THE INFLUENCE OF SOME VITAMIN DEFICIENT DIETS ON A
SUBSTANCE CHARACTERISTIC OF THE EPIDERMIS
OF THE MOUSE, RAT AND MAN* †

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Recent investigations have demonstrated that the epidermis of the mouse, rat and man contains a polarographically reducible substance not present in other tissues (mouse brain, liver, kidney, lung, heart, lymph nodes, skeletal muscle, spleen, pancreas and blood) (1, 2). This characteristic substance of epidermis also absorbs specifically in the ultraviolet and its absorption maximum is pH dependent (3). Since the reducible substance in the epidermis of the mouse, rat and man disappears when this tissue becomes carcinomatous, (3, 4) experiments were undertaken to ascertain whether other conditions such as various vitamin deficiencies, some of which affect the epidermis, would have any influence upon the properties of the epidermal substance.

MATERIALS AND METHODS

Groups of six to 50 rats were maintained on the various deficient diets1 until the symptoms characteristic of a particular vitamin deficiency became evident. The following vitamin deficient diets were employed: vitamin A (5), riboflavin (6), vitamin D, (5) and the essential fatty acids2 (7, 8). The vitamin E deficient diet3 contained Torula yeast which produced necrotic liver degeneration in the rat; L-cystine reduced the incidence of the necrotic degeneration, but it did not give full protection (9, 10). Since cystine has an influence on this deficiency the latter might be of some interest in a keratinizing tissue such as epidermis. This diet containing Torula yeast will simply be denoted (vitamin E deficient) in this paper.

Three days before the animals were sacrificed, the hair on the entire back was removed with a razor after thoroughly soaking the hair with distilled water. At the end of the 3 day period the rats were killed by decapitation, the shaved skin excised and then the epidermis was separated from the dermis at 50° C. by

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1 The rats deficient in vitamins A, D, E, and riboflavin were generously supplied by Mr. J. A. Crafa of the Central Research Department of Anheuser-Busch, Inc., St. Louis, Mo.
2 The essential fatty acid deficient diet was purchased from Nutritional Biochemicals, Cleveland, Ohio. This diet proved to be very effective in bringing about the essential fatty acid deficient state (7, 8) which responded to the administration of linoleic acid.
3 The vitamin E deficient diet contained the following components: Torula yeast 30, sucrose 59, Merck salt mixture #1, 5.0 and vitamin free animal lard 5; the vitamins other than E were supplied according to Schwarz (9, 10) at a level of 1 per cent.
the procedure of Baumberger et al. (11). The epidermis (1 to 3 grams) was extracted twice on a steam bath by refluxing for half-hour periods with 50 ml. of a mixture of 3 volumes of redistilled ethyl alcohol and one volume of peroxide-free ethyl ether. Partial purification of the reducible substance was accomplished by reextracting the alcohol-ether soluble material with anhydrous ether. The phospholipids were then separated from the ether soluble material as previously described, and the phospholipid-free fraction was partitioned between petroleum ether and a mixture of alcohol, acetone and water (3). The reducible substance concentrated largely in the latter mixture which, after drying at 60–70° C. in vacuo, was used for polarography with a Sargent Model XXI Polarograph and ultraviolet absorption measurements with a Beckman DU spectrophotometer (3).

RESULTS

The results on the absorption characteristics and half-wave potentials of the reducible substance from the epidermis of rats in various vitamin deficient states are shown in Table I. Since the absorption maximum of the compound is pH-dependent, the maximum at pH 1, 7 and 12 is given. Also the total maximum absorption (E) of each sample at the various pH levels is shown since this value was highest at pH 1 and lowest at pH 12. Finally, the half-wave potential, E½, a characteristic constant of a compound or group of compounds is tabulated. This potential is usually measured against the saturated calomel electrode (S.C.E.). Since the nature of the epidermal substance is unknown, there is no way of expressing its concentration in the usual way.

An inspection of Table I shows that the absorption maximum of the reducible substance from 2 samples of normal rat epidermis was 265, 274–76 and 284–85 mμ respectively at pH levels of 1, 7 and 12. The total absorption (E values)

<table>
<thead>
<tr>
<th>EPIDERMIS</th>
<th>ABSORPTION MAXIMUM (mμ) AND TOTAL ABSORPTION (E) AT VARIOUS pH LEVELS</th>
<th>HALF-WAVE POTENTIAL VS S.C.E. AT pH 5.0</th>
<th>E pH 1 at pH 3</th>
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<tr>
<td></td>
<td>pH 1.5</td>
<td>pH 7.0</td>
<td>pH 12.0</td>
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<tr>
<td>Normal</td>
<td>265</td>
<td>100</td>
<td>274–75</td>
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<tr>
<td>Normal</td>
<td>266</td>
<td>138</td>
<td>276</td>
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<tr>
<td>Riboflavin deficient</td>
<td>266</td>
<td>63</td>
<td>275</td>
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<tr>
<td>(Vitamin E deficient)</td>
<td>265–66</td>
<td>66</td>
<td>274</td>
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<tr>
<td>Vitamin A deficient</td>
<td>266</td>
<td>79</td>
<td>276</td>
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<tr>
<td>Vitamin A deficient</td>
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<td>35</td>
<td>273</td>
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<td>Vitamin D deficient</td>
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<tr>
<td>Essential fatty acid deficient</td>
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<td>39</td>
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<tr>
<td>Essential fatty acid deficient</td>
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<td>64</td>
<td>269–70</td>
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<tr>
<td>Essential fatty acid deficient</td>
<td>262</td>
<td>44</td>
<td>269</td>
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* Groups of rats showing increasing severity of essential fatty acid deficiency.
decreased as the pH of the solution was increased. The difference in total absorption between the two samples was due to the fact that unequal weights of epidermis were extracted, the sample having the larger total absorption weighted appreciably more than did the sample having less total absorption. The half-wave potentials of the substance from the 2 normal epidermis samples were $-1.44$ and $1.47$ volts vs S.C.E. The substance from the epidermis of rats on the riboflavin deficient and (vitamin E deficient) diets showed an absorption maximum as a function of pH and half-wave potentials in the same range as those obtained for the substance from normal epidermis. The substance from the epidermis of rats on vitamin A and D deficient diets showed no appreciable change from the substance in normal epidermis except that in one group of vitamin A deficient rats the material had an absorption maximum at pH 12 of 291 m.$\mu$ instead of 285 m.$\mu$.

The most interesting change in absorption properties was in the reducible substance from the epidermis of rats deficient in the essential fatty acids, arachidonic and linoleic. As the severity of the deficiency increased, the absorption maximum of the compound was shifted to shorter wave lengths at all pH levels examined. The half-wave potential was in the range of the substance present in normal rat epidermis.

FIG. 1. Histologic appearance of skin taken from a rat severely deficient in the essential fatty acids, $\times$ 110. Note differentiation of epidermis, enlargement and increased number of the sebaceous glands.
Another criterium of possible changes of the substance in epidermis is in the alteration of its ratio, total absorption \((E)\) and diffusion current \((i_d)\). The diffusion current is a quantitative measure of the amount of the reducible substance present in a sample. The ratio, \(E\), measured at pH 1 and the diffusion current, \(i_d\), determined at pH 5 showed some variation (Table I), but the lowest value was found for rat epidermis which had the most severe fatty acid deficiency. The diffusion current of the reducible substance is practically pH independent from pH 2 to 6, whereas, as previously stated, the absorption maximum is highest at pH 1.

The skin of the rats severely deficient in the essential fatty acids showed some interesting histological changes. The epidermis was well differentiated with six to seven layers of cells which were covered with a thick coat of keratin. The sebaceous glands were large and numerous (Figure 1). In contrast, the skin of the control rats showed a thin epidermis with less keratin and sebaceous glands of nearly normal size (Figure 2).

**DISCUSSION**

Although the chemical nature of the reducible substance characteristic of the epidermis of the mouse, rat and man is not known, it is reasonable to assume
that this compound has a function important to this tissue. Some of the properties of this compound may be briefly reviewed. It is polarographically reducible, and its reduction at the dropping mercury electrode resembles that of some pyridine compounds, but the substance from epidermis does not have the same half-wave potential as pyridine compounds known to occur in animal tissues. Furthermore its absorption spectrum is different from that of the pyridine compounds examined (2, 4). The absence of a tertiary or quaternary nitrogen in the reducible substance from epidermis was suggested by the application of the sensitive method of Kodicek and Reddi (12). This technic, however, does not rule out a tertiary or quaternary nitrogen with a substituent in the carbon adjacent to the pyridine nitrogen. Treatment of the reducible compound with 6 N hydrochloric acid in a sealed tube at 100° C. had no affect upon its half-wave potential, but reduced the absorption maximum to shorter wave lengths. The compound is destroyed by ashing at 450°C.

The substance from epidermis is soluble in water, methyl and ethyl alcohols and slightly soluble in ether. It is dialyzable and migrates freely on paper with several solvents (4). Although it is present in minute amounts in epidermis, attempts are underway to isolate this substance in crystalline form to facilitate further characterization by physico-chemical means.

The studies reported here reveal that the reducible substance of epidermis was not appreciably altered polarographically or in its absorption characteristics in rats deficient in riboflavin, vitamins A, D, or (E). Only in rats deficient in the essential fatty acids did the epidermal substance show significant changes in its absorption spectrum in that the maximum was shifted to shorter wave lengths at all pH levels tested and the extent of this shift varied directly with the severity of the deficiency. The half-wave potential was nearly the same as that obtained from the epidermal substance of normal rats. The possibility exist that the fatty acid deficiency in rats results in the appearance of other organic compounds which absorb maximally at shorter wave lengths thus lowering the absorption maximum of the reducible substance of epidermis. This possibility could be checked with paper partition chromatography to ascertain whether other ultraviolet absorbing compounds are present (4).

One of the interesting effects of the deficiency of the essential fatty acids on rat skin is the pronounced enlargement of the sebaceous glands. The increase in size of these appendages may be attributed to their need of linoleic or arachidonic acid for normal function. It has been known for some time that the sebaceous glands are involved in carcinogenesis of the epidermis of the mouse since these structures disappear following the topical application of methylcholanthrene (13). Concomitant with the loss of the sebaceous glands is a significant thickening of the epidermis. On the other hand rat epidermis is more resistant to the carcinogenic action of methylcholanthrene and even to the more potent 9:10-dimethyl -1:2 - benzantracene; furthermore the sebaceous glands are little affected following multiple applications of these carcinogens to rat epidermis (14). Since the sebaceous glands are signally enlarged and the epidermis is somewhat hyperplastic in rats on the fatty acid deficient diet, it would be of some
interest to determine the influence of several potent carcinogens on these structures in the skin of rats lacking the essential fatty acids.

The role of the essential fatty acids in human nutrition has been reviewed by Hansen and Burr (15). These investigators state that "the specific function of the essential fatty acids in maintaining a healthy skin, if such a function exists, is not known." The assumption that the sebaceous glands play an important role in regulating the state of health of the skin appears reasonable, and this role may be mediated through the essential fatty acids. Actually some investigators have reported beneficial effects of unsaturated fatty acids in eczema and steatorrhea (15). The latter disease is characterized by an increase in the amount and changes in the quality of the sebum excreted (16).

**SUMMARY**

1. The influence of various vitamin deficiencies on a polarographically reducible substance characteristic of epidermis is described. Some properties of this compound were not affected by deficiencies of vitamins A, D, (E) or riboflavin. A deficiency in the essential fatty acids, arachidonic or linoleic, altered the absorption maximum at various pH levels of the epidermal material; but this may have been due to the presence of other ultraviolet absorbing compounds in the epidermis as a result of the deficiency. This fat-free diet resulted in differentiation of the epidermis and pronounced enlargement of the sebaceous glands.

2. A brief resumé of some of the properties of the reducible compound of epidermis is given.

3. The only condition thus far found to cause the disappearance of the compound present in epidermis of the mouse, rat and man is the occurrence of squamous-cell carcinomas in this tissue; even when these carcinomas are highly differentiated, this substance is absent.

**REFERENCES**