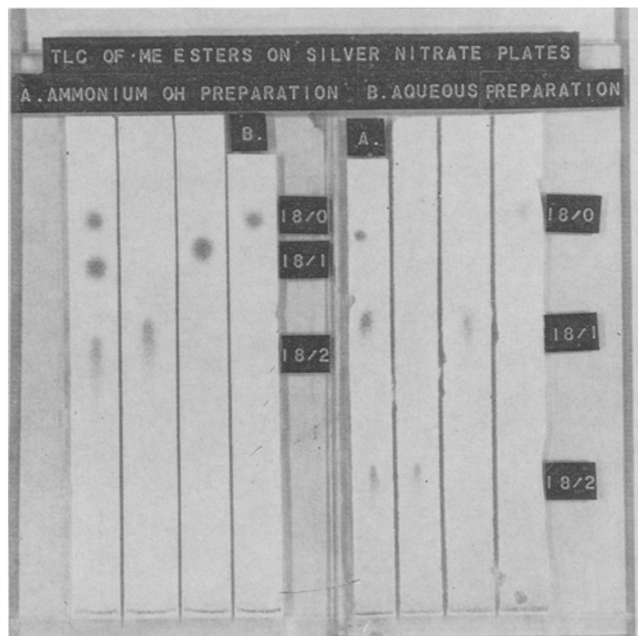

FIG. 1. Comparison of the resolution of methyl stearate, methyl oleate, and methyl linoleate on TLC plates impregnated with silver nitrate. *A*, plate prepared with an ammonium hydroxide solution. *B*, plate prepared with an aqueous solution.

do become darkened. Plates stored in a desiccator protected from light do not show discoloration even after four days.

In a comparison between the ammoniacal silver ion and the aqueous silver ion solutions for corrosiveness, the former was found to be less corrosive toward most metals used in the construction of TLC applicators and spreaders than the latter.

Kolthoff and Sandell (5) have reported that slightly soluble metal hydroxides dissolve in ammonia with the formation of complex ammino ions. From this supposition, the following set of equations was formulated:

1. $AgNO_3 + NH_4OH \leftrightarrows AgOH\downarrow + NH_4NO_3$
2. $NH_4OH \rightleftarrows NH_3 + H_2O$
3. $AgOH + 2NH_3 \rightleftarrows Ag(NH_3)_{2+} + OH^-$

When a stoichiometric amount of ammonium hydroxide was added to the silver nitrate, a precipitate was formed, in agreement with Equation 1. Upon the addition of more ammonium hydroxide, the precipitate dissolved, and is in agreement with Equation 3. The electrophilic ammino ion (Equation 3) apparently forms a stronger coordination complex with the nucleophilic π bonds than does the silver ion alone. This assumption is supported by the fact that improved resolution was obtained in the separation of the fatty acid methyl esters using TLC plates impregnated with the ammoniacal silver ion.

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On the Origin of the More Saturated Hydrocarbons of Skin Surface Lipid

HYDROCARBONS OTHER THAN SQUALENE or a hydrocarbon that gives the Liebermann Burchard test, have been consistently reported in studies of surface lipids of man and animals (1). Although a part of this material undoubtedly represents external contamination (1) (since the fraction resembles petroleum hydrocarbons), and although biosynthesis is unlikely since incubation of 1-C^{14} acetate with human skin slices failed to incorporate label into hydrocarbons under conditions where fatty acids, squalene and cholesterol became labelled (2), such material could still enter the body through the diet and be excreted intact through the skin. In support of this idea, cats fed hexadecane deposited it in the skin and in other tissue (3). Furthermore, rats fed a diet containing 50% mineral oil became greasy and appeared to excrete oil through the skin (4).

To determine whether the more saturated hydrocarbons of skin surface lipid could originate from excretion of dietary hydrocarbon, normally present at low levels, rats were fed 1-C^{14} octadecane, their skin surface lipid was wiped off at intervals, and the hydrocarbon fraction was separated and assayed for radioactivity as follows: Five male Sprague Dawley rats (about 290 g each) were individually stomach fed 1.8 mg 1-C^{14} octadecane (5.0×10^7 counts/min/g, New England Nuclear Corp., Boston, Mass.) in 0.50 ml corn oil. Paired control rats were stomach fed 0.50 ml corn oil only. Food was withheld on the day before stomach feeding but was available subsequently *ad libitum*. Several days before stomach feeding each rat was shaved from the neck to the base of the tail and from flank to flank (with clippers previously soaked in $CHCl_3$ to remove lubricating oil), then fitted with a large conical plastic collar which extended beyond the tip of the nose thus preventing oral contact with the body, feet and anus. Each rat was housed in a clean cage equipped with a wide net wire screen to minimize contamination from excrement.

At intervals as indicated in Figure 1, their backs were wiped with hexane soaked fat-free cotton pledgets. After each wiping the rat fed labelled hydrocarbon was moved to a new cage and his paired control was moved into the cage he vacated. If the animals could contaminate their backs from excrement on the cage, then the surface lipid of the paired control rats should have become radioactive. The surface lipid of the control rats had the same activity as background. This showed that the animals were not being contaminated with their excrement.

Wiping was performed by one person in a "standardized" way, i.e., the same area of the back was wiped with the same number and type of strokes. One person held the rat, another (wearing gloves previously soaked in hexane) wiped, while a third removed feces and urine from the table excreted during wiping.

The cotton pledgets were extracted with redistilled hexane, filtered, the solvent removed, the lipids weighed, (average yield per rat per wiping was 12 mg), and an aliquot assayed for radioactivity on a Nuclear Chicago scintillation counter by means of the channels ratio technique. Hydrocarbons (17.3 mg) were separated from the remaining lipid after application of 241 mg total lipid on a chromatographic column (2.5 × 10 cm) of silicic acid (Mallinckrodt) by elution with 70 ml of petroleum ether and counted.

The figure shows the rate of rise and fall of specific activity of rat skin surface lipid from the time of feeding of 1-C^{14} octadecane (zero days). The bars of the curve represent the range, the dots, average values. Since the initial appearance of activity was approximately 2 days after ingestion of 1-C^{14} octadecane and maximum activity appeared at about 6 days, it can be inferred that this activity arose from sebaceous gland excretion and not from keratinizing epithelia, for the cell renewal time of the malpighian layer of the back skin of adult rats is 15.9 days (5).

The radioactivity of the surface lipid, however, did not reside in the hydrocarbon fractions. It thus appeared unlikely that excretion of straight chain hydrocarbons when ingested at a low level can be a source of the hydrocarbons found in skin surface lipid. Whether other hydrocarbons of the petroleum type behave similarly remains to be determined.

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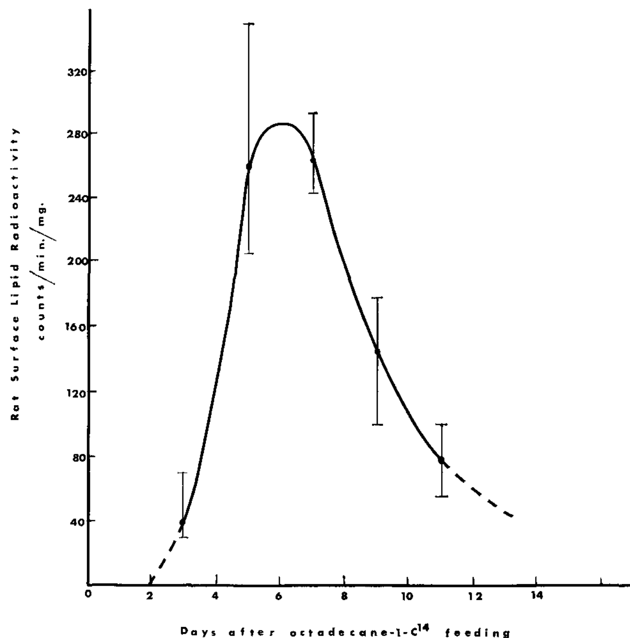

FIG. 1

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Effect of Storage Temperature on the Stability of Trivernolin

PREVIOUSLY WE REPORTED that under certain conditions of storage *Vernonia* oil and trivernolin undergo changes in their physical nature that are not always indicated by oxirane oxygen values (1). Of particular concern was the increase in viscosity that occurred during storage of trivernolin in a semisolid to solid state at 2–4C. This instability at 2–4C was further corroborated when trivernolin was stored and exposed to the surrounding atmosphere at −29C, −16C, 2C, and 15C for 6 months. The viscosity of the trivernolin stored at 2C increased 30%; no measurable changes were found in the other samples.

Since this behavior seemed unnatural, another study was made. The trivernolin used in this experiment had been refined by treatment with adsorbents and was a higher grade product than that previously used. Two samples were stored and exposed to the surrounding atmosphere; one at room temperature (25–27C)

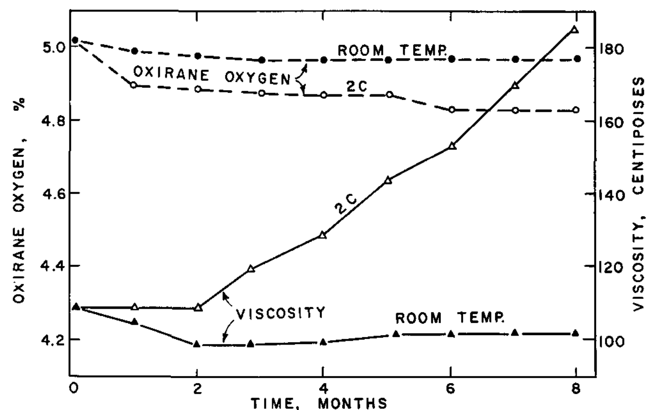

FIG. 1. Effect of storage on the viscosity and oxirane oxygen content of trivernolin.