transferred to newborn animals. Cells from uninjected rabbits, transferred to x-irradiated adults simultaneously with antigen, or after contact with antigen *in vitro,* produced antibodies against Shigella antigens(5), but not against protein antigens(7). Cells from uninjected animals in tissue culture are generally recognized not to produce antibodies(5), but one report(8) has indicated that cells from animals non-specifically stimulated by endotoxin produced antibodies following an *in vitro* contact with protein antigen.

Summary. Spleen cell suspensions from normal hens inoculated onto CAM's with BSA, or after *in vitro* contact with BSA, failed to produce demonstrable antibodies. Whole spleen cell and spleen white cell suspensions, taken from previously normal hens 36 to 48 hours after intravenous injection of BSA and inoculated onto CAM's, produced significant amounts of antibody without further contact with antigen 4 to 7 days after transfer. The white cell suspensions produced somewhat more antibody than did the whole spleen suspensions. Whole spleen cell suspensions, taken from hens 48 hours after intravenous reinjection of BSA, produced significant amounts of antibody, without further contact with antigen, 2 to 6 days after transfer to CAM's. Whole spleen cell suspensions, from hens stimulated by BSA injection more than 7 days previously, inoculated onto CAM's with BSA, or after *in vitro* contact with BSA, failed to produce demonstrable antibodies.

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Cholesterol Esterases. VII. Hydrolysis of Branched Chain Esters by Pancreatic Cholesterol Esterase.* (23811)

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In a study(1) on the relative specificity and activity of hydrolytic and esterifying cholesterol esterase in pancreatic extracts in a series of 13 fatty acids, it was observed that the enzyme was most active in hydrolysis with short chain fatty acid esters, and most active in esterification with long chain acids. An interesting exception to the high hydrolytic activity with short chain fatty acid esters was found with cholesterol isovalerate. This 5-carbon branched chain ester was hydrolyzed at 9% of the rate observed with the n-valeric acid ester. This markedly lower activity with a branched chain acid suggested the possibility of inhibition of the enzyme owing to steric hindrance effects. It was also shown that in a mixture of 2 esters the more slowly hydrolyzed ester competes with the more rapidly hydrolyzed one for the enzyme. In this paper we present data on the hydrolysis of a series of cholesterol esters, of fatty acids of varying chain length with differing degree, location, and size of branching by pancreatic cholesterol esterase.

Preparation and characteristics of branched chain esters. The esters were synthesized as described by Swell and Treadwell(1) and were recrystallized until melting points were

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TABLE I. Physical Constants of Cholesterol Esters.

Ester	Molecular wt: Theoretical	Molecular wt: Found	Melting point, °C*	$[\alpha]_D^{20}$†
n-Butyrate	456.7	458.8	111.0	−35.4
Isobutyrate	"	467.3	124.5	−31.8
n-Valerate	470.9	471.7	90.0	−34.3
Isovalerate	"	463.0	111.0	−32.2
DL-Methylethylacetate	"	484.1	107.5	−33.8
Trimethylacetate	"	469.3	161.0	−34.6
n-Caproate	484.9	479.0	94.5	−32.6
Isocaproate	"	476.9	99.5	−34.4
2-Methylvalerate	"	492.3	103.0	−29.0
Diethylacetate	"	470.2	93.0	−32.8
2-Ethylcaproate			53.0	

* Clear melting point(1).
† Determined on 5% solutions in chloroform.

constant and they gave no precipitate with digitonin. For determination of molecular weights of these branched chain esters, it was necessary to modify the saponification method described by Jamieson(2). In preliminary determinations it was found that less than 50% of some esters was saponified in the 2-hour period specified by Jamieson. A time study of saponification of cholesterol diethylacetate, the most resistant ester in the series, showed that refluxing for 8 hours gave complete saponification. The modified method included use of 200 mg quantities of the esters, a comparable decrease in volume of the alcoholic potassium hydroxide solution, and an 8 hour period of saponification. The molecular weight, melting point, and specific rotation of each of the esters used are shown in Table I. *Determination of hydrolytic activity of cholesterol esterase.* Composition and preparation of substrate mixtures, preparation of the pancreatic extract, and conditions for assay of the hydrolytic activity were the same as described previously(1). Samples were removed for analysis at 0, 2, 4, 6, 12 and 24 hours of incubation. The 0 and 24 hour samples were analyzed for total cholesterol to insure uniformity of the digests during incubation, and all samples were analyzed for free cholesterol. The free cholesterol content was used for calculating the degree of hydrolysis and determining the linear portion of the time curve. The activity (mg of cholesterol liberated/g of tissue/hour) was calculated from a point, usually 2 hours, on the linear portion of the time curve. The Schoenheimer and Sperry method(3) was used for determination of free cholesterol in the digest, but for total cholesterol it was necessary to modify the saponification step in the method. An aliquot of the (1-1) acetone-alcohol extract of the enzyme digest in a 15 ml centrifuge tube was evaporated to dryness, and 2 ml of 6% potassium hydroxide in aldehyde-free absolute alcohol added. After thorough mixing, the tube was placed in an oven at 67°C for 8 hours, neutralized with 10% hydrochloric acid using one drop of phenolphthalein as indi-

TABLE II. Hydrolysis of Cholesterol Esters by Pancreatic Cholesterol Esterase.

Ester	Structure of acid	Hydrolytic activity*	Hydrolysis ratio†
n-Butyrate	C–C–C–COOH	335	100
Isobutyrate	C–C(C)–COOH	17	5
n-Valerate	C–C–C–C–COOH	353	100
Isovalerate	C–C(C)–C–COOH	29	9
DL-Methylethylacetate	C–C(C)–C(C)–COOH	31	9
Trimethylacetate	C–C(C)(C)–COOH	1	0
n-Caproate	C–C–C–C–C–COOH	433	100
Isocaproate	C–C(C)–C–C–COOH	384	89
2-Methylvalerate	C–C–C(C)–C–COOH	14	3
Diethylacetate	C–C(C)(C)–C–COOH	7	2
2-Ethylcaproate	C–C–C–C(C)–C–COOH	1	0

* mg of cholesterol liberated per g pancreatic tissue per hr.
† Hydrolytic activity for ester/hydrolytic activity for ester of n-acid of same No. of carbons, × 100.

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

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