Percutaneous Absorption of $\alpha$-Tocopheryl Acetate

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By spraying $^{14}$C-labeled $\alpha$-tocopheryl acetate on the surface of the skin, and by conducting microradiographic investigations on the condition of its absorption in seven cases and 14 samples, the following observation have been acquired, and at the same time, some discussion have been made.

1. $\alpha$-Tocopheryl acetate is absorbed well through the healthy hairless skin.

2. There are two routes of absorption from the surface of the skin to the dermis. The first one leads through the horny layer, the epidermis and the borderline between the epidermis and the dermis. The second one goes through the pilo-sebaceous canal, the interior of hair follicles, inner and outer root sheaths and connective-tissue sheaths. No route through the sebaceous gland and sweat ducts has been detected.

3. The material has proven to have a high affinity for small blood vessels everywhere.

4. Hesitation in the absorption of the material has been observed in line with the lower part of the horny layer, the borderline between the epidermis and dermis, the borderline of inner and outer root sheaths, and the borderline between epidermal and connective-tissue hair follicles.

5. Noticeable observations on the study of microdistribution are as follows:

(a) In a comparatively short period of time, a large quantity of the material has appeared in hair papillae.

(b) Although a large quantity of the material is seen in the sebaceous gland and excretory ducts, it is scarcely detected in the environment of those systems.

(c) The material has not been seen in the sweat gland and sweat ducts. However, a large quantity of the agent has been witnessed in the environment of these systems and also in the blood vessels around them.

(d) Although the agent has not been observed in the fatty cell, it was seen in the fatty intercellular septum in large quantities.
There are already some papers dealing with the absorption of vitamin E. However, almost all of them recite the intestinal absorption of the vitamin, and no report whatsoever has been published regarding its percutaneous absorption. There are no investigations of the histological distribution of vitamin E except for few papers by Miyazaki (1) and Okada (2). As for the intradermal histological distribution of vitamin E, the fluorescence microscopic observation on α-tocopherol administered subcutaneously by Miyazaki is the one and only paper available.

Kamimura (3) and Yano (4) already have reported to some extent the topical effects of vitamin E, *i.e.*, the influence of vitamin E applied on the surface of the skin on the cutaneous microcirculatory system and also on the growth rate of hair (5) has been observed only at the directly topically applied region without the medium of central nerve systems.

The existence of such local effects of vitamin E proves that the vitamin has been absorbed into the skin through the hairless surface of the skin and reached vascular plexuses or around the hair papillae; and it is quite safe to assume that some concentration was maintained for a given period of time.

Primarily, most of the substances claimed to be vitamin E are liposoluble. Therefore, from a tradition the percutaneous absorption of vitamin E stands to reason, and, moreover, it must be assumed that a great portion of the vitamin is absorbed through the hair follicle. However, as mentioned above, no experiment whatsoever has been carried out on the absorption routes and intracutaneous histological distribution of vitamin E itself.

Therefore, the authors have carried out the present experiment in order to demonstrate the percutaneous absorption of α-tocopheryl acetate by means of microradiography using ^14^C-labeled α-tocopheryl acetate.

The experiment is directed to the confirmation of the vitamin in the cell components, thereby contributing directly to the establishment of a guide to the topical application of vitamin E.

**EXPERIMENTAL**

1. **Materials and Methods**

DL-α-Tocopheryl-1-5-methyl-^14^C-acetate, having a specific activity of 3 μCi per mg in 0.5% benzene solution, was kindly supplied to us by Eisai Co. for medical research.

This study was performed on 14 skin samples of 7 hospitalized patients for the plastic procedure of the head-scar. The patients had his hair cut short, and 0.5-1.0 ml benzene solution of the materials was sprayed topically on this regions. For the protection of contamination of radioactive substances, the solution were sprayed through a tiny hole of the paper, 0.5 cm in diameter. The solution was then dried rapidly by air blast and the region was occluded with "Saran Wrap".

**Preparation for Autoradiography** —— At various intervals of 4, 6 and 24 hours after application of ^14^C-labeled α-tocopheryl acetate, the region was sterilized with thimerosal and alkohol. Continuously the surgery was conducted, and necessary quantity of dermal fragment were excised.

For preparing autoradiography, Kukita and Matsuzawa's method (5) were used
as follows. The excised skin specimens were directly frozen at $-20^\circ$, and were cut 15-20 $\mu$ thick by the Cryostat freezing microtome. The slices were directly mounted on the emulsion layer of Fuji autoradiographic plate (EM. type ET-2E, 15 $\mu$) for contact method in dark room, and they were exposed for 2-4 weeks in the refrigerator. Then they were developed in Fuji-Rendol, fixed in Fuji-Fix, and washed in running water.

The histological layer was stained with cold lithium carmine, counter-stained with saturated picric acid alcholic solution, and were included with “Biolite”.

**Results**

Autoradiographic silver grains (thereafter referred to simply grains), showing the existence of the material, have been obtained from each sample at intervals of 4, 6 and 24 hours after the treatment. They are indicated by the degrees of concentrations and regions collectively in Table 1.

The results are described in due order below:

**Table 1**

*Deposition of Autoradiographic Silver Grains*

<table>
<thead>
<tr>
<th></th>
<th>Time after spraying</th>
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<tbody>
<tr>
<td></td>
<td>4 hr</td>
</tr>
<tr>
<td><strong>Epidermis</strong></td>
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</tr>
<tr>
<td>Horny layer</td>
<td>±</td>
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<tr>
<td>Prickle layer</td>
<td>-</td>
</tr>
<tr>
<td>Pilary canal</td>
<td>+</td>
</tr>
<tr>
<td>Inner root sheath</td>
<td>-</td>
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<tr>
<td>Outer root sheath</td>
<td>-</td>
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<tr>
<td>Hair papilla</td>
<td>-</td>
</tr>
<tr>
<td>Sebaceous gland</td>
<td>±</td>
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<tr>
<td><strong>Secretory portion</strong></td>
<td></td>
</tr>
<tr>
<td>Excretory duct</td>
<td>+</td>
</tr>
<tr>
<td><strong>Sweat gland</strong></td>
<td></td>
</tr>
<tr>
<td>Secretory gland</td>
<td>-</td>
</tr>
<tr>
<td>Excretory duct</td>
<td>-</td>
</tr>
<tr>
<td>Periglandular tissue</td>
<td>-</td>
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<tr>
<td>Periductal tissue</td>
<td>-</td>
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<tr>
<td><strong>Vessel</strong></td>
<td>-</td>
</tr>
<tr>
<td>Fatty cell</td>
<td>-</td>
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<tr>
<td>Intercellular septum</td>
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</table>

1. **Autoradiography 4 Hours after Treatment**

As shown in Fig. 1, a large quantity of grains exist diffusely and are more or less localized in the stratum corneum in the epidermis. In addition, a considerable quantity of grains are observed in hair follicles, excretory ducts and secretory portion of the sebaceous gland. Grains exist scarcely in other parts.

2. **Autoradiography 6 Hours after Treatment**

Fig. 2 shows a thick slice of 20 $\mu$, which has been exposed for 4 weeks. As can be seen from this, a great quantity of grains exist in all the layers of the epidermis, and the corium is distinguished having a fixed structure, *i.e.*, dense grains are connected with the epithel pegs and run along sweat gland.
On the other hand, grains taking the shape of densely diverged configuration are clearly observed in the surroundings of the middle dermal veniplex. Some of them go clearly with the disposition of venous capillaries and some others are arranged divergently towards the epidermodermal junction, although it is difficult to determine whether those grains are of the artery system or of the venous system. Fig. 3 shows hair follicles and papillae. Grains are almost diffusely arranged in internal and external root sheaths. However, they are arranged forming a rather thick layer outward, i.e., towards Henle's layer and the glassy membrane. Grains are arranged almost diffusely, though slightly thin, in hair papillae, and some of these grains, which take a rather thick band-like shape, exist near the hairmatrix, and fade away towards the upper part. In Fig. 4, grains are observed in the sebaceous excretory ducts and the sebaceous gland, as if they lie along the intercellular spaces. In the sweat gland, as shown in Figs. 5 and 6, grains are not seen in the interior of the gland, nor in sweat ducts. But dense grains, having a sort of run,
exist intermittently and take a sinuous course in the environment of these organs, and are observed as the pathway of the blood vessels surrounding the sweat gland and sweat ducts. A small quantity of narrow dense grains exist in groups in the environment of small blood vessels in other tissues inside the dermis. Grains are scarcely observed in the connective tissue. No grains exist in fatty cells in the subcutaneous tissue as shown in Fig. 7. However, some mass of grains are seen connectedly or separately in fatty intercellular septum.

3. Autoradiography 24 Hours after Treatment

Grains in the epidermis are small in quantity as compared with those observed six hours after treatment. Dense grains in small quantities are seen partially in the depressed parts of the epidermis. However, some ring-shaped grains are disposed densely along the borderline between the dermis and epidermis as shown in Fig. 8.

As shown in Fig. 9, ring-shaped clear grains lying somewhat irregularly and intermittently in line with the glassy membrane, and observed lightly in the greater part of root sheaths; they are different from those seen in the cases observed six
hours after treatment. In the surroundings of hair papillae, complicated string-shaped dense grains lie in a row perpendicular to the space between epithelial root and connective-tissue sheaths.

Similar string-shaped arrangements are seen in the vicinity of hair matrix below the hair bulb borderline, and ring-shaped arrangements which fade away upward along hair matrix in hair papillae are also detected in Fig. 10. Grains in the sebaceous gland appear to have decreased more than those observed in the cases six hours after treatment, and, further, they are not seen in the environment of the sebaceous gland. Grains are detected neither in the sweat gland nor in sweat duct. However, dense grains existing intermittently in the surroundings of the outer edges of both the sweat glands and sweat ducts have increased rather than those observed in the cases six hours after treatment. Grains in the fatty intercellular septum do not differ from those examined six hours after treatment. Grains merely exist around the small blood vessels in the dermis.

FIG. 8  Hesitate Figure Along the Borderline between Epidermis and Dermis
24 Hours after Treatment

FIG. 9  Autoradiography of Hairfollicle
24 Hours after Treatment

FIG. 10  Autoradiography of Hairpapilla
24 Hours after Treatment
DISCUSSION

It goes without saying that the condition of cutaneous absorption varies depending upon the agents administered. However, the same agent produces different conditions considerably according to the various aspects of individuals, skins and circumstances. It is, therefore, risky to consider that the experimental results obtained from different individuals indicate the successive changes of cutaneous absorption. However, it is not necessarily difficult to presume the outline of the process of absorption from many samples different in their respective time elements. It is also possible to suppose from the quantity of autoradiographic grains shown on the slices of the tissue at which the material administered has a high affinity, the regions where a large number of grains exist, or the obstacles to the absorption of the material.

On the other hand, as to the observation whether or not the material administered remains unchanged, there is much to be studied. The material used in the present experiment is \( \alpha \)-tocopheryl acetate. Judging from the fact that the agent can easily be changed into \( \alpha \)-tocopherol in the presence of alkaline phosphatase, it is quite possible to consider that the former has changed in part into the latter in the course of the present experiment. Since the separation of \( ^{14} \text{C} \) depends on its future position, such change is not easily made, however. That is to say, the grains which prove the existence of \( ^{14} \text{C} \), a radioactive substance, are not necessarily \( \alpha \)-tocopheryl acetate, and some of them might indicate the presence of \( \alpha \)-tocopherol. From the standpoints mentioned above, the results of the present experiment shall be discussed.

Needless to say, \( \alpha \)-tocopheryl acetate is absorbed into the healthy skin easily. This is confirmed by the fact that grains exist orderly in the skin tissue. Evidently there are two different routes of absorption, although it is difficult to compare them quantitatively; one of them is through the epidermis from the stratum corneum, while the other through hair follicles.

In case of the slices four hours after treatment where the hair follicles are few, grains are localized in the stratum corneum. In the samples six hours after treatment are observed the grains which exist all over the epidermis distinctly clearly from the dermis; grains which lie along the sweat duct running from the epithel pegs; and grains which exist in all the layers of the epidermis lying almost up to the hight of middle dermal veniplex. In the samples 24 hours after treatment, a decrease in the number of grains in all the layers of the epidermis, and a series of string-shaped grains which come up to the hight of the basement membrane are observed.

Those observations indicate that \( \alpha \)-tocopheryl acetate is absorbed into the stratum corneum through the surface of the skin in the first place.

Secondly, the agent is absorbed into all the layers of the epidermis, and then goes into the various tissues in the dermis. At the same time, they also indicate that the vasotropic character of \( \alpha \)-tocopheryl acetate is high and the absorption is somewhat hesitant.

Regarding the absorption by way of hair follicles, the absorption on each of the samples four hours after treatment shows that there are a considerable quantity
of grains in hair follicles and that α-tocopheryl acetate can easily go into hair follicles. In case of the slices six hours after treatment, grains spreading, as if permeating, over inner and outer root sheaths are seen. At the same time, some grains appear to become a little more dense towards Henle’s layer and glassy layer. Grains are scarcely seen in connective-tissue sheaths at this stage. In the samples 24 hours after treatment, a considerable number of grains are detected in connective-tissue sheaths. However, grains decrease in epithelial root sheaths. Dense ring-shaped grains exist in line with Henle’s layer and the grassy membrane. At the same time, they grow considerably feebly in part along the grassy membrane, and there are grains, each having both ends cut clearly and curled up along the grassy membrane; they are ring-shaped at a glance, but are not connected with one another. That is to say, these grains show the blood vessels located at the outer edges of the glassy membrane.

These observations indicate that α-tocopheryl acetate infiltrates into the hair follicles by way of pilo-sebaceous canal, and a part of it, through inner root sheaths, and another part of it being absorbed directly into outer root sheaths, are eventually absorbed into connective-tissue sheaths, which are the dermic tissue.

The absorption of α-tocopheryl acetate through the above mentioned two routes have been proven during the course of the present experiment. However, some discussions should be made with regard to other routes.

Since the existence of grains in the sebaceous ducts and the secretory gland was detected quite a long time ago, it is obvious that α-tocopheryl acetate can infiltrate easily into the sebaceous gland. However, the movement of α-tocopheryl acetate into the dermis or subcutaneous tissues through the sebaceous gland has not been confirmed for many reasons, i.e., grains are not hesitant surrounding the sebaceous gland and in the environment of excretory ducts during the entire period; the quantity of grains do not change in excretory ducts; no connective image of grains with the surrounding tissues appears; and grains are not observed around blood vessels.

Grains are not detected in the sweat gland. Thus the absorption of the material by way of the sweat gland is doubtful. However, as shown clearly in the case six hours after treatment, a considerable amount of grains are observed along the epithelpegs and sweat ducts at the stage when grains are detected in epidermic prickle layers. It is undeniable that these images help accelerate the absorption of α-tocopheryl acetate from the wall of sweat ducts in the dermis apart from the absorption from the interior of sweat ducts. At the same time, it is also quite safe to consider that α-tocopheryl acetate absorbed through this way has turned into a large quantity of grains in the surrounding blood vessels.

Some discussions will be made on the views obtained during the course of the study on the microdistribution of α-tocopheryl acetate.

First of all, comparatively dense grains are seen in hair papillae, and the movement of α-tocopheryl acetate into the region appears to have been made through two routes. The first route leads to the hairmatrix through the root sheaths or the hair itself, reaching the interior of hair papillae directly and going further through blood vessels. The second route goes into blood vessels in the papillae through the blood vessels surrounding the sweat gland or the blood vessels
in the connective-tissue sheaths, and leads further to papillae. The former can be considered as the main route judging from the following facts. Although there is no clear-cut distinction between them, grains in the outer root sheaths in the hair root differ scarcely from those in the root sheaths in the hair bulb in the case six hours after treatment; there are images suggestive of some hesitation at the outer edge in this stage; the quantity of grains at this stage is much more denser than the grains in hair papillae; and although images in hair papillae and connective blood vessels are comparatively clear, they are not clearly seen at distant regions.

Distribution in other tissues in the dermis is scarce. Only dense grains lying in the environment of blood vessels stand out clearly, and almost none of them is seen both in connective-tissue fibers and interstices.

As shown clearly in the experimental results, grains are scarcely observed in the fatty cell, but groups of dense grains are detected in the environment of the blood vessels in the fatty intercellular septums. Many experiments already have revealed that vitamin E has a high affinity for the adipose tissue. The view, however, is strange. The authors would like to regard it as the actual status of the microdistribution of \( \alpha \)-tocopheryl acetate which has not been clarified by the traditional way of experiment. On the other hand, it seemed more appropriate to assume that the route referred to above, although not clarified during the course of the present experiment, leads through the vascular system than to consider that it goes through the fatty intercellular septums or fatty cells. However, it is doubtful to think that the material has been carried by blood stream, judging from the anatomical point of view. But it can not be known that the material has been carried through lymph spaces.

The affinity of \( \alpha \)-tocopheryl acetate for nerves and blood vessels will be next. The study on nerves has failed in the present experiment due to some technological problems arising in the counterstain process. However, the affinity of \( \alpha \)-tocopheryl acetate for blood vessels is noticeable. Grains which lie in line with the pathway of the blood vessels underneath papillae, grains existing around the small blood vessels in the environment of the sweat gland, sweat ducts, hair papillae, the glassy membrane, the dermis and the fatty intercellular network, and, especially the existence of grains which are denser in the case 24 hours after treatment than in the case six hours after treatment, must be regarded as having a remarkable affinity regardless of whether they are \( \alpha \)-tocopheryl acetate or tocopherol. And this phenomenon might serve as a proven evidence of the strong local effects of vitamin E on the microcirculation system. However, although the affinity of \( \alpha \)-tocopheryl acetate for small blood vessels have been evidenced during the course of the present experiment, the question as to which layers show the affinity has not been answered. Nevertheless, since Miyazaki (1) witnessed that tocopherol has a high affinity for the lungs and capillary endothels in the dermis, it may be reasonable to consider that the affinity is located in the endothel cells.

Lastly, as to the cutaneous absorption of the substance, it is not appropriate to assume that all the tissues equally absorb the material. But as reported by Rein (7) et al., thought must be given to the question of the existence of barriers for fat-soluble substances. However, any image that serves to evidence the exis-
tence of absolute barriers, has not been detected in the present experiment, although a series of layers which act generally as barriers for absorption have been observed. In other words, the fact that clear-cut images of hesitation have been witnessed in the so-called water-barriers located at the lower edge of horny layers, in the basement membrane at the borderline between the epidermis and dermis, in Henle’s layer ordering inner and outer root sheaths in hair follicles, and in the glassy membrane at the border of epidermal hair follicles and connective-tissue sheaths, indicate that these images act as barriers, in general, for the absorption of the material, although the material has passed through in a short period of time as compared with the water-soluble substance reported by Witten et al. (8).

The aforementioned are what the authors have discovered regarding the condition of absorption, especially the routes of absorption, microdistribution, the affinity for blood vessels and layers which act as barriers tentatively in the cutaneous absorption of α-tocopheryl acetate.

References