Factors Which Affect the Activity of Glutaminase I
in the Guinea Pig Kidney*

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Davies and Yudkin (1) have reported that the administration
dilute hydrochloric acid to rats for three months produced a
2-fold increase in renal glutaminase activity, and Rector et al.
(2) found that administration of ammonium chloride to rats for
to six days caused a 4-fold rise in the activity of this enzyme.
Enzyme activity was also increased 2-fold in the guinea pig by
the administration of ammonium chloride for three weeks (3).

In the present study, renal and hepatic glutaminase I activity
have been measured in guinea pigs treated with the urinary
acidifying agents ammonium chloride and sulfur. dl-Ethionine
has been used in an attempt to inhibit the increase in renal
enzyme activity. The effects of adrenal steroid administration
and variation of dietary protein intake have also been examined.

EXPERIMENTAL

Methods

Adult male guinea pigs (400 to 600 g) were maintained on
Purina Guinea Pig Chow Checkers, water, and daily supplements
of fresh cabbage.

The substances under investigation were administered twice
daily at approximately 9:00 a.m. and 5:00 p.m. Ammonium
chloride and sucrose were given in solution, and enzymatic casein
hydrolysate and sulfur were suspended in water and administered
by stomach tube. Hydrocortisone (Merck Sharp and Dohme)
and deoxycorticosterone acetate (Ciba Pharmaceutical Products,
Inc.) were suspended in 0.9% sodium chloride and injected intra-
muscularly. DL-Ethionine and DL-methionine (Nutritional Bio-
chemicals Corporation) were dissolved in 0.9% sodium chloride
solution and administered by intraperitoneal injection.

The animals were placed in rodent metabolism cages 2 hours
before being killed, and the ammonia excreted in response to
intragastric administration of 10 mmoles of NH₄Cl per kg of
body weight was determined. Urinary ammonia concentration
was measured by modified Seligson (4) technique.

Enzyme Assay—The animals were killed by a blow on the head.
The kidneys were immediately removed, decapsulated and made
into a 5% homogenate in cold 0.9% NaCl solution according to
the technique of Potter and Elvehjem (5). The homogenate
was then assayed for glutaminase I activity. This was measured
by subtracting the activity of the inherent glutaminase from the
total glutaminase activity, the former being measured in the
absence of added phosphate and pyruvate ions (6). Each assay
was performed (in duplicate in the absence of pyruvate) with
and without added phosphate.

The following incubation mixture was found to be optimal for
the assay of glutaminase I activity for both kidney and liver:
0.2 ml of 5% tissue homogenate, 0.1 ml of 0.1 M L-glutamine,
0.1 ml of 0.5 M Na₂HPO₄-NaH₂PO₄ (pH 7.5), 0.5 ml of 0.05 M
Tris (pH 7.5), and 0.1 ml of 0.9% NaCl. Incubation was carried
out in 25-ml bottles under air at 37 to 38° for 20 minutes.

At the end of the incubation period, the bottles were closed
with a sleeve-type rubber stopper holding a ground-glass rod
moistened with 10 N H₂SO₄, and 1.0 ml of 20% Na₂CO₃ solution
was injected through the rubber stopper. The bottles were
then rotated horizontally on a motor-driven wheel for 30
minutes. The glass rod was removed and rinsed with 3.0 ml
of water into a separate vessel, and 2.0 ml of Koch-McMeekin
Nessler's reagent were added and maximal color was allowed to
develop. This was read in a Klett photocolorimeter at 420 μm
wavelength.

Enzyme activity is expressed as μmoles of NH₃ liberated per
100 mg of renal deoxyribonucleic acid per minute of incubation.
Deoxyribonucleic acid was measured in an aliquot of the same
homogenate by the method of Schneider (7), and its concen-
tration was determined by the color reaction of Dische (8) for
deoxyribose.

RESULTS

As shown in Table I, administration of 20 ml 1 M NH₄Cl per
kg per day for 2 days caused renal glutaminase I activity to
increase approximately 60% above control values. In 4 days,
enzyme activity was approximately 80% above zero day control
levels and remained at this level during 4 more days of treat-
ment. Administration of a similar volume of water alone pro-
duced no significant rise in renal glutaminase I activity.

Intragastric administration of 10 mmoles of NH₄Cl per kg
per day or less produced a temporary (at 2 days) rise in renal
glutaminase I activity which was less than that produced by 20
mmoles. Doses of 30 and 40 mmoles per kg per day caused
hemorrhagic pulmonary edema and could not be studied.

As shown in Table II, intragastric administration of 20 mmoles
of NH₄Cl or sulfur per kg per day for 4 days produced a signifi-
cant rise in renal glutaminase I activity. However, enzyme
activity in the liver was not elevated; it was slightly, but not
significantly, lowered. Intragastric administration of 20 mmoles
of sulfur per kg has been shown (unpublished observation) to produce a significant rise in the renal excretion of sulfate, hydro- 
drogen, and ammonium ions in the guinea pig.

The intramuscular administration of either hydrocortisone or 
and ammonium acetate (40 mg per kg per day) for 2 days 
had no significant effect on either basal renal glutaminase I 
activity or the response of the enzyme to the repeated adminis- 
tration of ammonium chloride (20 mmoles per kg per day). The 
mean and standard error (μmoles NH₃ per 100 mg of DNA per 
minute) of these groups were: control, 26 ± 2; hydrocortisone, 
30 ± 3; deoxycorticosterone acetate, 28 ± 3; ammonium chloride, 
40 ± 4; and ammonium chloride plus deoxycorticosterone 
acetate, 37 ± 4.

As shown in Table III, intragastric administration of either 
2.5 or 5 g of casein hydrolysate per day produced a significant 
rise in renal glutaminase I activity in 2 days. The decreased 
food consumption by the animals receiving 5 g of casein hy-
drolysate resulted in a daily protein intake by this group which 
was only slightly higher than that of the group which received 
2.5 g of casein hydrolysate. The increase in enzyme activity 
in these animals was not accompanied by any significant change 
in urinary pH.

As shown in Table IV, intraperitoneal injection of 2 mmoles 
DL-ethionine per kg per day had no significant effect on basal

<table>
<thead>
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<th>Table I</th>
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<tbody>
<tr>
<td><strong>Effect of repeated administration of ammonium chloride on renal glutaminase I activity</strong></td>
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<tr>
<td>Group</td>
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<tr>
<td></td>
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<tr>
<td>Control (20 ml 0.9% NaCl per kg per day)</td>
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<tr>
<td>NH₄Cl (20 mmoles per kg per day)</td>
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Values are Mean ± S. E.  
* Five animals in zero day control group, four animals in all other groups.  
† Significantly different from zero day controls (P < 0.05).

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<th>Table II</th>
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<td><strong>Effect of repeated (4 days) ammonium chloride or sulfur administration on renal and hepatic glutaminase I activity</strong></td>
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<td>Group</td>
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<tr>
<td>Control</td>
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<tr>
<td>NH₄Cl (20 mmoles per kg per day)</td>
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<tr>
<td>Sulfur (20 mmoles per kg per day)</td>
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Values are Mean ± S. E.  
* Significantly different from control group (P < 0.01).  
† Significantly different from control group (P < 0.05).  

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<td><strong>Effect of protein intake on renal glutaminase I activity</strong></td>
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<td>Group</td>
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<tr>
<td>Controls</td>
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<tr>
<td>Sucrose (2.5 g per animal per day for two days)</td>
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<td>Sucrose (5 g per animal per day for 2 days)</td>
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<tr>
<td>Casein hydrolysate (2.5 g per animal per day for 2 days)</td>
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<td>Casein hydrolysate (5 g per animal per day for 2 days)</td>
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Values are Mean ± S. E.  
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<th>Table IV</th>
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<tr>
<td><strong>Effect of injection of DL-ethionine and DL-ethionine plus DL-Methionine on the response of renal glutaminase I activity to repeated (2 days) ammonium chloride administration</strong></td>
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<tr>
<td>Group</td>
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<tr>
<td>Control (20 ml 0.9% NaCl per kg per day in- traperitoneally)</td>
</tr>
<tr>
<td>DL-Ethionine (2 mmoles per kg per day in- traperitoneally)</td>
</tr>
<tr>
<td>NH₄Cl (20 mmoles per kg per day) + 0.9% NaCl (20 ml per kg per day intraperitoneally)</td>
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<tr>
<td>NH₄Cl (20 mmoles per kg per day) + DL-ethionine (2 mmoles per kg per day intraperitoneally)</td>
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<tr>
<td>NH₄Cl (20 mmoles per kg per day) + DL-ethionine (2 mmoles per kg per day intraperitoneally) + DL-methionine (2 mmoles per kg per day intraperitoneally)</td>
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Values are Mean ± S. E.  
* Significantly different from control group (P < 0.001).
our data for the guinea pig have been plotted as a function of urinary ammonia excretion. As shown in Fig. 1, a positive correlation \( r = 0.67 \) was found between mean renal glutaminase I activity and mean ammonia excretion.

**DISCUSSION**

Renal glutaminase I activity was significantly elevated after 2 days of ammonium chloride administration, and maximal enzyme activity was observed by the fourth day of treatment. Thus, the "adaptation" period of this enzyme is between that of enzymes which are increased within a period of hours (9), and those which require more than a week (10). A similar time course has been observed for the "adaptation" of this enzyme in the rat (2).

Although repeated administration of either ammonium chloride or sulfur produced an increase in renal enzyme activity, enzyme activity in the liver was not elevated. The data thus support the positive correlation found between renal glutaminase I activity and urinary ammonia excretion. The reverse situation has been reported for the "adaptation" of xanthine oxidase activity in the mouse. Dietrich (10) found that repeated administration of xanthine to mice produced a rise in hepatic xanthine oxidase activity, but not in renal xanthine oxidase activity.

The administration of either deoxycorticosterone acetate or hydrocortisone had no effect on basal renal glutaminase I activity or the response of this enzyme to repeated ammonium chloride administration. These observations are in agreement with the results obtained by Iacobellis et al. (11) in the rat. Furthermore, Wilson and Selkin (12) showed that adrenalectomy in rats had no effect on the capacity of kidney to respond to repeated ammonium chloride administration with respect to the elevation of glutaminase I activity. Thus, the adrenal steroids do not seem to play a major role in the increase of activity of this enzyme.

It has been shown that enzyme activity may be increased by chemical compounds which are not substrates of the enzyme (13). The rise in renal glutaminase I activity after repeated administration of ammonium chloride does not seem to be substrate-induced, but is rather a nonspecific effect of such substances as urinary acidifying agents.

The increase in enzyme activity which follows the administration of a chemical compound may be due to activation of preexisting, inactive enzyme or to the synthesis of new enzyme. The amino acid antagonist \( \beta \)-ethionine has been used in differentiating between these two phenomena. Ethionine inhibits normal protein synthesis by preventing the incorporation of methionine (14) and other amino acids (15) into protein. Ethionine is itself incorporated into proteins (16). In previous studies with other enzyme systems (10, 13, 17, 18), ethionine was found to inhibit the increase in enzyme activity which resulted from the administration of the inducer, and, in each case, injection of an equimolar amount of methionine eliminated the effect of ethionine. \( \beta \)-Ethionine was used in all these experiments. It is assumed that the D isomer is as effective as the L isomer, since Wachstein and Meisel (19) have shown that both isomers are equally potent in producing tissue damage. In the present study, it was found that intraperitoneal injection of ethionine completely inhibited the rise in renal glutaminase I activity produced by the repeated administration of ammonium chloride. Furthermore, injection of an equimolar quantity of methionine eliminated the effect of ethionine. These results are consistent with the hypothesis that the increase in renal glutaminase I activity after repeated ammonium chloride administration is due to the formation of new enzyme. However, it is possible that ethionine had an effect on some system which regulates glutaminase I activity. For example, the synthesis of a renal glutaminase I activator may have been inhibited. Thus, although the data obtained with the use of ethionine and methionine suggest that new enzyme synthesis is responsible for the increase in renal glutaminase I activity, this point is not conclusively established.

Rector et al. (2) have shown that repeated administration of ammonium chloride to rats produces a parallel rise in both renal glutaminase I activity and ammonia excretion. These results suggest that the production of urinary ammonia is, at least in part, controlled by renal glutaminase activity. The positive correlation found between renal glutaminase I activity and ammonia excretion in the guinea pig supports this hypothesis.

**SUMMARY**

1. Intragastric administration of 20 mmol/l of NH₄Cl or sulfur per kg per day to guinea pigs increased renal glutaminase I activity 80% above control values in 4 days. Hepatic glutaminase I activity was not elevated by either treatment.

2. Intramuscular administration of either deoxycorticosterone acetate or hydrocortisone did not affect the rise in renal glutaminase I activity produced by NH₄Cl administration alone. Casein hydrolysate (2.5 g or 5 g per day), orally, for 2 days increased enzyme activity 50% above control values.

3. The rise in renal glutaminase I activity produced by intragastric administration of 20 mmol/l NH₄Cl per kg per day for 2 days was completely inhibited by the simultaneous intraperitoneal administration of \( \beta \)-ethionine (2 mmol/l per kg per day). Administration of \( \beta \)-methionine with \( \beta \)-ethionine (3 mmol/l per kg per day of each) and 20 mmol/l NH₄Cl per kg per day resulted in an increase in enzyme activity that was no different from that produced by NH₄Cl alone.

4. A positive correlation was found between renal glutaminase I activity and ammonia excretion.
REFERENCES

Factors Which Affect the Activity of Glutaminase I in the Guinea Pig Kidney
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