Enzymic studies on dystrophic mice and their littermates (lipogenesis and cholesterolgenesis)

This laboratory has been carrying out investigations using mice with muscular dystrophy and their littermates in attempts to define biochemical lesions[1-3]. Systematic enzyme assays have been performed utilizing tissues of both mice. The hereditary disease of these mice in many respects resembles human muscular dystrophy[4]. The enzyme systems to be reported are the ones concerned with lipogenesis and cholesterolgenesis[5,6].

Male dystrophic mice and their corresponding littermates were purchased during the winter from the Jackson Memorial Laboratory, Bar Harbon, Maine. The animals used were 5 to 6 weeks old. In all cases the dystrophic mice exhibited malfunction of the hind legs. The normal littermates were adjudged to be in satisfactory health by physical observation. The animals received an *ad libitum* diet of Purina chow and water. Attempts to utilize frozen sections for the study of these enzymes gave erratic results. For the above reason all the animals used were sacrificed prior to the enzymic assay. The brain, liver, kidney, spleen, and muscles from each individual animal were weighed and then homogenized in a loose fitting Potter-Elvehjem glass homogenizer. The crude homogenates were freed of debris by centrifugation for 6 min at 500 $\times$ *g*, and were incubated for 3 h with [^{14}C-*Me*]acetate obtained from a commercial source* (1 mC/mmole). The homogenates were previously fortified with 1 mg each of AMP, TPP, and DPN and were incubated under 100 % O_2. After incubation, carrier palmitic acid and cholesterol were added to each preparation. The cholesterol and fatty acids were recovered[5,6]. Digitonides and calcium salts were then used to purify the materials. The cholesterol-digitonide was then converted to the dibromo derivative and the calcium salts of the fatty acid recovered as free fatty acids. The recovered materials were radiometrically assayed in a flow counter to a standard deviation of $\pm$ 0.3 % and corrected for self-absorption.

TABLE I

LIPOGENESIS AND CHOLESTEROLGENESIS IN "NORMAL" AND "DYSTROPHIC-MICE" TISSUE

Recovered radioactivity (Average of 22 pairs of mice; Dystrophic and Littermate)

Organ	*Cholesterolgenesis*		*Lipogenesis*	
	Counts/min/mg C/mg N of original tissue from dystrophic mice	*Percentage ratio of radioactivity of dystrophic to littermate*	*Counts/min/mg C/mg N of original tissue from dystrophic mice*	*Percentage ratio of radioactivity of dystrophic to littermate*
Brain	8650	231	6430	187
Liver	7840	194	8970	197
Kidney	3490	184	3420	205
Spleen	4850	171	8440	201
Skin	6730	159	9730	219
Muscle	86	118	385	131

The radioactivity found for the recovered cholesterol and fatty acid of "littermates" has been designated as 100 % while the recovered radioactivity in cholesterol

Abbreviations: AMP, adenosine monophosphate; TPP, thiamin pyrophosphate; DPN, diphosphopyridine nucleotide.

* All radioactive materials were obtained on allocation from the United States Atomic Energy Commission.

and fatty acid for the dystrophic animal was the percentage obtained after comparison with values of the "normal littermate". These results are in Table I and represent the results obtained from 22 pairs of dystrophic mice and their corresponding littermates. The percentage standard deviation of the mean observed throughout these experiments was $\pm$ 21 %. The observed ratios are thus statistically significant except for muscle.

From Table I it can be concluded that homogenized and fortified tissues of dystrophic mice have a greater lipogenesis and cholesterolgenesis than their "normal" littermates. The brain shows the greatest difference, and is followed closely by the liver and kidney. The spleen and skin showed smaller increments. All values presented have been corrected to equal concentrations of nitrogen present in the original homogenate.

These results suggest that definite metabolic differences occur in the various fat-metabolizing centers of the dystrophic mouse.

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[1] J. L. RABINOWITZ AND T. SALL, *Biochim. Biophys. Acta*, 23 (1957) 1289; *J. Am. Chem. Soc.* 77 (1955) 5180.
[2] J. L. RABINOWITZ, *Atompraxis*, 4 (1958) 317; *Federation Proc.*, 18 (1959) 306.
[3] J. L. RABINOWITZ, J. BURNS, G. D. CHASE AND H. C. ALLEN, *Atompraxis*, 5 (1959) 487.
[4] A. M. MICHELSON, E. S. RUSSELL AND P. J. HARMAN, *Proc. Natl. Acad. Sc., U.S.*, 41 (1955) 1079.
[5] R. BRADY, J. L. RABINOWITZ, J. VAN BAALEN AND S. GURIN, *J. Biol. Chem.*, 193 (1951) 137.
[6] J. L. RABINOWITZ AND S. GURIN, *J. Biol. Chem.*, 208 (1954) 307.

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Direct ion-exchange chromatography of tissue extracts without precipitation of protein by use of non-ionic detergents

In general, the separation of constituents of a biological preparation by ion-exchange chromatography is done with protein-free filtrates because protein adheres to ion-exchange resins and clogs the columns. In the course of other experimental work, one of us (J.E.S.) chanced to discover that proteins in the presence of certain polyoxyethylene non-ionic detergents did not adhere to ion-exchange resins but flowed through resin columns directly. Small ionized molecules present in such protein-detergent mixtures did not seem to be affected and were retained by the resin column from which they could be afterward displaced sequentially in the usual manner, thereby permitting direct chromatography of tissue extracts.

Abbreviations: OPE_x, *p-t,t*-octylphenoxy-polyoxyethylene-ethanol of x ethylene oxide units; MDTA, methyldodecylbenzyltrimethyl-ammonium chloride; UMP, uridine monophosphate; TPN, triphosphopyridine nucleotide; IMP, inosine monophosphate; AMP, adenosine monophosphate; GMP, guanosine monophosphate; UDP, uridine diphosphate; ADP, adenosine diphosphate; UTP, uridine triphosphate; ATP, adenosine triphosphate; UDPG, uridine diphosphoglucose; PNA, pentose nucleic acid.