and sucrase also differed. This is in agreement with findings[14] on duodenal mucosa scrapings from diabetic mice. The different responses of these two enzymes after alloxan treatment are probably due to differences in their mechanisms of induction. Previously, we reported that in the chronic phase of diabetic rats[15], there is a positive correlation between food intake and alkaline phosphatase activity, but not disaccharidase activities.

To confirm the action of the effect of food consumption on enzyme induction, some diabetic rats were given restricted food, equivalent in amount to that consumed by control rats, from day 3 to 5. As shown in figure 2, on day 5, alkaline phosphatase did not increase in these animals, whereas the sucrase activity increased as in diabetic rats fed ad libitum. These results suggest that the induction of alkaline phosphatase activity in the diabetic intestine is caused by an increase in food intake resulting from insulin deficiency, whereas the induction of sucrase activity is caused by insulin deficiency in itself, or a humoral imbalance due to insulin deficiency.

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NADP-isocitric dehydrogenase of gerbil adrenal mitochondria: support of steroid hydroxylation

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Summary. In gerbil adrenal cortex the activity of intramitochondrial NADP-linked isocitric dehydrogenase (IDH) is up to 10-fold greater than the NAD-linked IDH. The NADP-IDH, apparent K_m 0.58 mM, V_{max} 280 nmoles/min/mg mitochondrial protein, appears to be the major source of reducing equivalents to support adrenal mitochondrial steroid 11B- and 19-hydroxylation in this species.

Key words. Gerbil, adrenal cortex; isocitric dehydrogenase; mitochondria; steroid; steroid hydroxylation.

Adrenal mitochondria contain cytochrome P-450 hemoproteins, cholesterol side chain cleavage and 11β-hydroxylase enzymes, which utilize oxygen and reducing equivalents transferred from NADPH to introduce hydroxyl groups into steroid hormone precursors[2]. Tricarboxylic acid cycle intermediates oxidized by NADP-linked enzymes support the greatest rate of steroid hydroxylations by adrenal mitochondria in vitro; e.g. NADP-isocitric dehydrogenase in rat adrenal[3] and malic enzyme in bovine adrenal[4] mitochondria. The adrenal gland of the gerbil, *Meriones unguiculatus*, secretes nearly equal amounts of 19-hydroxy-11-deoxycortisol and cortisol[5]. The 19-hydroxlating activity was shown to be localized, with the 11β-hydroxylase, in adrenal mitochondria where high rates of steroid hydroxylations in vitro occurred in the presence of isocitrate or malate[6]. In order to determine the source of intramitochondrial NADPH for steroid hydroxylation we studied the NADP vs NAD-linked dehydrogenation of isocitrate or malate and report gerbil adrenal mitochondria contain predominately NADP-isocitric dehydrogenase (NADP-IDH).

Materials and methods. Adrenal glands were removed from gerbils which had been allowed to expire in a carbon dioxide anesthesia chamber or from decapitated animals. Glands were homogenized at 4 °C in 0.25 M sucrose and 25 mM HEPES, pH 7.4 (buffered sucrose). Mitochondrial and microsomal fractions were obtained by differential centrifugation and washed by resuspension in buffered sucrose. Protein content was determined using the Bradford method[7]. Dehydrogenase activities were measured spectrophotometrically at 37 °C (340 nm, total volume 1.0 ml) in buffered sucrose with, as appropriate, 0.1–0.3 mM pyridine nucleotide, 7 mM $MgCl_2$, and 0.1–50 mM substrate. Steroid hydroxylation activities were determined using androstenedione (A) or testosterone (T) as precursor[6]. Steroids in the incubation media were extracted with dichloromethane and quantitated using High Performance Liquid Chromatography[8].

Table 1. Subcellular distribution of isocitric dehydrogenase and malate dehydrogenase in gerbil adrenal tissue

Fraction	Activity (nmoles/min/mg protein)*			
	Isocitric dehydrogenase NAD	Isocitric dehydrogenase NADP	Malate dehydrogenase NAD	Glucose-6 phosphate dehydrogenase NADP
Experiment 1				
Homogenate	–	480	2480	102
Nuclei/cell debris	–	118	934	–
Mitochondria	13	120	1220	–
+ ADP	20			
Microsomes	–	35	0	–
Cytosol	–	526	1220	160
Experiment 2				
Fresh mitochondria	19	43		
+ ADP	52	32		
Sonicate				
1 × 30 sec	20	154		
2 × 30 sec	64	206		
Freeze/thaw	–	247		

* The data presented for experiments 1 and 2 are results of replicate assays carried out on separate preparations of adrenal tissue. Enzyme activities in experiment 1 represent total activities determined after disruption of fractions by sequential sonication (30 s sonication on ice followed by 30 s cooling)

Table 2. Substrate support of 11β- and 19-hydroxylation of steroid by gerbil adrenal mitochondria in vitro

Precursor	Androstenedione (nmoles/15 min/mg mitochondrial protein)*		Testosterone	
Products	19-OHA	11β-OHA	19-OHT	11β-OHT
Additions**				
NADPH 1 mM	2.3	3.0	0.7	2.2
NADPH 1 mM + Ca^{2+} 10 mM	3.5	4.4	1.0	0.44
DL-Isocitrate	24.3	19.6	1.8	1.5
Malate	1.7	1.5	0.9	0.5
α-Ketogluterate	–	–	0.6	0.2
Succinate	–	–	0.3	0.2

* Average of duplicate incubations carried out in 0.15 KCl, 7 mM $MgCl_2$, 25 mM HEPES with 90 μM steroid; a separate experiment using testosterone as substrate gave results comparable to those listed.
** Substrate 10 mM final concentration.

Results and discussion. NADP-IDH activity was detected in all subcellular fractions tested (table 1). In fresh mitochondria the activity of NADP-IDH was 3-fold greater than that for NAD-IDH (table 1, exp. 2). Though mitochondrial disruption increased the activities of both IDH isozymes, the rise in activity of intramitochondrial NADP-IDH was the greater. NAD-IDH activity was modestly elevated by 1–10 mM ADP. NADP-IDH kinetics were studied in two separate experiments using different preparations of disrupted mitochondria; apparent K_m 0.58 mM, V_{max} 280 nmoles/min/mg mitochondrial protein. In frozen-thawed adrenal homogenate, the activity of malic enzyme was 2% that of NADP-IDH; virtually no malic enzyme was detectable in disrupted mitochondria. In contrast, in disrupted homogenate or mitochondria, high levels of malate dehydrogenase were evident (table 1). Thus mitochondrial dehydrogenations of isocitrate vs malate generate, respectively, NADPH vs NADH. Higher rates of mitochondrial steroid hydroxylation occur with the generation of intramitochondrial NADPH than with intramitochondrial NADH; reducing equivalents from NADH can be transferred to NADP with the aid of the energy dependent transhydrogenase[4,7]. These differences explain why rates of the 19- and 11β-hydroxylation of either A or T were greater in the presence of isocitrate than with malate (table 2). In this study A was a better precursor than T for steroid 19- and 11β-hydroxylation. Thus adrenal mitochondria of the gerbil, as in the rat[3] possess significant levels of NADP-IDH. In the intact adrenal cell it is possible that other substrates may gain entry to the mitochondria and be used to generate NADPH. However, the activity of NADP-IDH in the mitochondria is sufficient to support the levels of steroid 11β- and 19-hydroxylation.

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L-Gulonolactone oxidase is present in the invertebrate, *Limulus polyphemus*

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Summary. L-Gulonolactone oxidase (EC 1.1.3.8) which catalyzes oxidation of L-gulonolactone to L-ascorbic acid was detected in tissues of *Limulus polyphemus*.
Key words. Limulus polyphemus; horseshoe crab; invertebrate; L-gulonolactone oxidase; L-ascorbic acid.

Many vertebrates have the ability to synthesize L-ascorbic acid; those that do not do so have been shown to lack the enzyme L-gulonolactone oxidase (EC 1.1.3.8, GLO) which catalyzes the final step in the synthesis–oxidation of L-gulono-1,4-lactone to L-ascorbic acid[1–6]. Several invertebrate animals including certain insects[7–10] and penaeid shrimp[11], have been found to require dietary ascorbic acid and thus appear to be unable to synthesize it. Only one, the pink bollworm *(Pectinophora gossypiella)* has been reported to be an apparent synthesizer[10]; its content of L-ascorbic acid increased when it was maintained on a diet devoid of the vitamin. Possibly *Trogoderma granarium* and *Tribolium castaneum* also fall into this category[12]. GLO has been sought in homogenates of snails, prawns, crabs, spiders, annelids, centipedes and leeches but none of them was found to have it[3]. Claims of synthesis by insect tissue[13–15] have been discounted on methodological grounds[16]. The present study demonstrates that one invertebrate, the chelicerate arthropod, horseshoe crab *(Limulus polyphemus)* possesses an enzyme capable of synthesizing L-ascorbic acid from L-gulonolactone.

Materials and methods. Adult *Limulus* of both sexes were obtained from the Marine Biological Laboratory, Woods Hole, MA, in five separate shipments between 1976 and 1980. They were maintained at 12 °C without feeding in Instant Ocean, an artifical seawater preparation. Adult rats *(Rattus norvegicus,* Sprague-Dawley strain) were from stocks maintained in our facility and adult chickens *(Gallus gallus)* were obtained locally. All tissues were wrapped in parafilm immediately after removal and were frozen on dry ice or in a freezer and kept at −20 °C until assayed.

L-Gulono-1,4-lactone was synthesized by the method of Frush and Isbell[17] and the D-isomer was purchased from Sigma. Nuclear magnetic resonance spectra of these preparations were essentially identical and their optical rotations were of equal magnitude and opposite sign ($[\alpha]_D^{20} = +54°$ and $-54°$ for D- and L-respectively).

GLO activity was determined by a modification of the method of Ayaz et al.[18]. This method involves homogenization of the tissue in phosphate buffer, incubation of the homogenate with L-gulono-1,4-lactone at 37 °C, oxidation with charcoal, incubation with 2,4-dinitrophenylhydrazine (DNPH) at 47 °C and measurement of absorbance at 520 nm of the bis-dinitrophenylhydrazone of L-dehydroascorbic acid. Modifications in-