Activation of Cow Adrenal Glucose 6-Phosphate Dehydrogenase by Adrenocorticotropin*

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ABSTRACT: The activity of glucose 6-phosphate dehydrogenase (D-glucose 6-phosphate:NADP+ oxidoreductase, EC 1.1.1.49) crystallized from cow adrenal cortex was increased in the presence of adrenocorticotropin (ACTH). The degree of ACTH activation of the enzyme varied with buffer composition, pH, presence or absence of Mg^{2+}, and the level of substrates and ACTH. The apparent K_m for oxidized nicotinamide–adenine dinucleotide phosphate ($NADP^+$) at pH 7.0 in phosphate buffer in the presence of excess glucose 6-phosphate was 3.0×10^{-5} M in the absence of ACTH and 1.2×10^{-5} M in the presence of Ciba β^{1-24}-ACTH. Similarly, the apparent K_m for glucose 6-phosphate at pH 7.0 in phosphate buffer in the presence of excess $NADP^+$ was 12.5×10^{-4} M without ACTH and 6.0×10^{-4} M with ACTH. Near maximal increases in enzymatic activity occurred at pH 7.0 at approximately 1 mole of ACTH to 1 mole of enzyme. When enzyme was 4.2×10^{-9} M, glucose 6-phosphate 2.6×10^{-5} M, and $NADP^+$ 3.3×10^{-6} M, the rate of reduction of $NADP^+$ was only 0.5 mμmole compared with 311 mμmoles min^{-1} with excess glucose 6-phosphate and $NADP^+$. It was at the lower substrate concentrations that the greatest ACTH activation was observed. Thus, ACTH activation of adrenal glucose 6-phosphate dehydrogenase at cellular levels of substrates may be of major significance in regulating the pentose phosphate pathway for cell function and replication of the adrenal cortex. Various preparations of ACTH, both natural and synthetic, also reduced the K_m for both $NADP^+$ and glucose 6-phosphate. Other trophic hormones tested such as gonadotropins, vasopressin, and 3′,5′-cyclic adenosine monophosphate had little or no effect, indicating a unique adrenal species of the enzyme with receptor groups for ACTH activation.

Adrenocorticotropin (ACTH)[1] stimulates steroidogenesis in the adrenal cortex. The fact that steroidogenesis requires multiple enzyme systems makes the initial site of ACTH action difficult to separate from secondary sites of ACTH action (Haynes, 1968). Studies in our laboratories (McKerns, 1964a,b, 1966) have provided evidence that as a major mechanism, ACTH regulates steroidogenesis and other cell functions in the adrenal cortex by initially stimulating a species of adrenal glucose 6-phosphate dehydrogenase (D-glucose 6-phosphate:$NADP^+$ oxidoreductase, EC 1.1.1.49). Other possible regulatory mechanisms of the adrenal cortex by ACTH and other hormones have been discussed recently (McKerns, 1968). A part of this study has been reported in preliminary form (McKerns and Criss, 1968). The present report is a kinetic study demonstrating direct ACTH activation of a purified species (Criss and McKerns, 1968) of glucose 6-phosphate dehydrogenase from cow adrenal cortex.

Materials and Methods

Crystalline glucose 6-phosphate dehydrogenase was prepared from bovine adrenal cortex according to the procedure of Criss and McKerns (1968). The enzyme used in all of these studies had been purified 2500-fold. The crystals of purified enzyme dissolved in 0.01 M phosphate buffer at pH 4.0 with β-mercaptoethanol (2.7×10^{-3} M) and EDTA (2×10^{-4} M) was stable for over 6 months at 4°.

Dehydrogenase activity was measured by monitoring the initial rate of reduction of $NADP^+$ in Tris-HCl or phosphate buffer as described previously (Criss and McKerns, 1968). Substrate, buffers, and other chemicals were obtained from sources referred to previously (Criss and McKerns, 1968). Synthetic 23 AA ACTH preparations V-72 and AC-23 were obtained through the courtesy of Professor Klaus Hofman, University of Pittsburgh. β^{1-24}-Corticotropin was kindly provided by Dr. G. de Stevens, Ciba Pharmaceutical Co., Summit, N. J. A pentadecapeptide ACTH was kindly provided by Drs. A. Fanchamps and R. Boissonnas of Sandoz Ltd., Basle, Switzerland. ACTH was also purchased from Calbiochem, Los Angeles, Calif. We are indebted to Dr. J. Jewell, Ayerst Laboratories, New York, N. Y., for a supply of Equinex (PMSG). The pituitary gonadotropins (LH and FSH) and vasopressin were kindly supplied by Dr. M. M. Graff, Endocrine Study Section, National Institute of Health, Bethesda, Md. 3′,5′-Cyclic AMP was purchased from Schwarz BioResearch, Orangeburg, N. Y. The composition of buffers, pH, and concentrations

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[1] Abbreviations used that are not listed in *Biochemistry 5*, 1445 (1966), are: ACTH, adrenocorticotropin; LH, luteinizing hormone; FSH, follicle-stimulating hormone; PMSG, pregnant mares serum gonadotropin.

of Mg^{2+}, $NADP^+$, and glucose 6-phosphate vary throughout the study and are therefore given in the legends of the tables and figures.

Experimental Section

Effect of Buffer Composition, pH, and $NADP^+$ Concentration on ACTH Stimulation. The effect of ACTH on the initial velocity of 4.7×10^{-9} M glucose 6-phosphate dehydrogenase in the presence of high levels of glucose 6-phosphate (3×10^{-4} M) was studied with excess $NADP^+$ (10^{-4} M) and rate-limiting amounts of $NADP^+$ (10^{-6} M), in 0.1 M Tris-HCl or phosphate buffers, and in the presence or absence of Mg^{2+} (Table I). No increase in initial velocity was observed in Tris-HCl buffer at pH 8.0, in the presence of $MgCl_2$, when $NADP^+$ was saturating at 100 μM; while an increase of 74% was observed in phosphate buffer, at pH 7.0, in the absence of $MgCl_2$, and at 1 μM $NADP^+$. Various combinations of these factors produced various levels of increase in enzymatic activity.

TABLE I: Effects of the Buffer Composition, pH, Mg^{2+}, and Substrate Levels on the Activation of Adrenal Glucose 6-Phosphate Dehydrogenase by ACTH.[a]

Buffer	pH	Mg^{2+}	$NADP^+$ ($\times 10^{-6}$ M)	% Increase with ACTH
Tris-HCl	7.0	−	100	18.2
		−	1	51.6
		+	100	8.2
		+	1	38.9
	8.0	−	100	8.1
		−	1	16.2
		+	100	0.1
		+	1	2.4
Phosphate	7.0	−	100	31.7
		−	1	74.4
		+	100	10.3
		+	1	61.2
	8.0	−	100	12.2
		−	1	20.8
		+	100	2.3
		+	1	5.1

[a] Initial velocities were measured in 0.1 M buffers, at 37°, with 3×10^{-4} M glucose 6-phosphate and 4.7×10^{-9} M glucose 6-phosphate dehydrogenase. Mg^{2+} was 10^{-3} M $MgCl_2$. The per cent increase represents the increase in enzymatic activity which occurred when 7×10^{-9} M Ciba β^{1-24}-ACTH was added to the various assay mixtures.

ACTH Dose-Response Effect. The effect of various concentrations of ACTH on the activity of adrenal glucose 6-phosphate dehydrogenase is shown in Figure 1. When the enzyme concentration was 6.7×10^{-9} M, increasing concentrations of Ciba β^{1-24}-ACTH from 10^{-14} to 10^{-9} M stimulated an increasing enzyme activity. Further increase in the concentration of ACTH resulted in a progressive decrease of stimulation. The maximum stimulation occurred at approximately 1 mole of ACTH to 1 mole of enzyme.

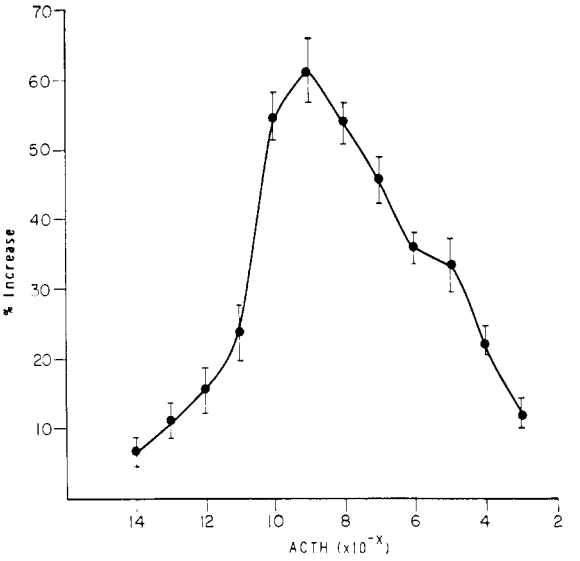

FIGURE 1: Change in the initial velocity of adrenal glucose 6-phosphate dehydrogenase in the presence of increasing amounts of ACTH. Activity measurements were made in 0.1 M phosphate buffer (pH 7.0) at 37°, with 3×10^{-4} M glucose 6-phosphate, 3×10^{-6} M $NADP^+$, and 6.7×10^{-9} M glucose 6-phosphate dehydrogenase. ACTH (Ciba β^{1-24}) was added at concentrations from 10^{-14} to 10^{-3} M, and is indicated as ($\times 10^{-x}$).

Specificity of ACTH Activation. In Table II it can be seen that various natural and synthetic ACTH preparations stimulated the initial velocity of adrenal glucose 6-phosphate dehydrogenase (4×10^{-9} M), when glucose 6-phosphate was 3×10^{-4} M and $NADP^+$ rate limiting at 3×10^{-6} M. No significant activation occurred with Hofman AC-23 ACTH where the N-terminal group was acetylated, the C-terminal aminated, and the lysine residues blocked with formyl groups. Hofman and Yajima (1962) had shown previously that this ACTH preparation is largely inactive in stimulating the adrenal cortex *in vivo* as judged by the ascorbic acid depletion test. The formyl-blocked compound was inactive in stimulating steroidogenesis in a homogenate of rat adrenal (McKerns, 1964b). It was suggested by McKerns (1964b) that ACTH activated adrenal glucose 6-phosphate dehydrogenase by hydrogen bonding or electrostatic attraction of the free amino end and the ϵ-amino groups of the lysine residues of the ACTH peptide to complementary groups (possibly carboxyl and hydroxy groups of amino acids) on the enzyme. 3′,5′-Cyclic AMP and vasopressin had no significant effect on the enzyme. The various gonadotropins tested had little or no effect and may have some contamination with ACTH.

TABLE II: Activity of Glucose 6-Phosphate Dehydrogenase in the Presence of ACTH and Other Hormones.[a]

Additive ($\times 10^{-9}$ M)	Velocity (mμmoles of NADPH min^{-1})	% Increase
None	6.03 ± 0.19	
4.0 ACTH (Ciba β^{1-24})	9.81 ± 0.43	62.7
2.6 ACTH (Calbiochem)	9.05 ± 0.51	50.1
2.9 ACTH (Hofman V-72)	9.24 ± 0.46	53.2
8.1 ACTH (Sandoz β^{1-25})	9.67 ± 0.62	60.4
2.4 ACTH (Hofman Ac-23)[b]	6.34 ± 0.94	4.9
2.1 3′,5′-cyclic AMP (Schwarz)	6.40 ± 0.60	6.1
6.7 LH (NIH-B2)	6.47 ± 0.36	7.3
8.4 PMS gonadotropin (Equinex)	6.04 ± 0.23	1.7
1.6 pituitary gonadotropin (NIH-B)	6.68 ± 0.43	10.8
3.2 FSH (NIH-S2)	6.35 ± 0.47	5.3
5.5 vasopressin (NIH LVP-1)	6.00 ± 3.0	0.0

[a] Activity measurements were performed in 0.1 M phosphate buffer, at pH 7.0, 37°, 3×10^{-4} M glucose 6-phosphate, 3×10^{-6} M $NADP^+$, and 4×10^{-9} M glucose 6-phosphate dehydrogenase. Velocity is given plus and minus standard error. [b] Nitrogen terminal of the 23 amino acid peptide is acetylated, COOH end is aminated, and the ε-amino groups of the lysine residues at positions 11, 15, 16, and 21 are blocked with formyl groups.

Lineweaver–Burk Plots of ACTH Activation of Adrenal Glucose 6-phosphate Dehydrogenase. Figure 2 shows that Ciba $^{1-24}$-ACTH caused a decrease in the apparent Michalis constants for both $NADP^+$ and glucose 6-phosphate. The K_m for $NADP^+$ at pH 7.0 in phosphate buffer was 3.0×10^{-5} M in the absence of ACTH and 1.2×10^{-5} M in the presence of ACTH. Similarly, the K_m for glucose 6-phosphate at pH 7.0 in phosphate buffer was 12.5×10^{-4} M without ACTH and 6.0×10^{-4} M with ACTH. A decrease in the apparent K_m for $NADP^+$ at pH 6.0 and 8.0 was also observed; however, the decrease at pH 8.0 was very small. ACTH caused little or no increase in the velocity when $NADP^+$ and glucose 6-phosphate were in excess.

Effect of Varying Substrate Concentrations on Enzyme Velocity. Since stimulation was more apparent at rate-limiting concentrations of both substrates, an examination of the initial velocity of glucose 6-phosphate dehydrogenase was made as both substrate levels were decreased (Table III). At an enzyme concentration of 4.2×10^{-9} M, the velocity was 311 mμmoles of NADPH min^{-1}, with 2.6×10^{-3} M glucose 6-phosphate and 3.3×10^{-4} M $NADP^+$. This was close to V_{max} at pH 6.8. Lowering just the $NADP^+$ concentration to 3.3×10^{-6} M, decreased the velocity to 12.6 mμmoles of NADPH min^{-1}. Lowering just the glucose 6-phosphate concentration to 2.6×10^{-5} M, decreased the velocity to 1.8 mμmoles of NADPH min^{-1}. However, decreasing $NADP^+$ to 3.3×10^{-6} M and glucose 6-phosphate to 2.6×10^{-5} M, decreased the velocity to 0.5 mμmole of NADPH min^{-1}. Thus, lowering the concentrations of both substrates greater than 10-fold below their calculated K_m levels decreased the initial velocity of the enzymatic reaction more than 600-fold. Similar effects were observed at a lower concentration of enzyme (1.4×10^{-9} M).

Discussion

One of the principal actions of ACTH would appear to be a stimulation of the preexisting adrenal enzyme glucose 6-phosphate dehydrogenase, by decreasing the apparent K_m for both substrates. This would represent a rapid response by the adrenal cortex to ACTH since it would immediately increase the rate of reduction of $NADP^+$ for steroidogenesis and other processes requiring reducing equivalents. It would also provide the necessary ribose sugars for synthesis of nucleotides and polynucleotides such as DNA and RNA. ACTH activation would also increase the metabolism of glucose 6-phosphate *via* the pentose phosphate pathway for other metabolic needs. Cell function and replication may depend upon hormone regulation of the pentose phosphate pathway through control of the activity of glucose 6-phosphate dehydrogenase (McKerns, 1964a,b, 1968).

These studies show that the activity of adrenal glucose 6-phosphate dehydrogenase and its stimulation by ACTH depends upon many factors. Maximum activity of the enzyme was obtained at pH 8.6 (Criss and McKerns, 1968). ACTH stimulation was more marked at lower pH. As suggested previously, ACTH may bind by hydrogen bonding or electrostatic attraction of charged amino groups on the N-terminal and the ε-amino groups on the lysine residues at positions 11, 15, 16, and 21, to complementary carboxyl or hydroxyl group on amino acids of the enzyme. This may induce a conformational change in enzyme structure for greater efficiency of hydrogen transfer from glucose 6-phosphate to $NADP^+$ (McKerns 1964a,b). In addition to inducing a possible conformational change in the enzyme, other mechanisms of ACTH activation are possible. The hydrogen bonding or electrostatic association of the positively charged ACTH to negatively charged amino acids on the enzyme may increase the net positive charge around the substrate sites for both glucose 6-phosphate and $NADP^+$. Both substrates presumably bind to their receptor groups on the enzyme through dissociated hydroxyls on the phosphate groups of glucose 6-phosphate or on $NADP^+$ (McKerns and Bell, 1960). In this regard enzyme activation especially for glucose 6-phosphate, was lower in buffer, high in phosphate, probably due to competition of the phosphate for substrate binding sites (Criss and McKerns, 1968).

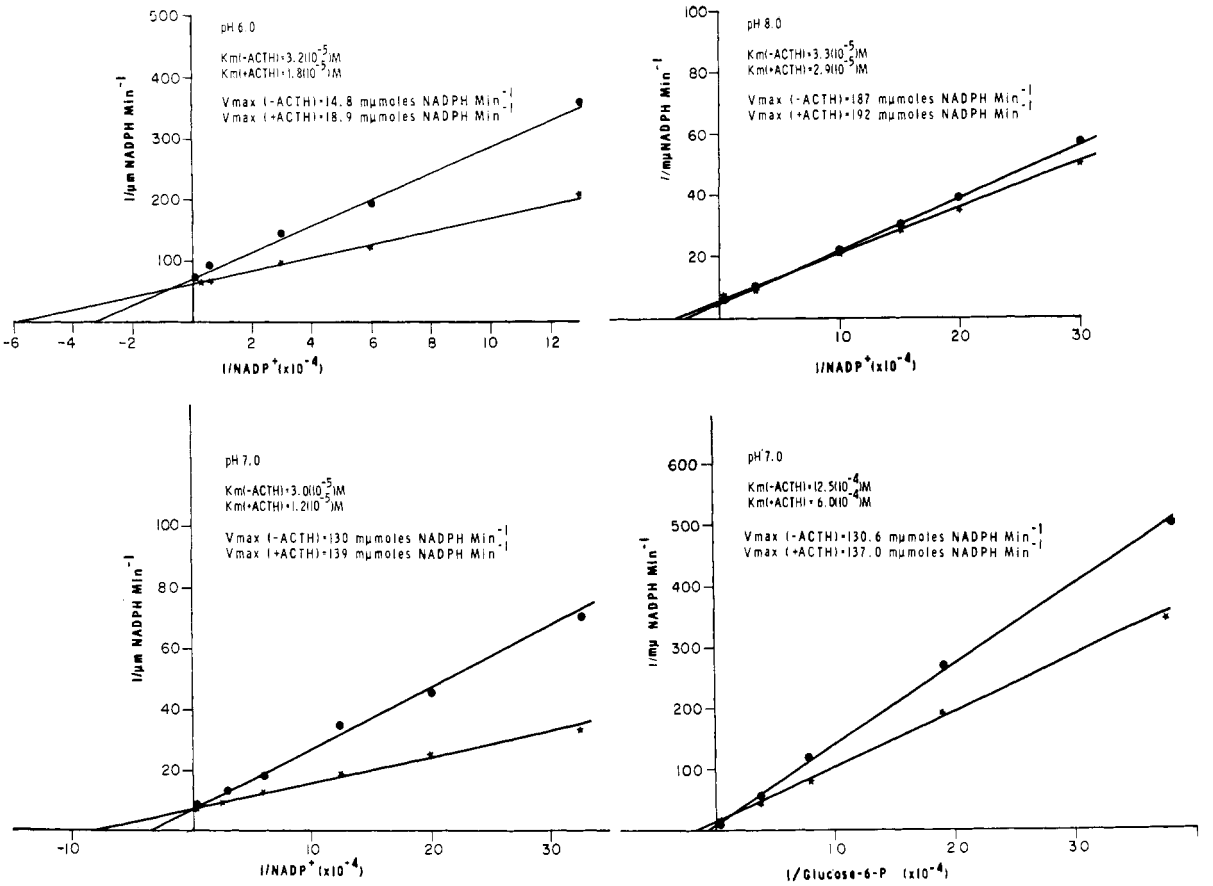

FIGURE 2: Lineweaver–Burk plots of adrenal glucose 6-phosphate dehydrogenase activity with and without ACTH. Activity measurements were performed in 0.1 M phosphate buffer, at 37°, with 1.8×10^{-9} M glucose 6-phosphate dehydrogenase, and 5.6×10^{-9} M Ciba β^{1-24}-ACTH. The plots with varying $NADP^+$ contain 8.6×10^{-3} M glucose 6-phosphate; the plot with varying glucose 6-phosphate contains 3.3×10^{-4} M $NADP^+$. Michalis constants were calculated from an equation which gives K_m as the negative reciprocal of the intercept of the line with the abscissa (McKerns, 1962). (●) Without ACTH and (*) with ACTH.

Changes in pH would be expected to change the efficiency of the hydrogen bonding or electrostatic attraction of ACTH to the enzyme. (H^+) may also influence the binding constants of the substrates. The pH of the intracellular environment of the enzyme around the mitochondria may be below 7.0 due to possible extrusion of H^+ from the mitochondria at the end of a metabolic cycle (McKerns, 1968). Mg^{2+} caused a 5% increase in the activity of glucose 6-phosphate dehydrogenase in the absence of ACTH and at pH 7.0 (Criss and McKerns, 1968). A greater ACTH stimulation was observed in the absence of Mg^{2+}. Increased levels of stimulation were observed at lower substrate concentrations and when the ACTH to enzyme ratio was about one. Therefore, greater ACTH stimulation of adrenal glucose 6-phosphate dehydrogenase occurs under conditions which would be considered suboptimal for this enzyme *in vitro*, but which may represent the *in vivo* environment.

One should consider other physiological factors to assess the possible *in vivo* role of ACTH activation of glucose 6-phosphate dehydrogenase. The main intracellular buffer is phosphate; excellent stimulation was observed in phosphate buffer, even in the presence of Mg^{2+}. One should consider the kinetics of the enzyme reaction at levels of enzyme and substrates to be found in the cell. Only at substrate levels of 2.6×10^{-3} M for glucose 6-phosphate and 3.3×10^{-4} M for $NADP^+$ in Table III was the rate velocity first order in terms of enzyme concentration (1.4–4.2 $\times 10^{-9}$ M glucose 6-phosphate dehydrogenase). There was little or no ACTH activation when the rate was first order. However, the physiological situation in the cell is not one of first-order kinetics for our calculated glucose 6-phosphate dehydrogenase concentration. Supernatant (cytoplasmic) $NADP^+$ is at a level of 1.7×10^{-6} M, with NADPH at 4.3×10^{-6} M in rat adrenal tissue (J. Purvis and F. Péron, personal communication). Since the maximum activity of glucose 6-phosphate dehydrogenase represents about one-third of the total maximum activity of $NADP^+$-specific dehydrogenases in high-speed supernatant from adrenal cortex (McKerns, 1962), it is probable that the level of $NADP^+$ available to glucose 6-phosphate dehydrogenase is less than 10^{-6} M. This is considerably below the K_m ($NADP^+$) of 3.0×10^{-5} M in phosphate buffer at pH 7.0 and near the level used where good ACTH stimulation was observed. The dynamics of the availability of glucose 6-phosphate from various metabolic reactions is unknown but is probably of the order of 10^{-5}–10^{-7} M. Enzymatic activity was extremely low (0.5 mμmole of NADPH min^{-1}) with enzyme 4.2×10^{-9} M, glucose 6-phosphate 2.6×10^{-5} M, and $NADP^+$ 3.3×10^{-6} M, at pH 6.8 in the absence

TABLE III: Effects of the Concentration of Enzyme and Substrates on the Initial Velocity of Glucose 6-Phosphate Dehydrogenase.[a]

Enzyme (M)	Glucose 6-Phosphate (M)	$NADP^+$ (M)	Velocity (mμmoles of NADPH min^{-1})
4.2×10^{-9}	2.6×10^{-3}	3.3×10^{-4}	311.0 ± 1.0
	2.6×10^{-3}	3.3×10^{-5}	183.1 ± 0.6
	2.6×10^{-3}	3.3×10^{-6}	12.6 ± 0.3
	2.6×10^{-3}	3.3×10^{-4}	311.0 ± 1.0
	2.6×10^{-4}	3.3×10^{-4}	19.9 ± 0.5
	2.6×10^{-5}	3.3×10^{-4}	1.8 ± 0.2
	2.6×10^{-3}	3.3×10^{-4}	311.0 ± 1.0
	2.6×10^{-4}	3.3×10^{-5}	6.8 ± 0.3
	2.6×10^{-5}	3.3×10^{-6}	0.5 ± 0.1
1.4×10^{-9}	2.6×10^{-3}	3.3×10^{-4}	101.9 ± 0.7
	2.6×10^{-3}	3.3×10^{-5}	7.1 ± 0.3
	2.6×10^{-3}	3.3×10^{-6}	8.3 ± 0.2
	2.6×10^{-3}	3.3×10^{-4}	101.9 ± 0.7
	2.6×10^{-4}	3.3×10^{-4}	12.7 ± 0.4
	2.6×10^{-5}	3.3×10^{-4}	1.1 ± 0.1
	2.6×10^{-3}	3.3×10^{-4}	101.9 ± 0.7
	2.6×10^{-4}	3.3×10^{-5}	3.4 ± 0.3
	2.6×10^{-5}	3.3×10^{-6}	0.5 ± 0.1

[a] Initial velocity measurements were performed in 0.1 M phosphate buffer at pH 6.8 and at 37°. Velocity is given plus and minus standard error.

of ACTH. This enzymatic activity can be contrasted with the near V_{max} potential of the same level of enzyme which was 311 mμmoles of NADPH min^{-1} when glucose 6-phosphate was 2.6×10^{-3} M and $NADP^+$ was 3.3×10^{-4} M. The amount of glucose 6-phosphate dehydrogenase in the cow adrenal cortex was calculated to be near 3.3×10^{-7} M (from Table I, Criss and McKerns, 1968). The level of ACTH in this gland has not been determined, so an evaluation of the significance of the hormone to enzyme ratio cannot be made. However, marked activation occurs at a 1:1 ratio. We do not have a rapid mixing device of sufficient sensitivity to measure the initial velocity of the reduction of $NADP^+$ and the degree of ACTH activation at tissue concentrations of enzyme and at the estimated cellular levels of $NADP^+$ and glucose 6-phosphate. However, since ACTH reduced the K_m for both glucose 6-phosphate and $NADP^+$, it is probable that at cellular levels of substrates, ACTH activation of adrenal glucose 6-phosphate dehydrogenase is of marked physiological importance.

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