Pyruvate and Glucose as Precursors of Acetoacetate. (23613)

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Although carbohydrate is known to be "antiketogenic," pyruvate has been shown to form acetoacetate in cell-free liver preparations(1,2,3). In the present series of studies, respiring homogenates of lactating guinea pig mammary gland were found to convert pyruvate and acetate to acetoacetate at rapid rates, in contrast with a slow, but measurable rate of conversion of glucose to acetoacetate(4).

The antiketogenicity of carbohydrate has been attributed to its ability to give rise to oxaloacetate, an explanation which is not accepted as entirely satisfactory(5). Since ketogenesis is an alternative to lipogenesis(6), and the latter is a synthetic process requiring the participation of reduced pyridine nucleotide(7), the possibility was considered that the ability of glucose to regenerate reduced pyridine nucleotide in respiring systems might be a key factor in determining its low ketogenicity. This hypothesis could be tested by studying the behavior of lactate, a metabolite closely related to pyruvate, but whose oxidation, like the breakdown of glucose, is coupled with the reduction of DPN.

Methods. Homogenates of mammary glands of lactating guinea pigs, prepared as described in a previous study of lipogenesis (8), and rabbit kidney cortex homogenates were incubated in a reaction mixture containing in addition to KCl: $MgCl_2$, 0.01 M; nicotinamide, 0.02 M; DPN, 3×10^{-4} M; ATP, 0.001 M; phosphate buffer, pH 7.4, 0.01 M aminotrishydroxymethylmethane (tris) buffer pH 7.4, 0.01 M and carrier acetoacetate, 0.01 M. After incubation with C^{14}-labeled substrates (0.005-0.01 M) in the Warburg apparatus, the reaction mixtures were acidified and the bound CO_2 collected in the NaOH in the center wells which had served to absorb the CO_2 during the measurements of O_2 consumption. The contents of the center wells were then removed, replaced by fresh NaOH and the acetoacetate was decarboxylated by the addition of aniline citrate. After addition of carrier K_2CO_3 the samples containing the respiratory CO_2 and the terminal CO_2 of acetoacetate respectively were precipitated with $BaCl_2$. Thin samples of $BaCO_3$ were mounted on planchets of 4.9 cm^2 area and counted with a gas-flow counter with 'Micromil' window. After correction for self-absorption, the 'total counts' were calculated and converted to mμmole-equivalents of the radioactive substrate oxidized. Although the radioactivity of only the terminal carboxyl group of acetoacetate was determined, it was assumed that both halves of the molecule were equally labelled and the radioactive acetoacetate was calculated as being derived from two molecules of pyruvate, lactate or acetate and from one molecule of randomly labeled glucose respectively.

Results. Experimental data representative of mammary and of kidney homogenates are shown in Table I. The high rates of conversion to acetoacetate of pyruvate and acetate are in contrast with the low ketogenicity of glucose and lactate. The addition of hexokinase, which accelerated the rate of aerobic glycolysis and increased the amount of glucose oxidized to CO_2, had no effect on the rate of its conversion to acetoacetate.

Since the oxidation of lactate differs from that of pyruvate in only one additional oxidative step coupled with the reduction of DPN, it may be concluded that the ability to regenerate reduced pyridine nucleotide, shared by glucose and lactate, is a factor of major importance in determining their low ketogenicity. In mammary homogenates, incubated with acetate or pyruvate, increasing amounts of fumarate have been found to cause progressive stimulation of lipogenesis(8) and progressively to depress acetoacetate formation (4). It seems probable that the effect of fumarate in large quantities, exceeding the small amounts required to catalyze Krebs cycle oxidations, is also due to its power to maintain pyridine nucleotides in the reduced form. Acetoacetyl-coenzyme A is obviously formed from pyruvate, and must also be formed from

TABLE I. Formation of Acetoacetate from Pyruvate, Lactate, Glucose and Acetate.

Exp. 1—Guinea pig mammary gland homogenate, dry wt 15.8 mg/vessel; incubation period 80 min.

Exp. 2—Rabbit kidney cortex homogenate (centrifuged to remove cell debris and nuclei), dry wt 19.8 mg/vessel; incubation period, 50 min.

Exp. No.	Additions	$-Q_{O_2}$	Labeled substrate (acetyl-coenzyme A equivalents), mμmoles/100 mg dry wt/hr, appearing in: Respiratory CO_2	Labeled substrate (acetyl-coenzyme A equivalents), mμmoles/100 mg dry wt/hr, appearing in: Aceto-acetate
1	2-C^{14}-pyruvate, 0.005 M	8.3	3010	5320
	2-C^{14}-DL-lactate, 0.005 M	5.9	3580	812
	C^{14}-glucose (r.l.), 0.005 M	8.8	6400	700
	Idem and hexokinase, 18 units	10.2	9000	780
2	2-C^{14}-pyruvate, 0.005 M	15.9	4770	11160
	2-C^{14}-DL-lactate, 0.01 M	17.0	7920	1430
	C^{14}-glucose (r.l.), 0.005 M	20.0	5820	2090
	1-C^{14}-acetate, 0.005 M	17.6	8620	9680

lactate and from glucose. In the presence of reduced pyridine nucleotides, as during the oxidation of the weakly ketogenic glucose or lactate, or of the ketogenic substrates supplemented with fumarate, acetoacetyl-coenzyme A appears to be reduced to β-hydroxybutyryl-coenzyme A, initiating the chain of reactions in the reversal of the fatty acid cycle(7). In the case of the ketogenic substrates undergoing oxidations in the absence of a source of reduced pyridine nucleotide, enzymic deacylation of acetoacetyl-coenzyme A would result in the formation of acetoacetate.

The ability of glucose to regenerate reduced pyridine nucleotide in an aerobic system, may explain not only its non-ketogenicity, but also its effectiveness in stimulating the synthesis of fatty acids from acetate, originally demonstrated in mammary slices(9) and later observed in actively respiring mammary homogenates metabolizing acetate in the presence of fumarate(8).

Whereas C^{14}-labeled glucose (randomly labeled), when present as the only precursor of acetyl coenzyme A, was virtually non-ketogenic, unlabeled glucose, when added to mammary homogenates converting 1-C^{14}-acetate to acetoacetate, showed no antiketogenic activity. In some preparations in which the rate of acetoacetate formation from acetate was relatively slow, glucose even accelerated the rate of incorporation of acetate carbon into acetoacetate (Table II). The 'ketogenic' effect of glucose persisted in the presence of fumarate in catalytic amounts, but was abolished when the concentration of fumarate was increased.

The increased rate of $C^{14}O_2$ production

TABLE II. Effect of Glucose on Formation of Acetoacetate from Acetate Guinea Pig Mammary Gland Homogenates. Exp. 1: dry wt 23.5 mg/vessel; incubation period 85 min. Exp. 2: dry wt 59 mg/vessel; incubation period 60 min. All flasks contained 1-C^{14}-acetate, 0.005 M.

Exp. No.	Additions	$-Q_{O_2}$	Acetate (mμmoles/100 mg dry wt/hr) appearing in: Respiratory CO_2	Acetate (mμmoles/100 mg dry wt/hr) appearing in: Aceto-acetate
1	None	4.0	2340	262
	Glucose, 0.02 M	8.0	3660	1520
	Fumarate, 0.001 M	7.5	3760	274
	Idem; glucose, 0.02 M	11.0	3660	1130
	Fumarate, 0.002 M	7.8	2140	156
	Idem; glucose, 0.02 M	9.2	1870	223
2	None	6.5	3650	1825
	Glucose, 0.005 M	7.3	2900	2670
	Idem; hexokinase, 18 units	6.6	2540	1615

from 1-C^{14}-acetate, observed on addition of glucose, indicates that the rate of activation of acetate was accelerated by the breakdown of glucose. It may be assumed that under the experimental conditions employed, the rate of formation of acetoacetyl-coenzyme A from the acetyl-coenzyme A derived from both acetate and glucose may have exceeded the rate of regeneration of reduced pyridine nucleotide coupled with the breakdown of glucose alone. As a result, a large part of the acetoacetyl-coenzyme A must have escaped reduction and have been deacylated.

Summary. 1. The ketogenic power of various C^{14}-labeled substrates, incubated with actively respiring homogenates of mammary gland and of kidney cortex, has been compared by measuring rate of incorporation of the substrate carbon into carrier-acetoacetate. Whereas acetate and pyruvate were strongly ketogenic, glucose and lactate gave rise to only small amounts of acetoacetate. The analogous behavior of glucose and lactate in contrast with that of pyruvate, indicates that the low ketogenicity of glucose and its ability to stimulate lipogenesis is associated with the regeneration of reduced pyridine nucleotides during its breakdown. 2. No antiketogenic action of glucose was observed when this was added to mammary homogenates metabolizing 1-C^{14} acetate. Under certain conditions, the presence of glucose resulted in the acceleration of the appearance of acetate-carbon in respiratory CO_2 and in acetoacetate. This 'ketogenic' action appeared to be due to stimulation by glucose of the activation of acetate and consequently the formation of acetoacetyl-coenzyme A at a rate exceeding the rate of regeneration of reduced pyridine nucleotide coupled with the breakdown of glucose.

1. Lehninger, A. L., *J. Biol. Chem.*, 1946, v164, 291.

2. Recknagel, R. O., and Potter, V. R., *ibid.*, 1951, v191, 263.

3. Crandall, D. I., and Gurin, S., *ibid.*, 1949, v181, 829.

4. Terner, C., *Fed. Proc.*, 1956, v15, 605.

5. Weinhouse, S., *Brookhaven Symp. in Biology*, 1952, v5, 201.

6. Chaikoff, I. L., and Brown. G. W., Jr., *Chemical Pathways of Metabolism*, 1954, v1, 277.

7. Lynen, F., and Ochoa, S., *Biochim. Biophys. Acta*, 1953, v12, 299.

8. Terner, C., *Biochem. J.*, 1956, v64, 532.

9. Balmain, J. H., Folley, S. J., and Glascock, R. F., *ibid.*, 1952, v52, 301.

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Effect of Trypsin on Agglutinability of Lipopolysaccharide Treated Erythrocytes.*† (23614)

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Crude somatic antigens of various enteric, Gram-negative bacilli as well as their lipopolysaccharide components become readily attached to erythrocytes of man and sheep, resulting in the acquisition of a new serologic specificity. These modified red blood cells thus become specifically agglutinable in the presence of homologous bacterial antibodies. With modified sheep cells lysis occurs upon the addition of antibody and guinea pig complement. It has been shown that erythrocytes from dog, rabbit, guinea pig, rat, and chicken are as readily modified by *Escherichia coli* antigen as human and sheep erythrocytes(1). Similarly, *Salmonella typhosa* Vi antigen be-

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