Metabolic Relationship Between Acetoacetate and Glucose. (19462)

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Glucose has long been known to lessen the extent of ketonuria(1-5). According to Harry *et al.*(6) ketolysis rather than antiketogenesis is apparently the primary mechanism whereby metabolism of carbohydrate brings about diminution of ketonuria. West(7) succeeded in condensing glucose with ethylacetoacetate, *in vitro,* to give rise to a product which was shown to be 2-tetra hydroxy butyl 5-methyl 4-carbethoxy furan (Gonzales (8,9)). Since Shaffer(10) put forward his interesting theory on ketolytic role of glucose, there have been many speculations as to whether glucose or some active derivative of it accelerates combustion or oxidation of the acetoacetate molecule by a definite chemical union of the nature of condensation or by merely exerting a catalytic effect. Soskin and Levine(11) reported that the rate of utilization of glucose is within certain limits proportional to the height of the blood sugar level in normal as well as diabetic animals. It has been found in this laboratory(12,13) that the intermediary fat metabolites when injected in the normal rabbits bring about a rise in blood sugar. As suggested earlier by Nath(14) and recently by Best and Taylor(15), this immediate rise in blood sugar as a result of the acetoacetate injection may be due to a physiological response to combat the disturbed situation and may, therefore, be considered as a protective phenomenon.

In vivo, as well as *in vitro* studies were, therefore, undertaken to throw some light on these points and to explore whether there is any metabolic relationship between glucose and acetoacetate that might exist in the living body as a natural mechanism for the diminution of ketone bodies.

Experimental. In vivo studies. A set of 36 healthy male rabbits, having fasting blood sugar values between 108 and 116 mg per 100 cc of blood, were selected. Blood was taken from the marginal ear veins and blood sugar estimated according to the method of Hagedorn and Jensen(16). Samples were collected after fasting for about 18 hours. All injections were subcutaneous. The Na acetoacetate and glucose injected were always in equimolecular proportions and calculated from the molecular weight of the condensation product of the two. As this compound is not highly soluble in water at 37°C, the aqueous suspension was administered subcutaneously by a bovine needle.

In vitro studies. Glucose 60 mg with 1 cc of whole blood and 20 cc of M/15 phosphate buffer of pH 7.3 was incubated at 37°C for 24 hours. The amount of glucose utilized was then found by estimating the amount of glucose remaining according to the method of Hagedorn and Jensen(16). The above experiment was next repeated with Na acetoacetate and condensation product respectively in different amounts.

Discussion. It is evident from Table I (A and B) that both acetoacetate as well as glucose, when injected in very low doses, cause a distinct rise in blood sugar values. But no such rise could be observed even when a high dose of acetoacetate (341.7 mg/kg) was followed immediately by an equimolecular amount of glucose (496 mg/kg), or, when the condensation product of the two (223.2 mg/kg) was administered subcutaneously, this indicated nicely that there is possibly some metabolic relationship between glucose and acetoacetate in the living body, a finding which is in accord with Hawk(17), that "It appears likely that glucose facilitates acetoacetate combustion in the living organism."

It was also observed that if acetoacetate alone is injected in the high dose, the animals showed signs of uneasiness and restlessness, but when the high dose was immediately followed by its equimolecular amount of glucose, these symptoms soon disappeared. It was further noted that the high dose of acetoacetate brings in the urine of a normal healthy rabbit a considerable increase in specific gravity. But no such harmful effect was ob-

TABLE I. Showing Glucose Tolerance of Animals (Rabbits) on Injection of Acetoacetate and Glucose, Administered in Their Equimolecular Proportions, Individually and One Immediately Followed by the Other, and Also Their Condensation Product.

Dose	No. of animals	Substance inj.	Dose, mg/kg body wt	Avg blood sugar values and stand. dev. (mg/100 cc) after 0 hr	½ hr	1½ hr	2½ hr
Low	5	Na acetoacetate	50	113 ±2	130.4±1.9	140.6±2.4	134 ±2.6
	2	Glucose	72.6	113.5±1.5	126.5±1.5	113.5± .5	113.5±1.5
	5	Na acetoacetate, immediately followed by glucose	50 72.6	115 ±2	106.6±1.9	109 ±2.9	100.6±2.7
	6	Condensation product of glucose and ethylacetoacetate	110.5	113 ±2.4	115 ±1.9	109 ±2.3	101 ±2.8
High	4	Na acetoacetate	341.7	112.8±1.9	175 ±2.7	183 ±3.1	179 ±2.6
	2	Glucose	496	113.5±1.5	169 ±1	102 ±1	113.5±1.5
	6	Na acetoacetate immediately followed by glucose	341.7 496	108 ±3.4	116 ±1.6	111 ±3.5	100 ±4.2
	6	Condensation product of glucose and ethylacetoacetate	223.2	111 ±2.2	115.8±2.4	115 ±2.1	119 ±1.9

TABLE II. Showing Glucose Utilization With and Without Na Acetoacetate or Condensation Product. (60 mg of glucose with 1 cc of whole blood, and 20 cc of M/15 phosphate buffer of pH 7.3 incubated at 37°C for 24 hr with different amount of condensation products and Na acetoacetate respectively).

	No. of experiments	Amt of condensation product (mg)	Amt of Na acetoacetate (mg)	Avg values for glucose utilized (mg) and stand. dev.	Avg % of glucose utilized*
A.	4	0	0	45 ± 2.2	73
B.	2	11.41	0	45 ± 4	73
	2	22.83	0	44 ± 0	72
	2	45.66	0	43 ± 0	71.8
	4	91.33	0	42 ± 3.1	70.6
	3	182.66	0	35 ± 2.9	56
C.	2	0	5.16	45 ± 0	73
	2	0	10.33	42 ± 2	70.6
	2	0	20.66	41 ± 0	70
	5	0	41.33	32 ± 7.2	49.6
	3	0	82.66	21 ± 2.8	29.8
	3	0	123.99	18 ± .255	23.4
D.	4	91.33	41.33	27 ± 5.9	40.6

* In calculating avg % of glucose utilization, blank values obtained in experiments without blood were subtracted from observed values.

served on immediately following the above high dose by its corresponding amount of glucose. Further, when the subcutaneous injections of both substances continued for some days there was no diuresis, but was observed in the animals receiving acetoacetate alone. Also the condensation product of glucose and ethyl acetoacetate administered subcutaneously to a normal rabbit, in the high dose of 223.2 mg/kg, had practically no abnormal effect on the system, *i.e.*, no glucose, acetone, albumin were observed in the urine of treated animals, neither was there diuresis or rise in specific gravity. Further, the high dose of condensation product (300 mg/kg) given subcutaneously to a normal rabbit, fasted for nearly 18 hours, did not raise the ketonemic level from its initial value. The estimation was done by the method of Behre and Benedict(18) at intervals of 2, 4, and 6 hours, respectively.

In vitro studies (Table II) indicated acetoacetate in small amounts of 5.16, 10.33, and 20.66 mg respectively, had practically no inhibitory effect on normal glucose utilization. But in appreciable amount (41.33 mg) it exhibited a distinct inhibitory effect on normal glucose utilization. On gradually increasing

the concentration of acetoacetate, such inhibition became more and more pronounced. On the other hand the condensation product of glucose and ethylacetoacetate was found to be without any inhibitory effect on the normal glucose utilization up to a concentration of 91.33 mg, *i.e.*, an amount equimolecular to 41.33 mg of acetoacetate. With amounts greater than 91.33 mg, the condensation product did show some inhibitory effect but very much less than that exhibited by corresponding amounts of free acetoacetate alone. In one set of experiments (Table II, D) wherein both condensation product and acetoacetate were kept, it was noted that the free acetoacetate together with the chemically combined form (condensation product) shows better utilization of glucose than the corresponding amount of free acetoacetate alone. Thus *in vitro* findings revealed the inhibition of normal glucose utilization by increased concentration of acetoacetate. It appears likely that increased concentration of acetoacetate very probably seriously impairs the metabolic relationship between glucose and acetoacetate, perhaps through the process of inactivation of the enzyme in the system, also obtained by Nath and Hatwalne(19).

Summary. 1. A metabolic relationship between glucose and acetoacetate has been shown to exist in the living body. 2. Increased concentration of acetoacetate (Na salt) distinctly inhibits normal glucose utilization when incubated at 37°C at a pH of 7.3 in the presence of whole blood (rabbit). 3. The condensation product of glucose and ethylacetoacetate has no effect on the normal ketonemic level and blood sugar tolerance curve of a rabbit and has neither any abnormal effect on the urine constituents of the treated animals.

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