Glucose-6-Phosphate as the Product of Glucose Phosphorylation in Testes.* (27568)

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Although ATP phosphotransferases are known which phosphorylate fructose and galactose at carbon 1(1,2), it has generally been assumed that phosphorylation of glucose by ATP yields glucose-6-P. However, Akaeda (3,4) has recently claimed to have identified glucose-1-P as the product of glucose phosphorylation in rabbit testes. On the other hand, this claim is disputed by Grillo(5) who identified glucose-6-P as a glucose phosphorylation product, and found no evidence of a labile phosphate ester after hexokinase assay in rabbit testes homogenate in the presence of 0.1 M fluoride which, he stated, inhibited phosphoglucomutase 100%.

By taking advantage of the differential inhibition of phosphoglucomutase by 0.06 M arsenate, we have recently shown that glucose-6-P is the primary phosphorylation product in rat liver(7) and we now report similar data which demonstrate clearly that glucose-6-P is the ATP-dependent glucose phosphorylation product in extracts of rabbit and rat testes.

Methods. Rabbits were sacrificed by a lethal intramuscular injection of phenobarbital and rats were decapitated. The testes were dissected, minced with scissors and homogenized, using a glass tube and Teflon pestle. The enzyme preparation was made exactly as described previously for rat liver glucokinase (6). The substrates, glucose-1-P, glucose-6-P, nicotinamide adenine dinucleotide-P ($NADP^+$) and adenosine triphosphate (ATP) were highest grade commercial preparations, and the glucose-6-P dehydrogenase was a high purity (A grade) crystalline yeast preparation, obtained from Calbiochem Inc. The use of this highly purified dehydrogenase ensured a high specificity of the assay procedure for glucose-6-P.

Results. Using an enzyme preparation of rabbit or rat testes, a series of 10 cuvettes was set up with the additions shown in Table I. No appreciable reduction of $NADP^+$ occurred with no addition (A); or with arsenate, (B), or glucose (C), or both (D). In the presence of glucose-1-P, reduction was very rapid, indicating a high phosphoglucomutase activity (E). Addition of arsenate inhibited phosphoglucomutase activity 90% (F), and the activity was not increased by glucose addition (G) or ATP addition (H). However, addition of both glucose and ATP to the arsenate-inhibited system (I) gave a $NADP^+$ reduction as high as in the cuvette containing glucose and ATP without arsenate (J). Exactly similar results were obtained with a similar preparation of rat testes (Table I).

In another experiment (Fig. 1) the relative

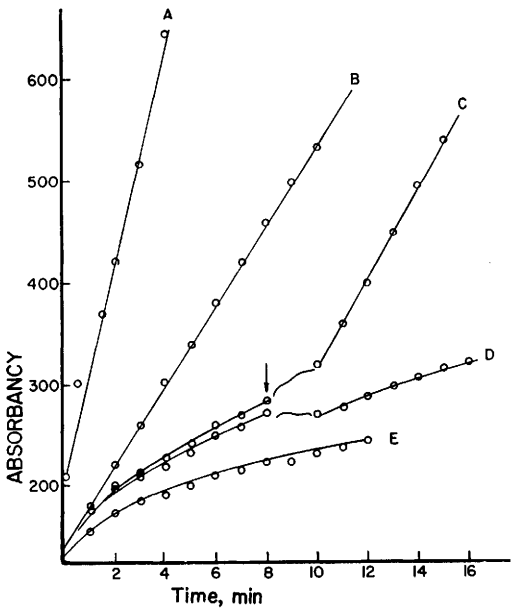

FIG. 1. Rates of phosphoglucomutase and glucokinase reactions. Experimental conditions as in Table I. Curve A, glucose-1-P; Curve B, glucose + ATP; Curve C, glucose, glucose-1-P, and AsO_4, ATP added at arrow; Curve D, glucose, glucose-1-P, AsO_4, water added at arrow; Curve E, glucose only.

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TABLE I. Assay of Phosphoglucomutase and Glucokinase in Testes Extracts.

Cuvettes	Additions				Tissues	
	ATP	Glucose	Glucose-1-phosphate	AsO_4	Rabbit testis	Rat testis
					$\Delta A/min. \times 10^3$	
A	—	—	—	—	—	—
B	—	—	—	+	3	2
C	—	+	—	—	6	4
D	—	+	—	+	5	3
E	—	—	+	—	106	210
F	—	—	+	+	10	22
G	—	+	+	+	11	23
H	+	—	+	+	12	24
I	+	+	+	+	42	95
J	+	+	—	—	40	80

Each microcuvette contained the following substances in the designated final concentrations: glycylglycine buffer, pH 7.4, 44 mM; $NADP^+$, 0.75 mM; $MgCl_2$, 7.5 mM; glucose-6-P dehydrogenase, 0.02 unit; enzyme preparation, 20 μl, and where indicated in the table, ATP, 3 mM, glucose, 0.1 M, glucose-1-P, 0.4 mM, and arsenate, 0.06 M. Final volume was 0.4 ml. Temperature was 25°. The reaction was started by addition of the tissue extract, and absorbancy changes noted each minute for at least 10 min., during which time the rates were strictly linear. Velocities were calculated from the ΔA between 5 and 10 min.

effects of arsenate on phosphoglucomutase and glucokinase reactions are portrayed graphically. Uninhibited phosphoglucomutase activity proceeded rapidly (curve A) but was inhibited 86% by arsenate (curves C and D). Two cuvettes, each containing glucose, glucose-1-P, and arsenate, were run for 8 minutes, at which time ATP was added to one and water to the other. The cuvette with ATP (curve C) showed immediate $NADP^+$ reduction at a rate slightly higher than the cuvette containing glucose and ATP from the beginning (curve B), whereas the blank continued at the inhibited rate (curve D). Although the experiments here described were carried out, for convenience of spectrophotometry, with the supernatant fraction after centrifugation at 100,000 $\times$ *g* in a Spinco model L ultracentrifuge, exactly similar results were obtained with a whole tissue homogenate.

Under the conditions of these experiments a change of one absorbancy unit corresponds to the reduction of 0.06 μmole $NADP^+$, and since the 20 μliters of enzyme preparation corresponds approximately to 6 mg fresh tissue (6) we can estimate from the absorbancy change of 0.040 per min that the specific activity of the rabbit testes preparation is about 0.4 μmoles $NADP^+$ reduced per g fresh tissue per min. This activity is comparable with that reported by Grillo(5). A determination of the Michaelis Constant according to the method of Lineweaver and Burk gave a value of approximately 1×10^{-4} M for the glucose Km.

The rapid reduction of $NADP^+$ during glucose phosphorylation by testes preparations in presence of glucose-6-P dehydrogenase under conditions which result in 90% inhibition of phosphoglucomutase precludes the formation of glucose-1-P as the primary phosphorylation product in this organ and leaves no doubt of the direct formation of glucose-6-P, as occurs in other tissues(8). Akaeda in his first paper(3) pointed out that his findings were not conclusive, and in his second paper (4), the identification of glucose-1-P by paper chromatography does not exclude its formation secondarily from glucose-6-P.

Summary. Extracts of rabbit and rat testes readily formed glucose-6-P (measured by $NADP^+$ reduction in presence of excess glucose-6-P dehydrogenase) from ATP and glucose in presence of 0.06 M arsenate, which inhibits phosphoglucomutase approximately 90%. These findings are considered to indicate that contrary to the reports of Akaeda, glucose-6-P is the primary phosphorylation product in this tissue.

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Tissue Antibody and Complement Levels in Runt Disease. (27569)

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Runt disease, first described by Billingham and Brent(1), is induced by injection of immunologically-competent lymphoid cells from a homologous donor into an immunologically-tolerant host. The syndrome generally is regarded as the result of immunological reactions of donor cells that have proliferated in the lymphoid tissues of the host. It was considered likely that the mature and immunologically competent grafted donor cells would produce antibodies against host tissues. Studies were undertaken, therefore, to determine whether tissue antibody levels were abnormally elevated in runt disease, and additionally to test the complement (C′) levels in the affected animals.

Materials and methods. Runt disease. Newborn albino rats were inoculated with about 10^6 lymphoid cells obtained from the spleen of an adult male Canadian hooded rat; the inoculum was suspended in Tissue Culture Medium #199(2), omitting the usually added horse serum. All animals of a given litter did not experience the disease with the same degree of severity. Nevertheless, one or more of the classic symptoms of the runt disease syndrome usually was evident. Most striking of these was the relatively small size of the runted animals. In addition, some developed alopecia around the eyes and nose, and the more severely affected walked with the mincing gait characteristic of the disorder. The latter condition has been attributed to a loss of skin elasticity and tonicity which restricts normal movement of the limbs(3). Two periods of crisis were observed, the first at about the tenth postinoculation day, the second at about day 20. Although some deaths occurred with each crisis, the majority of animals surviving the second crisis lived for at least 2 weeks, and some appeared to recover completely, eventually attaining the weight of control litter mates. Prior to exsanguination (25-35 days *post partum*), inoculated animals of a given litter were segregated according to size. Those significantly smaller than control litter mates were designated "small runts." Those that approached or equalled the size of the controls were categorized "hemi-runts." Sera from a given group were pooled and stored at –35°C until used in tests.

Serological procedures. Complement fixation tests(4) employing antigens derived from tissues and organs of various animal species were used to assay tissue antibody levels in the serum pools. C′ was assayed by a precise method developed in this laboratory(5).

Results. Representative experiments are summarized in Table I. Neither liver extract antigens of albino rat, rabbit, or chicken origin, nor albino rat spleen antigen revealed a significant increase of complement-fixing tissue antibody levels in the runted animals. In addition, tests with calf thymus nucleoprotein antigen were uniformly nonreactive. Tests with liver antigen from runted animals likewise showed no increase of tissue antibody.

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