cator, and the volume adjusted to 2 ml with acetone-alcohol. The remainder of the procedure for total cholesterol was as described by Schoenheimer and Sperry(3).

Results. The data are shown in Table II. The earlier single observation of Swell and Treadwell(1) on the markedly lower hydrolysis rate of a branched chain ester in comparison with the n-acid ester is confirmed and extended. All 8 of the branched chain esters studied were hydrolyzed at a slower rate than the corresponding n-acid esters, and with 2 of the esters the observed hydrolysis was within experimental error of the assay. The data also demonstrate effects of location, degree, and size of branching. Methyl-branches at carbons 2 and 3 of the acids have about the same effect in depressing the hydrolytic activity, while a methyl-branch at carbon 4 has little effect. A second methyl-branch on carbon 2 produces a further depression in activity over that with one methyl-branch. An ethyl-branch at carbon 2 gives a greater depression than a methyl at the same location. In general the closer the branching is to the ester bond and the greater the size of the branch, the greater the depression in hydrolytic activity. These effects are most likely due to spatial interference with the approach of substrate to the active site on the enzyme.

Summary. The synthesis and characteristics of a series of branched chain fatty acid ester of cholesterol are described. The hydrolysis of these esters by pancreatic cholesterol esterase was determined under standard conditions. The branched chain acid esters were hydrolyzed at lower rates than corresponding n-acid esters. The closer the location of the branch to the ester bond, the greater the depression in activity. Esters with ethyl groups on carbon 2 were hydrolyzed at a slower rate than those with methyl groups at this location.

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Total Lipid, Cholesterol, and Phospholipid Content of Non-Irradiated and Irradiated Skin (Rabbits).* (23812)

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Though the lipid composition of the skin may be important for its many functions, the present knowledge of skin lipids is still scanty (1). Only a few species have been examined. Furthermore, the methods which have been available for some comprehensive studies(2) have been inadequate to allow more or less complete characterization of the lipids. The present investigation has been aimed at an examination of the lipids of the rabbit skin with methods developed more recently, as well as at a study of the effect of ionizing irradiation. This paper reports the results of the examination of total lipids, cholesterol, and phospholipids.

Material and methods. We used young adult female albino rabbits about 1700 g weight. Animals were kept on balanced diet *ad libitum* for one week preceding experiments. Hair of both flanks was removed avoiding carefully damage to skin. "Superficial" irradiation was applied at 4000 r or 8000 r (Ritter self rectified machine; Machlett OEG60 Beryllium window tube; 22 cm focal distance; 30 KV; 10 MA; h.v.p.o. 06 mm). Calibration was performed with Victoreen r-meter. One flank of the rabbit was irradiated, the other serving as control, was protected with lead plate. Food was withdrawn following irradiation. Rabbits were

* This work was carried out under contract with U. S. Atomic Energy Commission.

killed by intravenous injection of air 24 hours or in some instances 48 hours after exposure. Immediately after sacrifice, the skin of irradiated and control sides was carefully removed with scalpel so that none of the subcutaneous tissue was included. Occasional histologic checks proved that specimens were free of subcutaneous material. The aliquots for lipid- and dry weight determinations were weighed immediately on a Gram Atic balance. Specimens for lipid examinations were kept frozen with dry ice for the short time required until lipid extraction could be carried out. Method for extraction and purification of lipids used followed the technic of Folch *et al.* (3) with the exception that the very small fluffy. proteolipid layer, which appeared on top of the chloroform phase after washing of lipid extract. was entirely removed. This small fluff. which usually contained less than 20 μg of lipid phosphorus, might otherwise contaminate the extracts sufficiently to cause erroneously high phosphatidylserine figures. The washed lipid extract was evaporated to dryness at 60°C under reduced pressure. The residue was dissolved in hot chloroform:methanol 2:1. The chloroform:methanol extract thus obtained was filtered through a Buchner funnel with a fritted disc of medium porosity. The funnel was washed with hot chloroform:methanol. The filtrate was evaporated again to dryness as described above and the residue was finally extracted with chloroform. This chloroform extract was sufficiently free of nitrogenous impurities. Additional passing of the material through a cellulose column(4) capable of removing even traces of such impurities as amino acids, did not affect the results to be described and was therefore omitted for these determinations. Total lipids were determined by weighing. An aliquot of the chloroform extract was evaporated at room temperature under reduced pressure. The evaporate was dried in vacuum desiccator over P_2O_5 for weight determination. The chemical analyses were carried out with the following procedures: Free- and total cholesterol by the methods of Brand and Sperry(5); total phospholipids by the method of Sperry(6); phospholipids easily hydrolysable with alkali by the method of Schmidt, Tannhauser *et al.*(7); ethanolamine and serine by the method of Axelrod, Reichenthal and Brodie(8); choline by the method of Hack(9); and aldehydes by the method of Feulgen and Gruenberg(10).

Results. The average percent lipid composition of non-radiated and irradiated skin of rabbits based on the dry weight or on the total phospholipid content of the skin are given in Table I and Table II respectively.

Table I shows that cholesterol of the non-

TABLE I. Percent Lipid Composition* of the Non-Irradiated and Irradiated Skin of Rabbits Based on Dry Weight.

	No. of rabbits†	Total	Cholesterol		P-lipids	
			Total	Free	Total	Easily hydrolyzed with alkali
Control	18-33	7.51 ± .93	.43 ± .02	.39 ± .02	1.32 ± .05	1.17 ± .05
Irradiated	18-33	7.89 ± .65	.47 ± .02	.41 ± .02	1.40 ± .06	1.22 ± .06

		"Cephalin"‡			Lecithin
		Total	Ethanolamine containing P-lipids	Serine containing P-lipids	
Control	18-33	.41 ± .016	.28 ± .012	.13 ± .013	.62 ± .045
Irradiated	18-33	.43 ± .026	.29 ± .014	.14 ± .013	.67 ± .048

* Means ± stand. error.

† Only lecithin values were determined in less than 25 rabbits.

‡ The term "cephalin" was used only to allow comparison with previous studies which did not distinguish between ethanolamine or serine containing phospholipids. The terms ethanolamine containing P-lipids or serine containing P-lipids were used instead of phosphatidyl ethanolamine and phosphatidyl serine respectively, because studies not given here have established that these fractions might contain also small amounts, up to 7%, of plasmologens, particularly ethanolamine-plasmologens.

TABLE II. Percent Phospholipid Composition* of Non-Irradiated and Irradiated Skin of Rabbits Based on Total Phospholipids.

		"Cephalin"‡			Lecithin	P-lipids	
	No. of rabbits†	Total	Ethanolamine containing P-lipids	Serine containing P-lipids		Sum of lecithin and cephalin	Easily hydrolyzed with alkali
Control	18-33	34.2 ± 1.3	21.8 ± .6	12.5 ± .8	48.1 ± 2.1	81.6 ± 3.0	87.7 ± 1.6
Irradiated	18-33	31.8 ± 1.6	20.7 ± .7	11.0 ± 1.1	52.9 ± 3.1	78.6 ± 4.0	82.1 ± 4.6

* Mean ± stand. error.
† Only lecithin values were determined in less than 25 rabbits.
‡ Use of terms "cephalin," ethanolamine containing P-lipids and serine containing P-lipids has been explained in Table I.

irradiated or irradiated skin of rabbits comprises mainly free cholesterol. The cholesteryl ester values are not included in the Table, but it can be calculated easily that they amount only to 9.3% or 12.8% of total cholesterol content of non-irradiated or irradiated skin respectively. Both free and total cholesterol values have not been affected significantly by the ionizing irradiation.

Phospholipids of the rabbit skin comprise 3 well defined fractions, *viz.*, lecithin, phosphatidyl ethanolamine and phosphatidyl serine. Occurrence of an additional but rather small plasmalogen fraction, ethanolamine-plasmalogen, has been indicated in Table I. The phospholipid composition of non-irradiated and irradiated rabbit skin based on total phospholipid content is given in Table II.

Table II demonstrates that the phospholipids of non-irradiated or irradiated skin of rabbits alike comprise about 50% lecithin and somewhat over 30% "cephalin." The "cephalin" itself consists of about two-thirds of ethanolamine containing phospholipids (mostly phosphatidyl ethanolamine and small amounts of plasmalogens) and of about one-third of phosphatidyl serine. The sum of all these phospholipid fractions, however, amounts to only about 80% of total phospholipid values. It must thus be assumed that other phosphorus containing compounds must have been present in addition. While no definite opinion about the exact chemical composition of these unidentified lipids can be expressed here, it may be pointed out that only about 82-88% of the phospholipids have been hydrolyzed easily with alkali. Since lecithins and "cephalins" usually can be hydrolyzed in such a way this is an indication that the unidentified lipids may be resistant to alkali-hydrolysis.

Conclusions. 1. Data have been presented on total cholesterol, free cholesterol, phospholipid, and total lipid content of non-irradiated and x-ray irradiated skin of rabbits. 2. Cholesterol of both non-irradiated or irradiated skin alike comprises mostly free cholesterol and only relatively small amounts, 9-13%, cholesteryl esters. 3. Phospholipids of both non-radiated or irradiated skin consists of about 48-53% lecithin, 21% phosphatidyl ethanolamine, 11-12% phosphatidyl serine, rather small amounts of ethanolamine-plasmagens, and a relatively large amount, 18-21%, of unidentified phospholipids. 4. Ionizing irradiation of the skin of rabbits has not significantly affected the lipids under study.

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