Lipids of chicken epidermis

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Abstract The lipids from chicken epidermis were analyzed by a combination of quantitative thin-layer and gas–liquid chromatography and by chemical and spectroscopic methods. The lipid groups present included wax diesters (34%), triglycerides (32%), sterols (11%), phospholipids (11%), nonphosphorus-containing sphingolipids (3%), β-D-glucosylsterols (3%), 6-O-acyl-β-D-glucosylsterols (2%), steryl esters (1%), cholesteryl sulfate (1%), and free fatty acids (1%). The major phospholipids were phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin, and the sphingolipids included ceramides, glucosylceramides, O-acylceramides, and O-acylglucosylceramides. Glucosylsterols and acylglucosylsterols have not been found in mammalian skin, and may be relevant to the evolutionary history of the epidermal water barrier. The wax diesters contained mainly 16-, 18-, and 20-carbon saturated fatty acids esterified to 20- through 24-carbon *threo* and *erythro* 2,3-diols, while the chicken epidermal triglycerides contained some very long-chain (26–40 carbon) saturated fatty acids. These wax diesters and unusual triglycerides may be of significance in human health. — **Wertz, P. W., P. M. Stover, W. Abraham, and D. T. Downing.** Lipids of chicken epidermis. *J. Lipid Res.* 1986. **27:** 427–435.

Supplementary key words epidermal lipids • epidermis • acylglucosylsterol • glucosylsterol • cholesterol glucoside • wax diester • long-chain fatty acid • triglyceride

Evolution of life on dry land required development of a mechanism for avoiding desiccation (1, 2). In terrestrial vertebrates, this mechanism seems to reside in a water barrier located in the outer portion of the integument (3, 4). Although it has long been thought that the water barrier is composed principally of lipids (4, 5), the details of barrier lipid composition and structure have only recently been revealed (6, 7). Most of the available information relating to barrier lipids comes from studies of mammals, where composition and structure seem to vary little among the several species that have been examined in detail (8–12). A survey of the literature revealed that very little has been published concerning epidermal lipids from other than mammalian species, and no analysis of avian epidermal lipids could be found. It was therefore of interest to examine in detail the epidermal lipid composition of a representative bird—the domestic chicken.

In both mammals and birds, lipids are synthesized in the viable epidermis (6), where they are packaged in membrane-enclosed lamellar granules (13). In mammals, the lamellar granules contain one or more stacks of flattened lipid vesicles. Avian lamellar granules differ in that they are larger and contain a greater number of stacks of lamellae. In both cases the contents of the lamellar granules are extruded from the uppermost viable cells into the extracellular spaces (14) where they spread to form broad multilamellar sheets (15) that constitute the water barrier (16, 17).

In mammalian epidermis, the lipids of the barrier layer consist mainly of ceramides, cholesterol, and free fatty acids, with small amounts of triglycerides and cholesteryl sulfate (6, 10). The viable portion of the epidermis contains, in addition, phospholipids and glucosylceramides (6). In recent investigations, the structures of seven series of mammalian epidermal ceramides (9) and an analogous set of glucosylceramides (18–20) were elucidated. Several of these sphingolipids proved to be structurally unusual and may function in assembly of lamellar granules and in maintenance of the water barrier (9, 21). Specifically, linoleate-rich O-acylglucosylceramides and O-acylceramides have been proposed as molecular rivets that hold together, respectively, the stacks of lamellae in the lamellar granules and the multiple extracellular sheets of the stratum corneum.

In the present study, the composition of the polar and nonpolar epidermal lipids of the chicken were investigated to determine their potential for forming the lamellar structures found in the epidermis of birds.

MATERIALS AND METHODS

Preparation and extraction of chicken epidermis

Three mature laying hens were shampooed with Johnson's Baby Shampoo (Johnson and Johnson, Inc., New Brunswick, NJ). After thorough drying under a hair

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography.

dryer, the chickens were anesthetized and the preen glands were surgically removed. The chickens were then individually caged and given a normal diet (Layena; Ralston-Purina, Inc., St. Louis, MO) and water ad libitum for 2 weeks prior to being killed by cervical dislocation. Feathers were removed by manual plucking, and epidermis was removed by gentle scraping with a stainless steel spatula after scalding with boiling water. The separated epidermis was dried in vacuo, and then subjected to three successive extractions with chloroform–methanol 2:1, 1:1, and 1:2. Each chloroform–methanol mixture was stirred with the epidermis for 2 hr at room temperature. The combined extracts were taken to dryness under reduced pressure. The dried epidermis and the extracted lipid were weighed.

Analytical TLC

All analytical TLC (22) was done with 0.25-mm thick layers of silica gel G (E. Merck, Darmstadt, West Germany). The silica gel layer was scored into 6-mm-wide lanes, and one sample or standard was applied to each lane, 2 cm from the bottom of the plate. Nonpolar lipids were resolved by multiple development with hexane, followed by toluene, followed by two developments half-way with hexane–ethyl ether–acetic acid 70:30:1. Ceramides and acylglucosylsterols were separated by two developments with chloroform–methanol–acetic acid 190:9:1. Development with chloroform–methanol–water 40:10:1 resolved the individual glucosylceramides and glucosylsterol from the total ceramides plus nonpolar lipids and the phospholipids plus cholesteryl sulfate. The individual phospholipids and cholesteryl sulfate were resolved using chloroform–methanol–isopropanol–triethylamine–aqueous 0.25 M KCl 30:9:25:18:6. Sphingolipids previously isolated from pig epidermis (9, 18, 19) were used as standards. Standard phospholipids and cholesterol were purchased from Sigma Chemical Company (St. Louis, MO).

After development, chromatograms were air dried, sprayed with 50% sulfuric acid and slowly heated to 220°C. After charring was complete, the chromatograms were cooled to room temperature and quantitated with a Shimadzu model CS-930 photodensitometer.

Preparative TLC

For preparative TLC, 0.5-mm silica gel H plates were used. Acylglucosylceramide and glucosylsterol were separated by development with chloroform–methanol–water 40:10:1. Triglycerides and wax diesters were separated by development with chloroform (HPLC grade containing 1.0% ethanol as preservative; Fisher Scientific, Pittsburgh, PA). Purification of acylglucosylsterols required two steps. First, a fraction containing acylglucosylsterol and a ceramide was collected after development with chloroform–methanol–acetic acid 190:9:1. This fraction was then separated into its constituents by development with chloroform–methanol–water 40:10:1.

Lipid bands on the preparative TLC plates were detected by visualization under ultraviolet light after the plate had been sprayed with an ethanolic solution (10 mg/100 ml) of 8-hydroxy-1,3,6-pyrenetrisulfonic acid trisodium salt (Eastman Kodak Co., Rochester, NY). This fluorescent indicator was largely left on the silica gel when the lipids were eluted with chloroform–methanol 2:1. The small amounts of indicator that eluted with the lipids did not interfere in subsequent analyses.

Wax diesters

The isolated wax diester fraction was saponified by treatment with 1 M KOH in 95% methanol at 60°C for 1 hr. The saponification mixture was then treated with excess BCl_3 in methanol at 60°C for an additional hour. Water was added and the products were recovered in chloroform and separated by preparative TLC with a mobile phase of toluene. The fatty acid methyl ester and diol-containing bands were separately scraped from the plate and the lipids were eluted with chloroform–methanol 2:1. A portion of the recovered diols was rechromatographed on silica gel H containing 5% boric acid, with ethyl ether as the developing solvent. This procedure separated *threo* from *erythro* isomers as described previously (23, 24).

Acetonides were formed from the 2,3-diols by treatment at room temperature with acetone containing a trace of HCl. After 1 hr, the reaction was stopped by addition of excess aqueous potassium carbonate, and the products were extracted into chloroform. The chloroform was removed under a stream of nitrogen prior to analysis of the acetonides by gas–liquid chromatography (GLC).

The fatty acid methyl esters also were analyzed by GLC as described below.

Triglycerides

The triglyceride fraction isolated as described above was resolved into two bands by preparative TLC with chloroform containing 1% ethanol as the mobile phase. This fractionation required three developments. The less mobile of the two components was chromatographically similar to standard triolein. The two bands were collected separately and the triglycerides were eluted with chloroform–methanol 2:1. Each of the two triglyceride fractions was treated with benzene–10% BCl_3 in methanol 1:4, at 70°C. The benzene seemed to be necessary to completely dissolve the more mobile component. After 2 hr, the reaction mixture was cooled, water was added, and the products were extracted into chloroform. Examination of the products by analytical TLC revealed one spot for the methyl esters from the less mobile starting material, similar to standard methyl oleate. The methyl esters from

the more mobile starting material yielded two spots, the more mobile of which ran slightly ahead of the methyl oleate standard. These methanolysis products were analyzed by GLC without further purification.

Glucosylsterols and acylglucosylsterols

Acylglucosylsterols were saponified by treatment with chloroform–methanol–10 M aqueous NaOH 2:7:1 at 60°C for 1 hr. The reaction mixture was acidified to pH 4 with 2 M HCl and the products were extracted into chloroform. Analytical TLC revealed two products corresponding to free fatty acid and glucosylsterol. These products were separated by preparative TLC with chloroform–methanol–water 40:10:1 as the developing solvent. The recovered fatty acids were converted to methyl esters by treatment with 10% BCl_3 in methanol at 60°C for 1 hr. The methyl esters were recovered in hexane and analyzed by GLC.

The glycosylsterols (ca. 2 mg) were hydrolyzed by treatment with 2 ml of 1 M HCl in methanol containing 20 M water (25). After 18 hr at 65°C, 4 ml of chloroform and 1 ml of water were added to the reaction mixture, and the chloroform layer was transferred to a clean tube and dried under nitrogen. Analytical TLC revealed only one lipid product, and this had a mobility identical to standard cholesterol.

The free sterols were acetylated by treatment with acetic anhydride–pyridine 1:1 for 1 hr at room temperature. These derivatives were analyzed by GLC and by electron impact mass spectrometry.

Spectroscopic methods

Proton magnetic resonance spectra were obtained with a Bruker Model WM-360 spectrometer. Samples were dissolved in either deuterochloroform or deuterochloroform–deuteromethanol 2:1. The residual chloroform signal at 7.20 was used as an internal reference for measurement of chemical shift values. Spectra were obtained for the acylglucosylceramide, acylglucosylsterol, and glucosylsterol from chicken skin. Acylglucosylceramide from pig skin has been characterized by the NMR method (20), and the spectra of this lipid were available for comparison.

Electron impact mass spectra were obtained with a Nermag R 10-10 C mass spectrometer coupled to a gas chromatograph with a 5% phenyl methyl silicone stationary phase capillary column operated isothermally at 260°C. The ionization voltage was 70 EV.

Gas–liquid chromatography

GLC was done with a Varian 3700 gas chromatograph equipped with a 50-m quartz capillary column (BP1, Scientific Glass Engineering, Inc., Austin, TX or CP SIL 88, Chrompack, Inc., Bridgewater, NJ) and an electronic integrator. Fatty acid methyl esters were chromatographed isothermally at 190°C or 260°C on the BP1 column. Methyl esters were also chromatographed on BP1 with an initial 10 min at 190°C followed by a 5°C/min increase in temperature until 300°C was attained and on CP SIL 88 with a temperature program starting at 160°C and increasing linearly at 5°C per min until a final temperature of 220°C was reached. A series of standard saturated fatty acid methyl esters ranging from C_{14} through C_{24} (kit KF, Applied Science, State College, PA) and a mixture including methyl palmitoleate, methyl oleate, and methyl linoleate (kit CE1-62, Nu-Chek-Prep, Elysian, MN) were used as standards. Acetonides of the 2,3-diols from the wax diesters were chromatographed isothermally at 200°C on CP SIL 88. Previously characterized acetonides of the 2,3-diols from chicken preen glands (24) were used as reference materials. Sterol acetates were chromatographed isothermally at 300°C on BP1, and cholesteryl acetate and cholestanyl acetate were used as standards.

RESULTS AND DISCUSSION

Fig. 1 shows a typical analytical thin-layer chromatographic separation of nonpolar lipids from chicken

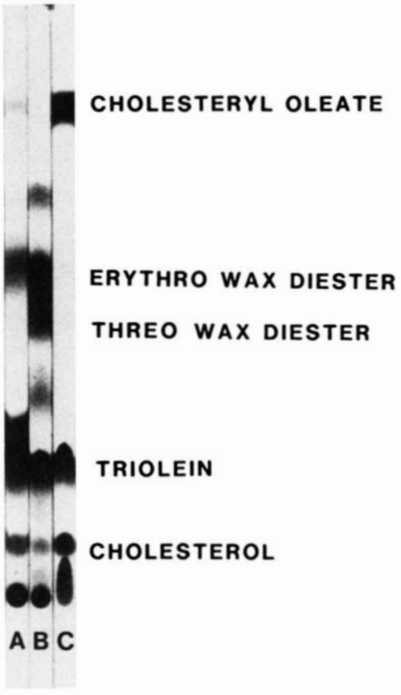

Fig. 1. Thin-layer chromatogram of chicken skin lipids and standards. Chicken epidermal lipid, chicken preen gland lipid, and reference standards were applied to lanes A, B, and C, respectively. The chromatogram was developed with chloroform, sprayed with 50% sulfuric acid, and charred at 220°C. Reference materials and the wax diesters from preen gland are identified on the figure.

epidermis, chicken preen gland, and several standards. Clearly, both the preen gland and epidermis contain a lipid class similar to the triolein standard. In addition, epidermis contains a slightly more mobile component which, although not completely resolved from the normal triglyceride component, can be appreciated in the densitometer tracing shown in **Fig. 2A.** It is also noteworthy that epidermal lipid contains only one major wax diester component (Fig. 1 and Fig. 2A). This is in contrast to the preen gland lipid, which contains nearly equal proportions of two diesters as indicated in Fig. 1. These two components of chicken preen gland lipid have been identified as the diesters of *threo* and *erythro* 2,3-diols (24, 26). It can be inferred from the distribution of lipids shown in Fig. 1 that epidermis contains major amounts of *erythro* wax diester but little of the *threo* isomer. This suggests that the epidermal lipids obtained in the present investigation were relatively free of preen gland lipids.

TABLE 1. Lipid composition of chicken epidermis

Lipid Class	Weight Percent
Wax diesters	34.0
Triglycerides 1	24.7
Triglycerides 2	7.7
Sterols	11.2
Phospholipids	11.2
Glucosylsterols	2.7
Acylglucosylsterols	1.5
Steryl esters	1.3
Cholesteryl sulfate	1.2
Acylglucosylceramides	0.9
Glucosylceramides	0.6
Acylceramides	0.6
Ceramides	0.6
Fatty acids	0.7
Unidentified	1.1

Epidermis from three individual hens was examined and judged to be qualitatively similar. The composition presented above was estimated from one set of chromatograms by photodensitometry.

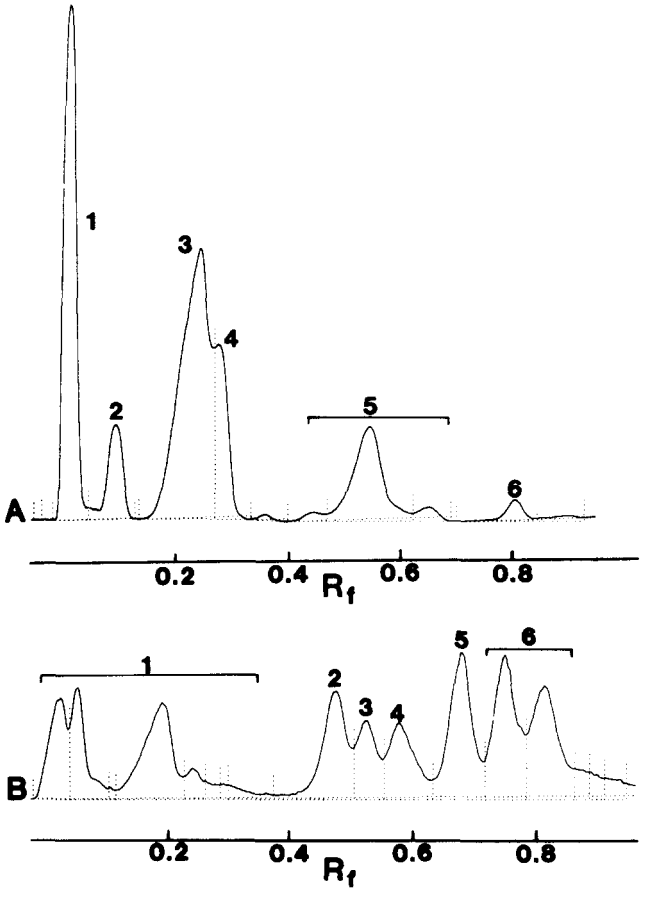

Fig. 2. Densitometer tracings of typical thin-layer chromatograms. Fig. 2A is the densitometer tracing of lane A from the chromatogram shown in Fig. 1. Peaks 1–6 correspond to fatty acids plus polar lipids, cholesterol, triglycerides #1, triglycerides #2, wax diesters, and cholesteryl esters, respectively. Fig. 2B is a densitometer record showing the chromatographic separation of the principal polar lipids from chicken epidermis. The chromatogram was developed with chloroform–methanol–water 40:10:1 and charred as described in the legend to Fig. 1. The peaks or groups of peaks numbered 1–6 on the densitometer tracing represent phospholipids plus cholesteryl sulfate, glucosylsterol, glucosylceramide, acylglucosylceramide, acylglucosylsterol, and ceramides plus acylceramides, respectively.

Chromatographic separation of the chicken epidermal polar lipids is illustrated in Fig. 2B. The lipid classes that are resolved in this chromatogram include phospholipids plus cholesteryl sulfate (peak 1), glucosylsterols (peak 2), glucosylceramides (peak 3), acylglucosylceramides (peak 4), acylglucosylsterols (peak 5), and ceramides plus acylceramides (peak 6). A less polar solvent system was used to further examine the ceramides and acylceramides, while a more polar system was necessary to identify individual phospholipids and cholesteryl sulfate. By combining the results obtained from such a set of chromatographic analyses, a complete composition was calculated.

Table 1 summarizes the lipid composition of chicken epidermis determined by quantitative TLC. This composition is similar to compositions reported for mammalian epidermis except for the acylglucosylsterols and glucosylsterols. These lipids have not been found in any mammalian source, although an acylglycosylsterol was tentatively identified in snake skin and frog skin (unpublished observations, Wertz, P. W., et al.) and similar materials have been reported to occur in millet (27) and other plant seeds (28).

It should be pointed out that the relatively high proportions of nonpolar lipids in chicken epidermis are probably not of significance to the water barrier function. Most of this nonpolar lipid is presumed to be located within the neutral lipid droplets that are retained within the epidermal cells. They would, therefore, not be available for service in the extracellular membranes that retard the movement of water through the skin.

Wax diesters

Also noteworthy are the wax diesters, which are prominent sebaceous lipids found on the skin surface in many mammals (29, 30), but which have not generally been identified as epidermal components. The one exception is the mouse, where wax diesters have been detected in both neonatal epidermis (12) and the epidermis from asebic mutants (31). Unfortunately, these murine wax diesters have not been completely characterized, and it is not known whether they are of the same type found in the chicken.

Wax diesters are major components of the preen gland lipids from the turkey (23) as well as the chicken (24, 26) and, as noted above, the 2,3-diols from the chicken preen gland diesters have been found to contain nearly equal proportions of *threo* and *erythro* isomers (24, 26). Although the animals used in the present study had had their uropygial glands removed 2 weeks before the epidermis was collected, it could not be assumed that the epidermis was free of residual preen gland lipids. However, the possibility of contamination by preen lipids did not seem likely in view of the unequal distribution of the *threo* and *erythro* isomers indicated by TLC (Fig. 1) and confirmed by GLC of the acetonides, as shown in **Fig. 3.** The principal esterified fatty acids from the wax diesters were the 16-, 18-, and 20-carbon saturated species as summarized in **Table 2.**

Triglycerides

The chicken epidermal lipids appear to contain two classes of triglycerides, one of which is more mobile than triolein (Fig. 1 and Fig. 2A). The more mobile component was isolated by preparative TLC employing multiple development. Saponification of the isolated lipid yielded fatty acids as the only charrable product, and treatment of these fatty acids with BCl_3 in methanol produced fatty acid methyl esters, which were analyzed by GLC. The results of these analyses as well as the fatty acid composition of the less mobile triglycerides are summarized in Table 2.

As can be seen in Table 2, the less mobile family of triglycerides (Triglycerides #1) contained mainly 14- through 20-carbon fatty acids; only small amounts of longer species were present in this sample. Similar chain length distributions have been reported for chicken liver triglycerides (32) and for abdominal fat (33).

In contrast, the triglyceride fraction that was more mobile on TLC (Triglycerides #2) contains significant proportions of 30- through 40-carbon fatty acids. It should be pointed out that since appropriate standards were not available these longer chain lengths were assigned entirely on the basis of plots of logs of retention times versus carbon number. Mass spectra were not obtained to verify the assignments, so there is still some uncertainty regarding details of the chain structures. Nevertheless, it is clear from the distribution of fatty acids found in this triglyceride fraction that this entity most probably contains two medium length fatty acids (14–20 carbons) and one very long chain fatty acid (24–40 carbons) per molecule. Presumably, this is the basis for the greater mobility of Triglycerides #2 on TLC.

It is noteworthy that the epidermal triglycerides were almost entirely saturated. Liver triglycerides (32) and body fat (33) both contain higher proportions of monoenoic fatty acids than the triglycerides of epidermis, and both of these internal tissues contain appreciable levels of linoleic and linolenic acids. The contrasting high degree of saturation found for the epidermal triglycerides is not unexpected. Rather, it is in accord with the fatty acid compositions previously reported for porcine (34) and

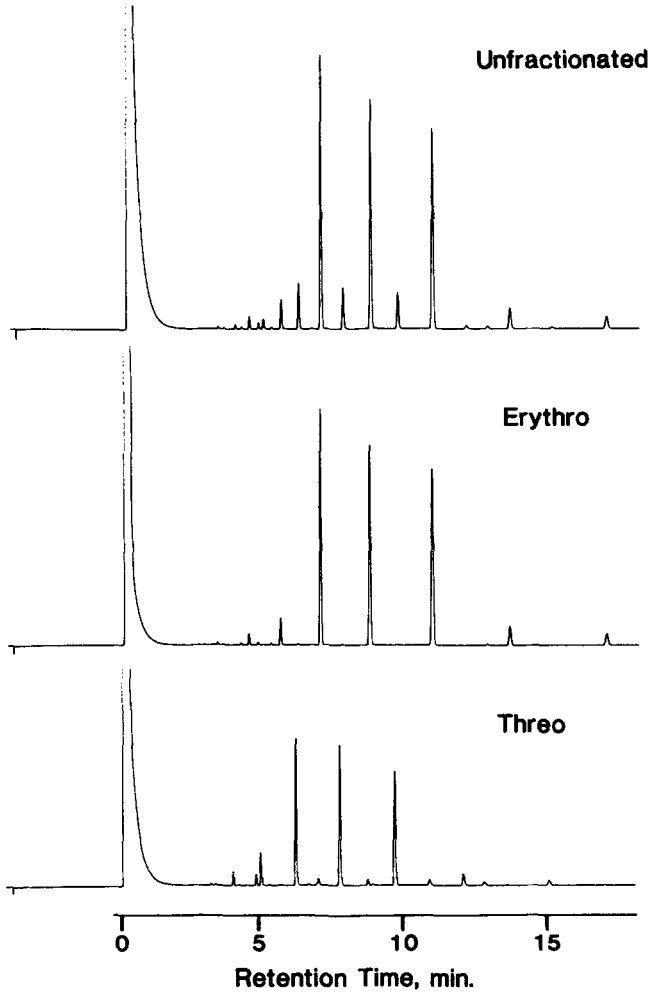

Fig. 3. GLC of acetonides of the 2,3-diols from chicken epidermal wax diesters. The top panel shows the chromatogram of acetonides prepared before fractionation of diols into *threo* and *erythro* isomers by TLC on silicic acid impregnated with boric acid. Chromatograms of the separated isomers are shown in the lower two panels. For each isomer, the major chain lengths were 21, 22, and 23.

TABLE 2. Fatty acid compositions of chicken epidermal lipids

Fatty Acid	Triglycerides #1	Triglycerides #2	Wax Diesters	Acylglucosylsterols
14:0	0.3	0.4	2.1	6.8
15:0	0.6	0.1	0.9	3.5
16:0	32.3	17.9	17.2	24.2
16:1	1.9	–	–	6.4
17:0	12.4	12.6	6.8	3.1
18:0	22.4	17.7	23.8	18.0
18:1	19.9	0.3	12.4	9.8
18:2	–	–	–	6.9
19:0	2.0	2.8	3.4	1.2
20:0	2.8	4.2	25.9	1.8
21:0	0.6	1.0	3.1	1.4
22:0	0.2	0.8	4.4	5.6
23:0	0.7	0.2	–	2.7
24:0	0.5	0.6	–	5.8
25:0	0.8	0.5	–	1.3
26:0	0.4	1.7	–	1.5
27:0	0.5	1.0	–	–
28:0	0.2	2.5	–	–
29:0	0.4	2.0	–	–
30:0	0.2	5.0	–	–
31:0	0.2	3.8	–	–
32:0	–	6.7	–	–
33:0	–	3.3	–	–
34:0	–	3.4	–	–
35:0	–	1.2	–	–
36:0	–	2.0	–	–
37:0	–	1.2	–	–
38:0	–	1.8	–	–
39:0	–	1.0	–	–
40:0	–	0.3	–	–
Others	0.7	4.0	–	–

Fatty acid compositions were determined by GLC of the fatty acid methyl esters. Peak assignments were based on comparison of retention times with those of authentic standards and, for the chains longer than 24 carbons, on linear plots of log of retention time versus carbon number from isothermal chromatograms. Each analysis was repeated two or three times with similar results. The compositions given above were determined from representative chromatograms by electronic integration. Proportions less than 0.1% are designated (–).

human (35) epidermal triglycerides. This high degree of saturation may reflect a requirement for these lipids in the outer portion of the skin to resist oxidative damage on exposure to the atmosphere.

Acylglucosylsterols and glucosylsterols

Included in Table 2 is the fatty acid composition of the acylglucosylsterols. The sterols found after hydrolysis of this fraction appear to be 62% cholesterol and 38% cholestanol as judged by GLC, and this is illustrated in **Fig. 4.** The identities of these sterols were confirmed by electron impact mass spectroscopy of the acetates. The acetate of the component assigned as cholesterol gave a mass spectrum identical to standard cholesteryl acetate. Although this spectrum did not include a molecular ion, an ion was observed at M/Z = 386, and other fragments with an ion abundance greater than 1.5% were found at M/Z = 370, 369, 368, 354, 353, 275, 274, 261, 260, 256, 255, 248, 247, 214, 213, and 173. The acetate of the second component gave a similar spectrum which included a molecular ion at M/Z = 430 and fragments with an ion abundance greater than 1.5% with M/Zs two units higher than each of the above-noted ions. The high proportion of cholestanol indicated by the present analyses was unexpected, but the relatively simple sterol composition is in accord with the sterol and sterol ester compositions reported for epidermal cells from the rat, pig, and human (36). Although it is possible that there are still unresolved steroids present in these sterol glycosides, the present results support the contention that the major components are cholesterol and cholestanol.

Identification of the sugar and the position of attachment of the ester-linked fatty acid were based on proton magnetic resonance spectra obtained from the native molecule, from the glycolipid produced on saponification, and from the acetylated derivative of the latter. After saponification and acetylation, the signals from all of the sugar ring protons were well resolved, and this region of the spectrum was essentially superimposable on that obtained for acetylated glucosylceramides. The anomeric proton appeared at 4.55 ppm as a sharp doublet with a coupling constant (J_{12}) of 8 Hz. indicating a β-glucosidic linkage. The protons on carbons 2, 3, and 4 all gave rise to multiplets between 4.9 and 5.2 ppm. Each of these

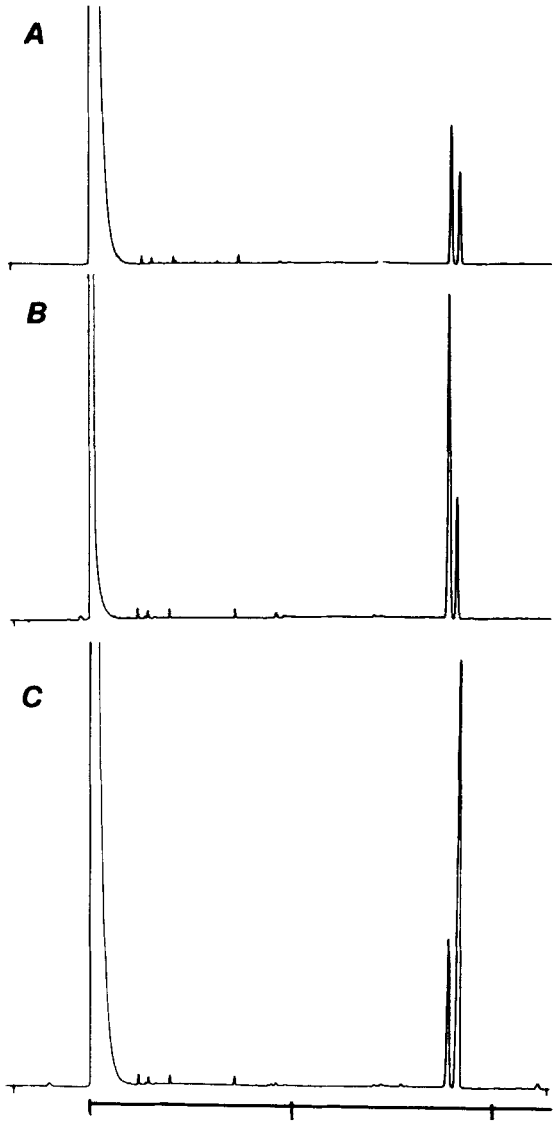

Fig. 4. Identification of the sterols from acylglucosylsterols by GLC. Panel A shows a chromatogram of the sterol acetates prepared from chicken epidermal acylglucosylsterols. B and C illustrate the results of cochromatography of the sterol acetates from A with cholesterol acetate and cholestanyl acetate, respectively.

multiplets appeared to be a doublet of doublets with all coupling constants approximately 8 Hz (J_{12}, J_{23}, J_{34}, J_{45}). The protons on carbon 6 were nonequivalent. One of these produced a doublet of doublets at 4.1 ppm with coupling constants of 10 Hz (J_{6A6B}) and 2 Hz (J_{56}) while the other gave rise to a doublet of doublets with coupling constants of 10 Hz (J_{6A6B}) and 4 Hz (J_{56}). These observations are consistent only with an assignment of a β-D-glucoside.

In the spectrum of the native acylglucosylsterol, the multiplets assignable to the protons 6A and 6B were shifted downfield to 4.35 and 4.45 by the presence of the ester. After saponification, these signals were found at 3.5 and 3.6 ppm. No other sugar ring proton signals were significantly altered by saponification. Therefore, the ester linkage appeared to be to the 6-hydroxyl group as shown in **Fig. 5.** The native glucosylsterol was similarly analyzed. It differed from the saponification product of the acylglucosylsterol only in the proportions of cholesterol and cholestanol, which in this case were 35% and 65%, respectively.

Acylglucosylceramide

The acylglucosylceramide was identified by comparison with authentic acylglucosylceramide from pig epidermis (18, 20). The material from chicken skin was indistinguishable from this material as judged by TLC mobility both before and after saponification. Also, these lipids were essentially identical as indicated by the proton magnetic resonance spectra of the native molecules and the acetylation products.

Lipids and the epidermal water barrier

The major degree of overlap between the epidermal lipid compositions of the chicken (Table 1) and mammals is in accord with the fundamental biological significance of the water barrier. Presumably, the forces of evolution selected an epidermal lipid composition suitable for retention of water in the relatively arid terrestrial environment, and only minor deviation from this primordial composition has been permitted. Thus, although the differences in appearance between avian and mammalian lamellar granules may be dictated by subtle differences in membrane lipid composition, a common acylglucosylceramide is present in both cases and may provide the driving force for assembly of the lamellae. It also appears that the structurally related acylceramide may function in holding together the extracellular lipid lamellae of the water barrier in both birds and mammals.

To speculate about the possible functions and the evolutionary significance of the acylglucosylsterols and glucosylsterols is irresistable. Although chicken epidermis is so far the only well-established source of acylglucosylsterols in the animal kingdom, a similar lipid has been tentatively identified in snake and frog epidermis. Since the acylglucosylsterol has two hydrophobic arms reaching outward from a central hydrophilic glucosyl group (Fig. 5), it would seem ideally suited to function as a molecular rivet in much the same fashion as has been proposed for the acylglucosylceramide. However, since frogs do not form lamellar granules (13), the acylglucosylsterol cannot be involved in this type of barrier-forming mechanism. Possibly acylglucosylsterols function in a more primitive barrier-forming scheme and may remain as a vestige in birds and possibly in snakes. They may be retained as the primary means of forming a water barrier

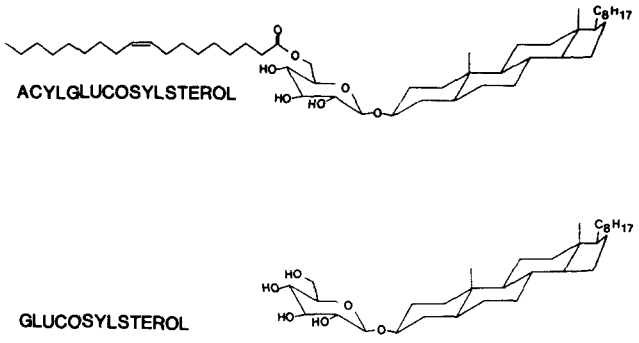

Fig. 5. Representative structures of the glucosylsterol and acylglucosylsterol from chicken epidermis.

in other less well land-adapted classes of vertebrates such as amphibians.

Chicken epidermal cells contain large lipid droplets, which are retained within the cells throughout the horny layer (37). Analogous structures are not formed in normal mammalian epidermis. Histological studies have indicated that these droplets contain principally neutral lipids. Presumably, these neutral lipid droplets would include much of the free sterols, the triglycerides, and the wax diesters that have been identified in the present study.

Since preliminary experiments indicated that the wax diesters found in chicken epidermis are chemically similar to the diesters isolated from preen glands, it was necessary to take extreme precautions to avoid contamination of the epidermis by preen gland lipids. Removal of surface lipids by shampooing and excision of the preen gland, 2 weeks prior to the collection of epidermis, was expected to ensure that any wax diesters were truly of epidermal rather than preen gland origin. A strong indication that this expectation was met is the fact that the diols from preen gland are composed of nearly equal proportions of *erythro* and *threo* isomers, while the diols recovered from the epidermis in the present experiments were predominantly *erythro* isomers.

Since chicken, including the skin, is a major part of the human diet, it is somewhat surprising that similar studies have not previously been reported. In this regard, it is noteworthy that chicken skin contains high proportions of both cholesterol and saturated fatty acids. Although dietary cholesterol and high proportions of long-chain saturated fatty acids have been thought to be related to the induction of cardiovascular disease in experimental animals and man, the effects on health of extraordinarily long-chain fatty acids such as those found in the epidermal triglycerides of the chicken (Table 2) are unexplored. It is also noteworthy that wax esters are known to serve as laxatives.

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