EXPERIMENTAL STUDIES OF THE TRANSCUTANEOUS ABSORPTION OF VITAMIN A*

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DURING THE past two decades, the topic of absorption of drugs and nutrients into and through intact skins of human subjects and the mechanisms involved in this process have been subjects of vital interest to cosmeticians, pharmacologists, and therapeutists. In his text, "The Principles and Practice of Modern Cosmetics," Harry (1) has presented a comprehensive and critical review of the extensive and, oftentimes, contradictory literature which has been accumulated on the cosmetic, biochemical, and dermatological phases of skin absorption and penetration.

Much of the confusion in both cosmetic and medical literature concerning the nutritional and the therapeutic merits of ingredients of cosmetics and of medicaments applied to cutaneous surfaces is attributable to (*a*) inadequate methods of testing and (*b*) the use of terms "absorption" and "penetration" as synonyms.

In this presentation, the authors have made no attempt to discuss limitations of experimental procedures which have been utilized by different investigators for determinations of comparative absorbabilities of drugs and nutrients into and through skin. A task of this magnitude and responsibility would extend far beyond the scope of a brief report of which the primary objective is the discussion of results of a series of experimental studies of absorption of vitamin A through skins of animals.

As a secondary objective of this communication, the authors wish to emphasize the need of the adoption of a uniform terminology for differentiation between (*a*) the passage into skin and (*b*) the passage through skin of drugs and of ingredients of cosmetics applied to the surface of the epidermis. The first of these two phenomena has been designated "intracutaneous absorption" and the latter "transcutaneous absorption."

Obviously, intracutaneous absorption is a prerequisite to transcutaneous absorption. Also, it is realized that some transcutaneous

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absorption will be an invariable "sequitur" of intracutaneous absorption. The purpose in the adoption of these two terms was not to distinguish between absorption into and absorption through skin as independent and separable phenomena. On the contrary, the sole reason for the introduction of the two qualifications of absorption was to enable an investigator to define clearly the objectives and results of his experimental or clinical researches. If the investigator's interest is limited to effects upon skin within areas of topical application, he is concerned only with intracutaneous absorption despite the fact that the product under test may pass through the skin. On the other hand, if the experimentalist or the clinician directs his attention to systemic evidences of either toxic or beneficial effects of the product applied to skin, then his objective is the demonstration of transcutaneous absorption.

The group of experiments described in this report was one of several series which were carried out, during a period of five years, for the purposes of assaying the comparative effects of different ingredients in skin creams and lotions upon the intracutaneous and transcutaneous absorption of vitamin A. In the series of experiments presented at this time, the objective was the determination of the transcutaneous absorbability of the vitamin, in a petrolatum base, applied to skins of rats, under carefully controlled conditions.

Experimental Procedure

In an experimental plan directed to determinations of transcutaneous absorption of any substance from sources applied to animals' skins, an obvious prerequisite is the elimination of possibilities of oral consumption of the product under test. Of the several methods which had been tried out, only one was found to be entirely satisfactory and this procedure has been adopted in all of the experiments on rats which are presented in this report.

Sources of vitamin A were applied to the abdominal and chest areas of rats' skins which had been clipped free of fur by means of an electric razor. Care was taken to avoid abrasions of the skin. The rats were held firmly on their backs in racks to which their feet and heads were securely fastened. Definite areas (1 sq. in.) for applications were outlined with surgeons' silk pasted on the skin. The purpose of this border of silk was to prevent seepage of the applied materials beyond the dehaired area of application.

Petrolatum, conforming with the specifications of the Pharmacopœia of the U. S. (2), was the base in which varying amounts of concentrates of vitamin A were incorporated by manual mixing. Potencies of the vitamin in these preparations were determined at regular intervals by the Carr-Price method (3). Results obtained by this colorimetric procedure were checked by both spectrophotometric analyses (4) and biologic assays (5). Samples of the preparations were utilized only so

long as the chemical method of assay showed that the vitamin potency was within a ±10 per cent range of the theoretical.

In the majority of the experiments, topical applications of vitamin A were made by spreading approximately 0.3 gm. of the petrolatum, fortified with the vitamin concentrate, over the defined, cutaneous areas and allowing it to remain in contact with the skin for two hours. At the end of this period, the preparation was removed, as completely as possible, with cleansing tissue, and the entire ventral surface of the chest and abdomen was scrubbed thoroughly with a sponge, which had been saturated with a soap solution. Then the skin surface was rinsed with warm water and dried, by wiping with a towel, before the rat was returned to its cage.

Throughout the test periods, all rats were maintained in individual cages and fed the vitamin A-deficient diet which has been prescribed for official use in biologic assays for vitamin A (5).

Topical applications of vitamin A in the petrolatum base were made to the same areas of skin, once daily, except Sundays, of each week of the test period. All test areas of skin were clipped free of fur at intervals of one week.

Judgments of systemic effects attributable to vitamin A applied to rats' intact skins have been based upon three criteria:

1. Gains in weights of young rats maintained on a vitamin A-deficient diet; these were recorded at intervals of one week during test periods.
2. Storage of vitamin A in the animals' tissues determined at the time of sacrifice.
3. Correction of the abnormal, morphological changes in the rats' tissues which are indicative of vitamin A deficiency.

Both preventive and curative types of experiment have been utilized in these studies of absorption of vitamin A through skin.

In the preventive experiment, rats at weaning age were placed upon the vitamin A-deficient diet. Applications on the base containing vitamin A were also begun at this time with the objective of determining whether sufficient vitamin A could be absorbed through the skin to influence either the rate of growth of the animals or the time of appearance of the characteristic gross signs of vitamin A deficiency. These animals formed the test group. At the same time, litter mates were placed upon the vitamin A-free diet and given applications of the base lacking vitamin A. These animals constituted a positive control group. Since this latter group received no source of vitamin A but otherwise were maintained under identical exprimental conditions, any difference in growth records between the test group and the control group could be attributed only to the effect of vitamin A absorbed through the skin.

The method employed in the cura-

tive experiments differed from that specified in the Pharmacopœia of the U. S. (5) for the biologic assay of vitamin A only in the fact that, in these experiments, the material containing vitamin A was applied to the intact skin instead of being fed by mouth.

Ten rats, at weaning age, were divided into two groups of litter mates. Each group of five rats included equal numbers of males and females. During an experimental period of eight weeks, the rats in one group (test group) received daily, a typical preventive experiment.

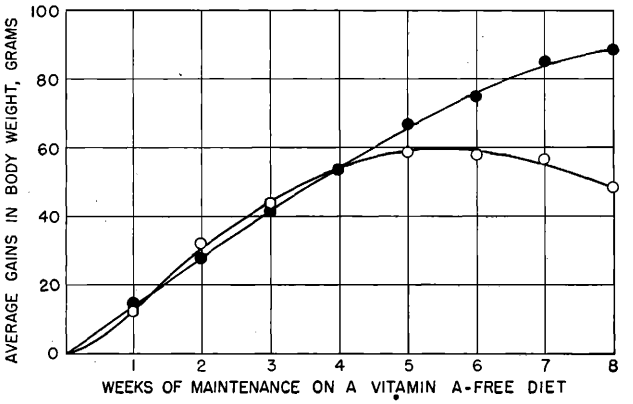

Chart I.—Comparative average gains in weight of rats maintained on a vitamin A-free diet and receiving daily topical applications of either petrolatum or petrolatum containing 2100 units of vitamin A per gram. ●, 5 rats receiving applications of base containing vitamin A; ○, 5 rats receiving applications of base without vitamin A.

At weaning age, the rats were placed on the vitamin A-free diet and allowed to deplete their bodies of vitamin to such an extent that their weights were either stationary or declining for a period of one week. At this time, the animals were considered vitamin A-deficient and topical applications of the vitamin were begun. In each case, one or more litter mate animals were maintained until death on the vitamin A-free diet and without applications of any kind, thus serving as negative controls.

Experimental Results

Chart I summarizes the results of topical applications of petrolatum containing 2100 units of vitamin A per gram, but their litter mates in the other group (control group) received comparable applications of the petrolatum without the vitamin concentrate.

Both groups grew at essentially the same rate for the first four weeks, but, after that period, the growth curve for the controls, receiving the plain petrolatum, leveled off and finally declined. However, the growth curve for the test rats, receiving the petrolatum containing vitamin A, continued to show practically constant weekly gains in weight. The slight change

in the slope of the curve during the last week is due largely to the loss in body weight, which was coincident with an attack of sniffles in one rat.

At the beginning of the test period, the average body weight of the control group was 50 gm. and that of the test group 46 gm. At the end of the experimental period of eight weeks, rats in the control group exhibited an average gain in weight of 49 gm. whereas, during the same period, their litter mates in the test group showed an average increase of 89 gm.

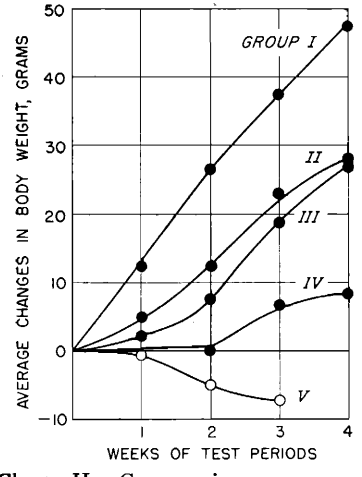

Chart II.—Comparative average gains in weight of vitamin A-deficient rats receiving daily topical applications of equivalent amounts of petrolatum containing varying concentrations of vitamin A.

Chart II presents a summary of results of a series of curative experiments.

For all of the curative experiments, a total of 45 rats, pooled from six litters, were divided into test groups at a time when growth curves indicated that their bodily stores of vitamin A had been depleted by the feeding, from weaning age, of the vitamin A-free diet.

Fourteen rats were continued on the diet without supplements of vitamin A until death or until they were sacrificed in a moribund state. These animals served as the negative controls which are included in the group designated as "V" in the chart.

Four groups, whose average gains in weight are depicted by the graphs in Chart II, received daily, throughout test periods of either four or five weeks, topical applications to their skins of equivalent amounts of petrolatum containing varying concentrations of vitamin A, as shown in the following tabulation.

Groups	No. of Rats in Group	Av. Body Wt. at Beginning of Test Period, Gm.	Concentrations of Vitamin A in Petrolatum Applied to Skins, I.U. per Gm.
I	3	124	2100
II	4	117	600
III	6	108	450
IV	4	119	100
V	14	120	0

Since all of the rats in the test groups were under observation for four weeks, but some were not continued through the fifth week, the graphs in the chart have been limited to the first four weeks of the test period.

The average curve for group V is representative of only 11 negative control rats which survived during the first three weeks of the test

period. All of these animals lost weight during the second and third weeks, but four survived through the fourth week.

In contrast with the negative controls, all of the four groups of rats, which received topical applications of petrolatum containing vitamin A, exhibited average gains in weight during the test period. Average results of this series of experiments point to some correlation between the concentration of vitamin A in the petrolatum base, which was applied to skins, and the growth responses of the vitamin A-deficient rats. It is evident, however, that this correlation is not quantitative. In fact, it is a matter of common knowledge that bioassays fail to yield quantitative relationships between dosages of vitamin A administered orally and growth responses of vitamin-deficient rats.

In all of the experiments on groups I to IV, inclusive, which have been summarized in Chart II, the source of vitamin A was maintained in static contact with rats' skins for two hours. A subsequent series of experiments was undertaken with the objective of determining the influence of massage upon the rate of transcutaneous absorption of vitamin A.

Results of this series of experiments are summarized in Chart III. Equivalent amounts of petrolatum, containing 450 units of vitamin A per gram, were applied daily and maintained in contact with skins of all rats for periods of two hours. In tests on one group of six rats (Group III of Chart II), this contact was entirely static. However, in experiments on the other group of four animals, the petrolatum containing the vitamin concentrate was rubbed gently with an index finger over the test area of skin at intervals during the period of contact with skin. The total time of rubbing was thirty minutes.

At the beginning of the test periods, both groups of rats showed approximately equivalent average body weights, *viz.*, 108 and 106 gm.

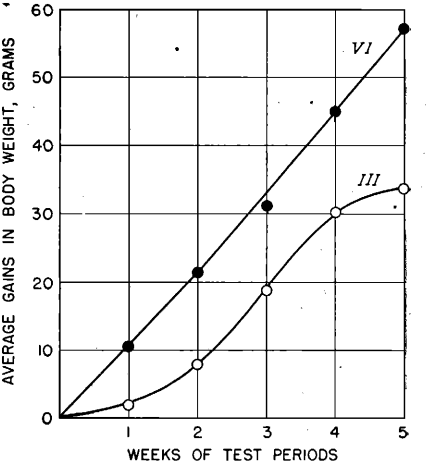

Chart III.—Comparative average gains in weight of rats receiving topical applications, with or without massage, of equivalent amounts of petrolatum containing 450 units of vitamin A per gram. ●, applications without massage to 6 rats; ○, applications with massage to 4 rats.

The curves of Chart III demonstrate that, throughout the test periods, vitamin A-deficient rats receiving topical applications of vitamin A in the petrolatum with massage made better gains in weight than did the animals to whose skins comparable amounts of the vita-

min were applied without massage. Total gains in weight during test periods of five weeks were 57 gm. for the former group of rats and 34 gm. for rats in the latter group.

During the first four weeks of the test period, the group of rats receiving topical applications of vitamin A with massage of the test areas of skin exhibited an average gain in weight which was approximately equivalent to the result shown in Chart II for the first group of rats which had received topical applications to their skins of petrolatum containing 2100 units of vitamin A per gram.

Results of the series of experiments which are presented in Chart III indicate that massage of the petrolatum, containing the concentrate of vitamin A, enhances the utilization of the vitamin for growth by young rats maintained on a vitamin-deficient diet.

The liver is the principal site of storage of the vitamin A which is in excess of the animal's immediate requirements. Two independent groups of investigators have reported that from 90 to 95 per cent of the total vitamin A of the rat's body may be found in the liver; the remainder was located in lung, kidney, and adrenal tissues (6, 7). One group of workers observed that the amount of vitamin A stored in the liver paralleled the quantities administered orally but only from 10 to 20 per cent could be accounted for (6).

In applications of the second criterion of transcutaneous absorption of vitamin A, particular attention was directed to the vitamin A contents of the test rats' livers and skins. After the animals had been anesthetized, at the time of sacrifice, their livers were removed *in toto* and weighed. After thorough mincing, the liver tissue was extracted with ethyl and petroleum ether under nitrogen. Sections of skin, weighing from approximately 1 to 2 gm. were dissected from the anterior abdominal wall and, in some experiments, from the backs of the rats and immediately frozen with liquid carbon dioxide. These frozen specimens were pulverized and then extracted as described for liver tissue.

Determinations of vitamin A in livers and skins were carried out by a modification of the method which has been described by McCoord and Luce-Clausen (7) but U.S.P. Reference Oil was utilized as the standard for colorimetric comparisons in place of the solutions of copper sulfate. All values for vitamin A in livers and skins are reported as International Units.

Table 1 presents data for vitamin A contents of livers and skins of three of the groups of rats which had been maintained on the vitamin A-free diet but had not received topical applications of vitamin A.

Five rats were sacrificed at the end of their depletion periods, i.e., after they had received no sources of vitamin for thirty-nine days and had exhibited declines in weight during the preceding seven days. Vitamin A contents of their livers varied from traces to 425 with an average of 216

TABLE 1—VITAMIN A IN LIVERS OF RATS MAINTAINED ON THE U.S.P. VITAMIN A TEST DIET WITH OR WITHOUT ORAL SUPPLEMENTS OF THE VITAMIN

Groups	Time of Sacrifice, Days After Depletion	No. of Rats in Group	Av. Change in Body Wt. During Test Period, Gm.	Vitamin A Units per 100 Gm.	
				Livers	Abdominal Skin
Depleted*	0	5	..	216	0†
Negative controls in Group V	24	6	−21	69	0‡
Positive controls receiving oral doses of U.S.P. Reference Oil	35	4	+28	168	3

* Sacrificed at the end of a period of 39 days, from weaning age, on the vitamin A-free diet.
† Four rats gave no evidence of vitamin A in their skins; 1 rat showed 6 units per 100 gm.
‡ Five rats gave no evidence of vitamin A in their skins; 1 rat showed 3 units per 100 gm.

units per 100 gm. Within this group, it was not possible to demonstrate any quantitative relationship between the amounts of vitamin stored in the livers of these rats and their gains in weight during the depletion period.

Six negative control rats in Group V (Chart II) were sacrificed in a moribund state. These rats had been continued on the vitamin-free diet for a longer period then their litter mates which were sacrificed at the time of depletion. Of the six negative controls, two gave evidences of only traces of vitamin A in their livers, and for the remaining four rats, the vitamin A contents of the livers varied from 80 to 112 with an average of 69 units per 100 gm.

Comparisons of the vitamin A contents of the livers of the negative controls with those of so-called depleted rats point to the conclusion that maintenance of rats on the basal, vitamin A-deficient diet for three weeks beyond the time of beginning the test periods reduced the liver stores of vitamin A coincident with losses in body weight.

The four animals included in the third group of Table 1 were representative of 12 rats in a reference group which had been used in a biologic assay of a preparation of petrolatum containing a concentrate of vitamin A. These four rats were continued on supplements of the Reference Oil for one week beyond the usual period of assay for vitamin A.

During a test period of thirty-five days, these rats had been fed an amount of the U.S.P. Reference Oil which was adequate to yield, during the first four weeks, total gains in weight within the Pharmacopœial requirements of 12 and 60 gm. (5).

At this point, it is deemed advisable to emphasize the fact that this range of gains in weight, prescribed by the Pharmacopœia of the U. S. do not represent either maximum or optimum rates of growth of young rats. It is simply a criterion of the nutritional status in which the rapidly growing young rat is most responsive to variations in intakes of vitamin A.

At the time of sacrifice, the livers of the four rats, which had received oral supplements of the Reference

Oil, varied from 112 to 210 with an average of 168 units of vitamin A per 100 gm. Although the intake of vitamin A was adequate to support rates of growth of these animals and prevent the development of gross signs of a dietary deficiency of vitamin A, it did not provide an excess of the vitamin for storage in the rats' livers.

Only one of the five depleted rats and one of the six negative controls gave evidence of vitamin A in their abdominal skins. In the group of four animals fed U.S.P. Reference Oil for five weeks, levels of vitamin A in skins ranged from traces to 4 mg. with an average of 3 mg. per 100 gm. The significance of these findings for vitamin A in skin will be discussed in a subsequent section of this report.

Data for vitamin A contents of livers and for total gains in weight of rats in the five groups which received, during test periods of five weeks, topical applications of petrolatum containing varying amounts of vitamin A are given in Table 2.

Livers were removed for analyses from only two animals in each of the first two groups. Carcasses of the other rats in these groups were preserved for histological examination of their viscera.

Among the four groups of rats which received static, topical applications of vitamin A, there is an apparent correlation between (1) concentrations of vitamin A applied to the skins and (2) total gains in weight and quantities of the vitamin stored in the animals' livers and skins within the following limits.

Both maximum gains in weight and maximum, hepatic reserves of the vitamin were found for the two rats in Group I which received the greatest amounts of vitamin A applied to their skins. On the other hand, the rats in Group IV, to whose skins petrolatum containing the smallest concentration of the vitamin was applied, exhibited minimum growth responses and minimum amounts of vitamin A retained in their livers. Also, this latter group showed the most variable results. Although total gains in weight were fairly uniform, i.e., from 6 to 15 with an average of 11 gm., two of the four rats gave no evidence of vitamin A in their livers, but the other two animals exhibited reserves of vitamin A equivalent to 325 and 425 units per 100 gm. of liver.

No significant difference was noted between the second and the third groups in respect to either total gains in weight or stores of vitamin in the rats' livers. This statement is applicable, also, to rates of growth for the first four weeks of the test period as summarized in Chart II. Comparative findings for these two groups suggest that the procedure, adopted in these experiments, is inadequate for demonstration of differences between the systemic effects of 135 units and 180 units of vitamin A applied to skin in equivalent amounts of petrolatum (0.3 gm.) daily during test periods of five weeks.

Average results reported in the

table do point to a striking difference between groups III and VI in reference to both gains in weight and hepatic stores of vitamin A. The sole variation in the experimental procedure, as applied to these two groups, concerned the method of application of the petrolatum base and the vitamin to the skin.

In the experiments on the third group of rats, the vitamin in the petrolatum base was spread, without rubbing, over test areas of skin (static contact) whereas, in tests on the sixth group of animals, the same amount of base containing equivalent concentrations of the vitamin was rubbed during thirty minutes of the period of contact.

The comparative experimental findings, which are presented in Table 2 and Chart II, suggest that the rats in Group VI, to whose skins vitamin A was applied with massage, utilized more of the vitamin for growth and, probably, also, for other metabolic processes dependent upon rapid rates of growth of young rats, than did the rats in Group III. Hence, rats in the former group had smaller reserves of the vitamin for storage in their livers.

A search of the biochemical, nutritional, and clinical literature reveals a paucity of quantitative data for vitamin A in human and animal skins. When due consideration is accorded the fact that the skin is the largest epithelial tissue of mammals, both in respect to weight and surface area, it is difficult to understand the persistent failures to include skin among the viscera analyzed in experimental studies of the distribution and storage of vitamin A. Only two publications, and both of these by one group of biochemists and nutritionists, give analytical data for vitamin A in skin. McCoord and Luce-Clausen (7) have reported values of vitamin A in skins of rats, and Clausen and McCoord (8) have recorded results of their studies of distribution of vitamin A among tissues of human subjects.

Table 2 presents a summary of results of analyses of sections of abdominal skins of rats in groups I to IV which received static appli-

TABLE 2—COMPARATIVE AVERAGE LEVELS OF VITAMIN A IN LIVERS OF RATS AFTER DAILY APPLICATIONS OF PETROLATUM CONTAINING VARYING CONCENTRATIONS OF VITAMIN A DURING FIVE WEEKS

Groups	Concentration of Vitamin A per Gm. of Petrolatum Applied to Skins	No. of Rats Analyzed in Each Group	Total Gains in Body Wt., Gm.	Units of Vitamin A per 100 Gm.	
				Livers	Abdominal Skins
I	2100	2	62	769	20
II	600	2	36	527	16
III	450	6	34	566	13
IV	100	4	11	187	3
VI	450*	4	58	191	..

* Applied to skins with massage.

cations of the petrolatum containing vitamin A. Sections of abdominal skin removed from rats in Group VI were not analyzed for vitamin A; they were utilized for histological studies.

Both levels of vitamin A found in skins of the two rats in Group I (21 and 19 units per 100 gm.) were at least equivalent to those reported by McCoord and Luce-Clausen (18.9 units) for rats sacrificed twenty-four hours after the administration of a dose of 1748 units of vitamin A in the form of halibut oil. Rats in groups II and III exhibited values for skin vitamin A which were significantly greater than the results tabulated by McCoord and Clausen for rats which had received, over a period of six weeks, a total of 2622 units of vitamin A divided into weekly doses. For these rats, the authors report levels of vitamin A varying from 4.27 to 9.60 units per 100 gm. of skin.

In order to obtain comparative data for vitamin A in skins of normal rats, which had been maintained on a nutritionally adequate diet, seven rats in the stock breeding colony were sacrificed and sections of their abdominal skins were removed for determinations of concentrations of vitamin. Results of these analyses showed variations from 10 to 25 with a mean of 16 units per 100 gm.

Sections of skin were removed from both the right and left sides of the abdomens of each of three normal rats. Comparative analyses of two skin sections from one rat gave checks within ± 5.7 per cent.

All of the data for vitamin A in skins of rats in the groups which are reported in Tables 1 and 2 represent levels for sections of abdominal skin. For rats in the test groups, which are included in Table 2, these sections included areas of skin to which the vitamin in the petrolatum base had been applied. At the time of carrying out these experiments on rats, it was not practically possible to exclude the possibility that amounts of vitamin A found in these sections may have included some of the vitamin retained in the skin from the preceding application of the petrolatum containing the concentrate of vitamin A. However, several later series of experiments on normal rabbits demonstrated that increments in vitamin A of the skin resulting from topical applications of a concentrate of the vitamin in petrolatum disappeared within periods of four to six hours after the preceding application.

Every precaution was taken to eliminate hangover, local increases in skin vitamin A attributable to either retention of the vitamin on the surface of the skin or the intracutaneous absorption of the vitamin. After each application, the skin was washed thoroughly with soap and water. Tests on rabbits showed this process of washing gave an average reduction of 12 per cent in the skin vitamin A. Also, an interval of twenty-four hours elapsed between the preceding application of vitamin A and the time of sacrifice of the rats.

However, in order to eliminate possibilities of intracutaneous absorption in appraisals of influence of transcutaneous absorption upon the vitamin A of skin, sections of skin were dissected from the backs of seven rats in groups I, II, and III.

TABLE 3—COMPARATIVE LEVELS OF VITAMIN A IN SKINS OF NORMAL RATS AND OF RATS RECEIVING TOPICAL APPLICATIONS OF VITAMIN A IN A PETROLATUM BASE AS THEIR SOLE SOURCE OF THIS VITAMIN

Groups	No. of Rats	Areas of Skin Analyzed	Units of Vitamin A per 100 Gm. of Skin: Mean	Units of Vitamin A per 100 Gm. of Skin: Standard Error of Mean
Normals	7*	Ventral	16†	1.67
		Dorsal	..	...
I, II, and III (Chart II and Table 2)	7	Ventral	17	0.86
		Dorsal	16	1.00

* Maintained on stock breeding diet—ages varied from 18 to 29 weeks with average of 24 weeks.

† Mean result of analyses of 10 sections of skin.

Results of comparative analyses of ventral and dorsal sections of skins of these seven rats are reported in Table 3. The data summarized in the table indicate approximately equivalent concentrations of vitamin A in areas of skin of both the abdomen and the backs of these animals.

At the time of sacrifice of the seven rats in the three test groups, samples of blood were drawn for analyses for vitamin A in the sera. In all instances, blood levels of the vitamin were slightly lower than the corresponding values for skin. The range of blood levels was from 11 to 15 with a mean of 12.6 units of vitamin A per 100 cc. of blood serum.

A group of 14 rats, litter mates of the animals in groups I, II, III, and IV, received oral supplements of vitamin A daily over a period of five weeks after depletion of their vitamin stores. These supplements were adequate to give total gains in weight between 12 and 60 gm. and to protect against the development of gross signs of deficiency of vitamin A. At the end of the test period, all animals were sacrificed and analyses were made for vitamin A in their livers and skins.

Table 4 summarizes the comparative data for the group of 14 rats which received oral supplements of the vitamin and the 14 rats whose sole source of the vitamin was that applied to their skins without massage.

The purpose of conducting these comparative experiments was not the evaluation of the relative nutritional or thereapeutic merits of vitamin A administered by the oral route or through the skin. On the contrary, the primary objective was the utilization of the results found for rats fed vitamin A as criteria of the systemic effects of the vitamin.

Data presented in Table 4 show that, during test periods, both groups of rats made approximately equivalent gains in weight. Hence, gains in weight have been adopted as common denominators in appraising the significance of differences between the two groups in respect

to (1) vitamin A stored in livers and (2) vitamin A in skins.

Values for vitamin A in both livers and skins were calculated and evaluated as units per total weight of these tissues.

Both groups of rats exhibited the three experimental findings which are indicative of systemic effects of vitamin A, *viz.*:

1. Gains in weight.
2. Levels of vitamin A in livers and skins above levels of depleted rats and negative controls (Table 1).
3. No evidence of metaplasia of specialized epithelial tissues, e.g., keratosis of the urinary tract, xerophthalmia, and localized infections.

Results of the comparative series of experiments summarized in Table 4, as well as those presented in Chart I and Table 3 are conclusive evidences of the transcutaneous absorption of vitamin A from the petrolatum base applied to the rats' skins.

The data summarized in Table 4 reveal some differences between the responses of the rats to vitamin A applied to skin and to the vitamin administered by mouth.

In proportion to amounts of vitamin A stored in livers, the rats receiving topical applications of the vitamin exhibited greater gains in weight than did the animals fed vitamin A. The coefficients of correlation between these two systemic effects of vitamin A were 0.71 for the 14 rats receiving topical applications of vitamin A and 0.36 for the 14 rats fed the vitamin.

Also, the rats to which vitamin A was applied topically showed larger amounts of vitamin in their skins, both ventral and dorsal, than did the animals receiving oral doses of the vitamin. Between these two results, the former group gave a coefficient of correlation of 0.75 and the latter a coefficient of 0.69.

TABLE 4—COMPARATIVE AMOUNTS OF VITAMIN A IN SKINS AND LIVERS OF RATS RECEIVING THE VITAMIN EITHER ORALLY OR IN TOPICAL APPLICATIONS TO SKINS

Groups	No. of Rats	Av. Body Wt., Gm. Initial	Av. Body Wt., Gm. Final	Units of Vitamin A per Wt. of Tissues: Skin Max.	Skin Min.	Skin Mean	Liver Max.	Liver Min.	Liver Mean
Vitamin A fed orally	14	115	144	3.5	Trace	1.6	81	6	36.3
Vitamin A applied to skins	14	116	144	5.8	Trace	2.7	63	Trace	32.4

CONCLUSION

Results of both preventive and curative series of experiments provide definite evidence of the transcutaneous absorption of vitamin A from a petrolatum base applied to rats' skins.

The findings of a small group of curative experiments suggested that

massage of the base over the skin enhances the transcutaneous absorption of vitamin A. This hypothesis was supported by results of subsequent series of experiments in which vitamin A in skin cream bases was either spread or massaged over rats' skins.

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