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Concerning the Subcellular Distribution of 3β-Hydroxysteroid Dehydrogenase/Isomerase in the Rat Adrenal[1] (38906)

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(Introduced by I. L. Schwartz)

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In their investigation of 3β-hydroxysteroid dehydrogenase in the beef adrenal Beyer and Samuels (1) found activity in all subcellular fractions, with the largest amount in the microsomal fraction. Experimental considerations of microsomal contamination in the other fractions led to the conclusion that the enzyme was located in the microsomes. This conclusion has been brought into question by Basch and Finegold who have reported that a substantial proportion of the enzyme activity is associated with the mitochondria of the rat adrenal (2). The discrepancies in these reports have prompted us to reexamine this problem.

Methods. Male Sprague–Dawley rats were killed by decapitation, the adrenals removed, cleaned of fat and cut into halves to facilitate homogenization. The glands were homogenized with three to six strokes of a motor-driven Teflon pestle in a medium consisting of 0.25 *M* sucrose, 1 m*M* EDTA and 12 m*M* Tris buffer at pH 7.5 and at a concentration of about 100 mg wet weight of tissue per ml of medium. The homogenate was centrifuged at 750 *g* for 10 min to obtain the cell-debris plus nuclei fraction. The supernatant was centrifuged at 3640 *g* for 10 min to obtain the heavy-mitochondrial fraction and the pellet obtained washed twice at the same speed. The supernatant fluid was discarded after each wash. The supernatant from the initial centrifugation for the heavy-mitochondrial pellet was centrifuged at 6600 *g* for 10 min to obtain the light-mitochondrial fraction and this was washed once at the same speed. The supernatant from the above fraction was centrifuged at 105,000 *g* for 60 min to obtain the microsomal and soluble fractions. All fractions were resuspended in the homogenization medium to the volume of the original homogenate.

The incubation conditions in the presence of pyruvate and lactic acid dehydrogenase and the assay for 3β-hydroxysteroid dehydrogenase/isomerase were those described previously (3) except that the temperature was 30°. In this coupled reaction the dehydrogenase is rate-limiting (4). The conditions for the determination of steroid 21-hydroxylase have been given (5) except that the incubation temperature was 37°. Cytochrome oxidase activity was measured by the method described by Wharton and Tzagoloff (6). Linear kinetics were obtained for the enzyme assays for the initial homogenate and for all subcellular fractions. Protein was determined by the method of Lowry *et al.* (7).

Results and Discussion. The distribution of 3β-hydroxysteroid dehydrogenase/isomerase, steroid 21-hydroxylase and cytochrome oxidase in the subcellular fractions of the rat adrenal is given in Table I. The distribution of the dehydrogenase is in excellent agreement with that reported by Beyer and Samuels (1). It is seen that the percentage of the dehydrogenase and the hydroxylase in the microsomal fraction are similar. In the heavy-mitochondrial fraction there is an increase in the dehydrogenase compared to the hydroxylase. A comparison of the specific activities of the hydroxylase in the microsomal and heavy-mitochondrial fractions indicates about a 6% contamination of the heavy-mitochondrial fraction by microsomes. Taking this contamination into account, indicates about 5% of the total activity of the dehydrogenase is present in the heavy-mitochondrial fraction. The discrepancies between these data and those of Basch and Finegold, who reported 32% of the recovered

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TABLE I. THE SUBCELLULAR DISTRIBUTION OF 3β-HYDROXYSTEROID DEHYDROGENASE, STEROID 21-HYDROXYLASE AND CYTOCHROME OXIDASE IN THE RAT ADRENAL.[a]

	Enzyme activity in subcellular fractions						
	WH	CD + N	HM	LM	Mc	S	Recovery
3 β-Hydroxysteroid	133	34.9	13.9	4.5	73.4	0	95%
	±7	±3.7	±1.8	±1.1	±5.9		
Dehydrogenase/isomerase							
% of total activity		26	10	3	55	0	
% of recovered activity		28	11	4	58	0	
Steroid 21-hydroxylase	28.8	8.3	0.9	0.5	15.1	0	86%
	±5.1	±1.6	±0.1	±0.1	±2.1	0	
% of total activity		29	3	2	53	0	
% of recovered activity		33	4	2	61	0	
Cytochrome oxidase	75.7	30.1	37.6	8.6	0.6	0	101%
	±1.9	±2.8	±1.1	±0.5	±0.3	0	
% of total activity		40	50	11	1	0	
% of recovered activity		40	50	11	1	0	
Protein	15.4	6.0	2.1	0.5	2.1	4.3	97%
	±0.5	±0.4	±0.2	±0.03	±0.1	±0.1	

[a] The enzyme activities are given as the means ± *SE* and represent five experiments for the hydroxysteroid dehydrogenase, four experiments for the steroid 21-hydroxylase and three experiments for cytochrome oxidase. The dehydrogenase and hydroxylase activities are expressed as nmoles of product/min/ml fraction and cytochrome oxidase in terms of the first order velocity constant per ml fraction. Protein is given as mg/ml fraction. Abbreviations: WH, whole homogenate; CD + N, cell debris and nuclei; HM, heavy mitochondrial fraction; LM, light mitochondrial fraction; Mc, microsomes; S, postmicrosomal supernatant.

activity in the heavy-mitochondrial fraction and 46% in the microsomal fraction (2), may be due, in part, to the low recovery (59%) of the total activity they obtained. Unequal losses in the various fractions would affect the percentage distribution of the enzyme.

The 5% of the dehydrogenase/isomerase activity found in the heavy-mitochondrial fraction may be due to a redistribution artifact following homogenization as suggested by Cowan *et al.* (8) who found that the activity present in the mitochondrial fraction is associated with the outer mitochondrial membrane. A small preferential increase in the dehydrogenase/isomerase over that of the hydroxylase in the mitochondrial fraction may reflect a functionally heterogeneous endoplasmic reticulum as has been found in the liver (9). However, regardless of the interpretation, it appears that the dehydrogenase/isomerase is located in the microsomal fraction.

Summary. The distribution of 3β-hydroxysteroid dehydrogenase/isomerase in the subcellular fractions of rat adrenal gland has been determined. Based on the total activity found, 55% was in the microsomal fraction, 10% in the heavy-mitochondrial fraction, 3% in the light-mitochondrial fraction and 26% in the fraction consisting of cell-debris plus nuclei. Ninety-five percent of the total activity was recovered in the fractions. Approximately half the activity in the heavy-mitochondrial fraction could be accounted for by microsomal contamination.

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