

DISTRIBUTION OF LIPID CLASSES WITH RESPECT TO AREA AND STRATIGRAPHIC LOCATION IN A STEERHIDE*

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ABSTRACT

Variability in the lipid content from one area of a hide to another has prevented accurate comparisons of the chemical compositions of hides. Since the various lipid constituents are the result of different biological processes and serve different purposes within the hide, the contents of certain lipids vary more than others within the hide. In these studies the lipid components were determined in each of five stratigraphic layers taken from 11 areas which represented about two-thirds of the area of the right side of a steerhide. There was considerable variability in the content for each lipid class, both areawise and stratigraphically.

The lipids on the coarse-clipped and fine-clipped hair were determined for nine areas, and these also showed considerable variability.

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INTRODUCTION

The variability of the lipid components of animal hides has been of interest to leather chemists for many years. As early as 1924, McLaughlin and Theis (1) showed that the total lipids varied both areawise and throughout the thickness of the hide. The areawise variability was confirmed by Clarke and Frey (2) for both greasy and non-greasy hides. Koppenhoefer and Highberger (3) showed that the variability of the lipids was still more complicated when they detected differences between the epidermal and corium lipids. This layerwise variability was further defined by Mellon et al. (4) in a study of the fatty acid components of the lipids of the different layers of a fresh steerhide. This study indicated that different wax esters, glycerides, and phospholipids predominate in different strata of the hide. This tends to confirm the belief of Koppenhoefer (5) that the lipids of a hide are intimately associated with its physiology. The hide surface and the hair are also covered with a lipid film. According to Nicolaidis (6), the main sources of these lipids are the sebaceous glands and the keratinizing epidermis.

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Since previous work has been done with a limited number of areas and layers, it has not been possible to tell whether the distribution of the lipid components is uniform over the whole hide. To establish more fully the lipid distribution in a hide, the lipid materials were studied in five layers from 11 areas comprising approximately two-thirds of the entire area of a side. The lipids on the hair were studied from nine alternate areas representing approximately one-half the area of the side.

EXPERIMENTAL

Preparation of Material.—A Black Angus steerhide weighing 72 lb. was obtained, washed, cleaned, and fleshed within four hours of the killing. The hide was split along the backbone, and each side was divided into 18 areas, each ten inches square, six areas from neck to butt, and three from back to belly. Nine alternate areas and two intervening areas on the right side of the hide were selected for analysis. The locations of the areas selected for analysis are shown in Figure 1. Each area was clipped with sheep-shearing clippers to separate the coarse hair. The fine hair was removed with a small animal clipper having a very close cutting blade. The short stubble remaining was then removed with a dry safety razor. The coarse-clipped hair and fine-clipped hair clippings were kept separate. The piece of hide from each area was then split into five layers with a band knife splitting machine. The first and fifth layers were sliced about 0.050 inch in thickness. The remainder of each area was divided into three layers of approximately equal thickness.

Extraction of Lipids.—Each piece of hide or sample of hair was placed into a quart-size fruit jar fitted with a rubber ring seal, and extracted under an atmosphere of nitrogen by mechanical shaking with 500 ml. of Bloor's Solvent, which consists of three parts by volume of 95 percent ethanol, and one part diethyl ether. The first extraction period was six hours and was followed by three additional extractions with fresh solvent for 16 hours each. The extracts were combined and evaporated to small volume on a steam bath and then taken to dryness under nitrogen atmosphere with a rotary evaporator. The residues were extracted twice with 25 ml. portions of n-hexane and once with 25 ml. of diethyl ether, and the solutions were filtered into 100 ml. round-bottom flasks. The extracts were again evaporated to dryness under nitrogen on the rotary evaporator, and the resulting residues were weighed as total lipids.

The efficacy of the extraction procedure was checked by determining the amount of lipids removed in each of the four extraction steps from one of the grain layers. The percentages of the total lipids in each of the four extracts respectively were: 39, 46, 9, and 4 percent. After the fourth extraction, the hide residue was hydrolyzed with 1.5 N HCl for five hours and extracted with ether. The ether layer was purified by washing with water and evaporated to dryness. This residue was extracted with a hexane-ether mixture. The extract

obtained after evaporation was weighed and amounted to only two percent of the total lipid. This represents the residual lipid remaining unextracted in the hide residue.

Separation of Lipid Classes.—The lipids were fractionated into the major class components (wax and sterol esters, glycerides, free sterols plus free fatty acids, and phospholipids) on a silicic acid-celite column according to the procedure of Luddy et al. (6), except that chloroform-methanol (1:1, V/V) was used as the final solvent to elute the phospholipids. The solvents were removed from the eluted fractions in a rotary evaporator, and the lipid residues were dissolved as before with n-hexane and diethyl ether, and transferred to tared flasks. The solvents were completely evaporated, and the weight of each lipid class was determined.

Solvents.—All of the solvents were carefully purified. The diethyl ether was distilled in the presence of metallic sodium. The ethanol and methanol were distilled in the presence of potassium hydroxide. The n-hexane and Skellysolve F (a petroleum ether with a boiling range of 39–49°C.) were distilled twice.

RESULTS AND DISCUSSION

Preliminary experiments showed that for some hide areas approximately 100 square inches of hide are required to obtain sufficient amounts of certain components for significant results. Since the hide available was approximately 60 inches square a pattern of test areas shown in Figure 1 was chosen. Because of the labor involved in the extractions and fractionations, only the odd-numbered areas on the right side of the hide were used for the first set of experiments. Later it appeared that data from areas 8 and 14 would also be desirable.

The numerical data obtained on each sample are recorded in Table I. The first column shows the thickness in mils of the piece extracted. The second column presents the original weight of the wet unextracted sample. The third column gives the weight of the dry extracted residue. The remaining columns contain the lipid data. These are reported as grams per thousand grams of dry fat-free sample to eliminate decimal numbers. For each area, the coarse-clipped hair is designated by C, the fine-clipped hair by F, and the five stratigraphic layers starting with 1 for the grain layer and progressing in order to the flesh layer 5.

It is difficult to picture the actual distribution of the lipid materials from these numerical data. Therefore, to simplify the presentation of the data a composite picture depicting the distribution of each component was prepared by arranging graphs of the layerwise distribution of the component in each area according to the location of the area on the hide. In each of the graphs the five layers are represented in order beginning with the grain layer on the left and ending with the flesh layer on the right of each area graph. All data are presented as grams of lipids per 100 grams of the fat-free dry weight of that area.

TABLE I
STRATIGRAPHIC COMPOSITION OF THE LIPID
CONSTITUENTS OF A STEERHIDE

Area and Layer	Original Thickness mils	Original Weight gm.	Fat-free Dry Weight gm.	Concentration of Lipid Classes g./kg. Fat-free Dry Weight				
				Total Lipids	Wax and Sterol Esters	Glycerides	Sterols and Free Fatty Acids	Phospholipids
1-Coarse Hair		2.5	2.3	256	19	184	58	Nil
Fine Hair		6.5	4.7	172	10	127	35	Nil
1	56	162	48	37	5	10	13	10
2	27	56	20	16	4	5	1	6
3	20	22	8	38	3	18	3	15
4	30	22	8	28	3	18	3	5
5	49	70	22	185	6	172	1	5
3-Coarse Hair		4.5	3.0	99	11	69	20	2
Fine Hair		6.0	5.0	95	8	67	19	Nil
1	53	53	13	107	19	31	22	35
2	16	7	2	114	50	20	35	10
3	15	30	9	36	8	7	8	14
4	15	28	10	24	6	5	6	5
5	55	92	29	142	Nil	134	8	Nil
5-Coarse Hair		2.0	1.1	229	27	161	39	Nil
Fine Hair		4.5	3.8	121	15	81	26	Nil
1	54	85	22	56	9	16	5	26
2	33	52	19	14	3	3	1	8
3	32	35	13	16	8	3	2	3
4	32	42	15	18	4	8	2	4
5	52	69	23	72	Nil	7	1	Nil
7-Coarse Hair		4.0	2.4	128	16	84	29	Nil
Fine Hair		6.0	4.4	121	16	79	26	Nil
1	54	114	31	66	8	31	20	7
2	26	47	18	17	2	9	5	2
3	26	50	19	30	1	25	1	3
4	23	34	13	34	3	27	3	1
5	52	89	32	80	Nil	75	2	3
8-1	50	73	13	93	23	24	34	11
2	35	74	26	17	2	9	5	1
3	35	51	19	56	1	50	5	Nil
4	27	37	16	35	3	28	4	Nil
5	45	51	27	86	Nil	80	6	Nil
9-Coarse Hair		4.5	3.1	97	12	58	27	Nil
Fine Hair		5.5	4.4	98	16	49	33	Nil
1	45	81	20	73	11	19	5	38
2	35	40	14	22	2	9	6	6
3	37	61	23	21	3	14	3	1
4	40	67	25	39	Nil	36	2	2
5	46	55	20	73	Nil	62	11	Nil

DISTRIBUTION OF LIPID CLASSES IN A STEERHIDE

TABLE I (Continued)

Area and Layer	Original Thickness mils	Original Weight gm.	Fat-free Dry Weight gm.	Concentration of Lipid Classes g./kg. Fat-free Dry Weight				
				Total Lipids	Wax and Sterol Esters	Glycerides	Sterols and Free Fatty Acids	Phospholipids
11-Coarse Hair		4.5	3.1	114	24	63	26	Nil
Fine Hair		5.0	3.7	74	13	40	20	Nil
1	57	194	58	30	4	13	6	8
2	33	73	27	59	1	44	7	8
3	35	78	28	96	Nil	91	5	Nil
4	33	46	16	166	Nil	150	16	Nil
5	45	65	21	202	Nil	199	3	Nil
13-Coarse Hair		5.0	2.0	108	12	76	22	Nil
Fine Hair		5.0	3.4	143	17	93	35	Nil
1	42	75	18	120	9	65	22	24
2	48	77	27	47	Nil	33	4	10
3	55	97	36	146	5	136	3	2
4	50	84	28	436	Nil	424	11	1
5	54	85	28	405	Nil	391	12	3
14-1	48	69	19	104	9	58	15	23
2	35	49	17	23	1	15	4	3
3	43	83	31	91	Nil	83	8	Nil
4	45	84	30	80	Nil	74	6	Nil
5	50	96	33	183	Nil	177	7	Nil
15-Coarse Hair		8.0	6.1	78	7	39	31	Nil
Fine Hair		6.0	4.0	76	14	31	33	Nil
1	51	97	28	41	7	14	15	6
2	35	39	13	54	8	28	10	9
3	39	69	24	42	5	28	6	3
4	35	63	22	56	5	42	5	3
5	50	86	30	72	8	58	4	4
17-Coarse Hair		7.0	4.0	128	21	76	25	1
Fine Hair		6.0	3.3	113	15	68	29	Nil
1	56	135	39	31	5	10	9	8
2	39	91	33	38	1	30	2	5
3	38	47	17	89	Nil	84	3	3
4	41	100	36	107	Nil	98	6	4
5	48	68	23	140	Nil	131	7	2

The detailed description of the distribution of the total lipids is shown in Figure 2. The range between the high and low values for an area varies from area to area. Some areas, especially those in the central and belly regions, show only a small variation of lipid content from layer to layer. The greatest variation comes in the kidney area where there is a tremendous increase in the amount of lipids in the three layers approaching the flesh side. This same trend is also shown in lesser degree in the adjacent areas. Most of the other areas show

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increased amounts of lipids in the grain and flesh layers over the amount found in the intermediate layers. For the neck and fore shank areas this increase in the flesh layer is considerable.

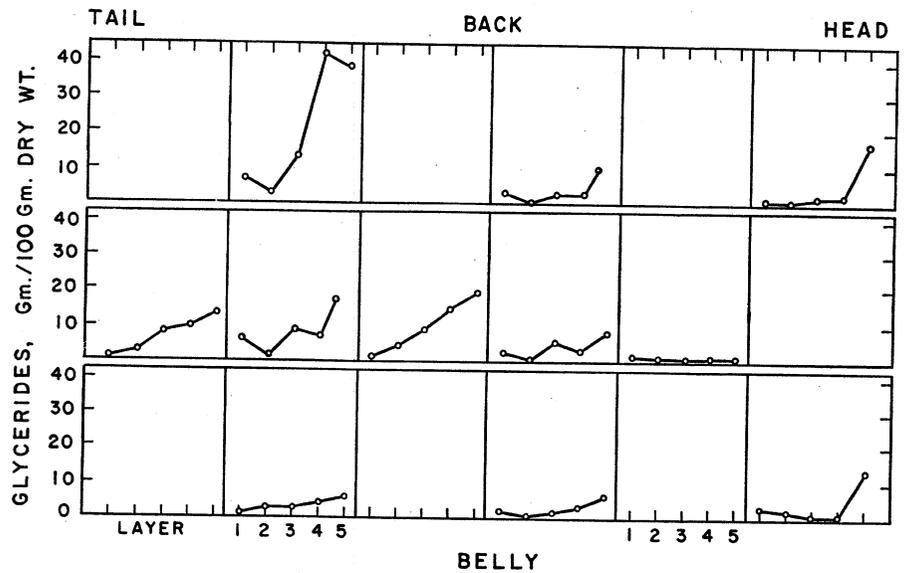


FIGURE 3.—Distribution of glycerides in selected areas of the right side of a steerhide.

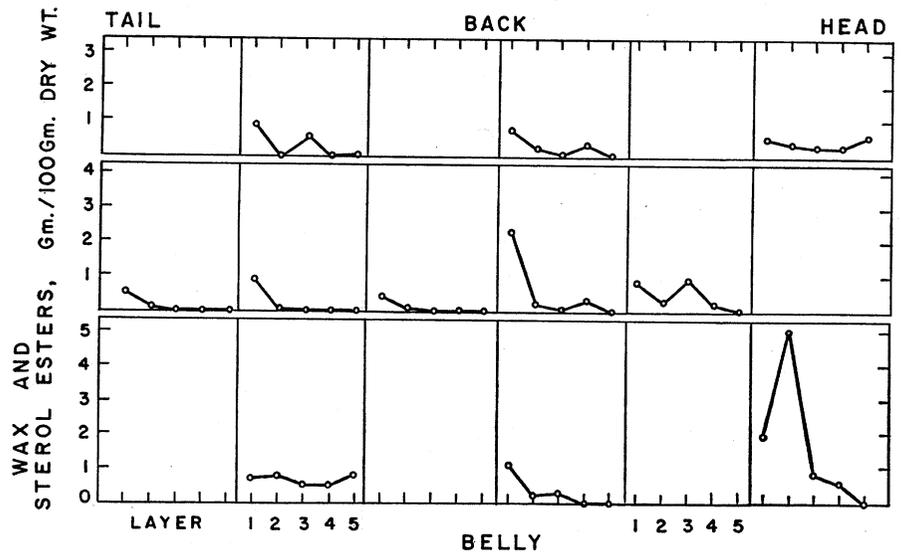


FIGURE 4.—Distribution of wax and sterol esters in selected areas of the right side of a steerhide.

These data support in large degree the findings of McLaughlin and Theis (1) that the lipid content of a steerhide is greater in the grain and flesh layers than in the corium, although there are a few areas where this trend apparently does not exist or is extremely limited.

When the total lipids are fractionated into the major lipid classes, the distribution patterns for the components show considerable variation. Since most of the lipid material in hides is of a glyceride nature, it is not surprising that the pattern (Figure 3) for glycerides follows closely the pattern for the total lipids.

The distribution of the wax and sterol esters is shown in Figure 4. Since there are considerably less wax and sterol esters than glycerides, the ordinate axis scale is only one tenth that used for the previous figures. In general, most of the wax and sterol esters are found in the grain layer with most of the other layers having negligible amounts. There is only one marked exception, and this is in the right fore shank where there are about three times as much wax and sterol esters in the second layer as are present in the first layer. The grain layer of this area as well as that in the middle of the side have much greater wax and sterol ester contents than the other grain areas.

With the separation technique employed the free sterols and free fatty acids are determined together, and the distribution of these components is shown in Figure 5. In many respects the distribution is the same as that for the wax and sterol esters, and this would lead to the assumption that the free sterols predominate in the same areas where the wax and sterol esters predominate. Most of the additional variations might be explained by assuming that the free fatty acids are higher in all layers of the kidney region, in the grain layer along the backbone, and in the flesh layer of the belly and fore shank.

The distribution of the phospholipids, which is shown in Figure 6, exhibits considerable variability. In most areas the amount of phospholipids is higher in the grain and the adjacent layer than it is in the remaining three layers. There is very little phospholipid in the flesh layer. The presence of an appreciable portion of the phospholipids in the second layer indicates that the phospholipids either have a function beyond the grain layer or are capable of migrating more deeply into the hide than most of the other lipids. This is in agreement with the findings of Nicolaides (6) who reported that the phospholipids are higher in the basal layer of the dermis and decrease toward the upper layer. There are two regions of high concentration of phospholipids. One is in the kidney region, and the other is in the belly and fore shank region. The amount of phospholipids decreases gradually with distance from these two regions.

If the data on the distribution of the individual lipids were averaged for all the areas, the results would agree quite favorably with the findings of Koppenhoefer and Highberger (3, 5), on their composite samples representing chiefly

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the bend area of a hide. Although their data show the major trends described here, their description is not accurate for certain areas which show little or no variation from layer to layer.

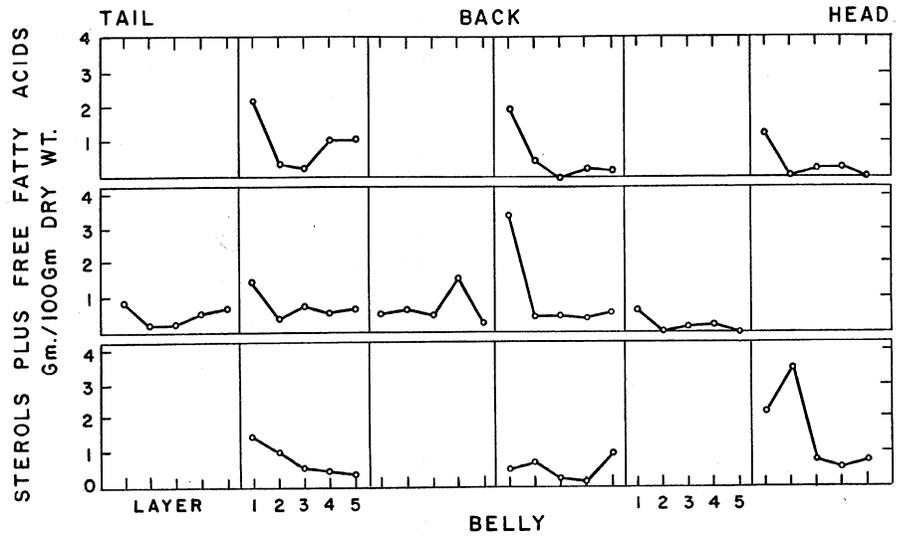


FIGURE 5.—Distribution of sterols plus free fatty acids in selected areas of the right side of a steerhide.

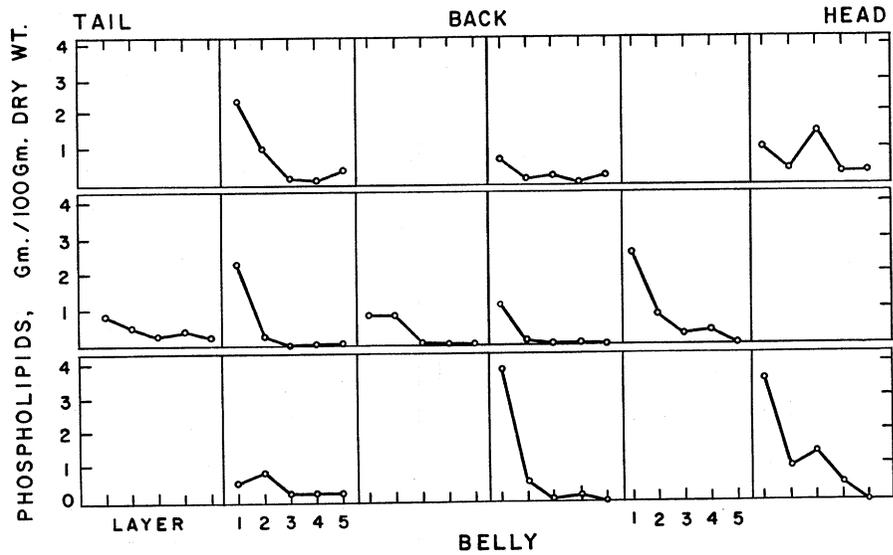


FIGURE 6.—Distribution of phospholipids in selected areas of the right side of a steerhide.

Although the graphs show that the large amounts of any particular lipid occur in only a few areas or stratigraphic layers, one cannot say that the lipid components are relatively constant in the other areas. Even though the trend line for the graphs may look to be quite horizontal, the minor variations represent two- or three-fold increments of the lowest values. Therefore, it is doubtful whether a correction for fat content of a hide piece can be valid unless the fat is determined in the same sample being analyzed.

The distribution of the total lipids on the hair bears little resemblance to the distribution of total lipids in the grain layer. For most of the areas, the total lipid concentrations for the coarse-clipped and fine-clipped hair are quite similar. However, in the shoulder area the amount of total lipids on the coarse hair is considerably greater than the amount on the fine hair. This trend is reversed for the kidney area. There is also less lipid on both the coarse and fine-clipped hair of the belly region than there is along the backbone of the animal.

The glycerides have a similar trend, since they constitute the greatest portion of the total lipids. The distribution of the glycerides on the hair does not follow the distribution of the glycerides in the grain layer.

The wax and sterol esters appear to have a much different distribution. Their highest values on the coarse-clipped hair occur in the middle of the side, whereas the values for the fine-clipped hair agree quite well with those over the rest of the hide. The wax and sterol ester concentration of the fine-clipped or the coarse-clipped hair is not related to the concentration in the grain layer.

Since the free sterols and free fatty acids are probably related to the wax ester, sterol ester and glyceride concentrations, it is not surprising to find that these components also are not related to the concentrations in the grain layer.

There appears to be no phospholipid on either the coarse-clipped or the fine-clipped hair. Apparently, the phospholipids are not excretory products of the skin.

CONCLUSIONS

1. There is considerable variability in the concentrations of the lipid components throughout the hide, even between contiguous areas and stratigraphic layers. Because of this, corrections made for the fat content of a hide sample must be based on the lipid analysis of that particular sample.

2. In the hide the glycerides occur in highest amounts in the kidney region where they increase from moderate levels in the grain to very high levels in the flesh side. For the other regions of the hide, the glycerides attain moderate amounts only in the flesh layer.

3. The wax and sterol esters are found principally in the grain layer. The fore shank area has the highest concentration, and here the wax and sterol esters predominate in the second layer. The free sterols appear to follow the same trend.

4. The free fatty acids appear to be concentrated in the kidney region, in the grain layer along the backbone, and in the flesh layer of the belly region.
5. The phospholipids are found primarily in the two layers near the grain and have comparatively small values elsewhere in the hide. The highest concentrations are in the kidney, fore shank and belly regions.
6. The distribution of the lipids on the hair is quite different from that within the hide. On the hair the highest concentrations of lipids occur in the shoulder area. However, the concentration of lipids on the coarse-clipped hair is greater than on the fine-clipped hair, except in the kidney region, where the reverse is true. The phospholipids are notably absent from the hair.

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DISCUSSION

PROFESSOR RODDY: After the paper presented by Mrs. Tancous, where we are talking in terms of something like 1700 hides, and now in this particular case one hide, in justification to the two authors, I should like to bring up this particular point. I can remember when Koppenhoefer and Highberger did original work on lipid fractionation of the kidney areas of hides. In those days the only thing used to split hides was the old straight hand razor. This represented a tremendous amount of work.

However in this present case while we have only one hide, we certainly have a number of layers. Fortunately at the present time we have a very nice device to cut it into the five layers. But it is still a very tedious operation and I should like to say that Dr. Bitcover and Dr. Mellon are to be congratulated for their fortitude in going through this tremendous amount of work to give us further information on the various lipids as they are located in different areas of the hide. Only those who have worked with splitting hides in any shape or form, much less fresh hides, have any appreciation of the technique required in addition to the procedures of extraction and analysis that are involved in doing this type of work. I say this, having a little background in the way of just preparing

hides for analysis, and I should like to congratulate them on the work they have presented at this time.

In discussion of the work presented in this paper, it is well to keep in mind the problems involved in the techniques used. To begin the discussion, I should like to ask Dr. Bitcover, first of all, a question on the weight of the hide and, in addition, the technique of splitting it into five layers. This is important to anyone wanting to go into this particular type of work.

DR. BITCOVER: As Mr. Roddy said, this work was quite tedious. We obtained an Angus hide, which weighed about 70 pounds and we brought it back to the laboratory as soon as we could. Within four hours we had shaved it, cut it into strips and we cut the strips into the ten-inch squares that I referred to. We weighed the squares and split them on a band knife splitter into five layers, and put them into Bloor's solvent mixture which had been prepared ahead of time and had been chilled in the refrigerator.

We did all of this to minimize any changes that could happen to the fat materials. We worked as fast as we could to get all these lipids into the solvent mixtures, which we then stored under nitrogen in a cold room until we analyzed them. We prepared 55 samples, altogether, for analysis.

PROFESSOR RODDY: Thank you. I should like to have had this discussion presented in the formal paper, in regard to the extensive technique involved. I think it is well to call the attention of the audience to the magnitude of the work involved.

In the graph comparing total lipids versus glycerides, you made the point that they were very similar. Of course this is true. You can almost superimpose the total lipid graph on the glycerides graph. Is this actually the true picture of what you had for the cross-section of the hide, or is this a matter involving the splitting technique?

I think you mentioned in the formal paper that both the hair side layer and flesh side layer were taken off at 0.050 inch. The center three layers would naturally vary. Would you like to comment on this?

DR. BITCOVER: The top and bottom layers were split to 50/1000 of an inch, as Mr. Roddy says. We did not attempt to follow the structure of the hide. We took the remainder and split it into approximately three equal thicknesses. Thus, the belly would be much thinner than the backbone, so naturally that difference would be reflected in the thickness of the middle layers. However, the analysis of the glycerides was quite similar to the total lipids, because glycerides compose most of the lipids in the hide.

DR. LOLLAR: With respect to that particular point, the 50/1000th inch grain layer, in general, where did that go with reference to the depth of penetration of the hair follicle?

DR. BITCOVER: We didn't measure that. We just wanted to get areas of equal volume on the top and bottom layers so that we could compare the lipids from the top and bottom layers on the basis of equal volume. With the middle layers, we couldn't do that precisely.

DR. LOLLAR: Would I be correct in assuming, however, that there were still hair follicles penetrating into the second layer, or was the second layer in general free, by visual observation?

DR. BITCOVER: This second layer was generally free.

PROFESSOR RODDY: That was the point I was trying to get at in regard to splitting into five layers. Did you in every case go below or include the sebaceous or oil gland? In most cases you indicated you did run into a fair amount of the phospholipids, also sterols?

DR. BITCOVER: Yes.

PROFESSOR RODDY: Are there any questions from the floor on this research? If not, I'd like to turn the meeting back to Dominic Meo, and I thank you very much for your interest.
