injected male dog. Cholestyramine resin decreased the elevated cholesterol level of a progesterone-injected male dog.

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Kinetic Properties and Adaptations of Liver Glucose-6-Phosphatase.* (28230)

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The adaptation of liver glucose-6-phosphatase has been observed in response to both dietary(1) and hormonal(2) manipulations, as well as in diabetes(3). It appears that the adaptation results in a synthesis of more enzyme since the increase in activity can be prevented by ethionine, both in the case of response to diet(4) and to hormone administration(5). In both of these experiments, it was shown that the suppression of the adaptation by ethionine could be counteracted by additional methionine. There has been little work on the nature of the new or "induced" glucose-6-phosphatase. One report on the sub-cellular localization of the enzyme after cortisone treatment indicated no differences in distribution of the enzyme in control and cortisone treated rats(6). However, the widespread appearance of activity in all fractions appears to leave something to be desired in establishing a single sub-cellular localization. It has also been reported that in diabetes, which increases the liver glucose-6-phosphatase activity, this enzyme has altered kinetic properties(7). This report indicated an increase in apparent K_m (K_m') of the enzyme in both whole homogenates and with isolated microsomal fractions. All differences between the enzyme from normal and diabetic animals disappeared after solubilization with digitonin or desoxycholate(8). There has also been a report indicating no differences in the kinetic properties of the enzyme in normal and diabetic rats(9).

This report concerns studies of the kinetic properties of glucose-6-phosphatase in normal rats and in animals with increased total liver glucose-6-phosphatase activities. Male Sprague-Dawley rats were used. The diets have been described previously(1,10). Diabetes was produced by injection of alloxan and hydrocortisone injected as a suspension of the acetate (5 mg/rat/day). Diabetes was confirmed by blood glucose levels of 300 mg% or greater. The following methods were used: glucose-6-phosphatase assay(1), determination of K_m'(11), tissue fractionation (12), and blood glucose(13). All animals

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TABLE I. Effect of Diets and Hormones on Activity and Kinetic Constants of Liver Glucose-6-Phosphatase.

Diet or treatment	No. of rats	Glucose-6-phosphatase: Activity μm G-6-P split per min per 100 g body wt	Glucose-6-phosphatase: Apparent $K_m \times 10^{-3}$	Glucose-6-phosphatase: Apparent $K_m \times 10^{-3}$ of corresponding control group	Probability that K_m' values of the 2 groups are the same
Dextrin (control)	67	64.0 ± 1.4	2.76 ± .06*		
Cortisone	20	132.6 ± 6.4	2.38 ± .19	2.74 ± .12	.10
Hydrocortisone	18	132.0 ± 7.0	2.49 ± .14	2.74 ± .12	.20
Fasting	8	69.8 ± 5.1	2.84 ± .28	2.87 ± .27	.50
High protein	12	108.9 ± 7.7	3.65 ± .35	2.91 ± .17	.05
Sucrose	18	90.9 ± 3.8	2.06 ± .12	2.93 ± .09	.001
Fructose	15	105.0 ± 3.2	1.58 ± .10	2.55 ± .16	.001
Margarine	8	105.6 ± 6.4	2.57 ± .12	2.65 ± .14	.50
Glycerol	8	121.6 ± 4.5	2.61 ± .16	2.65 ± .14	.50
Diabetics	15	115.3 ± 5.1	5.92 ± .50	2.61 ± .18	.001

* Standard error of mean.

were maintained on the diets or hormone treatment for 4-5 days before sacrifice.

Table I shows the effect of diets and hormone treatments on total liver glucose-6-phosphatase activity per 100 g of body weight and on the K_m' of this enzyme. It was also found that isolated microsomal fractions from 12 normal, 20 sucrose and 4 protein rats had the following K_m' values respectively: 2.81 ± 0.16, 1.99 ± 0.09, and 3.26 ± 0.16, all $\times 10^{-3}$. It is evident that the enzyme from the fructose and sucrose fed rats has a lower K_m' than that from normal animals regardless of whether this parameter is measured in whole homogenates or isolated microsomes. In confirmation of the work of Segal *et al*, a marked increase in the K_m' was observed in diabetic animals. There was no significant difference in the K_m's of normal and high margarine fed rats. Although there was a tendency of lower than normal K_m' values after cortisone or hydrocortisone treatment and an increase after the high protein regimen the differences were not significant. This difference in the action of fructose-containing diets and the high protein or high margarine diets is especially interesting in view of the fact that the adaptations to these 2 types of diet have been shown to differ in certain other aspects(14,15).

Since it has been reported that solubilization of glucose-6-phosphatase results in lowering of the K_m'(8), an attempt was made to determine if the fructose or sucrose diets caused the appearance of soluble enzyme. No significant amounts of glucose-6-phosphatase activity could be found in 100,000 $\times$ *g* supernatant solutions after any of the above treatments. Thus, it is not the appearance of soluble enzyme that is the cause of the lower K_m' after fructose or sucrose feeding. The possibility exists that the changes in apparent kinetic constants may be due to alterations in the microsome enzyme complex. The term K_m' has been used throughout since the measurement of a true K_m of a particulate bound enzyme, especially in the presence of certain competing enzymes, is at the least very difficult, if not impossible. The possibility that the enzyme, in this case the glucose-6-phosphatase bound to the microsomal fraction, may exist as one or more isozymes with diabetes causing an increase in one and a fructose diet causing an increase in a different isozyme is highly improbable. In studies of glycogen storage disease (Type I), which appear to be due to a simple recessive, there is a complete or nearly complete absence of liver glucose-6-phosphatase(16). It is unlikely that there is more than one liver glucose-6-phosphatase, as this would require one gene to control several isozymes.

Summary. The activities and apparent kinetic constants (K_m') of liver glucose-6-phosphatase were measured following treatments causing increases in activity. Differences in K_m' values were observed after fructose feeding, either as fructose or sucrose, and after diabetes produced by alloxan. The liver glucose-6-phosphatase obtained from fructose

fed animals had significantly lower Km′ values than from glucose fed animals. This was noticeable in both the whole homogenate and isolated microsomal fraction. K_m' values obtained from diabetic animals were significantly higher than those from normal animals. All other treatments increasing the liver glucose-6-phosphatase activity produced no significant changes in the kinetic properties of this enzyme.

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Aldosterone and Corticosterone in Maintenance of Lactation in Adrenalectomized Rats.*† (28231)

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Earlier investigations have established that rats adrenalectomized early in lactation are unable to lactate sufficiently to support young (1,2). Maintenance of normal lactation in rats adrenalectomized on day 4 of lactation has been accomplished with daily subcutaneous injections of 500 μg hydrocorticosterone acetate plus 250 μg desoxycorticosterone acetate(3). However, the predominant secretions of the adrenal cortex of the rat are aldosterone and corticosterone(4). Cowie and Tindal(5) found that 50 μg aldosterone per day maintained lactation in adrenalectomized rats at approximately 70% of normal. Corticosterone alone or in combination with other adrenal steroids has not been tested for maintenance of lactation. This investigation was designed to determine minimal levels of these steroids which would maintain lactation in adrenalectomized rats when injected subcutaneously each day.

Materials and methods. Pregnant Sprague-Dawley-Rolfsmeyer rats were maintained in individual litter cages with free access to pelleted feed and water. The animal room temperature was maintained at 78 ± 1°F. A total of 105 lactating rats was used in this study. On day 4 of lactation the mothers were adrenalectomized or adrenal-ovariectomized in a single operation and litters were standardized to 6 pups. Injections were made subcutaneously each day beginning on day of operation.

Aldosterone-21-acetate‡ was dissolved in sesame oil and injected in a volume of 0.1 ml per day. Corticosterone-21-acetate was suspended in physiological saline with the aid of

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