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Polyethoxy cholesterols

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Synopsis—Individual ETHOXY CHOLESTEROLS have been isolated from SYNTHETIC mixtures by laminar CHROMATOGRAPHY, and their effects on the benzene/water INTERFACIAL TENSION has been measured by the pendant drop method. The results indicate that the PROPERTIES of ethoxy condensates of WOOL WAX ALCOHOLS, which have been analysed briefly, could be improved by fractionation.

Introduction

Nonionic surface-active substances derived by the condensation of ethylene oxide with suitable hydrophobic compounds have many applications as o/w emulsifying agents. Polyethoxy derivatives of wool wax alcohols (1) have many uses in cosmetics; several grades are available under the name *Polychol®N*, where N is the molar ratio of ethylene oxide to alcohol in the mixture when the condensation reaction is initiated. The concept of the hydrophil-lipophil (H/L) balance suggests that the *Polychols* should possess a wide range of properties. In reality, however, the *Polychols* in which N may have selected values up to 40, have surprisingly similar properties, and are only moderately good emulsifying agents. One reason for their indifferent performance may lie in the heterogeneity of the parent wool wax alcohols (*Hartolan®*) which contain three main classes of compounds; aliphatic long-chain alcohols, cholesterol and its congeners, and lanosterol and its congeners. If these classes of alcohols react with ethylene

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oxide at different rates, the H/L balances for the three series will be different. Consequently, when one series has been brought to its optimum H/L balance, the other series will be off balance. Another possible source of imperfection is that each parent alcohol will be distributed among several homologues having a range of N-values and, therefore, a range of H/L balances. If the range is too wide, the surface-active properties of the series will be adversely affected.

One object of this study was to examine the way in which ethoxy units are distributed in the products of the reaction of ethylene oxide with an alcohol. A second objective was to explore the effect of molecular structure on surface activity by measuring the decrease in the benzene/water interfacial tension caused by individual ethoxy compounds.

Experimental

Preparation of ethoxylates

(a) Ethylene oxide (1 ml; about 5 equivalents) was added to a cold (—10°C) mixture of cholesterol (2 g), benzene (20 ml) and sodium (0.1 g), and the tube was sealed and set aside at room temperature. After 4 months the tube was opened and the contents were evaporated to dryness under reduced pressure.

(b) A second preparation differed only in the quantity of ethylene oxide (5 ml; 25 equivalents) and the reaction time (6 weeks).

Similar preparations containing lanostenol (=dihydro-lanosterol) (2 g) plus ethylene oxide (1 ml) and *n*-octadecanol (3 g) plus ethylene oxide (1 ml) were also set aside for 4 months.

Chromatography

Standard methods of laminar chromatography were used except where specified. Glass plates were coated with *Silicagel G* (Merck) using the Shandon apparatus. For normal work the spreader gap was 0.3 mm and for preparative chromatography it was 0.6 mm.

Chromatograms were developed in tanks which had been lined with filter paper soaked in solvent; all eluent compositions are expressed in volumes. Sulphuric acid was the usual chromogenic reagent; in the cold, cholesterol derivatives were easily distinguished because they gave reddish-purple spots whereas derivatives of the other alcohols remained colourless.

On heating the chromatoplate, lanosterol derivatives exhibited a transient yellow colour; eventually, all the organic compounds charred.

Isolation of ethoxy cholesterols

Product (a) (1.5 g) was chromatographed on 15 preparative chromatoplates in three batches of five. Four elution was performed with benzene/acetone/water; for the first two the volume ratio was 400+100+1 and for the next two it was 140+60+1. Recovery of the components, using ether/ethanol/water (14+5+1) as the extracting solvent, yielded:-

N:	0+1	2	3	4	5	6	7	8 and more
mg:	457	160	184	182	139	92	71	132

Product (b) (2.9 g) was chromatographed on 20 preparative plates in exactly the same way to yield:-

N:	0+1+2	3	4	5	6	7	8+9	8-22
mg:	88	35	51	68	79	103	353	1 090

(from N=7 upwards the resolution became progressively poorer)

The mixture N=8-22 (800 mg) was chromatographed on 8 large (20× 40 cm) preparative chromatoplates in one batch, using a combination of the gradient- and multiple-elution techniques. The tank was charged with benzene/acetone/water (140+60+1; 1 500 ml). After the first elution, the solvent-composition in the tank was changed by the *addition* of a different solvent, and the chromatogram was developed again. This sequence of operations was repeated as follows:-

After the nth development v ml of benzene/acetone/water was added.

1	100	5+5+2
2	100	5+5+2
3	100	5+5+2
4	500	6+4+1
5	100	5+5+2
6	250	5+5+2
7	0	—
8	250	5+5+2
9	250	5+5+2
10	250	5+5+2
11	250	5+5+2
12	500	4+6+3

Recovery of the components using ethanol/water (4+1) as the extractive solvent gave the following yields:-

N:	8	9	10	11	12	13	14	15	16	17-20
mg:	23	42	79	94	91	78	67	58	53	95

The mixture N=0+1 (300 mg) was chromatographed on ten preparative plates by the technique of continuous development using cyclohexane/ethyl acetate (4+1) as the eluent. Recovery of the compounds using ether as the extractive solvent afforded cholesterol (222 mg), m.p. and mixed m.p. with an authentic sample, 148°C, and mono-ethoxy cholesterol (68 mg).

All compounds were purified by further chromatography by the multiple development technique or by continuous elution. For example, penta-ethoxy cholesterol was subjected to continuous elution for 6 h in benzene/acetone/water (400+100+1) and it was recovered using ether/ethanol/water (190+9+1) as the extractive solvent.

Analytical data

Satisfactory elemental analyses were obtained only for mono-ethoxy cholesterol, (found: C, 80.7; H, 11.6%; $C_{29}H_{50}O_2$ requires: C, 81.0; H, 11.6 %) and di-ethoxy cholesterol (found: C, 78.2; H, 11.4%; $C_{31}H_{54}O_3$ requires: C, 78.5; H, 11.4%).

Although all the higher homologues were dried by storage over phosphorous pentoxide, *in vacuo*, for 4 weeks, elemental analyses indicated that they could not be dehydrated completely. For example, for N=3 the calculated carbon content is 76.55%, for the hemihydrate it is 75.1% and for the monohydrate it is 73.8%. On prolonged drying, the carbon content increased from 74.1% to 75.7%. Concurrently, the melting point increased from 80° to 101°C, and the optical rotation changed from —23° to —27°.

Analyses indicated, for tetra-ethoxy cholesterol (C, 72.6; H, 11.0%) the monohydrate, and for tetradeca-ethoxy cholesterol (C, 63.4; H, 9.9%) the dihydrate. Intermediate members contained non-stoichiometric amounts of water which increased with the number of ethoxy units in the compound.

Melting points and optical rotations of the lower members of the series are:

N	1	2	3	4	5	6	7	9	13	16
mp(°C)	116	108	101	90	84	76	70	60	46	44
$[\alpha]_D(CHCl_3)$	—33	—29	—27	—23	—21	—20	—19		—9 (in ethanol)	—6

Interfacial tension

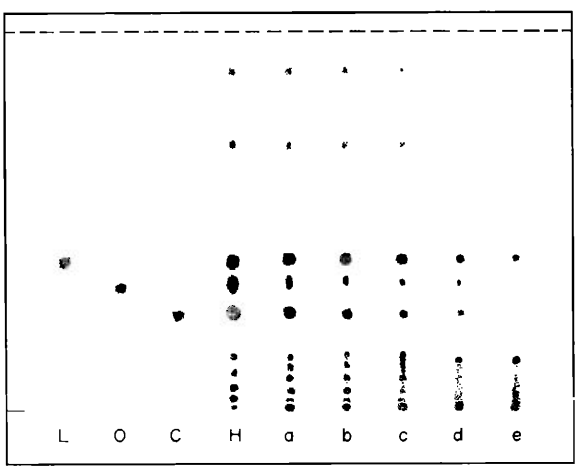
Measurement of the interfacial tensions were made by the drop-weight technique (2) using a micrometer syringe (3) fitted with a stainless steel capillary tube. Calculations were based on the equation:

$$\gamma = \frac{\mu(\rho_1 - \rho_2)\, g\, \beta}{r}$$

where γ is the interfacial tension
μ is the volume of the drop at the moment of its detachment.
ρ_1 and ρ_2 are the densities of the phases.
β is the correction factor of Harkins and Brown
r is the detachment radius.

Densities were determined with a Perkins pyknometer; the capillary radius was 0.626 mm. Water was double-distilled in a *Barraglass* still; the temperature was 20°C; benzene was A.R. grade which had been redistilled in an all-glass apparatus; it had b.p. 80.1°C.

Results



Substrate: Silicagel G
Solvent: Cyclohexane/acetone (9+1)
Key: L = Lanostenol
O = *n*-Octadecanol
C = Cholesterol
H = *Hartolan*
a = *Polychol 5*
b = *Polychol 10*
c = *Polychol 15*
d = *Polychol 20*
e = *Polychol 40*

Figure 1. Chromatogram of the *Polychols*

When *Polychols* were examined by laminar chromatography, the resulting chromatograms showed a surprisingly good degree of resolution, specially in the region of low N-values (*Fig. 1*). These chromatograms showed the presence of each of the three main starting alcohols. Estimates of the amounts of unreacted alcohols were made by the area/weight

Table I.

Proportions of free alcohols in *Polychols*, expressed as percentages of the amounts in the starting material (*Super Hartolan*)

	Average "N"	Lanosterol	Cholesterol	Long-chain alcohols
Polychol 5	12	95	50	30
Polychol 10	14	35	20	10
Polychol 20	23	15	5	5
Polychol 40	41	15	0	0

method (4). The results, expressed as percentages of the amount in the starting material, are shown in *Table I*. It is clear that the rate of reaction of ethylene oxide is fastest with the long chain alcohols and slowest with lanosterol. It follows that the derivatives of the long-chain alcohols are more extensively ethoxylated than those of lanosterol. Because the *Polychols* contain free alcohol, their N-values are misleading. *Table I* shows, approximately, the average "N" for the total alcohol that has actually reacted, i.e., after allowance has been made for free alcohol.

Fig. 2 indicates that even the simplest of the *Polychols* is too complex a

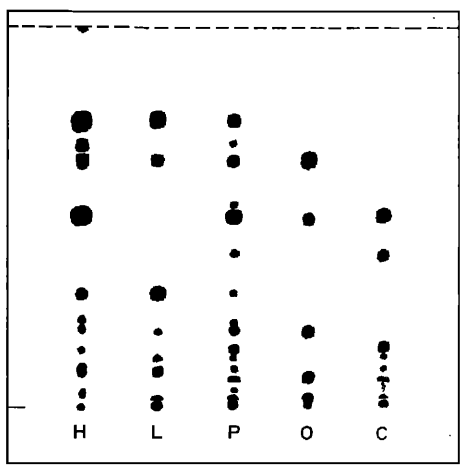


Figure 2. Chromatogram of polyethoxy derivatives of various alcohols.

Substrate: Silicagel G
Solvent: Cyclohexane/ethyl acetate (9+1); four elutions

Key: H=*Hartolan*
L=Product of the reaction between: Lanostenol (1 mole) and ethylene oxide (5 moles)
P=*Polychol 5*
O=Product of the reaction between: *n*-Octadecanol (1 mole) and ethylene oxide (5 moles)
C=Product of the reaction between: Cholesterol (1 mole) and ethylene oxide (5 moles)

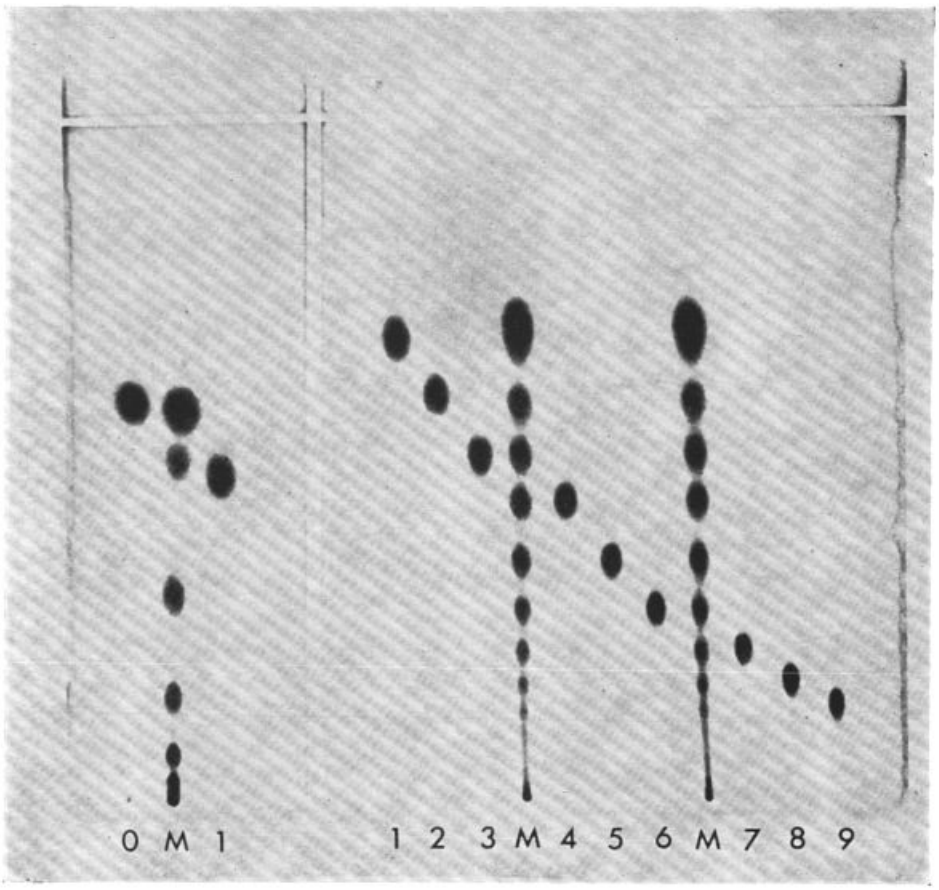


Figure 3. Chromatograms of ethoxy cholesterols

Substrate: Silicagel G
Solvents: Left—Cyclohexane/ethyl acetate (3+2)
Right—Benzene/acetone/water (85+40+1)
Loads: M, 100 µg; others, 10 µg.

Key: M=Product of the reaction between: Cholesterol (1 mole) and ethylene oxide (5 moles)

0=Cholesterol
1=Mono-ethoxy cholesterol
2=Bi-ethoxy cholesterol
3=Tri-ethoxy cholesterol
4=Tetra-ethoxy cholesterol
5=Penta-ethoxy cholesterol
6=Hexa-ethoxy cholesterol
7=Hepta-ethoxy cholesterol
8=Octa-ethoxy cholesterol
9=Nona-ethoxy cholesterol

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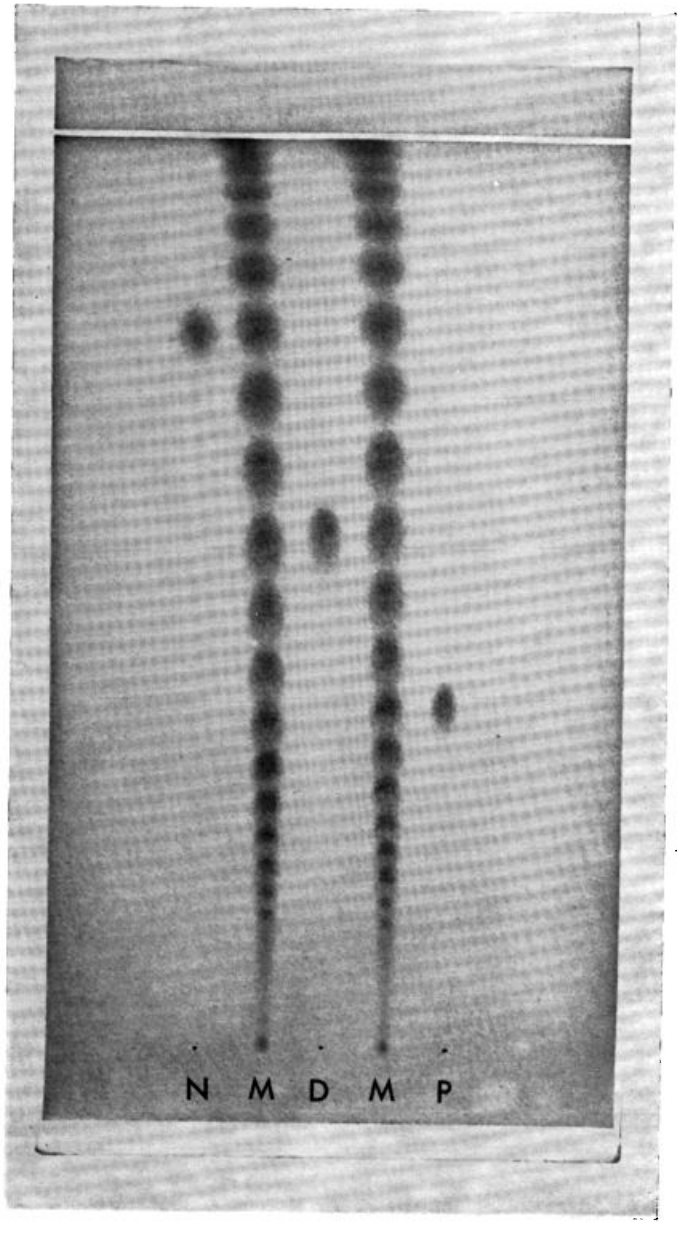

Figure 4. Chromatogram of polyethoxy cholesterols

Substrate: Silicagel G
Solvent: Acetone/benzene/water (60+40+3); three elutions
Loads: M, 200 µg: others, 10 µg

Key: M=Product of the reaction between: Cholesterol (1 mole) and ethylene oxide (25 moles)
N=Nona-ethoxy cholesterol D=Dodeca-ethoxy cholesterol
P=Pentadeca-ethoxy cholesterol

Facing page 207

mixture for detailed study. Accordingly, the reaction between ethylene oxide and cholesterol was used as a model. Examination of the products by laminar chromatography showed that the number of ethoxy units per alcohol molecule depended on the proportion of ethylene oxide in the system. Similar experiments, in which the alcohol had been omitted, showed that ethylene oxide undergoes self-condensation to form a series of polyethylene glycols. For lanostenol the reaction proceeded more slowly than for cholesterol; use of metallic potassium instead of sodium scarcely affected the rate of condensation.

Fig. 3 illustrates several aspects of the reaction of cholesterol with 5 moles of ethylene oxide. First, free cholesterol is still present in appreciable amounts. Secondly, some material remains at the origin; presumably this is polyethylene glycol. Next, the most plentiful component is that for which N=3. Finally, the highest member that can be distinguished with certainty, is that for which N=9. The reaction of cholesterol with 25 moles of ethylene oxide follows a similar pattern (*Fig. 4*). Free cholesterol is still present (about 2%), the most abundant member is that for which N=11 and the highest member that can be distinguished is that for which N=23. These observations indicate that the number of ethoxy units attached to a cholesteryl radical is well below expectation; for reactants having a molar ratio of N:1, the most abundant products are those containing about N/2 ethoxy units.

Mixtures containing nominal proportions of 5 and 25 ethoxy units per cholesterol molecule were used for the isolation of individual compounds by preparative laminar chromatography. Individual compounds were identified by their positions on the chromatogram (*Fig. 3* and *4*), and the identities of the first two members of the homologous series were confirmed by elemental analyses.

The interfacial tension between water and a solution of the sample in benzene, was determined by the pendant drop method. By slowly expelling drops of water from an *Agla* micrometer syringe into the benzene solution, it was possible to study interfacial ageing effects. For cholesterol no ageing effect was observed, but for the ethoxy homologues there was barely a perceptible drift. For example, for N=3 the interfacial tension decreased by 0.3 mN m^{-1} during 30 min.

Fig. 5 shows the equilibrium interfacial tensions as a function of concentration for the individual polyethoxy cholesterols. Pentadeca-ethoxy cholesterol was the highest individual member of the series for which an adequate quantity of material could conveniently be isolated. It is evident

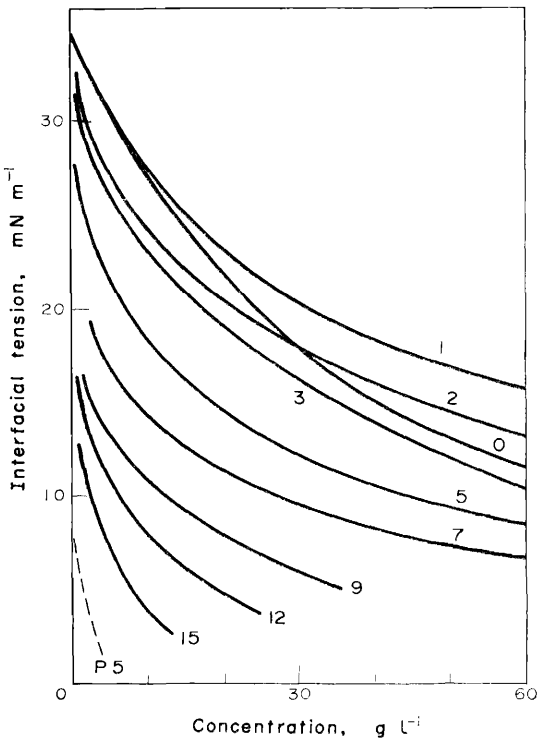

Figure 5. Effect of individual polyethoxy cholesterols on the benzene/water interfacial tension at 20°. The numbers are the number of ethoxy units per cholesterol radical in the molecule. P5=*Polychol 5*

that the optimum H/L balance for the series lies somewhere above N=15, probably near N=20.

Fig. 6 shows the results for some of the *Polychols*. A well established characteristic of surface-active agents is that mixtures of homologues are generally more efficient than pure compounds. The behaviour of *Polychol 5* conforms with this pattern. On the other hand, *Polychol 40* is much less effective than the behaviour of other *Polychols* would suggest; it seems that the hydrophilic contribution to *Polychol 40* is too large. An arbitrary guide to satisfactory surface activity is that the agent should lower the interfacial tension to less than 1mN m^{-1} at a concentration not exceeding 0.5 g l^{-1}. By this criterion, none of the individual polyethoxy cholesterols which have been tested are satisfactory. *Polychol 40* is just acceptable but is less

effective than the simple mixture of ethoxy cholesterols for which N = 17 —20.

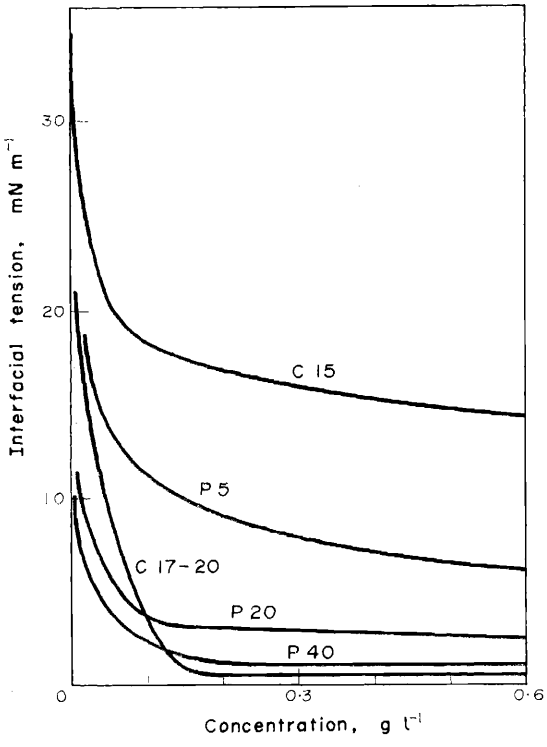

Figure 6. Effect of *Polychols* on the benzene/water interfacial tension at 20°.

Key: P 5 = *Polychol 5*
P 20 = *Polychol 20*
P 40 = *Polychol 40*
C 15 = Pentadeca-ethoxy cholesterol
C 17-20 = Mixture of heptadeca-, octadeca-, nonadeca- and eicosa-ethoxy cholesterols.

Conclusion

Chromatographic analyses indicate that for polyethoxy cholesterol N and for *Polychol N*, the symbol N is a very approximate guide to composition because the number of ethoxy units attached to an alcohol varies over a wide range. In *Polychol 40* the lowest number is 0 (free lanosterol is still present) and the highest number most certainly exceeds 40 (for the long-chain alcohols). Comparison of the surface-activity of the *Polychols* with

that of ethoxy cholesterol 17-20 strongly suggests that the inferior performance of the former arises from its heterogeneous composition and, in particular, from its wide range of N-values. Fractionation of *Polychol* into narrow cuts is likely to give products having improved surface activity.

Because *Polychol* contains many compounds which vary over a wide range of N-values, the activities of its most useful components are diminished by dilution with less useful components. Several advantages may be foreseen if *Polychol* were to be fractionated. For example, the cuts will cover a range of fairly specific surface-activities, and at least one cut should exhibit a surface-activity superior to that of the parent *Polychol;* the relatively homogeneous composition of the individual cuts will eliminate problems of incompatibility in blending caused by the extreme components of the original range.

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