Acetate-Replacing Factors for Lactic Acid Bacteria: Intracellular Distribution in Rat Liver. (18906)

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The existence in biological preparations of a group of chemically related substances that can replace acetate in its growth stimulatory effect for certain lactic acid bacteria has been demonstrated(1). Natural materials contain a microbiologically inactive form or forms that can be converted to 2 active substances by acid hydrolysis. Purification of one of these substances from liver has revealed that in catalytic amounts it is effective as an acetate-replacing factor(2). Concentrates of this factor are very active in the pyruvate oxidase factor assay of O'Kane and Gunsalus(3), suggesting that it is involved in oxidative decarboxylation of pyruvate.*

It has become aparent that certain types of enzymatic activity are associated with different parts of cells(4). Localization of acetate-replacing factor activity within liver cells would serve as a starting-point for a more detailed study of the function of these factors in animal tissues.

Experimental. Fractionation of cell components. Stock rats originally of the Wistar strain were fasted overnight, killed by decapitation, and the livers were quickly removed and chilled in ice-cold isotonic KCl. The livers were blotted, weighed, and homogenized in isotonic (0.25 M) sucrose in an all-glass apparatus(5). The homogenate was separated by the method of Schneider(6) into three fractions—nuclei, mitochondria, and a third fraction consisting of the submicroscopic particles and soluble components of the cells. The fractionation was carried out below 10°C.

TABLE I. Distribution of Acetate-Replacing Factor Activity in Fractions of Rat Liver Homogenates.

Liver fraction	Acetate-replacing factor activity: Total,* acetate units	Fraction of homogenate, %
Homogenate	7.5 (6 –8.9)	(100)
Nuclear fraction	1.3 (1 –1.6)	17.3
Mitochondrial fraction	4.8 (3.5–6)	64 (56–70)
Unfractionated residue	.9 (.7–1.2)	12
Unaccounted for	.5	6.7

* Per mg of fresh liver or its equivalent. One acetate unit is equivalent to the growth response of 1 mg of sodium acetate(1). Average values obtained with the livers of 4 rats are presented and the range of the experimental determinations is given in parentheses.

Assay of acetate-replacing factor activity. The medium and procedure employing a strain of *Streptococcus lactis* as the assay organism have been described(1). Samples of the various fractions and of the homogenate were autoclaved at 120° for 3 hours with an equal volume of 6 *N* sulfuric acid to release acetate-replacing factor activity(1). The acid hydrolysates were neutralized and assayed. The results are presented in Table I.

Discussion. In order to determine the distribution of acetate-replacing factor activity within liver cells, the latter were separated into 3 fractions by means of the cell fractionation technic. These fractions were the nuclei, mitochondria and a third fraction consisting of the submicroscopic particles and soluble components of the cell. Cytological examination indicated that the fractions consisted mainly of the components mentioned. Moreover, it was shown that the succinoxidase

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3. O'Kane, D. J., and Gunsalus, I. C., *J. Bact.*, 1948, v56, 499.

* Personal communication from Dr. Gunsalus.

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activity of the rat liver homogenates was associated mainly with the mitochondria, as had been previously demonstrated(7).

Having established that the liver cells had been separated into distinct particulate components, it was possible to determine the distribution of acetate-replacing factor activity in the cells. The results reported in Table I indicate that approximately 64% of the acetate-replacing factor activity of rat liver is associated with the mitochondria. This finding is in conformity with the apparent role of acetate-replacing factors in pyruvate oxidation and the fact that mitochondria are centers of respiratory activity in liver cells(7).

Summary. The major portion of the acetate-replacing factor activity of rat liver homogenates has been demonstrated to be associated with the mitochondria.

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Endocrine Influences on Proteinuria in the Rat: Effect of ACTH.* (18907)

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The kidney enzyme renin produces an intense though transient proteinuria when injected intraperitoneally into the normal rat (1). It has been shown that bilateral adrenalectomy abolishes this proteinuria(2), and that it is restored in the adrenalectomized rat by the injection of suitable doses of cortisone, desoxycorticosterone acetate and aqueous adrenal cortex extract(3). Hypophysectomy similarly abolishes renin proteinuria. Cortisone is effective in restoring the ability of the hypophysectomized rat to respond to renin with proteinuria while desoxycorticosterone acetate is not(4). The normal, spontaneous proteinuria of the male rat is reduced by bilateral adrenalectomy(3), and by hypophysectomy (4). Cortisone and desoxycorticosterone acetate restore this proteinuria to normal levels in the adrenalectomized rat but fail to do so in the hypophysectomized animal.

In order to define further the endocrine influences on certain types of proteinuria in the rat, the effect of various doses of pituitary adrenocorticotropic hormone (ACTH) on renin proteinuria and the spontaneous proteinuria of normal male rats has been studied.

Methods. Animals: Male albino rats of the Slonaker-Addis strain were used in these experiments.

Urine collections: All animals were taken from the colony at 4 P.M. and placed in individual urine collection cages overnight. Seventeen hours later the overnight urine collection period was terminated. The protein excreted during this overnight period is referred to as the animal's spontaneous or "base" proteinuria. During the overnight period the animals received only a 15% solution of glucose in 0.4% sodium chloride containing 0.5% of a solution of B vitamins (Betaplexin). Following the termination of the overnight urine

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