resin (such as Nalcite HCR) in the acid cycle followed by washing with water to remove anions, and displacement of the amino acids with 1 *N* ammonium hydroxide.

Sensitivity was increased by virtue of the smaller spots obtained with smaller chromatograms. Quantities of 0.005 μM gave good dark spots.

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THE BIOSYNTHESIS OF RADIOACTIVE CHOLESTEROL BY PARTICLE-FREE EXTRACTS OF RAT LIVER*

by

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The biosynthesis of cholesterol has hitherto been accomplished *in vitro* by the use of tissue slices[1,2,3] or homogenates[4,5]. It is the purpose of this communication to report on the successful fractionation of rat liver homogenate, and the preparation from it of a water-soluble enzyme system capable of incorporating ^{14}C-labelled acetate into cholesterol.

Rat livers (2 g) were rapidly homogenized for 15–20 sec at 0° C in a loose-fitting Potter-Elvehjem glass homogenizer with two volumes of buffer (K_2HPO_4 0.019 *M*, KH_2PO_4 0.005 *M*, $MgCl_2$ 0.007 *M*, nicotinamide 0.03 *M*, sucrose 0.18 *M* pH 7.6). The homogenate was centrifuged at 3000 r.p.m. for 10 minutes (600 × *g*) to remove cell debris, cells, and nuclei. A mitochondrial fraction, which was obtained from the supernatant homogenate by centrifugation at 20,000 r.p.m. for 40 minutes (33,000 × *g*), was washed with cold buffer solution and resedimented. The supernatant fraction was centrifuged at 37,000 r.p.m. for 30 minutes (100,000 × *g*) to remove any remaining particles. The packed mitochondria were lysed by the addition of an equal volume of cold water, followed by occasional and gentle stirring for one hour at 0° C. The complete water-soluble enzyme system was prepared by the addition of clear supernatant fluid (4–5 volumes) to the lysed suspension followed by centrifugation at 37,000 r.p.m. for 30 minutes.

After incubation, the cholesterol in each sample was recovered as the digitonide[3] and plated. In some instances the cholesterol-digitonide obtained from the water-soluble enzyme system was converted into cholesterol dibromide and assayed again for radioactivity, with essentially no change.

Washed mitochondria plus supernatant fluid also provides a system which appears to incorporate acetate into cholesterol as efficiently as does the water-soluble enzyme system. Further details will be reported in a later communication.

TABLE I

INCORPORATION OF 1-^{14}C-ACETATE INTO CHOLESTEROL BY HOMOGENATES AND WATER SOLUBLE EXTRACTS

Total volume in each flask was 5 ml. Additions were 1 mg each of adenosine triphosphate, diphosphopyridine nucleotide and 1-^{14}C sodium acetate ($3 \cdot 10^5$ cpm/mg C). Gas phase 95% O_2 —5% CO_2; incubated with shaking at 34° C for 3 hours. To all preparations except whole homogenate, 0.5 mg of carrier cholesterol was added prior to saponification. Each experiment was done in duplicate or triplicate. Male Wistar Albino rats were used. In Experiments 1 and 2 the animals were fasted prior to sacrifice. In each experiment, several livers were pooled to provide sufficient homogenate for the preparation of subsequent enzyme fractions.

Exp.	*System*	*Recovered cholesterol cpm/mg C* Range	*Recovered cholesterol digitonide mg* Range	*Acetate incorporated in recovered cholesterol* μ moles
	Whole Homogenate	90–100	4.0–4.8	0.014
	Washed mitochondria and buffer	0–4	2.0–4.0	0.0004
	Supernatant Fluid (Particle-free)	6–10	0.5–2.0	0.0005
1	Mitochondria and Supernatant Fluid	28–38	2.0–4.0	0.004
	Mitochondria and Supernatant Fluid and Microsomes	18–43	2.0–4.0	0.005
	Water-Soluble Enzyme System	32–47	0.5–2.5	0.006
	Whole Homogenate	189–280	4.0–4.3	0.034
	Mitochondria and Supernatant Fluid	39–57	2.0–3.5	0.005
2	Mitochondria and Supernatant Fluid and Microsomes	40–53	2.0–3.5	0.005
	Water-Soluble Enzyme System	32–50	1.0–2.0	0.005
	Whole Homogenate	40–55	2.0–3.0	0.004
3	Mitochondria and Supernatant Fluid	12–24	1.5–2.0	0.001
	Water-Soluble Enzyme System	17–29	0.5–1.0	0.001

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BOOK REVIEWS

Advances in Protein Chemistry, Herausgeber: M. L. ANSON, JOHN T. EDSALL UND KENNETH BAILEY. Volum VI, Academic Press Inc. New York, 1951, xi + 549 Seiten, 87 Figuren, $ 9,50.

Mit jedem Band mehr kommen die Advances of Protein Chemistry dem Ziel näher, das den Herausgebern vorschwebt: Ein Werk zu schaffen, das unsere Erkenntnisse über Proteine vollständig, souverän und angepasst an den jeweiligen Stand der Forschung wiedergibt. Dies beruht darauf, dass die Herausgeber es verstehen, hervorragende Autoritäten aller Nationen als Autoren für die einzelnen Probleme zu gewinnen und ferner auf der Treffsicherheit, mit der die Probleme ausgewählt werden, die gerade besonders aktuell und entwicklungsfähig sind.

So beginnt der VI. Band mit einem vorzüglichen Beitrag von R. W. G. WYCKOFF über die