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I hereby recommend that the thesis prepared under my supervision by Roland M. Koppenhaffer.

entitled Animal Skin Lipids

The Distribution of the Lipids in Frog Skin Hide.

be accepted as fulfilling this part of the requirements for the degree of Ph. D.

Approved by:

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ANIMAL SKIN LIPIDS

The Distribution of Lipids in Fresh Steer Skin

A dissertation submitted to the

Graduate School
of the University of Cincinnati

in partial fulfillment of the
requirements for the degree of

DOCTOR OF PHILOSOPHY

1936

by

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CONTENTS.

INTRODUCTION.

The skin is not only one of the largest organs of the animal body, but its varied physiologic functions and its specialized metabolism make it one of the important organs of the body. The lipids of the skin, because of their involvement in the formation of the sebaceous secretion, and their role in the keratinization processes of the epidermal cells, are intimately associated with the physiology of the skin. Yet comparatively few studies in recent years have been directed toward skin lipids, and our knowledge of these constituents is due to the researches of a few men.

The early work of Unna and his co-workers (21,22) still represents the most comprehensive investigations of skin lipids. Their work was particularly concerned with the cholesterol and phospholipid distribution in human skin and with the changes these lipids undergo during skin metabolism. More recently Eckstein and Wile (8) have studied the lipid distribution of exfoliated human skin. They reported that cholesterol constitutes 13 to 24 per cent of the total lipid derived from such material, 90 per cent of which exists as free cholesterol, while phospholipid comprised only 2.5 to 3.15 per cent of the lipid obtained. The low phospholipid content of their material was undoubtedly due to the keratinous nature of the

material analysed. Roffo (19) has observed the increase in the skin cholesterol of rats subjected to ultraviolet radiation and Kawaguchie (9) has performed similar experiments with guinea pigs and rabbits. Pachur (18) has analysed the lipids of the human skin surface. The recent papers of Kooyman (10,11) have reported the changes which the epidermal lipids undergo during keratinization and the character of the surface lipids of the human skin.

It has been difficult in attempting to study the lipids of the human skin to obtain normal tissue in sufficient quantity to analyse. The investigators have, at times, resorted to peculiar sources to obtain lipids representative of various skin structures or of various skin processes. The use of the skin of the steer in studying the metabolism of skin lipids offers obvious advantages. It can be obtained in a normal state immediately after slaughter and post mortem activity can be reduced to the time required to prepare the material for alcoholic dehydration. Furthermore, it is of such a quantity and thickness that it can be separated mechanically into representative horizontal divisions. A study of the distribution and character of the lipids of these divisions yields, as will be seen, much information concerning the general lipid metabolism of the skin.

A previous publication (12) has indicated the nature and distribution of the various lipids of the steer skin. The epidermal region was found to contain the preponderance of the

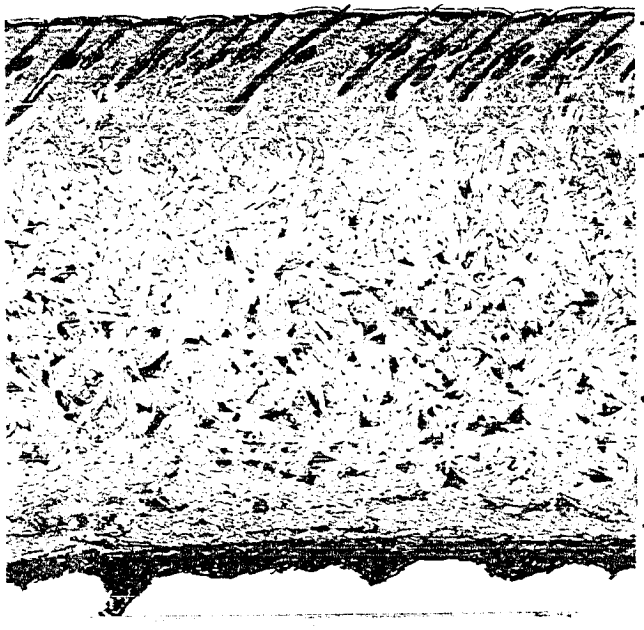
phospholipids and cholesterol. In addition a wax composed of long chain, saturated, hydroxy-acids in ester combination with aliphatic alcohols was isolated in this region. The corium contained smaller amounts of phospholipid and cholesterol but varying and often large deposits of triglycerides similar in chemical nature to those contained in the reserve lipid deposits of the subcutaneous tissue. It was evident from the data obtained that a striking similarity existed between the lipids of human skin and those of the steer skin, particularly in regard to their nature and distribution.

Based upon some preliminary tests correlated with histologic evidence, the separation of the following divisions seemed desirable to illustrate the metabolic activity of the skin lipids. After clipping the hair, the skin was divided into six horizontal divisions each representing loosely defined skin structures. The separation is shown diagrammatically in the figure. The analyses of lipids of the subcutaneous layer, having been reported previously was not attempted.

The uppermost division, consisting of the hair, needs no description. The next division, termed the "horn" because it embraces that portion of the epidermal system resulting from the keratinization processes, together with the adhering surface lipids, contains the end products of the lipid metabolism of the epidermal region. The adjacent division, the "basal" division, includes the region extending from nucleated epidermal cells to the base of the hair roots. The mechanical separation of this division is easily possible because the hair roots af-

Figure

Illustrating the Separation of the Various Divisions



Section of Normal Steer Skin
Stained with Scarlet R
Magnification 10 X

ford a sharp contrast to the white background of the underlying material. This division contains the following structures - the hair pockets, the sebaceous and suderiferous glands, the nucleated cells of the stratum germinativum together with the preponderance of the wandering cells interspersed throughout the corium minor. The histologic structure of this division as well as the chemical analyses of its lipid indicate it to be a region of comparatively intense physiologic activity. The next division removed constitutes the upper portion of the corium adjoining the base of the deepest hair roots. It will be referred to as the "transition" division since its removal incured a complete separation of epidermal structures from the corium division. It is in this region that the transition of the connective tissue structure from the fine fibres of the corium minor to the large fibre bundles of the corium major occurs. Furthermore, preliminary experiments indicated that this region contained a sphingomyelin-like lipid which is present in some skins.

The corium was separated into two divisions, the corium major and the corium base. The corium major comprised sixty per cent of the dry weight of the skin. It is composed, mainly, of bundles of white connective tissue fibres, together with smaller quantities of reticular tissue sheaths and interfibrous material. The solidity of this region and the intricate manner in which its constituent fibres are interwoven to give the skin the physical toughness it possesses, make this

the most important leather producing region of the skin. The lower corium division, or corium base, differs from the upper corium division particularly in the increased concentration of fat cells which are present.

EXPERIMENTAL PROCEDURE

The skin of a two year old, grade steer was obtained immediately after slaughter and cut into strips parallel to the median line. Each strip was mechenically separated with a sharp razor into the various divisions outlined above. Obviously the precision of such a separation is limited by the variable structure of the skin. However, with careful technique and close observation of the structural demarcations of the various divisions it is possible to obtain satisfactorily consistent separations. This was further checked by microscopic examination of the various divisions. This method is preferable to any chemical means of separation since it avoids changes in the character of the lipids due to their reactivity.

The material obtained was cubed, if necessary, and plunged into cold ethyl alcohol. The average time elapsing between slaughter and alcohol immersion was three hours. After complete dehydration had been obtained through several changes of alcohol the material was ground in a Wiley mill and extracted with successive changes of hot alcohol until negligible amounts of lipids remained. The completeness of the extraction was

was further tested by hydrolysing a portion with strong alkali, acidifying, and extracting the acidified solution with ethyl ether. Previous publications from this laboratory (16) have attributed the failure to completely remove, by solvent extraction, all the lipid from the steer skin to the presence of a lipid-protein complex. With more adequate facilities for subdivision and extraction of hide material, it has been possible, with solvent extraction, to remove 98 per cent of the total skin lipid. On filtering the hot alcoholic epidermal extracts, a slightly soluble wax separated from the warm alcohol. In order to obtain a maximum separation of this wax, the epidermal extracts were cooled to 5° C overnight and the wax recovered by filtration. The respective alcoholic solutions of the lipids, including the dehydration solutions, were evaporated in a vacuum still and the residual lipids almost completely recovered by solution in ethyl ether. Weights and iodine numbers were then determined on aliquots of the ether solution. The ether soluble lipids were further fractionated, after removal of the ethyl ether, by twice dissolving them in about 750 cc. of hot alcohol and allowing the less soluble lipids to precipitate overnight at 5° C. By this procedure, most of the complex lipids, as the cholesterol and phospholipids, remained in solution in the cold alcohol, while the triglycerides of the corium and the remaining waxes of the epidermis were obtained as insoluble material. The phospholipids were then removed from the cold alcohol soluble lipids according to Bloor's procedure (4).

In this manner five fractions were obtained, diluted to volume, and aliquots removed for analyses. They were the waxes, the cold alcohol insoluble lipids, the alcohol-acetone soluble lipids, lecithin, and cephalin.

The usual constants were determined on these fractions using the standard procedures of the American Association of Agricultural Chemists (2). Free cholesterol was determined colorimetrically by a modification of the Leiberman-Burchard reaction (13), while cholesterol ester was determined by the method of Bloor and Knudson (6). Oxycholesterol was approximated by application of the Lipschutz reaction.

RESULTS

From a summation of the detailed analyses of various fractions presented in Tables 2 - 7, a compilation of the more significant variables was prepared. These are presented in Table 1. These data, together with the analyses of the various fractions, demonstrate not only the character and distribution of the various lipid entities, but also indicate the processes of the lipid metabolism in the skin.

The Distribution of the Lipids in the Skin

The data presented in Table 1 show that the lipid concentration is greatest toward the upper and lower extremities of the skin. In the epidermal region this deposition of lipid consists of the phospholipids, cholesterol and waxes, while the

Table 1.

The Lipid Distribution in Fresh Steer Skin

Division	Epidermal Region			
	Hair	Horn	Basal	Tran
Weight Dry Material (gms)	93	194	396	22
Weight Lipid in Dry Material (gms)	4.28	16.9	28.8	
Per cent Lipid in Dry Material	4.60	8.72	7.28	
Per cent of Total Skin Lipid	3.39	13.4	22.8	
Weight Cholesterol (gms)	0.62	1.96	3.86	
Per cent Cholesterol in Dry Material	0.67	1.01	0.97	
Per cent Cholesterol of Division Lipid	14.5	11.6	13.4	1
Weight Lipid Phosphorus (mgm)	0.0	61.0	373.0	3
Per cent Phosphorus in Dry Material		0.031	0.094	
Weight Acetone Insoluble Phospholipid (gms)	0.0	1.02	7.81	
Per cent Phosphorus precipitated as lecithin		41.3	67.0	
Per cent Phosphorus precipitated as cephalin		6.2	9.9	1
Phosphorus-Cholesterol Ratio		1-32.2	1-10.3	1-
Weight Free Fatty Acid (as stearic acid)(gms)	1.15	4.06	1.93	
Per cent Free Acid in Dry Material	1.24	2.09	0.49	
Per cent Free Acid of Division Lipid	26.8	17.7	8.3	1
Weight Wax Fraction (gms)	0.90	4.28	10.1	
Per cent Wax in Dry Material	0.97	2.21	2.55	
Per cent Wax of Division Lipid	21.0	25.3	35.1	
Weight Alcohol Insoluble Fraction (gms)		1.53	3.96	
Per cent of Dry Material Weight		0.79	1.00	
Per cent of Division Lipid		9.0	13.7	
Weight Alcohol-Acetone Soluble Fraction (gms)	3.38	9.92	7.07	
Per cent of Dry Material Weight	3.63	5.11	1.78	
Iodine Number of Ether Soluble Lipids	50.5	49.8	63.4	5
Acetyl Value of the Total Fatty Acids	40.7	47.0	56.8	3

Table 1.

The Lipid Distribution in Fresh Steer Skin

	Epidermal Region				Corium Region	
	Hair	Horn	Basal	Transition	Corium Major	Corium Base
	93	194	396	228	1839	378
al (gms)	4.28	16.9	28.8	4.45	45.0	27.0
terial	4.60	8.72	7.28	1.95	2.44	7.15
ipid	3.39	13.4	22.8	3.52	35.6	21.4
	0.62	1.96	3.86	0.50	1.17	0.30
Dry Material	0.67	1.01	0.97	0.22	0.063	0.079
Division Lipid	14.5	11.6	13.4	11.2	2.60	1.11
gm)	0.0	61.0	373.0	39.3	72.2	16.1
ry Material		0.031	0.094	0.017	0.0039	0.0042
nospholipid (gms)	0.0	1.02	7.81	0.38	1.43	0.18
ipitated as lecithin		41.3	67.0	7.2	68.9	34.5
ipitated as cephalin		6.2	9.9	13.0	4.2	
io		1-32.2	1-10.3	1-12.7	1-16.3	1-18.6
stearic acid)(gms)	1.15	4.06	1.93	0.49	1.09	0.56
y Material	1.24	2.09	0.49	0.21	0.06	0.15
vision Lipid	26.8	17.7	8.3	11.1	4.0	2.05
	0.90	4.28	10.1			
rial	0.97	2.21	2.55			
Lipid	21.0	25.3	35.1			
Fraction (gms)		1.53	3.96		34.5	21.3
Weight		0.79	1.00		1.88	5.65
pid		9.0	13.7		77.4	80.7
luble Fraction (gms)	3.38	9.92	7.07	3.82	8.70	4.92
Weight	3.63	5.11	1.78	1.68	0.47	1.30
luble Lipids	50.5	49.8	63.4	58.4	55.6	54.2
Fatty Acids	40.7	47.0	56.8	37.8	2.4	1.4

increased lipid content of the corium base is due to the triglyceride deposition. The subcutaneous fat tissues were carefully removed before the mechanical separation and they are not to be confused with the deposition of lipid within the corium. Histologic examination of skin sections demonstrates, in a qualitative manner, that such a distribution of lipid exists in the skin.

The Triglycerides of the Corium

The preponderance of the lipids of the corium, demonstrable by histochemical technique, occur as cellular deposits distributed among and between the corium fibre elements. This deposition has been shown (17) to be more pronounced toward the subcutaneous layer and to be identical in histologic structure to the cells of this layer. The analyses of the corium lipids, recorded in Table 1, show that the corium base contains 7.15% lipid and the corium major 2.44% lipid, 77-80% of which are cold alcohol insoluble triglycerides. The quantity of such triglycerides isolated from the corium is approximately proportional to the histologically demonstrable lipid in the corium. Both chemical data and histologic evidence indicate the increased deposition of this material toward the subcutaneous region, and the analyses of these triglycerides and the lipid of the subcutaneous deposits are almost identical. Hence these lipids result from the deposition of triglycerides in the skin as well as in the subcutaneous lipid reserves. The amount of such deposits varies considerably from skin to skin depending evidently upon the nutrition of the animal. When present in large

amounts, such lipids are the major factor in the production of detrimental grease stains often found on finished leather.

The chemical characteristics of the corium triglycerides are listed in Table 2. The seperation of the fatty acids by the lead salt-ether procedure shows that they consist of 65 per cent liquid acids and 35 per cent solid acids. The liquid acid is oleic acid and the solid acids are mixtures consisting principally of palmitic and stearic acids. Other workers have determined the constituent acids of tallow prepared from this material (3). We have isolated a saturated triglyceride which appears to be tripalmitin from these corium lipids (12).

The Phospholipids of the Skin

The lipid phosphorus distribution in the skin is likewise presented in Table 1. The phospholipid distribution in the two corium divisions is constant. This is particularly significant in view of the disproportionate distribution of triglyceride among these divisions. The corium phospholipid content of many skins examined in other phases of this work has been observed not to follow the triglyceride content of the corium but to remain fairly constant. It is evidently associated with the fundamental protoplasmic structure, the fibreblasts of the corium. Phospholipids appear to be a necessary constituent of living cells and Bloor (5) has correlated the degree of physiologic acticity of the cells with their phospholipid content. The passive activity of the corium region as compared to the epidermal region might well be associated with the difference in

Table 2.

Analyses of Cold Alcohol Insoluble Fractions

Division ++	Epidermal Region		Corium Major	
	Horn	Basal	Corium Major	Corium Base
Iodine Number	15.0	43.6	54.7	51.8
Acid Value	5.0	6.3	0.2	0.2
Per cent Fatty Acid	60.7	61.0	93.2	92.4
Mean Molecular Weight	323	301.5	272.8	274.1
Iodine Number	10.2	45.9	55.6	53.6
Saponification Number	187.6	199.0	209.6	209.4
Acetyl Value	82.6	37.8	0.0	0.0
Per cent Unsaponifiable	40.6	35.1	1.7	2.0
Iodine Number	25.1	40.9	55.1	59.0
Acetyl Value	191.5	178.2	-	-
Per cent Cholesterol	14.9	41.1	-	-
Per cent Lipid Phosphorus	0.0	0.56	0.0	0.0

++ This fraction was not isolated from the lipids of the hair and transition divisions because of the small quantity of total lipid available.

their phospholipid content.

The rapidly multiplying cells of the stratum germinativum of the epidermal layer and the wandering cells which are found in the region directly beneath the epidermal layer, exhibit characteristic staining reactions with basic dyes which are attributed to the acidic constituents of the cells. These cells, particularly those of the epidermal layers, contain large numbers of mitrochondria. The studies of Mayer, Rathery and Shaeffer (15) have shown the similarity between the chemical and physical properties of the cell mitrochondria and the phospholipids. The acidic nature of the phospholipids may explain, in part, the characteristic stain reaction of these cells with basic dyes. The mitrochondria are active in the metabolic processes of the cell and their number can be correlated with the degree of physiologic activity of the cell, as can the phospholipid content.

The lipid phosphorus concentration in the epidermal region as recorded in Table 1 reaches a maximum in the basal epidermal region. This is also the region in which the nucleated cells of the epidermal layers show their maximum development. There is an appreciable decrease in lipid phosphorus in the horn division and none was identified in the lipid isolated from the hair. It is evident that the phospholipid molecule is destroyed during keratinization. The analyses of the phospholipid fractions in Table 3 record the gradual decomposition of the phospholipids of the epidermal cells.

Table 3.

Analyses of Phospholipid Fractions

Division	Lecithin			Cephalin
	Epidermal Region			
	Horn	Basal	Corium Major	Basal
Weight of Lipid (gms)	0.89	6.54	1.29	1.27
Iodine Number	60.6	67.7	71.5	72.4
Per cent Fatty Acid	59.7	68.4	61.6	79.4
Mean Molecular Weight	398.5	360.0	347	412.5
Iodine Number	66.5	77.2	83.0	90.4
Saponification Number	191.5	171.7	183.5	196.0
Acetyl Value	74.9	52.1	51.2	45.9
Per cent Unsaponifiable	13.0	8.5	7.0	7.7
Iodine Number	45.4	77.1	102.3	77.2
Per cent Cholesterol	4.4	6.4	1.0	1.8
Per cent Lipid Phosphorus	2.83	3.82	3.85	2.90

The cells of the sebaceous glands are originally derived from the epidermis and they likewise undergo a degenerative process with the resultant formation of the sebum. The analyses of the basal epidermal region indicates that phospholipid and free cholesterol must be the principal lipid constituents of these active cells. Using the Golodetz reaction on human skin, Unna (20) found the most cholesterol was contained in the embryonic cells of the epidermis, the hair pockets, and the sebaceous glands. The large quantity of lipid which the cells of the sebaceous glands accumulate during degeneration is deposited within the sebaceous gland to form the essential constituents of the sebum. The analyses of this sebum, presented in Table 5, shows it is composed of cholesterol esters and waxes of hydroxylated fatty acids in ester combination with aliphatic alcohols. In this respect, the hydroxylated nature of the phospholipid fatty acids is significant in indicating that they are the probable source of the acid constituents of the wax described above. Evidently the transformation of the cholesterol and phospholipid of epidermal cells, occurring during the degenerative processes resulting in sebum formation, is effected by a concurrent hydroxylation and esterification of the lipid constituents to form the characteristic waxes of the sebaceous secretion. The manner of formation of the aliphatic alcohols present in the sebum waxes is uncertain.

The phospholipids represent an easily separated entity in the various divisions and the analyses of the lecithin fractions of the epidermal divisions show the changes which are effected in these lipids during keratinization. Particularly significant are the decreases in the iodine number and the increase in the hydroxylation of the constituent fatty acids recorded in Table 3. Sufficient lecithin was not obtainable to permit complete analyses in all the divisions. Cephalin was obtained in quantity sufficient for analysis only from the basal epidermal division. The cephalin is more unsaturated than the lecithin and its fatty acids are of higher molecular weight. The high molecular weight of the acids of the phospholipids, calculated from their alkali equivalence, indicates that polymerization or lactone formation occurs. Saponification following the titration of the fatty acids in hot alcohol resulted in an increased alkali absorption as these complexes are broken down. Only liquid fatty acids are separated by application of the lead-salt ether procedure to the phospholipid fatty acids. The double precipitation procedures employed in the separation of the phospholipids failed to remove an appreciable amount of the non-saponifiable material. The preparation of pure lipid entities was not considered necessary in the evaluation of the general characteristics required.

The Cholesterol of the Skin

The work of the early German investigators (20) as well

as the recent publications of Eckstein and Wile (8) and Kooyman (10,11) have associated the greater proportion of the skin cholesterol with the epidermal region. Particular attention has been devoted to the cholesterol esterification which is believed to occur during keratinization of the epidermal cells. The presence of cholesterol in the sebum is disputed.

The total cholesterol distribution is given in Table 1. A more detailed examination of the various forms of cholesterol and their distribution was made upon a second skin. These results are presented in Table 4. The corium cholesterol, like the lipid phosphorus, is uniformly distributed throughout the corium. The close association between cholesterol and the phospholipids in living tissues has led to hypotheses concerning their mutual physiologic relation. The uniform distribution of cholesterol in the corium and the constancy of its weight relation to the dry corium weight, as observed in several skins, suggest that it, like the phospholipid molecule, is associated in the cellular elements of the corium. The cholesterol of the corium exists in the uncombined state.

The increase in the cholesterol content beginning in the transition division and reaching a maximum in the epidermal region follows in part the phospholipid distribution in the same regions. Thus the cholesterol and phospholipid distribution, as illustrated by the phosphorus-cholesterol ratio,

Table 4.

Cholesterol Distribution

(determined on a second skin)

Division	Epidermal Region	
	Horn	Basal
Weight Cholesterol (gms)	2.46	2.68
Per cent Cholesterol in Dry Material	1.62	0.92
Weight Esterified Cholesterol (gms)	0.81	1.04
Per cent Cholesterol as Ester in Dry Material	0.54	0.36
Per cent Cholesterol Esterified	33.1	38.8
Oxycholesterol Test	Positive	Negative

shows some degree of uniformity in those divisions in which keratinization processes are absent. However, the decrease in phospholipid in the epidermal horn division is not accompanied by a concurrent decrease in cholesterol, and cholesterol but no lipid phosphorus was present in the lipids of the hair division. This indicates that a more rapid destruction of phospholipid than of cholesterol occurs at the skin surface. Kooyman (11) has also observed this decrease in phospholipid during keratinization in human skin but he observed a concurrent less rapid removal of cholesterol, a characteristic which these results do not show. The high cholesterol content of the skin surface, resulting because of its more stable nature, must evidently depend upon those factors which would tend to retain an intact surface such as long hair. This would explain the variations in the cholesterol content of the horn layer of the two skins as expressed in Tables 1 and 4.

A considerable percentage of the epidermal cholesterol exists as cholesterol ester. The identification of only combined cholesterol in the sebaceous secretion (see Table 5) accounts for its presence in the epidermal divisions. Since the sebum is the predominant source of surface lipids, it is probable that the preponderance of the cholesterol esters on the surface arise from this source. Whether cholesterol esters are likewise produced during keratinization cannot be concluded from these experiments. The identification of oxycholesterol in the horn division and its absence in the under-

lying basal division suggests the manner of cholesterol destruction on the surface.

The free cholesterol was isolated principally from the acetone-alcohol soluble fraction (see Table 7), where it may comprise as high as 80% of the non-saponidiable material. The cholesterol esters, because of their lesser solubility, are present in the cold alcohol insoluble fraction and in the wax fraction where they represent the total cholesterol present. It is difficult to remove the cholesterol completely from the phospholipid fractions.

The Waxes of the Epidermis

A previous publication has reported the presence and character of the waxes present in the epidermal region of the steer skin. Linser (14) has reported the presence of such waxes on the human skin surface and Ameseder (1) has isolated n-eicosanol (arachyl alcohol) from such a source. We have isolated the same alcohol from the epidermal waxes of the steer skin. Because of the saturated nature of the wax and its distribution it was first believed to result from oxidation processes at the epidermal surface, and to constitute the waxy sheath which protects the skin surface. However, in this experiment, the horn division contained only 30 per cent of the total wax present, while the basal epidermal division contained 65 per cent of the total wax. This definitely demonstrated that this wax was not entirely of surface origin.

The procedure with a second skin was slightly altered to determine the reason for such a distribution. After removal

of the horn division the sebaceous glands were clearly visible imbedded just below the surface. By rotating the razor so that scraping instead of cutting action was attained, it was possible to press the oily glanduler secretion to the surface where it could be collected. Histologic sections of the scraped skin showed that a definite removal of sebaceous lipid was obtained by this procedure. It is significant that lipid was pressed from the glands only when the force was applied in the direction of the grain, i.e., with the slope of the hair follicle.

The analyses of this lipid, presented in Table 5, as compared to the analyses of the total lipid of the entire basal division from which it was expressed, indicate the nature of the unaltered sebaceous secretion. Obviously, material separated in such a manner would be contaminated to a small extent by the lipids of the cut surface which might be picked up during the removal of this fraction. Fortunately certain pronounced differences exist between the lipid removed from the gland and that of the basal division, from which the extent of such contamination might be judged. Thus the esterified nature of the cholesterol of the sebaceous secretion compared to the high free cholesterol content of this division, the comparatively low free fatty acid content and the low lipid phosphorus content of this lipid as compared to the lipid of the basal division are significant factors in indicating the small degree of contamination of the

Table 5.

Analysis of Sebaceous Secretion

(as isolated)

Iodine Number	32.6
Acid Value	10.1
Saponification Number	154.8
Per cent Fatty Acid	57.4
Mean Molecular Weight	249.1
Iodine Number	27.3
Acetyl Value	74.9
Per cent Unsaponifiable	42.7
Iodine Number	56.0
Per cent Total Cholesterol	14.4
Per cent Esterified Cholesterol	13.7
Per cent Lipid Phosphorus	0.159

sebaceous lipid isolated. The analyses of the secretion, recorded in Table 5, shows the nature of its lipid contents. A direct comparison with the saturated wax of the basal epidermal division (see Table 6), from which the cholesterol ester has been removed, indicates that the sebum contains two distinct types of waxes. The one group conprises the aliphatic alcohol - saturated hydroxy-acid esters which are present in great quantities in the epidermal region. The other group are the esters of cholesterol and evidently more unsaturated acids. This letter group would be effective in producing the iodine numbers recorded for the various lipid fractions. One recrystallization from alcohol removed the free fatty acids and lipid phosphorus, which contaminated the lipid, along with some of the constituent cholesterol esters and left a waxy residue constituting 75% of the lipid pressed from the skin. It seems certain then that the origin of the epidermal wax is the sebaceous secretion and that this secretion is composed of the esters of fatty acids and aliphatic alcohols or cholesterol to yield a low melting, waxy material.

The analyses of the waxes isolated from the three epidermal divisions are presented in Table 6. The cholesterol of this fraction is completely esterified and its partial separation in this fraction is due to the insolubility of such compounds. From such waxes we have already reported the isolation of i-hydroxy-stearic acid, stearic acid and n-

Table 6.

Analyses of Wax Fractions

Division	Epidermal Region		
	Hair	Horn	Basal
Iodine Number	9.3	4.9	6.5
Saponification Number	149.5	156.0	175.6
Acid Value	1.2	0.9	0.3
Per cent Fatty Acid	75.4	59.2	55.5
Mean Molecular Weight	351.0	261.0	252.0
Iodine Number	--	3.9	5.3
Acetyl Value	91.1	85.4	79.2
Per cent Unsaponifiable	26.9	44.1	45.4
Iodine Number	--	6.2	7.4
Acetyl Value	--	182.2	175.0
Per cent Cholesterol	7.2	6.2	8.9

eicosanol (arachyl alcohol)(12). The analyses of the non-saponifiable material suggests the presence of lower chain alcohols other than the C_{20} alcohol isolated. The fatty acids have been subjected to fractional distillation and the results, yet incomplete, indicate that shorter chain acids of the saturated series, probably lauric and myristic, are also present.

It is not possible to conclude from these experiments whether the disintegration of the epidermal cells represents an additional source of waxes. A slight difference exists between the character of the waxes of the basal and horn divisions which might be accounted for by such an additional location of wax formation in the epidermis. The concentration of waxes on the surface of several skins has been found to vary in the same relation as the surface cholesterol, with which it is associated. The wax concentration of the basal division depends upon the sebaceous activity of the animal.

The Free Fatty Acids of the Skin

The high free fatty acid content of the lipids of the human skin surface has been reported by Kooyman (11), who attributed it to the lipolytic action of bacteria and enzymes and to atmospheric oxidation at the skin surface. The data in Table 1 show the progressive increase in free fatty acid toward the surface of the skin. In an attempt to associate the increase in free acid with the concurrent decrease in phospholipid, the free fatty acids were isolated. The analyses of the acids of the horn division were similar to those of

alcohol-acetone soluble fraction except that the free acids had a slightly higher acetyl value. No conclusion could be made as to the origin of the acids, but that some hydroxylation occurred after hydrolysis seemed evident. The free fatty acids of the basal division, however, vary considerably in character from the combined acids of this region. They possess an acetyl value of 56.2 compared to 25.6 for that of the combined acid of the acetone soluble fraction and 34.8 for the phospholipid fatty acids. The mean molecular weight of 311 of the free fatty acids compares with a molecular weight of 270 for the combined acid. These characteristics tend toward those of the acids of the sebum waxes and it is possible that these acids of the basal division are intermediates of the wax metabolism occurring in the sebaceous glands.

The Composite Iodine and Acetyl Values

After separation of the insoluble waxes, all the alcohol used for extraction was evaporated and the lipid taken up in ethyl ether. The iodine numbers of these ether soluble lipids were determined and they are recorded in Table 1. The iodine numbers of the corium lipids are nearly identical with those of the corium triglycerides which represent the preponderance of the corium lipids. The increase in iodine number in the transition and basal divisions reflects, as does the phospholipid distribution, the state of increased activity existing around the sebaceous glands. The decreased iodine

number of the lipids of the horn division is the result of oxidation occurring during keratinization.

The hydroxylation of the fatty acids of the various divisions is presented. Since the fatty acids of the corium triglycerides are not hydroxylated the acetyl value of the acids of the corium lipids is due to the acids of the phospholipid and alcohol-acetone soluble fractions. There is a decided increase in the acetyl values of the transition and basal division acids. The composite value varies with the activity of the sebaceous glands and with the amount of sebum formed. However the acetyl value of the wax free ether soluble lipids is also a maximum in the basal division. Such hydroxylation is certainly associated with the sebum formation in yielding the waxes which comprise this secretion. The phospholipids represent a definite skin entity whose evolution to the surface can be traced. A comparison of the values in Table 3 indicates that concurrent hydroxylation and an increased saturation occurs in the phospholipid fatty acids during keratinization. It is impossible to make such a comparison with the lipids of other fractions or from the composite values recorded in Table 1 because of the varied distribution and solubility of the sebum waxes.

The Analysis of the Cold Alcohol Insoluble Lipids

The analyses of the lipids precipitated in cold alcohol solution is presented in Table 2. The triglycerides of the corium, separated in this manner, have already been dis-

cussed. The epidermal lipids of this fraction consist of the more soluble waxes, cholesterol esters, and small amounts of phospholipids and triglycerides. The waxy nature of the fraction from the horn division is particularly evident. The alcohols of these waxes, judging by the acetyl value of the non-saponifiable material, must be C_{14}- C_{16} alcohols, which probably accounts for their failure to precipitate with the less soluble wax fraction already discussed. The cholesterol of this fraction is 80 - 100 per cent esterified. The fatty acids from the horn division present in this fraction are similar to those of the wax fraction while those of the basal division show accentuated oxidation characteristics.

The Analysis of the Alcohol-Acetone Soluble Lipids

This fraction comprises the balance of the skin lipids. In it are found the free fatty acids, the free cholesterol, some cholesterol esters, an appreciable percentage of acetone soluble phosphorus containing lipids, and the alcohol soluble triglycerides. An application of Channon's procedure (7) for the isolation of phosphatidic acid salts from three grams of this lipid resulted in the isolation of 33 mg. of lead phosphatide, indicating that the acetone soluble phosphorus cannot be due to the presence of phosphatidic acid. From the hair and transition divisions, the lipids of this fraction constitute the total ether soluble lipids of the division.

Table 7.

Analyses of Alcohol -- Acetone Soluble Fractions

Division	Epidermal Region			
	Hair	Horn	Basal	Transition
Iodine Number	48.4	51.8	65.0	59.6
Acid Value	66.8	75.6	28.5	24.7
Per cent Fatty Acid	56.2	57.5	45.6	61.2
Mean Molecular Weight	271.5	270.3	286.0	309.0
Iodine Number	41.4	49.5	85.1	60.6
Saponification Number	215.1	218.8	213.5	186.0
Acetyl Value	22.6	21.7	48.2	37.8
Per cent Unsaponifiable	38.1	33.7	43.6	24.5
Iodine Number	56.1	63.5	68.1	65.0
Acetyl Value	159.3	167.0	145.5	157.8
Per cent Cholesterol	44.0	49.6	79.1	47.5
Per cent Total Cholesterol	16.8	16.7	34.5	11.6
Per cent Lipid Phosphorus	0.05	0.30	0.82	0.77

Table 7.

Analyses of Alcohol -- Acetone Soluble Fractions

	Epidermal Region				Corium Region	
	Hair	Horn	Basal	Transition	Corium Major	Corium Base
	48.4	51.8	65.0	59.6	65.3	65.9
	66.8	75.6	28.5	24.7	22.0	21.8
d	56.2	57.5	45.6	61.2	61.5	78.5
eight	271.5	270.3	286.0	309.0	280.2	277.0
	41.4	42.5	65.1	60.6	69.1	67.4
mber	215.1	218.8	213.5	186.0	207.4	207.6
	22.6	21.7	42.2	57.6	9.7	8.4
lable	38.1	33.7	43.6	24.5	25.1	13.4
	56.1	63.5	68.1	65.0	73.8	80.1
	159.3	167.0	145.5	157.8	156.9	145.1
erol	44.0	49.6	79.1	47.5	49.0	41.4
lesterol	16.8	16.7	34.5	11.6	12.3	5.55
sphorus	0.05	0.30	0.82	0.77	0.22	0.21

The Analyses of the Transition Division Lipids

The transition division was isolated to insure a complete separation of epidermal and corium material. Furthermore, it was thought that this division, comprising 7 per cent of the dry weight of the skin, might contain a sphingomyelin-like lipid fraction which has been isolated from some skins and which appears to exist in this region. Its absence in this skin, an irregularity which has been previously observed with other skins, leaves this point undetermined. The analyses of the lipid of this fraction is interesting for it indicates by its character a transition from corium to epidermal lipid. Particularly significant is the increase in lipid phosphorus and cholesterol as lipid constituents and the higher iodine number and acetyl value as compared to the lipid of the corium material. Whether this is characteristic of this region or the result of insufficient separation of epidermal material is not clear.

The Analysis of the Lipid of the Hair

The lipid material adhering to the hair is similar to that of the horn division from which it is undoubtedly derived. Such differences as do exist between these two divisions consist in the total disappearance of lipid phosphorus and the continued increase in the free fatty acids as a constituent of the lipid of the hair. The presence of cholesterol suggests its stability as compared to the phos-

pholipid molecule. The cholesterol ester content of the hair was not detetmined.

SUMMARY

A study has been made of the lipids of the skin of the steer. The work has been directed toward examining the general nature and distribution of the lipid constituents of the skin. This has been facilitated by a mechanical separation of six approximate horizontal layers of the skin. The following conclusions may be drawn.

The lipids of the corium may be classified into two groups, one composed of the complex lipids and sterols, the other comprising the triglycerides. The former are associated in the skin as active constituents of the protoplasm and they are intimately involved in the physiologic activity of the tissue. The uniform distribution of the phospholipids and cholesterol in the corium region, in spite of large variations in the triglyceride distribution, is in accord with such an interpretation. The triglycerides of the corium are deposited in fat cells which are more concentrated in the region near the subcutaneous tissues. Their quantity varies considerably with the individual skin and is evidently influenced by the diet of the animal.

Whereas the triglyceride content of the corium is characteristic of that region, the wax deposition is equally

characteristic of the epidermal region. In addition, the complex phospholipids and cholesterol constitute a high percentage of the lipids of this region. The sebaceous glands and the nucleated epidermal cells represent the two major sources of lipid in this region. The lipids from these two sources collect upon the epidermal surface where they serve as a protective agency for the skin.

The 'basal' epidermal division is the site of the skin's most active metabolic processes. In this region the sebum results from the degeneration of the nucleated cells which line the sebaceous glands and the phospholipids and cholesterol of these cells are transformed into the waxes and cholesterol esters of the sebum. The sebum is exuded upon the skin surface. During keratinization of the epidermal cells the lipids of these cells undergo a concurrent degeneration, the suggested nature of which appears similar to that occurring in the sebaceous glands (20). A differentiation between two such processes involving degeneration of epidermal cells is not possible from this experiment but the results do indicate the lipid degeneration in the horn division. Thus we have shown the destruction of the phospholipids, the accumulation and gradual oxidation of cholesterol, and a slight change in the character of the waxes of this 'horn' division. Increased saturation, hydroxy-acid formation, and the liberation of free fatty acid at the skin surface have been demonstrated. Oxy-cholesterol is present in the epidermal horn

division but not in the underlying basal division.

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BIBLIOGRAPHY

1. Ameseder, F., Z. physiol. Chem., 52, 121, 1907.
2. Assoc. of Official Agric. Chem., Methods - 2nd Edition, 1921.
3. Banks, A., and Hilditch, T. P., Biochem. J., 25, 1168, 1931.
4. Bloor, W. R., J. Biol. Chem., 68, 33, 1926.
5. Bloor, W. R., J. Biol. Chem., 80, 443, 1928.
6. Bloor, W. R., and Knudson, A., J. Biol. Chem., 27, 107, 1916.
7. Channon, H. J., and Foster, C., Biochem. J., 28, 853, 1934.
8. Eckstein, H. C., and Wile, U. J., J. Biol. Chem., 69, 181, 1926.
9. Kawaguchi, S., Biochem. Z., 221, 232, 1920.
10. Kooyman, D. J., Archiv. Derm. and Syph., 25, 444, 1932.
11. Kooyman, D. J., Archiv. Derm. and Syph., 29, 12, 1934.
12. Koppenhoefer, R. M., and Highberger, J. H., J. Am. Leath. Chem. Assoc., 29, 598, 1934.
13. Leiboff, S. L., J. Biol. Chem., 61, 177, 1924.
14. Linser, P., Dtsch. Arch. klin. Med., 80, 201, 1904.
15. Mayer, A., Rathery, F., and Shaeffer, G., J. de physiol et de path. gen., 16, 581, 1914.
16. McLaughlin, G. D., and Theis, E. R., J. Am. Leath. Chem. Assoc., 19, 428, 1924.
17. O'Flaherty, F., and Roddy, W. T., J. Am. Leath. Chem. Assoc., 30, 290, 1935.
18. Pachur, R., Dermat. Ztschr., 60, 486, 1931.
19. Roffo, A. H., J. de physiol. et de path. gen., 29, 739, 1931.
20. Rothman, S., and Schaaf, F., Handbuch der Haut und Geschlechts Krankheiten, Vol. 1, Part 2, Julius Springer, Berlin, 1929.

BIBLIOGRAPHY
(continued)

21. Unna, P. G., and Golodetz, L., Biochem. Z., 20, 469, 1909.

22. Unna, P. G., and Golodetz, L., Biochem. Z., 25, 425, 1910.