the glassware in the first 2 experiments, or of the skin by feces in the third. It would seem reasonable to assume that any dermal loss of iron would be mediated in part at least by the plasma transport mechanism, yet in the intravenous experiments in which the plasma iron was raised to very high levels, no appreciable loss through the skin occurred.

It should be noted that an oral dose of 10 mg of iron to a 10 kilo dog corresponds roughly to a 70 mg dose to an average man, a level relatively higher than the iron fed daily by Mitchell and Hamilton(5).

It is difficult to reconcile the final conclusion of Mitchell and Hamilton(5) that the skin and sweat glands are important excretory organs for iron with the finding that the absorption of iron occurs with difficulty and can be influenced by bodily needs. This has been amply demonstrated with radioiron in the dog(1), rat(3), and human(2) subject. The reutilization of iron released from destroyed erythrocytes following the administration of phenylhydrazine(9), transfusion of non-viable red cells(10), and normal wear and tear(11), has been shown repeatedly. The occurrence of transfusion hemosiderosis, both clinically and in normal dogs(12), indicates little or no ability on the part of humans and dogs to excrete iron. Moreover, the high tissue iron found by Finch and his co-workers(13-15) in rats and dogs fed a corn grit and lard diet is incompatible with much loss of iron from the body by any route, including the skin.

Summary. Following intravenous or oral administration of single doses of radioiron to dogs, little or no radioiron can be washed from the skin and hair, thus indicating that no significant amounts of iron are excreted through the skin in 1 to 7 days.

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Some Effects of Subcutaneous Tocopherols in Normal and Tocopherol-Deficient Mice. (17718)

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Pregnant mice will resorb their fetuses when fed a tocopherol-deficient diet long enough to deplete the body stores of this vitamin; such animals, however, when treated with tocopherols will have normal litters(1,2). Although parenteral administration of tocopherols has been used effectively in preventing or repairing the histopathologic changes in voluntary muscle due to tocopherol deficiency in several species, the effect of treatment by this route on the maintenance of pregnancy has not been fully explored, due primarily to the greater effectiveness of the oral over the parenteral route of administration(3-8). To supplement

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TABLE I.
Subcutaneous Tocopherols in Normal Mice.

Preparation	Amt, mg	Days treated	Animals No.	Animals Sex	Effect on reproductive organs
Alpha phosphate	15-25	7-65	8	F	None
" succinate A	12-27	4-100	11	M	"
" "	12-28	19-89	10	F	Continuous vaginal cornification
" "	4-8†	25-46	2	F	Continuous vaginal cornification
" "	18-19	52-69	5	SF	Continuous vaginal cornification
" " B	20-27	30-35	5	F	Initial vaginal cornification, 2 to 6 days
" " C	20-28	46-50	5	F	None
" palmitate	18-24	15-37	7	F	Limited intermittent vaginal cornification
" "	15-34	44-50	7	M	None
Mixed natural concentrate, 34%	.25 cc/ daily, 4 days	14	4	F	"

† Pellet implanted in omentum.
SF, spayed female.

TABLE II.
Subcutaneous Tocopherols in Pregnant Tocopherol-deficient Mice.

No. animals	Tocopherol preparation			Litters
5	Alpha palmitate		19-23 mg	Normal
5	" phosphate		20-29 "	"
5	" succinate (C)		14-26 "	"
5	delta phosphate		20-82 "	"
6	Mixed natural concentrate		.05-.25 cc	"
14	—	None	—	Dead

the available data on the reaction of mice to tocopherols, experiments were undertaken: to determine the effect of several tocopherol preparations administered subcutaneously on the local reaction of the tissue adjacent to the site of administration in animals fed a standard, stock diet or a tocopherol-deficient diet; and to test the effectiveness of these preparations on maintenance of pregnancy during tocopherol deficiency.

Methods. Mice of a homogeneous, inbred strain (C57) were kept on wood shavings in individual cages and given food and water *ad libitum*. Stock animals were reared on a commercial diet (Wayne Dog Blox or Rockland Complete Rat Diet); whereas those used for tocopherol-deficiency studies were prepared by transferring the pregnant mothers from the stock to a tocopherol-deficient diet* and continuing this diet subsequently for the young. The following crystalline esters of tocopherol were made into cylindrical pellets 2 mm in diameter and 2 to 8 mm long for implantation: d-alpha-tocopheryl palmitate, phosphate, or succinate, and d-delta-tocoph-

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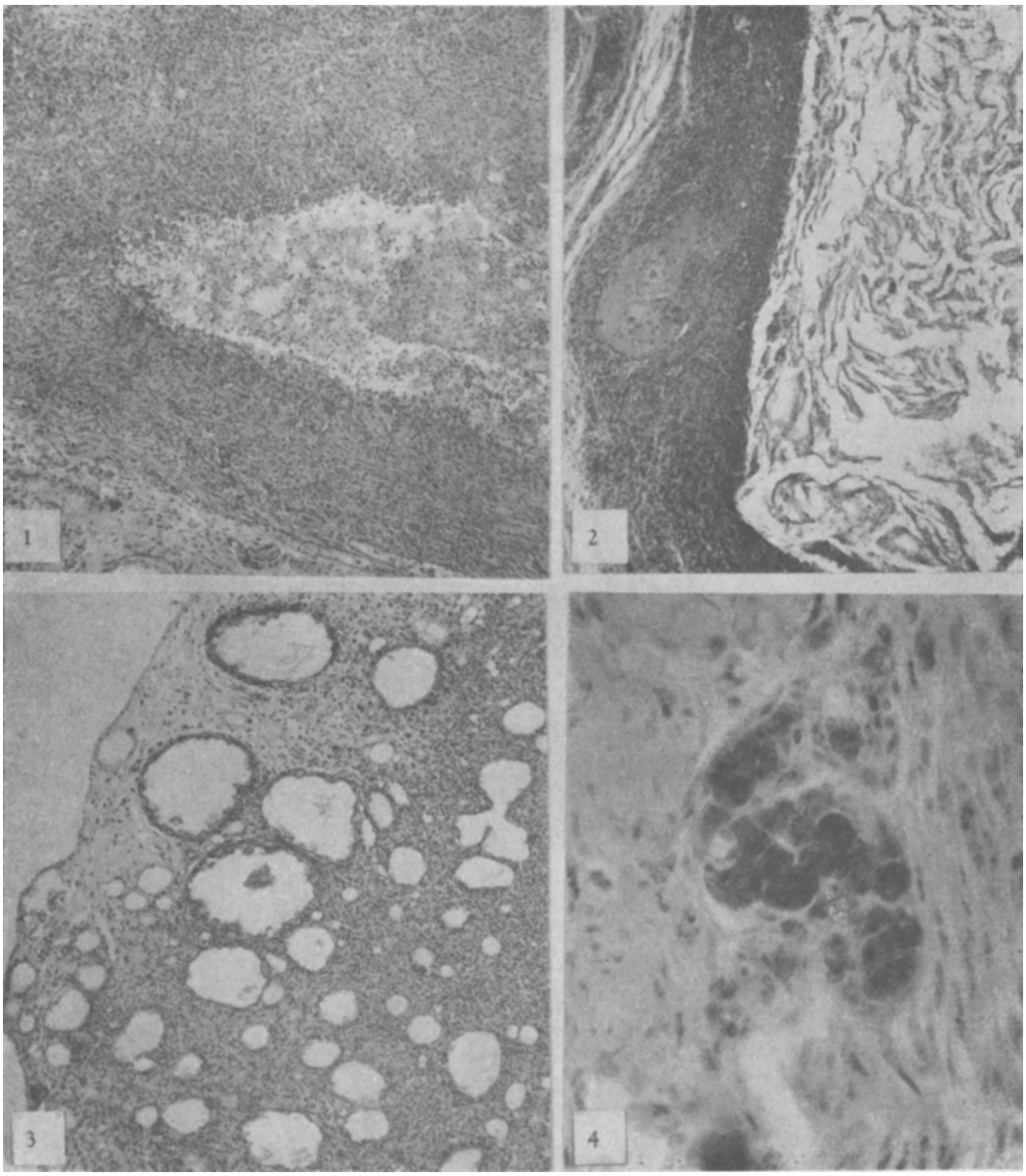
* The tocopherol-deficient diet consisted of: casein 20%, starch 50%, salts 2.5%, yeast 7.5%, lard 18.0%, cod liver oil 2.0%.



Photomicrographs of paraffin sections, 10 μ. Ehrlich's hematoxylin and Kinyouin's acid-fast stains. Figs. 1-3, 67×; Fig. 4, 290×.

FIG. 1. Area of subcutaneous tissue which surrounded a pellet of alpha-tocopheryl succinate (A) in a female mouse for 52 days. Necrotic tissue is near the central pellet site which is enclosed by a connective tissue capsule and infiltrated with leucocytes.

FIG. 2. Pellet area removed from a female 28 days after implantation, showing the emulsified alpha-tocopheryl palmitate pellet (right) and connective tissue capsule with leucocytic infiltration (left).

FIG. 3. Multilocular cysts of mixed natural tocopherol solution in the subcutaneous tissue of a female for 14 days. The area is infiltrated with leucocytes and connective tissue. The tocopherol solution in the peripheral part of the cysts was acid-fast.

FIG. 4. Section from the peripheral part of the connective tissue capsule shown in Fig. 1, showing histiocytes of various size containing acid-fast pigment.

eryl phosphate. A mixed natural tocopherol solution† was injected. The subcutaneous tissue over the dorsal shoulder region was chosen as the site for administration in both instances. The pellets were implanted by means of a metal, tubular applicator inserted through an incision at the base of the tail and passed cephalad in the subcutaneous tissue. The tocopherol solution was injected via a tuberculin syringe and 27 gauge needle inserted through the skin and directed an inch or more from the site of entrance. Daily vaginal smears were made to determine the regularity of the estrous cycles, and to verify the time of insemination and course of gestation. The animals were killed by illuminating gas at various intervals after treatment. The tissues were fixed in Zenker-acetic, cut in paraffin at 10 μ, and stained with Ehrlich's hematoxylin and Kinyouin's acid-fast stain.

Local reaction to subcutaneous tocopherols. Males and females (30 to 50 days of age) fed the stock commercial diet, and pregnant tocopherol-deficient females (75-125 days of age) were treated with tocopherols as shown in Tables I and II. All the tocopherol preparations produced local reactions in the subcutaneous tissue (Fig. 1-4). The alpha-tocopheryl palmitate and the concentrate of mixed natural tocopherols appeared to produce the least amount of local reaction. The alpha-tocopheryl succinate and delta-tocopheryl phosphate were more irritating, whereas the alpha-tocopheryl phosphate usually resulted in ulceration of the overlying skin and occasional loss of the pellet.

Pellets of alpha-tocopheryl succinate which could be compressed into a firm mass were removed from the implantation area, dissected free of tissue, dried in a desiccator and weighed. The other esters, which emulsified in the subcutaneous tissue, and the mixed natural tocopherol concentrate, which formed multi-locular cysts, could not be recovered with sufficient accuracy to determine the amount absorbed by the animals. Some of the tocopherol was absorbed from these sites, as indicated both by the maintenance of gestation (described below) in animals fed the tocopherol-deficient diet, and by a decrease of 1-2 mg in the weight of those pellets which could be recovered and weighed accurately.

Two other reactions to these tocopherols were noted: the presence of an acid-fast pigment in the cells of the fibrous capsule around the pellet and the prolonged vaginal cornification found after implantation of certain tocopherol pellets. A pigment, similar in its staining to that found in the adrenals of mice(9), was found adjacent to the subcutaneous tocopherol deposits. Whether this pigment was of a different nature from that found in the tissues of tocopherol-deficient animals remains to be determined.

Estrogenic activity of certain tocopherols has been claimed by certain investigators and denied by others(10-13). As shown in Table I, vaginal cornification was found after implanting some of the crystalline tocopherols.‡ The weights and histological structure of the testes and accessory reproductive organs of the males were not affected by the various tocopherols. The various contradictory results concerning the estrogenic properties of

† The author is indebted to Distillation Products, Inc., Rochester, N. Y., for the crystalline tocopherols and for the mixed natural tocopherol, concentrate solution (340 mg tocopherols/g solution: containing 170 mg of alpha-tocopherol/g and 170 mg/g of varying amounts of beta, gamma, and delta tocopherol).

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‡ Three different preparations of *d*-alpha-tocopheryl succinate were used. The first (A) resulted in continuous vaginal cornification. Histological sections of the vaginae and uteri from the animals showed changes characteristic of estrogen stimulation. The second (B) caused vaginal cornification lasting only from the 2nd to the 6-8th day after implanting the pellets. The third (C) had no effect on the reproductive system of normal or spayed animals. This last preparation was given to the animals used for the gestation studies.

tocopherols may be due primarily to a contaminant in the particular tocopherol preparation used. Since the mixed natural tocopherol concentrate did not produce any estrogenic effects, whereas some of the crystalline preparations obtained from the concentrate did, may have been due to some substance in the tocopherol concentrate capable of inhibiting the estrogenic material.

Gestation with subcutaneous tocopherols. To provide assurance of uniformly early and severe tocopherol depletion, the experimental animals used in this study represent offspring of mothers kept throughout pregnancy and lactation on the tocopherol-deficient diet. The young were continued on this diet and mated, when 75-100 days of age, with males reared on the stock diet. The untreated animals resorbed their young, whereas those given the tocopherol preparations during the first week of gestation had normal litters. (Table II).

Summary and conclusions. 1. Pellets of crystalline tocopherol esters (d-alpha-tocopheryl palmitate, phosphate, or succinate and d-delta-tocopheryl phosphate) were implanted into the subcutaneous tissue over the dorsal shoulder region of male and female mice. A solution of mixed natural tocopherols was injected into the same region in other animals.

2. All the tocopherol preparations produced local tissue changes resembling a foreign body reaction. Qualitatively, alpha-tocopheryl palmitate and mixed natural tocopherol concentrate produced slight local reaction. The reaction to alpha-tocopheryl succinate and delta-tocopheryl phosphate was greater, and that to alpha-tocopheryl phosphate the most pronounced.

3. All the tocopherol preparations maintained gestation in tocopherol-deficient mice.

4. Two of the alpha-tocopheryl succinate and the alpha-tocopheryl palmitate preparations produced intermittent or continuous vaginal cornification.

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Turnover of P^{32} in Different Types of Nuclei from Rabbit Liver.* (17719)

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Hitherto investigations into nuclear metabolism have been carried out with mixtures of nuclei isolated from the various cells which make up the tissue being studied. By differential centrifugation it has been possible to separate nuclei isolated from rabbit liver into two fractions, one made up predominantly of hepatic cell nuclei, the other containing nuclei of other types of cells present in the liver and to compare P^{32}-turnover in hepatic cell nuclei with other types of nuclei found in the liver.

Nuclei were isolated in 5% citric acid as previously described(1). They were then subjected to centrifugation at 900 g for 3 minutes, after which successive layers were removed with a pipette and examined microscopically. The nuclei of the upper layers were predominantly spherical, while those near the bottom of the centrifuge tube were predominantly ellipsoidal, fusiform and cylindrical, together with occasional ones which were constricted and lobed. By repeated centrifugation (5x) of the upper and lower layers, about half of the nuclei could be separated into the two groups mentioned above (Fig. 1 and 2). In each group counts were made of the frequency of the spherical and non-spherical types. In Group 1, 90-95% of the nuclei were spherical, while in group 2, 98-99% were non-spherical. On the basis of morphology, the predominant type of nucleus in Group 1 could readily be identified as that of the hepatic cell. Those of Group 2, could be recognized as fibroblast,

* Supported by a grant from the U.S. Public Health Service.

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