consist of long chain aliphatic alcohols(1) determinable by gas-liquid chromatography(3).

Several different samples of epidermal phosphatides were chromatographed on silicic acid impregnated paper(10). The following phosphatides were identified: phosphatidyl serine, phosphatidyl ethanolamine, phosphatidyl choline, inositol phosphatide, phosphatidic acids and sphingomyelin along with other phosphatides. These same phosphatides together with plasmalogens have been found in human epidermis by Bloomstrand *et al*(16) and by Gerstein(17).

Summary. In a limited number of analyses the lipid composition of human male and female epidermis of extremities shows differences in that female epidermis has much more triglycerides than does the male epidermis while the latter has more cholesterol, ether eluate and phosphatides than does female epidermis. The fatty acid composition of the triglyceride fraction is characterized in part by containing about 73% unsaturated fatty acids while this figure for the phosphatides is 50%. The phosphatides contain 2 unsaturated acids with 20 carbons and small amounts of pentadecanoic, heptadecanoic, behenic, lignoceric acids as well as the more common fatty acids.

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Biochemistry of the α-Glyceryl Ethers. I. Distribution in Mammalian Tissues and in Starfish.* (28874)

DAVID TODD AND GEORGE P. RIZZI (Introduced by R. I. Dorfman)
Department of Chemical Engineering and Chemistry, Worcester Polytechnic Institute, Worcester, Mass.

A report(1) on the distribution of α-glyceryl ethers in the tissues of the dog has recently appeared. During our work on the α-glyceryl ethers we have carried out a survey of the occurrence of these ethers in various tissues of the rabbit, in a number of mammalian marrows and in the starfish.

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Methods. Preparation of non-saponifiable (NS) and phospholipid non-saponifiable (PNS) fractions: Tissue lipid extracts were obtained following the method of Folch(2). Usually 10-20 g of the wet tissue were used, the tissue being fresh or newly thawed from −20° storage. The total lipids were then separated into neutral lipid and phospholipid fractions by acetone precipitation as follows:

for every gram of lipid 4 g of ether is added and to the resulting clear solution is added 100 cc of acetone. The suspension is left at 4° overnight, the supernatant solution filtered, and the phospholipid precipitate immediately treated as before, the solvent volumes being adjusted to the approximate weight of phospholipid. After a third precipitation the combined filtrates, which certainly contain some phospholipids, are evaporated to give neutral lipids. It has been found that the lipid recovery is always better than 90%.

The neutral lipids were saponified with alcoholic-aqueous KOH(3) to obtain NS, the bulk of which usually consists of cholesterol. PNS was obtained as follows: a phospholipid fraction was boiled 8 hours with excess acetic acid-acetic anhydride (3:1 v/v)(4), the resulting dark solution diluted with water, most of the acetic acid removed *in vacuo* at 50°, the mixture extracted twice with ether and the residue left on removal of the ether saponified as above. Ether work-up gave the PNS as a pale yellow viscous oil. The weight of this PNS is usually about 10-20% of that of the phospholipids, and seems to be always free of cholesterol. We regard it as probable that the PNS consists, aside from glyceryl ethers, mainly of the aldol condensation products of aldehydes derived from plasmalogens, and possibly sphingosine. Paper chromatography fails to reveal any free aldehydes in the PNS.

Analysis of NS and PNS for glyceryl ethers: NS: At first the periodic acid procedure of Karnovsky(5) was applied directly to aliquots of the NS.† When it was found that large amounts of cholesterol present gave apparent glyceryl ether values as high as 5% (when paper chromatography showed no glyceryl ethers were present), it was decided to remove the bulk of the cholesterol by crystallization from methanol. By use of 6 cc of hot methanol for every 100 mg of NS, filtration at 0°, followed by a rinse with 1 cc of ice-cold methanol, about three-quarters of the cholesterol can be removed. Experiments with known mixtures showed that if as much as 10% of glyceryl ethers is present, all of it remains in the methanol filtrate. While even this purification does not do away with the false glyceryl ether phenomenon, it is a useful procedure for obtaining a concentrated NS for paper work. PNS: The direct HIO_4 method can be used, though in most of the work reported here the glyceryl ether values are so low that paper chromatography has been the tool principally employed.

Column chromatography: It has been found that chromatography on Davison silica gel can be used effectively to separate the total glyceryl ethers present in an NS from other material. Elution with hexane-ether 3:1 rapidly removes cholesterol, and hexane-ether 1:1 removes all the glyceryl ethers. If large amounts of cholesterol are present it is expedient to use the methanol crystallization step prior to chromatography in order to minimize tailing of the cholesterol.

Paper chromatography:‡ It has been found that the glyceryl ethers can be very conveniently separated from other lipid hydrolysate components and, with one limitation, from one another, by using kerosene-impregnated paper(7) developed with ethanol-water 60-40 v/v). The glyceryl ether spots on the dried paper are detected by HIO_4 treatment followed by application of Schiff reagent. By comparison of the intensity of a given spot color with those of standards, one can make a rough estimate of the relative amounts of material in the several zones. About 50 μg of glyceryl ether per 2 cm of running front represents the maximum load for good separation. For detection of the glyceryl ether spots, the air-dried strip is passed quickly through a dilute (1-2%) aqueous solution of periodic acid, allowed to hang for about one

† While it is conceivable that substances other than α-glyceryl ethers in lipid hydrolysates might give rise to formaldehyde under the conditions of the method employed, the presence of such compounds has never been established; the method is a specific one as far as is now known.

‡ Emmerie(6) has reported the paper chromatographic separation of the alkoxyacetaldehydes obtained by HIO_4 oxidation of the glyceryl ethers. We have found this method rather tedious and sensitive to temperature changes, and therefore worked out the method described.

TABLE I. α-Glyceryl Ethers of *Asterias forbesi.*

	% glyceryl ethers* (as wt % of total NS or PNS)	Composition of glyceryl ethers (as % of total glyceryl ethers) 18:0†	16:0	18:1
PNS	27	35	65	0
NS	36–42	55	45	0

* Calculated as batyl alcohol as determined by HIO_4 oxidation, followed by HCHO determination.
† The symbol 18:0 is a shorthand representation for the glyceryl ether (batyl alcohol) containing the 18 carbon alkyl group with no double bonds.

minute, then placed in aqueous H_2SO_3 solution (2-5%). The paper is rinsed briefly in tap water, then pulled quickly through Schiff reagent (which can be obtained water-white by a simple charcoal treatment) and held in the air till maximum color contrast of the pink spots has developed—about 20-30 seconds. The strip is immersed 10 minutes in dil. (0.5-1.0 *N*) HCl, then washed in running tap water for an hour. It is essential to remove all of the Schiff reagent if it is desired to preserve the paper, as otherwise the reagent slowly reacts with the paper to produce a pink background.

We have found that the R_f of batyl alcohol is usually about 0.5, but because of variations in temperature, running solvent composition and amount of kerosene applied, this figure may vary considerably. We have found it convenient to use batyl alcohol as a standard and refer other spots to this one. Usually the R_{bat} of chimyl alcohol is about 1.35-1.40 (referring always to spot centers).

It was found that selachyl alcohol always runs with chimyl alcohol, therefore a method had to be devised to measure the two when they occur together. The hydrogenation technique developed originally to solve the same problem in the fatty acid series(7) was used. The zone in question is cut out; the paper pieces placed in a glass-stoppered 25 cc Erlenmeyer flask with 2-3 cc of ethyl acetate and several mg of PtO_2; the air displaced by hydrogen; the closed flask swirled for 30 minutes. The recovered material is rerun on paper. Any material originally selachyl alcohol is now converted to batyl alcohol and is separated from the unaltered chimyl alcohol. Recovery, as gauged by color intensity, in this overall procedure is about 50%.

The method was checked using commercially available (Lights) selachyl alcohol; on reduction and rerunning most of the material ran as batyl alcohol but some still ran as chimyl alcohol. Repetition of the hydrogenation on the re-eluted chimyl zone failed to alter the material further. It is concluded, and this has been confirmed by recent gas chromatographic analysis, that the "selachyl alcohol" is a mixture of selachyl, chimyl and batyl alcohols.

The presence of large amounts of cholesterol does not noticeably disturb the running properties of the glyceryl ethers. The batyl alcohol in a mixture of 50 μg of batyl alcohol and 4 mg of cholesterol runs exactly as the uncontaminated ether, the cholesterol moving barely a centimeter from the origin.

The advantage of the paper chromatographic method is its great sensitivity, 5 μg of batyl alcohol are easily observed by this method. However, the recovery in the hydrogenation-rerunning procedure is so poor that clearly the method gives only gross information about relative amounts of saturated and unsaturated ethers.

Discussion. Since starfish have been so carefully studied with respect to the glyceryl ethers(5), we first investigated this source. We have found that the lipids of mature (7-8 in. diam.) *Asterias forbesi* consist of about

TABLE II. α-Glyceryl Ethers of Mammalian Yellow Marrow.

Source	NS as wt % of marrow	Gl ether as wt % of NS	Gl ether composition (as % of total gl ethers) 18:0	16:0	18:1
Rabbit	c. 2	13	0	100	0
Cow	.2–.4	12–25*	50	50	0
Sheep	.36	11	—	—	—
Pig	.5–.6	4–8	—	—	—
Rat	—	0	—	—	—
Man†	—	11.3, 16.6	25	50	25

* Four samples have been found to give values of 12, 17.5, 24 and 25%.
† Two red marrow (from the last 3 vertebrae of the coccyx) were examined; the composition of only one of these samples was determined.

TABLE III. The α-Glyceryl Ethers* in the Organs of the Rabbit.

Organ	Lipid as wt % of wet organ	NS as wt % of lipids	Gl ethers as wt % of NS	PNS as wt % of lipids	Gl ethers as wt % of PNS
Blood	.44	33	<.1	12	<.1
Liver	5.3	7.0	<.1	2.0	.5
Heart	6.1, 8.1	7.0	<.1	3.2	3
Brain	12.7	21	<.1	9.7	2
G.I. tract	17.6	2.8	2	.5	1
I.P. fat	88	1.6	4	.045	8
Spleen	7.7	9.0	<.2	2.5	5
Kidney	9.8	5.5	1	2.7	1
Marrow	—	2.0	2†	.7	6

* Chimyl alcohol is the only glyceryl ether found in the rabbit.
† Note the low value, compared to 13% found by the HIO_4 method in 2 other specimens.

66% neutral and 34% phospholipids. The distribution of the glyceryl ethers of starfish lipids is given in Table I.

The starfish produces only saturated glyceryl ethers and 40% of these are bound in phospholipid form. While the same two ethers are found bound in the 2 forms, the proportions vary. This fact speaks against any rapid equilibration involving the glyceryl ethers in the 2 forms of conjugation.

Since bovine bone marrow NS has been found to contain batyl alcohol(8), a number of bone marrows were investigated. In all cases only the NS was investigated since it has been shown(8), and confirmed by us, that phospholipids are totally absent from bovine yellow marrow. The results are shown in Table II.

The organs of the rabbit were next examined in an attempt to determine the distribution of glyceryl ethers, bound both as neutral esters and as phospholipids, in a typical mammal.§ The trace amounts (Table III) found in organs other than the marrow were estimated visually from paper chromatograms. In the rabbit the only glyceryl ether found is chimyl alcohol.

It is clear from Table III that in the rabbit the glyceryl ethers occur bound in both possible forms. While the marrow is the most important site of occurrence, smaller amounts also occur in the heart, brain and spleen.

§ It has recently been shown that free glyceryl ethers as well as glyceryl ether diesters occur in ratfish liver oil and in basking shark liver oil(9). Our isolation technique does not distinguish between these possibilities.

The relation between plasmalogens and glyceryl ether compounds would appear to be very close, as has been pointed out(10), since the latter are formally derived from the former by addition of two hydrogen atoms. Evidence is available for the existence of non-phospholipid plasmalogens(5,11) paralleling the usual phospholipid type. Indeed one can reasonably suspect that these two classes make up a lipid-soluble hydrogen donor-acceptor system:

$$\begin{array}{l} \quad H \quad H \\ CH_2\text{–}O\text{–}C = C\_ \\ | \end{array} \quad \begin{array}{c} +2(H) \\ \rightleftarrows \\ -2(H) \end{array} \quad \begin{array}{l} \\ CH_2\text{–}O\text{–}CH_2\text{–}CH_2\text{–} \\ | \end{array}$$

If this is the case, it is clear that since in tissues like liver, brain and heart the plasmalogens frequently comprise 40 to 50% of the total phospholipids, because of the established low level of glyceryl ether esters, the position of equilibrium normally lies far to the left.

There seems little doubt that the glyceryl ethers play a "bottleneck" role in the synthesis of leucocytes in the marrow(12).

Summary. 1. A number of animal lipids have been separated into neutral and phospholipids, and each of these fractions examined, after hydrolysis, for glyceryl ethers. 2. A paper chromatographic method of separating the various α-glyceryl ethers from one another is described. 3. In mammals the richest site of bound glyceryl ethers is in the marrow.

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Behavior of Microorganisms and Endotoxin Perfused Through the Isolated Rat Liver.* (28875)

JAMES P. NOLAN,† ROBERT A. LEVINE‡ AND VINCENT T. ANDRIOLE
(Introduced by Gerald Klatskin)
Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn.

The mechanisms by which enteric organisms gain access to the bile is not known. Three routes are theoretically reasonable; hematogenous, lymphatic and direct ascending infection. This problem has been investigated with conflicting results since the early part of this century. Experiments utilizing common duct fistulae in dogs and rabbits have demonstrated the appearance of a variety of enteric organisms and of the *Staphylococcus aureus* in the bile within 5 to 10 minutes following an intravenous or endoportal inoculation. On the basis of these observations, it was concluded that the passage of bacteria from the blood to the bile was almost instantaneous(1). A number of other investigators, however, using similar organisms in the rabbit and guinea pig, observed no such rapid transit of bacilli from blood to bile. Indeed, positive bile cultures after intravenous inoculation were obtained only inconstantly, and then after a latent period varying from 3 hours to a week(2,3).

It occurred to us that perfusion of microorganisms through the isolated rat liver might help clarify the issue of whether or not bacteria are rapidly filtered from the blood into the bile. The main advantages of this approach would be: (1) the system is isolated from the predominant lymphatic flow along deep lymphatic vessels that follow the course of the portal veins and bile ducts draining toward the hilum of the liver, (2) organisms could be constantly perfused at a known rate directly into the portal vein and (3) hemodynamic effects could be directly observed without the influence of other tissues and organs.

Materials and methods. The isolated perfused rat liver preparation essentially involves rapidly removing the liver from an animal and placing it in a system where it is perfused by oxygenated, heparinized donor blood. The liver maintains its histologic and metabolic integrity for a period of some 5 to 6 hours and bile is produced at a rate comparable to that observed in the intact animal. The procedure used for removal and perfusion of the liver was that described by Miller *et al*(4). Male Sprague-Dawley rats were

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† Present address: Dept. of Medicine, State Univ. of New York at Buffalo Med. School, Buffalo, N. Y.

‡ Present address: Metabolic Division, U. S. Army Med. Research and Nutrition Laboratory, Fitzsimons Gen. Hospital, Denver, Colo.