cles were sedimented as previously described (3). Autoradiograms obtained showed that the major incorporation of radioactivity into triglycerides occurred in the fraction rich in these lipoprotein particles. These results are identical to those obtained by Patton et al. (3) with freshly secreted milk synthesizing systems from cows and goats.

Since results presented herein were readily duplicated with sows of different breeds, they suggest that freshly secreted swine milk contains a well organized system for glyceride synthesis. Apparently this system contains all enzymes and cofactors necessary for net synthesis of glycerides. Although much further work is required to determine the relationship of this system to in vivo milk fat synthesis, the ease of obtaining this physiologically available enzyme source should facilitate the task. These data confirm the earlier work of McCarthy and Patton (1) and suggest that this glyceride synthesizing activity is widespread in freshly secreted milks.

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The Structure of Alkane Diols of Diesters in Vernix Caseosa Lipids

Human skin is notorious for producing many types of fatty chains in the form of acids and alcohols (ref. 1 and 2 and others quoted there). Fatty chains with 2 OH groups (diols) have also been found in vernix caseosa in two independent investigations (3,4). Kärkkäinen et al. (4) reported the fatty portions of the diols as straight chain whereas Downing (3) reported them as branched but did not further identify them. Neither study located the two OH groups in the chains. In wool wax the OH groups are in the 1 and 2 positions (5-7) whereas in preen gland lipids, they are in the 2 and 3 positions (8). α-Hydroxy fatty acids and 1,2-diols of wool wax are configurationally related (6), and possibly arise from a common biosynthetic mechanism different from that of the 2,3-diols (8). We undertook this study to determine which of the two diol types, if either, human skin produced, and to determine whether the chains were straight or branched, and if branched, the type of branching.

Figure 1 outlines the preparation and analysis of the diols. The diols were obtained from the unsaponifiable part of material eluted where diesters should emerge (chromatogram I fractions 13 to 19). In diol diesters both OH groups are esterified with fatty acids. One would expect these diesters to emerge after elution of sterol esters plus wax esters (monoesters) but before elution of triglycerides (triesters). The intermediate polarity of fractions 13 to 19 relative to mono- and triesters is readily seen in TLC (Fig. 2a, b). Infrared spectra of these fractions has prominent carbonyl absorptions, no free OH absorptions and, in general, were very similar to spectra of monoesters of fatty acids with fatty alcohols.

Since fraction 15 was Liebermann-Burchard negative and showed only one spot by TLC, we saponified this fraction first and worked it up by our usual techniques. Although the separation of saponification products was incomplete and required additional separation (Fig. 1), only two types of substances were found: saponifiables with the same R_F as palmitic acid (Fig. 2d) and unsaponifiables with the same R_F as 1,2-diols (Fig. 2d) but not 2,3-diols (Fig. 2e.) Infrared spectra of the unsaponifiables also matched closely with synthetic straight chain 1,2-diols although some differences were noted in the 1400 to 1360 cm^{-1} region (discussed below). Thus, apparently fraction 15 was solely 1,2-diol diesters.

Fraction 13 gave a negative Liebermann-Burchard test when we removed material mi-

TABLE I
Analysis of Alkane Diols of Vernix Caseosa Diesters

Peak No.	Per cent of total fraction	GLC[a] of diol acetonides[b] Carbon No.[c] based on 1,2-diol standard[d] (JXR)	1,2-diol standard[d] (EGSSX)	2,3-cis diol standard (EGSSX)	2,3-trans diol standard[e] (EGSSX)	GLC[a] of aldehydes from HIO_4 oxidation of diols[f,g] Carbon No.[c] based on (EGSSX)	Per cent of total
1	11.6	19.65	19.58	21.08	20.35	18.56	11.8
2	1.9	20.00	20.00	21.49	20.79	19.00	2.2
3	3.1	20.65	20.59	22.08	21.32	19.55	3.3
4	6.4	20.72	20.73	22.19	21.50	19.70	5.3
5	1.1	21.00	21.00	22.46	21.73	20.00	1.0
6	47.5	21.65	21.58	23.01	22.31	20.57	47.3
7	Trace	21.72 (?)	21.72 (?)	23.10 (?)	22.40 (?)	20.70 (?)	Trace
8	3.7	22.00	22.00	23.40	22.70	21.00	3.4
9	Trace	22.40 (?)	22.30	23.70	23.00	21.40	Trace
10	3.8	22.55	22.58	24.00	23.30	21.55	3.5
11	12.9	22.72	22.72	24.12	23.44	21.70	12.9
12	0.6	23.00	23.00	24.40	23.70	22.00	0.5
13	5.2	23.65	23.59	25.00	24.29	22.54	5.8
14	Trace	23.72 (?)	23.72 (?)	25.10 (?)	24.40 (?)	22.70 (?)	Trace
15	1.2	24.00	24.00	25.40	24.70	23.00	0.8
16	Trace	24.40 (?)	24.40	25.80	25.10	23.40	Trace
17	Trace	24.55 (?)	24.50	25.90	25.20	23.50	Trace
18	0.5	24.71	24.70	26.05	25.35	23.70	0.4

[a] GLC performed on a Loe Model 160 gas chromatograph equipped with H_2 ionization detector. Acetonides were examined on three different columns: a 1/8 in. × 1.5 ft stainless steel column packed with 3% JXR on silanized Gas Chrom. Q 100–200 mesh, 280 C, He at 60 ml/min for chain lengths up to C_{30}; a 1/8 in. × 8 ft stainless steel column packed with 8% EGSSX on silanized Gas Chrom. P, 100–200 mesh, He at 60 ml/min, 190 C; and a 1/8 in × 9 ft stainless steel column packed with 3% JXR on Gas Chrom. Q, 220 C, He at 60 ml/min. (Applied Sciences Inc. State College, Pa. supplied all phases and supports.) Aldehydes were examined on the EGSSX column at 170 C, 60 ml He/min.

[b] Acetonides were from fraction 15 Chromatogram I Figure 1. To establish the iso and ante-iso structures of the diol chain three fractions of acetonides were collected by preparative GLC procedures as described in (2): peak No. 1–5, peak No. 6 and peak No. 7–18. The acetonides were hydrolyzed with 6N H_2SO_4 for 14 hr, the diols extracted with ether, washed with water, and dried over KOH in a vacuum dessicator. The diols were then oxidized with $KMnO_4$ (12). Peak No. 6 gave only acetone which showed it to have the iso structure whereas collected peaks 1–5 and 7–18 gave both acetone and 2-butanone showing that both iso and anteiso structures were present.

[c] Carbon numbers, determined by the method of Woodford and Van Ghent, were measured as previously described (2). Entries followed by (?) were minor peaks whose carbon numbers were difficult to determine accurately.

[d] 1,2-Diol standards were prepared by $LiAlH_4$ reduction of C_{16}, C_{18}, C_{22} and C_{26} α-hydroxy fatty acids (Applied Sciences, Inc.).

[e] 2,3-*Cis* and 2-3-*trans* diol acetonide standards were from preen gland lipids of the hen as in (8). Homologues obtained were C_{22} through C_{25}.

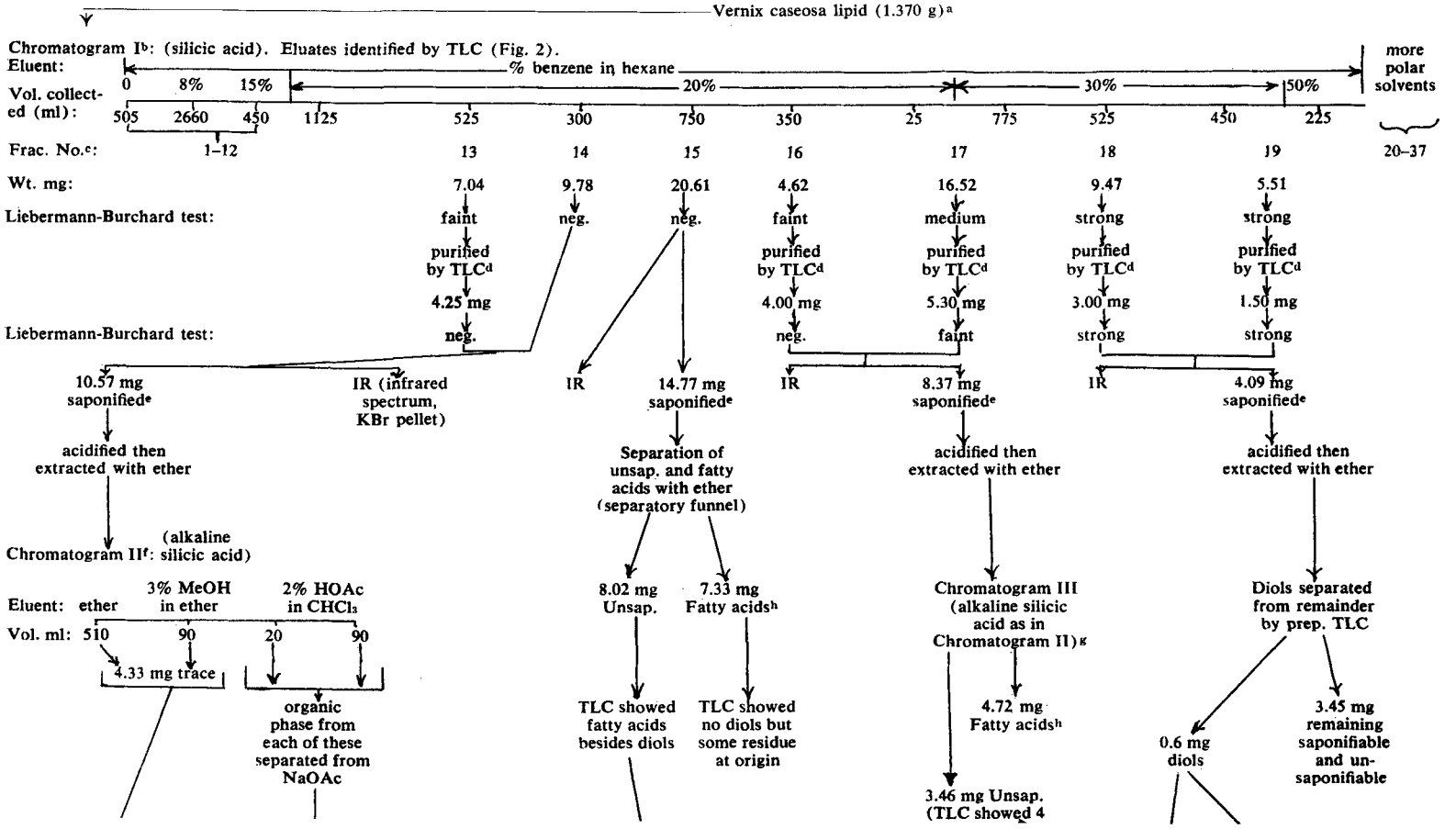
[f] Aldehydes obtained as in footnote 1 Figure 1. Aldehyde standards C_{17}, C_{21} and C_{25} were prepared similarly from C_{18}, C_{22} and C_{26} synthetic 1,2-diols, and C_{20} to C_{23} aldehydes were also obtained from the periodic acid oxidation of hen 2,3-diols (8).

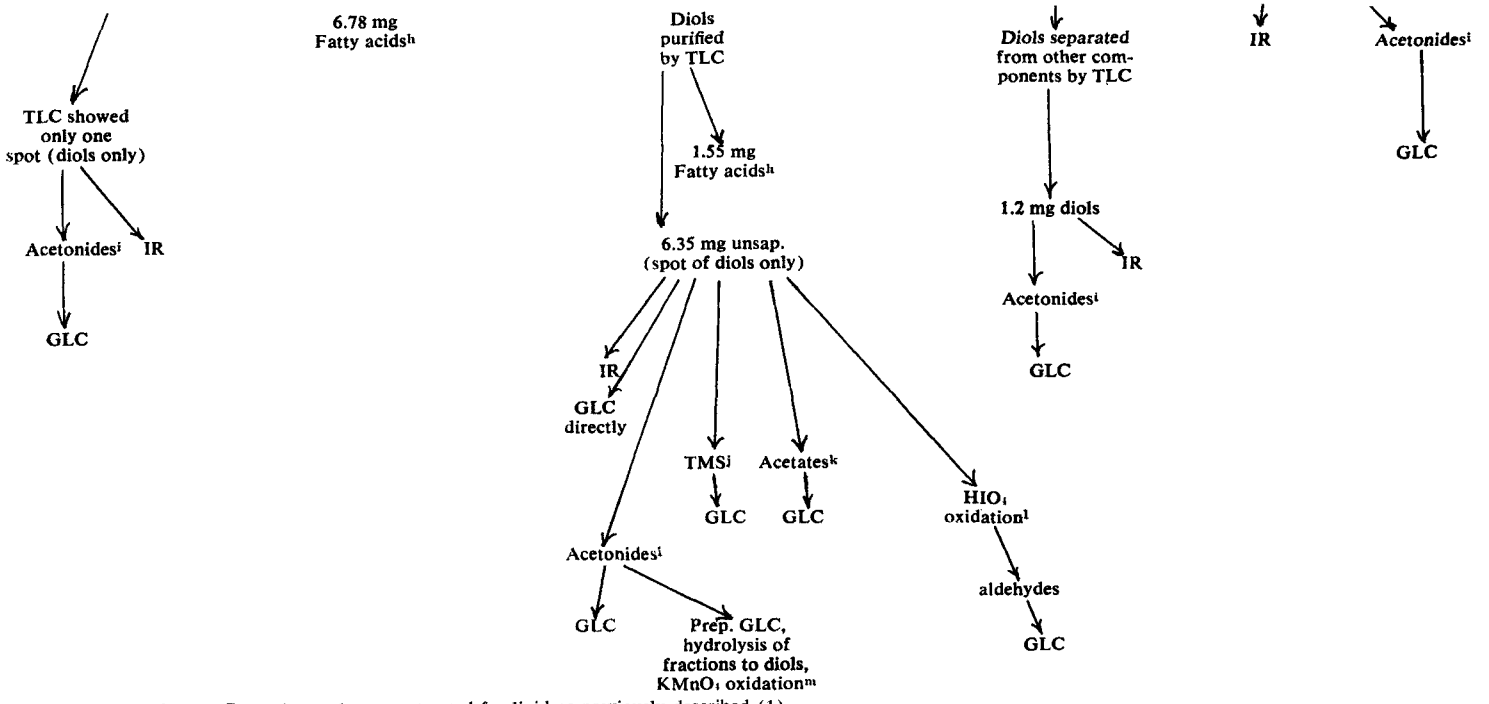
grating above the bulk of the fraction by preparative TLC (Fig. 2a and c). Apparently some very polar sterol esters had overlapped with early diester fractions. The saponification products of combined fractions 13 and 14 were separated quantitatively on alkaline silicic acid (chromatogram II Fig. 1). Again only 1,2-diols and fatty acids were recovered showing that these fractions, too, were diesters of 1,2-diols.

Later fractions (17 to 19) gave increasingly positive Liebermann-Burchard tests which persisted even after each fraction was purified to give one spot by TLC (Fig. 2c). Saponification products of fractions 18 and 19 showed by TLC in two systems, besides fatty acids and 1,2-diols, material migrating where α-hydroxy fatty acids, sterols and fatty alcohols migrated. Two additional types of diesters could account for these products: α-hydroxy fatty acids esterified on the OH group with an unsubstituted fatty acid and on the COOH group with either a sterol or a fatty alcohol. Kärkkäinen et al. (4) also found evidence for the latter type of diester. Of the three types of diesters apparently present in vernix caseosa, diesters of diols are in greatest abundance.

The alkane diols of fractions 13-19 formed acetonides, which on hydrogenation and GLC analysis on a polyester (EGSSX) column, showed no GLC pattern change from the original. Thus the alkane chains must have been saturated, in confirmation of earlier work (3,4). The free diols underwent periodic acid oxida-

FIG. 1. Isolation and analysis of diols from diesters of vernix caseosa.





[a] Vernix Caseosa from a Caucasian male was extracted for lipid as previously described (1).

[b] 110 g silicic acid (Unisil 100-200 mesh, Clarkson Chemical Co., Inc., Williamsport, Pa.) packed into a column 4.5 cm i.d. to 16.5 cm bed height.

[c] Only the work-up of fractions 13-19 (Fig. 2a,b) are discussed in this paper.

[d] TLC as in Figure 2 except 2 mg lipid were streaked on each plate, the plates developed, sprayed with water to make spots visible, diester region scraped, scrapings dried and lipid extracted with freshly redistilled ether.

[e] Diesters were saponified for 2 hr. with 10% KOH (wt/vol) in ethanol-water (9:1 v/v) under reflux in a N_2 atmosphere.

[f] Silicic acid (Unisil) made alkaline as in (9) and 2.5 g packed in a column 1 cm i.d. to a bed height of 8 cm.

[g] Same conditions as in f except 2% formic acid (as recommended in ref. 9) rather than 2% acetic acid was used to elute fatty acids. This avoided the necessity of removing a salt (e.g. NaOAc) with a water wash.

[h] Analysis of these fatty acids showed unusual double bond patterns to be reported subsequently.

[i] Acetonides prepared as in (8).

[j] TMS derivatives prepared as in (10).

[k] Acetates prepared as in (2); purity checked by TLC with hexane/ether (80/20) solvent.

[l] HIO_4 oxidation carried out essentially as in (12): 0.5 mg (—1.7 μmoles) diol treated in the dark with 0.034 ml 1M H_5IO_6 (3.4 μmoles) plus 0.2 ml redistilled tetrahydrofuran (J. T. Baker Chem. Co., Phillipsburg, Pa.) for 4 hr. at room temperature, then 2 ml water added and aldehydes extracted twice with 2 ml portions of hexane and the pooled extracts washed with water.

[m] See footnote b Table I.

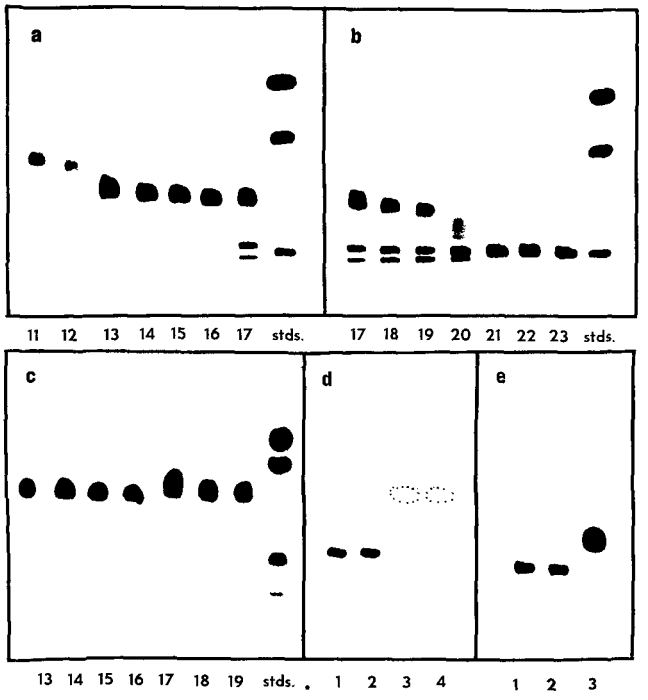


FIG. 2. a and b are photographs of TLC plates of fractions 11 through 23 of Chromatogram I (Fig. 1). TLC procedures as used by us (13) were those of Rouser et al. Sixty micrograms of each fraction, and standards consisting of 30 μg each of squalene, cholesteryl oleate and triolein were applied to each plate and the plate developed with hexane/ether (95/5 by volume). The "diester region" is the area between cholesteryl oleate (R_F = 0.5) and triolein (R_F = 0.1).

2c. Procedures as in 2a and b except development solvent was hexane-ether (92:8); 50 μg each of fractions 13 through 19, previously purified by preparative TLC where indicated in Figure 1 were applied to the plate. Standards were as in 2a and b.

2d. Procedures as in 2a and b except that the development solvent was $CHCL_3$-acetone -HOAc (80/20/1 by volume); 30 μg respectively applied in lanes (1) 1,2-hexacosane diol, (2) unsaponifiable of fraction 15, (3) saponifiable of fraction 15, (4) palmitic acid.

2e. Procedures as in 2a and b except that the solvent was ether, lanes (1) 40 μg unsaponifiable of fraction 15, (2) 40 μg of 1,2-eicosane diol, (3) 100 μg of 2,3-diols.

tion and GLC analysis of the resultant aldehydes showed material with carbon numbers corresponding to four homologous series (Table I): iso (71.7%) anteiso (19.1%) normal (8.7%) and branched chain of an unknown type (0.5%). That the chains were truly iso and anteiso was proved by $KMnO_4$ oxidation of free diols (obtained from hydrolysis of collected fractions of acetonides) which yielded acetone and 2-butanone respectively (Table I footnote b). Infrared spectra of the original diols showed a doublet at 1365 and 1380 cm^{-1} also consistent with the iso structure.

GLC retention data of the acetate, trimethyl silyl ether (TMS), and especially the acetonide derivatives of the diols support the TLC data that the positions of the OH groups are 1,2- rather than 2,3-. Table I shows that when the peaks of the diol acetonides of fraction 15 were plotted on three different standard curves (i.e. either 1,2-; 2,3- *cis;* or 2,3-*trans* diols), only the 1,2-diol standard curve gave retention data that matched exactly the aldehyde retention data predicted for periodic acid degradation of each chain by one C-atom. If 2,3-*cis* or 2,3-*trans* diols standard curves were used, and degradation of each chain by 2 C-atom assumed, the retention data of the acetonides did not match those of the aldehydes.

GLC of the diol acetonides from fractions 13 to 19 showed that the later fractions generally had shorter diol chain lengths (Table II). A calculated homologue distribution of all the 1,2-diols of these diesters fractions corresponded better to the data of Kärkkäinen et al. (4) (assuming that their homologues

TABLE II

Percentage Composition of Homologues of Alkane Diols from Chromatographic Fractions of Vernix Caseosa Diesters[a]

Peak numbers	1,2-Diol carbon numbers[b]	Fraction numbers of chromatogram I Figure 1				Calculated composition of total diols[c]
		13 and 14	15	16 and 17	18 and 19	
		%	%	%	%	%
1	19.65	9.8	11.6	15.5	39.6	12.4
2	20.00	1.5	1.9	3.1	4.0	2.0
3	20.65	2.1	3.1	2.8	2.5	2.7
4	20.75	4.3	6.4	5.0	6.8	5.6
5	21.00	0.9	1.1	3.5	1.7	1.3
6	21.65	48.4	47.5	44.0	23.2	46.5
7	21.72	trace	trace	trace	trace	trace
8	22.00	3.8	3.7	3.5	2.7	3.7
9	22.40	trace	trace	trace	trace	trace
10	22.55	3.8	3.8	3.9	2.2	3.7
11	22.72	14.1	12.9	10.8	7.0	12.9
12	23.00	0.8	0.6	0.9	0.5	0.7
13	23.65	8.5	5.2	4.7	6.8	6.4
14	23.72	trace	trace	trace	trace	trace
15	24.00	0.7	1.2	1.1	1.2	1.0
16	24.40	trace	trace	trace	trace	trace
17	24.55	trace	trace	trace	trace	trace
18	24.72	0.8	0.5	0.5	0.6	0.6

[a] Area percent calculated with assistance of Dupont 310 Curve Resolver.

[b] As acetonides (Table I). GLC performed on 1/8 in. × 9 ft column packed with 3% JXR programmed from 218 C to 260 C at 2°/min, He flow at 60 ml/min.

[c] Calculation based on weight recovery of diols for fractions 13 to 19.

were iso and anteiso instead of straight as they reported) rather than to the data of Downing (3).

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