ciates at the Saint Vincent and Sarah Fisher Home for Children, Farmington, Michigan, for the excellent manner in which the feeding and care of the children were carried out; to Melvin Feiner, Robert Morse, and Joseph Catania, for chemical and radiometric analyses; to Jack Dowdall for essential logistic support; to Edward P. Hardy, Jr. and John H. Harley for counsel, guidance and supervision.

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Received December 10, 1962. P.S.E.B.M., 1963, v112.

Effect of Diet and Sex on Kidney Transamidinase Activity in the Rat.* (28125)

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Transamidinase, which catalyzes the synthesis of glycocyamine, is the first enzyme in the biosynthetic pathway leading to creatine (1). In the rat it occurs in highest concentration in the kidney, but as much as 20% of the glycocyamine required by the animal may come from other tissues(2). The importance of this enzyme in control of creatine synthesis is suggested by an increase in creatine synthesis following administration of glycocyamine(3). This implies that availability of glycocyamine normally limits rate of creatine synthesis.

Various experimental conditions alter kidney transamidinase activity(4-10). Notably creatine feeding depresses the *in vitro* transamidinase activity of normal animals(4-9). Neither the explanation for this effect of creatine nor its significance in physiological control of transamidinase activity are known. Further insight into the mechanisms controlling transamidinase activity might be gained by determining the causes of its variability in normal animals. This variability may be quite large. For instance, results from several separate experiments indicate that the *in vitro* transamidinase activities of female rats receiving a purified diet(4) and of chow-fed male rats(8) are approximately 50% of the activity of male rats receiving a purified diet (9).

This report concerns controlled experiments which demonstrate the differences in kidney transamidinase activity due to diet and sex. Evidence that these differences are not secondary to an effect of creatine is also presented.

Methods. Weanling Sprague-Dawley rats of both sexes were used in these experiments. The male rats were divided into 5 groups: the first group was supplied with a complete purified diet(4), the second group received the

* Supported by research grant from Nat. Inst. Health, U. S. Public Health Service.

TABLE I. Effect of Sex and Diet on Creatine Excretion and Transamidinase Activity.

Diet	Sex	Transamidinase activity*	Urinary Creatine/Creatinine
Purified	♂	42.3 ± 1.1†(7)‡	.35 ± .01 (9)
"	♀	25.7 ± 1.9 (9)	.27 ± .01 (5)
Chow	♂	19.6 ± 1.2 (8)	.84 ± .14 (8)
Chow + glycine	♂	20.4 ± .7 (5)	.61 ± .09 (5)
Purified + glycine	♀	27.1 ± 2.8 (10)	.29 ± .01 (5)
Purified + casein	♂	40.7 ± 1.1 (5)	.34 ± .01 (5)
Chow + casein	♂	22.8 ± 1.8 (5)	.48 ± .06 (5)

* μmoles glycocyamine formed per hr per g wet weight of kidney.
† ± standard error.
‡ No. of animals in each group is given in parentheses.

same diet in which 10% of casein replaced a like weight of sucrose, the third group received pulverized Purina Laboratory Chow, the fourth group received the chow to which 10% of casein was added, and the fifth group received the chow to which 6% of glycine was added. The female rats received either the complete purified diet or the same diet in which 6% of glycine replaced a like weight of sucrose. After one month, several animals from each group were killed and kidney homogenates were assayed for transamidinase activity. Urinary excretion of creatine and creatinine was measured in 24 hour urine collections obtained during the last few days of the experiment.

In a separate experiment the effect of prolonged feeding of glycine and creatine on transamidinase was tested. Groups of weanling rats received either the purified diet, the purified diet containing one per cent creatine, or the same diet containing a combination of 6% of glycine and one per cent of creatine. Glycine and creatine replaced like weights of sucrose in the diet. The animals were killed for transamidinase assay after receiving the diets for one month.

In the last experiment groups of weanling male rats were supplied with the purified diet in which various concentrations of creatine and glycine replaced similar amounts of sucrose. Concentrations of creatine and creatinine were measured in a single urine collection from each of these rats during the second week of the experiment. Special care was taken to prevent contamination of the urine by food. The ratio of creatine to creatinine was used to evaluate the degree of creatinuria since certain of the collection periods for these animals were less than 24 hours.

Urinary creatine and creatinine concentrations were measured by Folin's method(11) with minor modifications. The transamidinase activity of kidney homogenates was measured in duplicate by the Van Pilsum procedure(10), and the Sims method for developing the Sakaguchi color was used(12).

Results. The data in Table I show that female rats receiving the purified diet and chow-fed male rats have approximately 50% of the kidney transamidinase activity of male rats supplied with the purified diet. The transamidinase activities of the male chow-fed rats are in agreement with the control activities reported for rats receiving a diet of Purina fox chow meal(8). Similar effects of comparable diets on kidney transamidinase have been reported for mice with hereditary muscular dystrophy, but the differences did not occur in normal mice of the same strain (13). The relatively low transamidinase activity of the female rats confirms the observations of Walker(14). Glycine supplementation had no effects on the transamidinase activities of the female or chow-fed rats (Table I). By contrast the same concentration of glycine for the same length of time doubled the kidney transamidinase activity of rats fed the purified diet plus creatine (Table II). Similar effects of creatine and glycine have been noted in chow-fed rats(8). The only evidence of glycine toxicity was slight growth depression in the female rats.

The urinary creatine to creatinine ratio was low in the female rats and only moderately elevated in the chow-fed rats (Table I). The increase in the creatine to creatinine ratio and the decrease in transamidinase activity in the

TABLE II. Effect of Dietary Glycine and Creatine on Transamidinase Activity.

Addition to diet	No. of animals	Transamidinase activity*
None	3	45.4 ± 1.5†
1% creatine	4	10.5 ± 1.0
idem + 6% glycine	5	21.2 ± 1.3

* μmoles glycocyamine formed per hr per g wet weight of kidney.
† ± standard error.

chow-fed rats are similar in magnitude to the changes produced in male rats by a diet containing 0.1% creatine (Table IV)(9). Increasing protein concentration of the chow diet by adding casein did not affect transamidinase activity although it appeared to slightly reduce creatine excretion (Table I). Extra casein in the purified diet did not alter creatine excretion or transamidinase activity.

The results of 2 experiments where homogenates of kidneys from male chow-fed rats were mixed with similar homogenates from male rats receiving the purified diet are shown in Table III. The transamidinase activities were additive. Thus there is no evidence for the presence of an inhibitor or absence of a cofactor in the kidneys of chow-fed rats.

Supplemental dietary glycine did not significantly affect the creatinuria of creatine-fed rats (Table IV) and did not increase creatine excretion by the rats receiving the purified diet. Kidney transamidinase activities of similar groups have been reported(9), and it was found that glycine partially prevents the depression of transamidinase even in the groups with extreme creatinuria.

Discussion. Kidney transamidinase activity is low in a variety of conditions that cause creatinuria. The relationship between the creatinuria and the low enzyme activity is unknown in most of these conditions, but in 2 cases, hyperthyroidism and Vit. E deficiency, the loss of transamidinase activity has been attributed to increased passage of creatine through the kidney(4,5). Other mechanisms that might also limit transamidinase activity have been difficult to identify because of the common occurrence of creatinuria.

A means of recognizing low activities caused by creatine is suggested by the observations that glycine(7-9) and citrulline(8) reverse the effect of creatine on transamidinase. The reasons for the antagonism between these compounds are not clear, but glycine and creatine are known to compete for reabsorption by the renal tubule(15) and might compete for transport across other cell membranes. One site for competition would be the intestinal mucosa since the effects of these compounds have been demonstrated by feeding experiments. If glycine blocks the absorption of creatine it could reverse the effect of dietary creatine and then have no influence on a low transamidinase activity caused by excess endogenous creatine. In studying this possibility the effect of glycine on creatine excretion was measured. The failure of glycine to reduce urinary excretion of creatine both in the control and creatine-fed rats clearly shows that glycine does not significantly impair intestinal absorption of creatine.

The lack of an effect of glycine on the transamidinase activity of chow-fed rats therefore supports the hypothesis that factors other than creatine are responsible for the relatively low activity. The reason for the low activity is not known, but it may be re-

TABLE III. Effect of Mixing Homogenates from Control and Chow-fed Rats.

The incubation mixture consisted of 20 μmoles of glycine, 20 μmoles of L-arginine, and either 0.5 ml of 4% kidney homogenate from control or chow-fed rats or a mixture of 0.25 ml from a chow-fed and 0.25 ml from a control rat in a total volume of 1.5 ml of 0.067 M phosphate buffer at pH 7.4. The reaction was carried out under air at 37°C for 1 hr.

Glycocyamine formed		
Control	Chow-fed, μmoles/g wet wt kidney/hr	1:1 mixture
45.2	21.4	33.8
39.6	22.0	28.9

TABLE IV. Effect of Glycine on Creatine Excretion in Creatine-fed Rats.

% creatine in diet	Urinary creatine to creatinine ratio: Without supplemental glycine	With 6% glycine added to diet
0	.49 ± .06*(6)†	.44 ± .05 (9)
.1	.63 ± .13 (4)	.82 ± .10 (5)
.5	2.9 ± .78 (4)	3.4 ± .90 (5)
1.0	4.7 ± .65 (4)	3.1 ± .59 (5)

* ± standard error.
† No. of animals in each group is given in parentheses.

lated to the amino acid composition of the diet. In contrast to the observation of Van Pilsum and Canfield in chow-fed rats(8) arginine feeding does not stimulate the *in vitro* transamidinase activity of rats supplied with a purified diet(9). This difference due to diet and the stimulatory effect of several other amino acids on transamidinase of chow-fed rats(8) suggests that the enzyme may require a particular balance of amino acids to maintain maximal activity.

In spite of the low *in vitro* transamidinase activity there is an increase in the urinary creatine to creatinine ratio in the chow-fed rats. The explanation for this observation is not known, but it should be pointed out that low *in vitro* transamidinase activity is compatible with normal or increased creatine synthesis *in vivo*(5).

Glycine feeding also has no effect on the transamidinase activity of female rats. The interpretation of this finding is complicated by the apparent toxicity of glycine for the female, but the absence of significant creatinuria supports the hypothesis that factors other than creatine limit the activity of kidney transamidinase. The possibilities that estrogens depress or that androgens increase transamidinase activity should be considered. Stimulation of transamidinase activity could account for the increased rate of creatine synthesis that is produced by methyltestosterone in man(16).

Summary. The *in vitro* kidney transamidinase activity of female rats supplied with a purified diet and of chow-fed male rats was found to be approximately 50% of that of male rats supplied with the purified diet. This accounts for much of the variability that has previously been found in normal kidney transamidinase activity. The female rats did not have a greater creatinuria than the comparable male rats, but the chow-fed male rats exhibited moderate creatinuria. Extra dietary glycine did not increase the transamidinase activity of the female or chow-fed rats. Also, glycine had no effect on urinary excretion of creatine by creatine-fed rats. These observations indicate that there are factors other than creatine which limit the activity of kidney transamidinase.

The author is indebted to Miss Cecilia Hsu and Mr. Richard E. Gale for valuable technical assistance.

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Received December 10, 1962. P.S.E.B.M., 1963, v112.