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Esterification of Cholesterol by Canine Adrenal Cell Fractions.* (30101)

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The origin and function of the adrenal cholesterol esters has not been adequately elucidated. The adrenal cholesterol esters may be an important reservoir for synthesis of the corticoids. A recent report(1) has shown that cholesterol esters can serve as precursors for synthesis of steroids by canine adrenal homogenates either directly or after the liberation of free cholesterol by hydrolysis of the esters. The mechanisms involved in the formation and hydrolysis of the adrenal cholesterol esters are particularly relevant to utilization of the esters in steroid synthesis. The adrenal cholesterol esters appear to be synthesized primarily *in situ*. It has been shown(2) that the adrenal cholesterol esters possess a characteristic fatty acid spectrum which is different than that of the serum esters. Also, *in vivo* studies(3) with injected C^{14}-cholesterol suggest that the adrenal gland removes cholesterol esters from the blood, hydrolyzes them, and then re-esterifies the liberated free cholesterol to form the characteristic adrenal cholesterol esters.

More direct evidence for the synthesis of adrenal cholesterol esters has been provided by recent reports(4-7) which have shown that adrenal homogenates from a number of species (rat, dog, bovine, guinea pig, rabbit) can esterify cholesterol. The reaction requires ATP and CoA and is inhibited by free fatty acid. A hydrolytic cholesterol ester system has also been shown to be present in canine adrenal(1).

The purpose of the present report was to provide additional information on the canine adrenal cholesterol esterifying system. Data are presented on the occurrence of cholesterol esterifying systems in adrenal cell fractions and on the nature of the cholesterol esters synthesized.

Methods and materials. Adrenal glands were removed from dogs immediately after sacrifice, demedullated, chilled on ice, and homogenized in a medium (100 mg/ml) containing 0.1 M phosphate buffer, pH 7.2, 0.03 M nicotinamide, 0.004 M magnesium chloride, and 0.01 M glutathione as described by Frantz and Bucher(8). The homogenate was centrifuged at 600 $\times$ *g* for 10 minutes to remove cells and debris. The supernatant solution was centrifuged at 10,000 $\times$ *g* for 30 minutes to obtain mitochondria and then at 104,000 $\times$ *g* for 60 minutes to obtain microsomes. The final supernatant solution is referred to in Table I as soluble fraction. Both mitochondria and microsomes were washed with buffer-medium and the solutions centrifuged at 10,000 $\times$ *g* and 104,000 $\times$ *g* for 10 minutes respectively; both fractions were made to a volume equal to that of the 600 $\times$ *g* supernatant. One ml aliquots of the cell fractions or homogenate were incumated with cholesterol-4-C^{14} (added in 0.1 ml acetone) and co-factors as indicated in

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TABLE I. Conversion of Cholesterol-4-C^{14} to C^{14}-Esters and C^{14} Steroids by Adrenal Cell Fractions.

Cell fraction	Exp 1 Sterol ester	Exp 1 Steroid zone	Exp 2 Sterol ester	Exp 2 Steroid zone	Exp 3 Sterol ester
	CPM × 10^3 recovered				
Homogenate	81.5	45.6	53.0	76.6	74.0
Mitochondria	35.0	35.6	42.5	68.8	59.5
Microsomes	23.0	35.3	17.0	41.6	40.5
Soluble fraction	18.0	24.4	12.5	26.9	17.5
Boiled homogenate	.0	.0	.0	.0	.0

Incubation mixture contained 1 ml cell fraction, 0.05 μmole, cholesterol-4-C^{14} (1 μc), 1.25 μmoles TPN, 1.35 μmoles DPN, 5 μmoles ATP, 1 μmole CoA, 10 μmoles glucose-6-phosphate, final volume 3 ml with phosphate-buffer medium; incubation time 2 hr.

Table I. The incubations were carried out in duplicate in a Dubnoff incu-shaker under an atmosphere of 95% O_2-5%CO_2 for 2 hours. The digests were extracted with 2:1 chloroform-methanol as described earlier(9).

Free and esterified sterols were separated by silicic acid column chromatography(10), and free and ester cholesterol determined by the method of Sperry and Webb(11). The fatty acid composition of the cholesterol esters was determined by gas-liquid chromatography(12). The cholesterol esters were further separated into 4 major classes (saturated, monounsaturated, linoleate, and arachidonate) by silicid acid column chromatography(13). The gas-liquid chromatography data provided information on the fatty acid composition (by mass) of the total cholesterol esters, whereas the isolation of the cholesterol esters by the silicic column procedure afforded an opportunity to determine the C^{14}-activity associated with each cholesterol ester class. Steroids were isolated and chromatographed on paper as described earlier(14). The C^{14}-activity of the various lipids was determined in a Nuclear-Chicago liquid scintillation system.

Results. Several representative experiments on the conversion of cholesterol-4-C^{14} to C^{14}-esters and C^{14}-steroids are shown in Table I. All of the cell fractions examined were active in the synthesis of cholesterol esters and steroids. Mitochondria were found to be most active followed by microsomes and soluble fractions.

A comparison of the fatty acid composition of the cholesterol esters (gas-liquid chromatography) of the cell fractions with the composition of C^{14}-cholesterol esters synthesized is shown in Table II. The C^{14}-cholesterol esters synthesized were very similar in composition to those cholesterol esters originally present in the cell fractions. The cholesterol esters synthesized by mitochondria and microsomes were similar and contained a high proportion of linoleate and other polyunsaturated fatty acids (41-48%). Soluble fraction and the original homogenate synthesized cholesterol esters containing a slightly larger proportion of monounsaturated fatty acids than mitochondria or microsomes.

Analysis of the homogenate and cell fractions for free and ester cholesterol (Table III) showed that most of cholesterol was in the esterified form. The major part (74%) of the cholesterol ester in the original homo-

TABLE II. Nature of Cholesterol Esters Synthesized by Adrenal Cell Fractions.

	Cholesterol esters							
	Saturated		Monounsaturated		Linoleate		Polyunsaturated	
Cell fraction	Mass	C^{14}	Mass	C^{14}	Mass	C^{14}	Mass	C^{14}
	(% total C^{14} esters or fatty acids)							
Homogenate	24.3	19.8	35.1	34.2	13.8	14.6	26.8	31.4
Mitochondria	24.7	20.1	32.0	28.3	14.8	15.3	28.5	36.3
Microsomes	29.8	25.6	27.4	26.2	10.5	13.7	32.3	34.5
Soluble fraction		24.5		32.2		19.3		24.0

TABLE III. Free and Ester Cholesterol of Adrenal Cell Fractions.

Cell fraction*	Free	Ester	Ester
	(mg/100 mg adrenal tissue)		(total %)
Homogenate	.52	4.68	90.0
Mitochondria	.35	3.50	91.0
Microsomes	.08	.55	87.3
Soluble fraction	trace	<.10	

* Represents analysis of adrenals from 2 dogs.

genate was recovered in the mitochondria fraction.

Discussion. The results of the present study confirm and extend the earlier observations(4) on the canine adrenal cholesterol esterifying system. The major portion of the esterifying activity of canine adrenal gland is associated with the mitochondria. Chemical analysis of the adrenal cell fractions for cholesterol esters also show that most of the adrenal esterified cholesterol is also associated with the mitochondria fraction. It thus appears that canine adrenal mitochondria synthesize and contain most of the cholesterol esters of that tissue. Also, as shown here, mitochondria are very active in conversion of cholesterol to steroids. Longcope and Williams(6) recently reported that rat adrenal cell fractions also esterify cholesterol. A major part of the activity was sedimented between 20,000 and 85,000 $\times$ *g* and the cholesterol esters synthesized contained a high proportion of polyunsaturated fatty acids. These and the present findings suggest that rat and dog adrenals differ in the cell location of the most active cholesterol esterifying system, but that the adrenals of both species synthesize highly unsaturated cholesterol esters.

The similarity in composition of the cholesterol esters synthesized with those esters present in the adrenal tissue further supports the view(4,6,7) that most of the cholesterol esters of that tissue are synthesized *in situ*. The specific function of the canine adrenal mitochondria cholesterol esterifying system and the cholesterol esters synthesized remains to be elucidated.

Summary. Canine adrenal cell fraction (mitochondria, microsomes, and soluble fraction) were found to convert cholesterol-4-C^{14} to esters and adrenal steroids. The most active cell fraction was mitochondria; mitochondria also contained most of the adrenal cholesterol (as ester). The cholesterol esters synthesized by mitochondria and microsomes contained a high proportion of polyunsaturated fatty acids. The esters synthesized were similar in composition to those present in mitochondria and microsomes.

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