dogs. The quantitative ratio of virus particles in blood to that in either of these fluids was similar (approximately 10^2 or 10^3:1).

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Esterification of Cholesterol and Hydrolysis of Cholesterol Oleate by Homogenates of Bovine Adrenal Glands and Their Subcellular Components.* (29779)

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In an earlier study(1) rats were injected intravenously with a very low-density lipoprotein fraction prepared from the chyle of donor rats that had been fed ^{14}C-labeled cholesterol. About 75% of the labeled cholesterol in this chyle preparation was in the esterified form. Twenty-four hours after the injection about 90% of the ^{14}C-sterol in liver, small intestine, adipose tissue, muscle, bone and spleen of the recipient rats was in the free form. The situation in the adrenal glands was unique: the proportion of the injected ^{14}C-sterol in the free form was higher than that of the injected preparation at the earliest intervals; later, however, the proportion in the free form decreased, and by 24 hours about 90% of the sterol ^{14}C in the gland was esterified. This finding suggested that the adrenal gland first hydrolyzed the cholesterol esters of the chyle lipoproteins, but subsequently resynthesized cholesterol esters characteristic of the gland. In a later study(2) in which chyle lipoproteins were incubated with a variety of rat tissues, it was shown that the adrenal gland was among the tissues possessing a very high hydrolytic activity. Cholesterol esterifying activity has been demonstrated in adrenal homogenates of dogs(3), hogs(4) and rats(5). The present report deals with some characteristics of the esterifying and hydrolytic reactions in the bovine adrenal gland and with their intracellular localization.

Materials and methods. Preparation of tissue homogenates and subcellular fractions. Bovine adrenal glands, obtained from local abattoirs, were cleared of visible adipose tissue and homogenized with three volumes of an ice-cold 0.9% NaCl† solution in a motor-driven, Potter-Elvehjem type homogenizer equipped with a tight-fitting teflon pestle. The homogenate was first centrifuged at 350 $\times$ g for 15 minutes to sediment the cellular debris, which was discarded. The supernatant fraction was designated supernatant A. Supernatant A was centrifuged at 10,000 $\times$ g for 10 minutes to sediment Pellet I, and the supernatant from this centrifugation was again centrifuged at 100,000 $\times$ g for 45 minutes to sediment Pellet II. The supernatant fraction obtained by centrifugation at 100,000 $\times$ g for 45 minutes was designated cell sap. Pellets I and II were separately resuspended by homogenizing them gently by hand in the Potter-Elvehjem homogenizer in a volume of 0.9% NaCl equal to the original volume of supernatant A. En-

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† In some experiments 0.25 M sucrose was used in place of 0.9% NaCl and the same results were obtained.

TABLE I. Esterification of Cholesterol-4-^{14}C by Bovine Adrenal Preparations. Average and ranges of 4 determinations are recorded below. Each incubation mixture contained 0.5 ml of the cell fraction, 4.5 ml of acetate buffer, pH 5.0,* 0.1 M and 10,000 cpm of cholesterol-4-^{14}C. When cofactors were added they were dissolved in buffer and consisted of the following: 50 μmoles of ATP, 1 μmole of coenzyme A and 50 μmoles of $MgCl_2$ per incubation flask. The final volume of the incubation mixture was 5.0 ml. Incubation was carried out for 3 hr at 37°C.

	% of cholesterol-4-^{14}C esterified			
	Whole gland		Adrenal cortex	
Cell fraction	No cofactors added	Cofactors added	No cofactors added	Protein, mg/flask
Supernatant A	4.67 (4.37-5.03)	5.00 (4.90-5.10)	4.45 (4.37- 4.53)	11.2 (10.7 -13.7)
Pellet I	2.44 (2.42-2.45)	2.61 (2.51-2.71)	2.50 (2.01- 2.99)	5.35 (4.27- 6.25)
" II	1.76 (1.53-2.00)	1.67 (1.64-1.71)	1.86 (1.60- 2.12)	2.62 (2.25- 3.57)
Cell sap	7.48 (7.00-8.06)	6.16 (5.75-6.37)	8.27 (6.00-10.5)	7.80 (6.25- 8.75)

* In preliminary studies on whole bovine adrenal homogenates, the pH optimum for esterification of cholesterol-4-^{14}C was found to be pH 5.0.

zymatic activities were determined in the subcellular fractions immediately after their preparation. All the above operations were carried out in a 4°C cold room.

Radioactive substrates and cofactors. The labeled cholesterol-4-^{14}C (specific activity: 13.7 mc/mmole) was purchased from the New England Nuclear Corp., Boston, Mass. Cholesterol-7-α-^{3}H-oleate (specific activity: 0.34 mc/mmole) was synthesized by the procedure of Page and Rudy(6). Both labeled substrates were purified on silicic acid columns(7).

Adenosine triphosphate (ATP-Disodium salt) and coenzyme A were purchased from Pabst Laboratories, Milwaukee, Wis.

Incubation procedures. Aliquots of the homogenates and of subcellular fractions were incubated for 3 hours at 37°C with continuous shaking in stoppered flasks containing either 0.1 M Na acetate (pH 5.0 or below) or 0.1 M K phosphate (pH 6.0 or above) buffer and 10,000 cpm of the radioactive substrate dissolved in 0.1 ml of acetone. The final volume of the incubation mixture was 5 ml. Air was used as the gas phase, and the reaction was terminated by addition of 15 ml of a 3:1 (v/v) ethanol-ethyl ether mixture.

Analytic procedures. Total lipids were extracted from the incubation mixtures as described in(1). Esterified and free sterol fractions were separated from total lipid extracts by silicic acid chromatography(7). The dried lipid fractions eluted from the column were dissolved in 15 ml of toluene containing 45 mg of 2,5-diphenyl-oxazole and 1.5 mg of 1-4-bis-2(5-phenyl-oxazolyl)-benzene and assayed for ^{14}C in a Packard Tri-carb liquid scintillation spectrometer.

For determination of sterol content, digitonin-precipitable sterols were isolated from esterified (after hydrolysis) and free sterol fractions and purified according to Sperry and Webb(8). The purified digitonides were dissolved in known volumes of anhydrous methanol, and the sterol contents in aliquots of the methanol solution were determined by the ferric chloride reaction(9).

Protein was determined by the biuret method(10).

Results. Esterifying enzyme in bovine adrenals. Table I shows that the cell fractions prepared from whole glands had the same capacity for esterifying labeled cholesterol as did those prepared from the cortex. Hence, in subsequent studies whole adrenal glands were used.

The subcellular distribution of the cholesterol-esterifying enzyme is also shown in Table I. Although activity was detected in both Pellet I and Pellet II, the percentages for esterification of cholesterol-4-^{14}C were highest in the cell sap. Since the concentrations of endogenous cholesterol differed in each subcellular fraction (Table II), the extent of the dilution of the labeled cholesterol with the endogenous cholesterol would not be the same for each fraction. We assumed complete mixing of the added free cholesterol with all the endogenous free cholesterol in order to calculate the amount of free choles-

TABLE II. Cholesterol Content of Subcellular Fractions of Bovine Adrenal Glands. Averages and ranges of 4 determinations are recorded below.

Fraction	Cholesterol (μg/ml)		
	Total	Unesterified	Esterified
Supernatant A	449 (448 -450)	419 (417 -420)	30.2 (28.5 -31.3)
Pellet I	204 (201 -206)	195 (192 -198)	8.80 (8.75-10.0)
" II	48.0 (46.0- 50.4)	42.7 (41.2- 44.5)	5.20 (4.80- 5.91)
Cell sap	191 (188 -194)	175 (173 -178)	16.1 (15.5 -16.9)

terol esterified by one ml of each subcellular fraction. These calculated values were 19.6 mμmoles for supernatant A, 4.73 for Pellet I, 0.756 for Pellet II and 13.1 for the cell sap. It is apparent, then, that the cell sap had the highest cholesterol-esterifying activity.

Effect of substrate concentration. When the free cholesterol added to the incubation mixture was raised from 12.5 to 52.0 mμmoles, no increase was observed in the amount of the added cholesterol esterified. This indicates that the amount of endogenous free cholesterol present in the cell sap (Table II) was probably sufficient to saturate the esterifying enzyme.

Cofactor requirements. Esterification occurred in the absence of added ATP, CoA and Mg^{++}, and the addition of these cofactors failed to increase esterification (Table I). In subsequent experiments concentrations of added ATP and $MgCl_2$ were varied from 5 to 50 μmoles. Again, the added cofactors had no effect on the esterification of cholesterol by the cell sap. It is conceivable, however, that the concentration of these cofactors endogenously present was sufficient for the esterification. To test this possibility, cell sap was dialyzed overnight at 4°C against distilled water containing 0.1% EDTA (ethylenediamine tetraacetic acid). Incorporation of labeled cholesterol into cholesterol esters by both dialyzed and undialyzed samples in the absence and in the presence of ATP, CoA and Mg^{++} was then studied. Dialysis did not significantly alter the esterification of labeled cholesterol, and addition of the cofactors after dialysis did not enhance the esterification (Table III).

The effect of pH on esterification of the labeled cholesterol by cell sap is shown in Fig. 1. The optimum pH for the esterification was 5.0.

The hydrolytic enzyme in the bovine adrenal gland. Hydrolysis of labeled cholesterol oleate occurred in all the subcellular fractions of bovine adrenal homogenates (Table IV). The specific activities (per cent of labeled cholesterol oleate hydrolyzed per g protein) of both Pellet I and Pellet II were higher than that of the cell sap.

As shown in Table II, the concentration of endogenous cholesterol esters was different in each of the subcellular fractions. Since the concentration of endogenous cholesterol esters was higher in the cell sap than in either Pellet I or Pellet II, the lower values obtained for the hydrolysis of labeled cholesterol in the cell sap might be accounted for, in part at least, by a greater dilution of the added tracer cholesterol oleate in that fraction. However, calculations of the possible extent of dilution of the added cholesterol oleate in each subcellular fraction cannot be made,

TABLE III. Effect of Dialysis on Esterification of Labeled Cholesterol by Bovine Adrenal Cell Sap in Presence and Absence of Added Cofactors. Averages and ranges of 4 determinations are recorded below. Each incubation flask contained 0.5 ml of the enzyme preparation, 4.5 ml of acetate buffer, pH 5.0, 0.1 M and 10,000 cpm of cholesterol-4-^{14}C. The cofactors, when added, were dissolved in the buffer and consisted of the following: 50 μmoles of ATP, 1 μmole of coenzyme A and 50 μmoles of $MgCl_2$ per incubation flask. Final volume of the incubation mixture was 5.0 ml. Incubation was carried out for 3 hr at 37°C.

Enzyme preparation	Protein, mg/ml	% of cholesterol-4-^{14}C esterified	
		No cofactors added	Cofactors added
Undialyzed cell sap	17.5	5.42 (5.17-5.75)	4.74 (4.71-4.77)
Dialyzed cell sap	15.0	4.54 (4.45-4.64)	3.25 (3.12-3.38)

since it is not known whether or not the ratio of cholesterol oleate to total cholesterol esters is the same in each fraction.

The optimum pH for hydrolysis of labeled cholesterol oleate by the combined particulate fraction was 7.5 (Fig. 2).

Discussion. The results of our studies with homogenates and subcellular fractions show that in bovine adrenal glands the most active subcellular fraction and the pH optimum for esterification of cholesterol are not the same as those for hydrolysis of cholesterol esters. Esterifying activity was associated chiefly with the nonparticulate fraction, and the optimum pH for this reaction was 5.0. Hydrolytic activity, as measured by the hydrolysis of labeled cholesterol oleate, seemed to reside to a greater extent in the particulate fraction, and the pH optimum was 7.5. An earlier statement(11) that bovine adrenals, as com-

TABLE IV. Hydrolysis of Cholesterol-7-α-^{3}H-oleate by Bovine Adrenal Preparations. Averages and ranges of 4 determinations are recorded below. Each incubation mixture contained 0.5 ml of the enzyme preparation, 4.5 ml of K phosphate buffer, pH 7.5* 0.1 M and 10,000 cpm of cholesterol-7-α-^{3}H-oleate. Incubation was carried out for 3 hr at 37°C.

Cell fraction	Protein, mg/flask	% of cholesterol-7-α-^{3}H-oleate hydrolyzed	Specific activity†
Supernatant A	11.2 (10.7 -13.7)	79.8 (76.9-83.7)	7.12
Pellet I	5.35 (4.27- 6.25)	68.1 (62.9-74.5)	12.7
" II	2.62 (2.25- 3.57)	41.2 (36.6-46.5)	15.7
Cell sap	7.80 (6.25- 8.75)	44.6 (40.7-49.5)	5.72

* In preliminary studies on whole bovine adrenal homogenates, the pH optimum for the hydrolysis of cholesterol-7-α-^{3}H-oleate was found to be 7.5.

† Specific activity is defined as % of cholesterol-7-α-^{3}H-oleate hydrolyzed per mg protein.

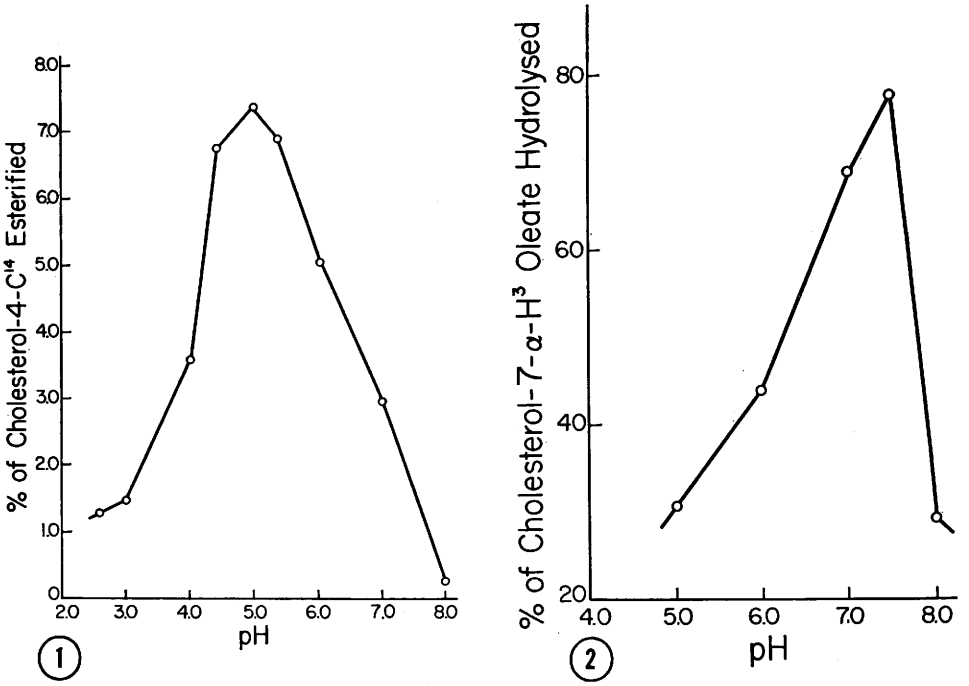

FIG. 1. Effect of pH on esterification of cholesterol-4-^{14}C by cell sap of bovine adrenal homogenates. Citrate-phosphate buffers were used for pH below 4.0, Na acetate buffers for pH 4.0 and pH 5.0, and K phosphate buffers for pH 6.0 and above. Incubation conditions (with the exception of addition of cofactors) were the same as given in Table I.

FIG. 2. Effect of pH on hydrolysis of cholesterol-7-α-^{3}H-oleate by combined particulate fraction (Pellets I and II) of bovine adrenal homogenates. Na acetate buffer was used for pH 5.0 and K phosphate buffers were used for pH 6.0 and above. Incubation conditions as given in Table IV.

pared to those of rats, rabbits and guinea pigs, have an extremely low capacity for esterifying cholesterol can be accounted for by the fact that esterification was studied in the particulate fraction (corresponding to our Pellet II), which we have shown possesses low esterifying and high hydrolyzing activity. In addition, the enzymatic assay in this earlier work(11) was carried out at a pH that favored hydrolysis of cholesterol esters rather than their synthesis.

It is interesting that cell sap prepared from bovine adrenals does not appear to require an exogenous source of ATP, CoA or Mg^{++} for incorporation of labeled free cholesterol into esters. In this respect this tissue resembles rat(12) and hog pancreas(13) and rat small intestine.‡

Summary. The presence of enzymes capable of both synthesizing and hydrolyzing cholesterol esters has been demonstrated in bovine adrenal gland homogenates. Most of the esterifying activity was associated with the cell sap, whereas the bulk of the hydrolytic activity seemed to reside in Pellet I and Pellet II of the homogenate. The optimum pH for esterification of cholesterol by the cell sap was 5.0; that for hydrolysis of cholesterol oleate by the combined particulate fraction was 7.5. Dialysis of the cell sap did not significantly alter its capacity to esterify labeled cholesterol, nor did the addition of ATP, CoA and Mg^{++} enhance the esterification reaction in this fraction.

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Absorption of Fructose in Man. (29780)

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The mechanism of fructose absorption is unknown. Active transport has not been demonstrated in any species either *in vivo* or *in vitro* but conversion of the fructose to glucose within the intestinal mucosa has been demonstrated in the dog(1), guinea pig(2), and hamster(3). Glucose, which appears to be formed from fructose by a fructokinase pathway similar to that found in liver(4,5) may then be actively transported(3).

In rat intestine, on the other hand, very little conversion of fructose to glucose occurs (6), possibly due to absence of glucose-6-phosphatase. Instead, a considerable proportion of absorbed fructose appears in the portal vein as lactic acid. No observations on this subject in man have been reported. Ginsberg and Hers(5) speculated that in man little conversion of fructose to glucose would occur, as they did not detect microsomal glu-

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