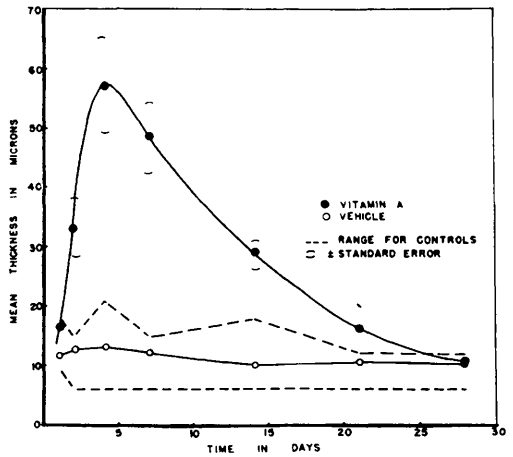

FIG. 5. Effect of a single inj. of vit. A on thickness of rabbit epidermis excluding corneum. Range only is given for the controls because of the relatively high measurement error in layers only a few cells thick.

Microscopic sections show minor changes in the dermis suggesting slight irritation. A dramatic change in the architecture of the epidermis occurs after vit. A but not after vehicle (Fig. 1 to 4). These changes may be summarized as follows: (a) *Epidermal thickness*: The epidermis (excluding corneum) increases about 5 fold in thickness within 4 days and gradually returns to its original dimensions about 2 weeks later (Fig. 5). Thickness of epidermis is unchanged by vehicle injections. (b) *Cyto-architecture*: Normal rabbit epidermis (or epidermis after injection of vehicle) is rarely more than 2 cells thick and consists in a basal layer over which more highly differentiated cells are distributed sporadically. The corneum is diffuse and basophilic. After vit. A injection the epithelium shows distinct layers of cells in various stages of maturation. Thus germinative, spinous and granular layers are clearly evident. The corneum is now thinner, denser and decidedly eosinophilic. No stratum lucidum can be seen in either control or treated tissues. It is of interest that the nuclei in the germinative layer after vit. A injection are larger and younger in appearance than in control epithelium. The increase in thickness of the epidermis is due to an increase in both size and number of cells. Formation of rete pegs has not been observed. (c) *Occurrence of mitosis*: In order to determine whether vit. A increases rate of cell division or slows down rate of maturation and death of cells, counts were made of the number of nuclei undergoing mitosis in the basal layer and spinous layer of tissues taken 2, 4 and 7 days after injection. As can be seen in the table, there is no striking difference between the number of nuclei undergoing mitosis in any group. However, minor differences in mitotic rate would hardly be detected because of the relatively small number of nuclei counted. (d) *Distribution of cell types*: It will be clear from the counts in the table that vit. A causes a change in the ratio of nuclei in the basal layer to the spinous layer from 9 to 1 to about 1 to 1.

Discussion. The effects of vit. A on the skin by topical application or systemic administration have been studied by others(5-8) and somewhat similar, but less dramatic and reproducible changes have been reported. It seems most likely that this is due to the comparatively low concentration which can reach the cells by these routes since still more profound changes in the epidermis have been reported by Fell and Mellanby(9) who found that very high concentrations of the vitamin, such as can be achieved only in tissue culture, will suppress keratinization altogether and change the epidermis to mucous secreting epithelium. After systemic administration (oral, intramuscular, intravenous, subcutaneous) storage of vitamin in the liver would operate

TABLE I. Occurrence of Mitosis in Rabbit Epidermis after Injection of Vit. A.

Time after inj., days	No. of slides counted	Mean % of various nuclei observed out of 250 cells counted: Basal layer Normal nuclei	Basal layer Mitotic nuclei	Spinous layer Normal nuclei	Spinous layer Mitotic nuclei
		Vitamin A			
2	8	62.5	.4	37.1	0
4	4	46.4	0	53.6	0
7	12	46.7	.2	53.0	.03
		Vehicle			
2	8	89.1	.3	10.6	0
4	4	88.3	.1	11.6	0
7	12	89.2	.1	10.7	0
		Uninj. controls			
2	8	90.0	.3	9.7	0
4	4	88.8	0	11.2	0
7	12	88.0	.2	11.8	0

against attaining a high concentration in the skin. Topical application would also appear to be an unreliable approach to the epidermis especially in the case of human skin lesions since the conditions of interest present an unusually thick keratin barrier. Furthermore, the absence of sebaceous glands in plantar skin would almost preclude penetration of vit. A at this most common site of verrucae and other hyperkeratoses since the sebaceous glands form the normal pathway for absorption of the vitamin. Intraepidermal injection of vit. A is, of course, a practical impossibility, so that, intracutaneous injection would appear to be the only efficient method of introducing vit. A at the site of an epidermal lesion.

There seems to be only one possible interpretation for the results here reported. The presence of large (hence young appearing) nuclei in the basal and spinous cell layers of the epidermis coupled with a normal rate of mitosis, indicates that the physiologic sequence of maturation and death of these cells has been slowed. Therefore, cells of all types have accumulated and less keratin has been laid down. If these changes occur also in human skin it is reasonable to suppose that injection of vit. A at the site of an heloma would reverse the pathologic process since this lesion shows a central area of atrophy of the epidermis with an overlying plug of horny material. The mechanism of action in the case of verruca, a proliferative virus disease, is still obscure and future work will be directed toward its clarification.

Summary. It has been found that a single intracutaneous injection of vit. A in the rabbit causes an increase in thickness of the epidermis (excluding corneum) which is due to an increase in both number and size of the cells in the germinative and granular layers. The corneum becomes thinner and denser. The occurrence of large, young appearing nuclei in the absence of an increase in mitotic rate indicates that the maturation process is slowed.

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Resistance of Human Gingival Collagen to Human Gingival Bacteria.* (23421)

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Since breakdown of the collagen fibers that anchor the teeth is a principal feature of periodontal disease, the demonstration that microorganisms present in the gingival sulcus or periodontal pocket produce collagenases assumes importance. Investigators using collagen paper or azocoll as substrates(1,2) have reported isolation of oral and gingival organisms that exhibited either collagenase-like or true collagenase activity. However, other proteolytic enzymes, such as trypsin, are capable

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