UREASE TOXICITY IN GROWING SWINE 1, 2

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It has been postulated by Dang and Visek (1960) and Visek (1962) that the improvement in gain and feed efficiency of rats and chicks immunized with jackbean urease is due to a decrease in ureolytic activity of the gastrointestinal tract. This reduction in urea hydrolysis, caused by an antiurease-urease reaction, results in a decreased amount of ammonia which the body must detoxify. Therefore, energy is conserved for growth. Initial planning for a study of the effects of urease immunization upon the performance and hematology of growing swine disclosed the need for preliminary research on the toxicity of injected urease.

Parenteral injections of jackbean urease have been reported to be toxic to mice (Dang and Visek, 1963; Tauber and Kleiner, 1931), rats (Dang and Visek, 1963), rabbits (Dang and Visek, 1963; Kirk and Sumner, 1931; Tauber and Kleiner, 1931), dogs (Handford, 1961) and guinea pigs (Dang and Visek, 1963; Kirk and Sumner, 1931). Elevated blood ammonia and depressed blood urea levels were observed in these animals. Ammonia was suggested as the toxic agent in urease toxicity, as the symptoms closely resembled those exhibited in ammonia toxicity (Tauber and Kleiner, 1931; Kirk and Sumner, 1931).

This study was initiated to determine the effects of a large intraperitoneal injection of jackbean urease upon growing swine and its relationship to ammonia toxicity.

Experimental Procedure

Twenty Hampshire-Yorkshire crossbred pigs weighing 20 to 30 lb. were used in three trials. A control and a treatment pig were used in Trial I to determine if a single large injection of 100 modified Sumner units 6 (SU) per pound of body weight (BW) was toxic to the pig, and if the plasma urea and ammonia levels were changed. In Trial II with two pigs per treatment, 0 (physiological saline), 50 and 100 modified SU/lb. BW, were given intraperitoneally in a single dose. Trial III consisted of two treatments, 0 (physiological saline) and 75 modified SU/lb. BW, with six pigs per treatment. Twice-recrystallized urease, extracted using Kirk and Sumner's (1934) method and recrystallized using Dounce's (1941) procedure, was used in Trials I and II, while Sigma urease type II powder was used in Trial III.

All pigs were placed in metal metabolism cages approximately 20 hr. before they were injected. Pigs were fasted during all trials, but were allowed access to water three times daily. Urine was collected in large bottles containing 10 ml. of 25% H₂SO₄ or HCl, before and after the injection of urease.

Blood samples were taken from the anterior vena cava before the pigs were placed in the cages, before the urease was injected, and at specific intervals thereafter. A combination of NaF and EDTA was used as an anticoagulant for plasma samples. Sterile tubes, corks and vials were used for handling and storing serum to avoid possible bacterial contamination which might influence urease activity. Separation of both serum and plasma from cells was completed by centrifuging at 2000 g for 20 min. Serum was removed, placed in vials and frozen for later determinations of serum urease activity. Rectal temperature of the pigs were recorded at the time of blood sampling.

The hematocrit values were determined by the procedure outlined by McGovern et al. (1955). Hemoglobin was determined by the cyanmethemoglobin method (Crosby et al., 1954). Serum sodium and potassium determinations were made with a Beckman DU spectrophotometer equipped with a flame attachment using an oxygen-acetylene burner.

6 A modified Sumner unit is that amount of urease which will produce 1 mg. of ammonia N from a 3.0% urea-phosphate buffer at pH 7.0 at 27° C. in 5 min.
Determination of serum glutamic-oxaloacetic and glutamic-pyruvic transaminase activity was made according to the procedure outlined in Sigma Technical Bulletin No. 410.

Brown's (1959) p-dimethylaminobenzaldehyde method was used for determining urea N. Serum and urine ammonia N were determined by the microdiffusion method (Conway, 1957) using the Obrink (1955) modified Conway units. A 1:50 dilution of urine was used for both urinary ammonia N and urea N.

Liver samples which had been frozen after removal from the pigs were prepared for determinations of ammonia and urease activity as follows. Frozen samples were placed in the cold room (4°C) 15 to 30 min. before they were cut into small pieces and placed in a semimicro container. A minimum amount of deionized water was added, and the sample was homogenized in the cold room for 1 min. at high speed on a two-speed Waring Blender. The homogenized sample was removed and the container was rinsed with a minimum amount of deionized water. The homogenate including washings was centrifuged at 2000 g for 50 min. The supernatant was saved for assay.

One-half ml. was homogenized in the cold room for 1 min. in the cold room. Only the supernatant washings was centrifuged at 2000 g for 50 min. in the cold room. The supernatant was saved for assay. Visek et al. (1959) indicated no difference per volume in ureolytic activity between the entire homogenate of intestine or its supernatant. A 10-ml. aliquot of the supernatant was taken for dry matter. The homogenate including washings was centrifuged at 2000 g for 50 min. in the cold room. Only the supernatant was saved for assay. Visek et al. (1959) indicated no difference per volume in ureolytic activity between the entire homogenate of intestine or its supernatant. A 10-ml. aliquot of the supernatant was taken for dry matter determination at 100°C. for 15 hr. Urease activity and ammonia were determined as rapidly as possible after thawing the samples.

The determination of liver ammonia and urease activity was as follows. One-half ml. of liver supernatant was placed in the middle chamber of the Obrink modified Conway unit which contained 1.0 ml. of 0.5% boric acid indicator in the center chamber. Then 0.5 ml. of 3% urea-phosphate buffer was added and mixed with the liver supernatant. The reaction was allowed to proceed for 5 min. at room temperature. One milliliter of 45% potassium carbonate was then added carefully and quickly to the mixture. The top, which fitted into the outer chamber, was then sealed with 1.5 ml. of the potassium carbonate, and the sample was mixed with the potassium carbonate and placed in the warm room (40°C.) for 1 hr. The ammonia produced was determined by titrating the contents of the center chamber with standardized 0.002 N H₂SO₄. Controls using phosphate buffer instead of urea-phosphate buffer were carried out to determine the ammonia content of the intestinal supernatant and to correct urease activity values. Liver urease activity and ammonia were expressed on the basis of supernatant dry matter. The same method, using 0.25 ml. of serum, was used in determining serum urease activity. The method of Sumner (1951) was used for determining the activity of recrystallized urease. Pigs which died were autopsied.

The data were tested by analysis of variance (Snedecor, 1956). Treatment means were compared by Duncan's (1955) multiple range test.

Results and Discussion

Trial I. The plasma ammonia N level rose from a pretreatment value of 0.50 mg. of ammonia N/100 ml. to a value of 3.5 mg. of ammonia N/100 ml., 9 hr. after the urease was injected. The plasma ammonia N level of the control pig remained relatively constant. A decrease in plasma urea concentration followed the rise in plasma ammonia. The plasma urea N level dropped from 12 to less than 1 mg. of urea N/100 ml. following the injection of urease.

There was an elevation of urine ammonia in the pig injected with urease, whereas the level in the control pig decreased. The urine urea N level was lower in both pigs during the post-injection phase, but there was no apparent difference between the two pigs.

The urease injected pig went into convulsions with severe twitching and tetanic spasms of the skeletal muscles after 8 hr. This continued intermittently for 2 hr. at the end of which time the pig died. There were no overt manifestations of pain. A rise in rectal temperature was recorded, reaching 43°C. 9 hr. after the injection.

Trial II. A summary of results obtained in this trial is presented in figure 1 and table 1. Plasma ammonia N rose significantly following the injection of either 50 or 100 SU/lb. BW (figure 1). The maximum mean level in mg./100 ml. of plasma was 1.7 and 3.0 for the 50- and 100-unit groups, respectively. As shown in figure 1, plasma urea N levels were significantly lower following urease injection. Note that the plasma urea N level of all three groups was lower after 22 hr. of fasting. Plasma urea N levels of control and urease-injected pigs began to increase about 7 hr. after injection. This initial decrease in plasma urea after approximately 20 hr. of fasting and then the rise approximately 10 hr. later have been observed previously (Chance et al., 1962; Henneman et al., 1962).

Urease activity was found in the serum following intraperitoneal injections of urease.
UREASE TOXICITY IN SWINE

TABLE 1. UREASE ACTIVITY AND AMMONIA N LEVEL OF LIVER, AND URINE FLOW, UREA N AND AMMONIA N LEVEL OF GROWING PIGS INJECTED INTRAPERITONEALLY WITH 50 AND 100 SU OF UREASE PER POUND OF BODY WEIGHT (TRIAL II) a, b

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>50 units</th>
<th>100 units a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver urease activity, units/g. DM x 1000</td>
<td>2.7±5.5*</td>
<td>7.2±7.2</td>
<td>144.5±111.7</td>
</tr>
<tr>
<td>Liver ammonia N, mcg./g. DM</td>
<td>883±50</td>
<td>1228±164</td>
<td>1511±32</td>
</tr>
<tr>
<td>Urine flow, ml./hr.</td>
<td>8.6±2.3</td>
<td>7.3±0.2</td>
<td>6.6±1.0</td>
</tr>
<tr>
<td>Pre-injection</td>
<td>6.4±1.0</td>
<td>11.7±1.1 e</td>
<td>12.4±1.5 e</td>
</tr>
<tr>
<td>Post-injection</td>
<td>90.3±16.1</td>
<td>85.9±7.1</td>
<td>79.2±16.7</td>
</tr>
<tr>
<td>Urine urea N, mg./hr.</td>
<td>42.7±3.9</td>
<td>39.3±5.7</td>
<td>41.4±4.2</td>
</tr>
<tr>
<td>Pre-injection</td>
<td>47.6±19.9</td>
<td>46.6±12.8</td>
<td>37.8±20.9</td>
</tr>
<tr>
<td>Post-injection</td>
<td>6.7±1.2</td>
<td>4.2±2.4</td>
<td>5.3±0.2</td>
</tr>
<tr>
<td>Change</td>
<td>5.7±1.2</td>
<td>8.3±3.5</td>
<td>6.3±1.2</td>
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<td>Urine ammonia N, mg./hr.</td>
<td>-1.0±0.0</td>
<td>4.1±1.1</td>
<td>1.0±1.2</td>
</tr>
</tbody>
</table>

a Jackbean urease prepared by the authors.
b Two pigs per treatment.
c One pig died 25 hr. after injection.
d Both pigs died, one after 15 hr. and one after 20 hr.
e Standard Error.

The activity (SU/ml. of serum x 1000) was greater 6 hr. after the urease was injected than 10 hr. after the injection (37.9 and 7.8, respectively, for the 50-unit group, and 83.8 and 33.6, respectively, for the 100-unit group). This finding agrees with work reported by Kirk and Sumner (1931), who found urease in rabbit blood after intraperitoneal injections.

Figure 1. Effect of an intraperitoneal injection of 50 and 100 SU of urease per lb. of BW on plasma ammonia and urea levels of growing pigs (trial II).
Figure 2. Effect of an intraperitoneal injection of 75 SU of urease per lb. of BW on plasma ammonia and urea levels of growing pigs (trial III)

Serum potassium levels were significantly increased in pigs receiving the urease injection at both 6 and 10 hr. after the injection (23.5, 28.3, 28.6, mg./100 ml., respectively, for the 0-, 50- and 100-unit levels at 6 hr.). Serum sodium (av. 323 mg./100 ml.) levels were not different between control and urease-injected pigs.

Liver urease activity and ammonia N level of urease-injected pigs were larger than for control pigs, although the difference was not significant (table 1). It appears that urine urea N was not affected by the levels of urease injected. Urine ammonia N and urine flow were significantly increased following urease injection.

Both pigs receiving the 100-unit level of urease died—one after 15 hr. and the other after 20 hr. Only one pig on the 50-unit level died (25 hr. after the urease injection). Pigs injected with 100 SU/lb. BW of urease showed intermittent tetany starting about 4 hr. after the injection. The rectal temperature was elevated in only one of the pigs receiving the 100-unit level. The pig which died on the 50-unit level appeared to have no tetany, was very limp, and had a subnormal rectal temperature (36.1° C.) before death. Again, no overt sign of pain was observed.

Trial III. As indicated in figure 2, plasma ammonia N was significantly elevated, and plasma urea N was significantly decreased following the injection of 75 SU of Sigma urease type II powder per pound BW, compared to pigs receiving a saline injection. The maximum ammonia N level was 2.5 mg./100 ml. of plasma. All pigs injected with urease died.

Analysis of hematocrit and hemoglobin data indicates a significant difference between control and urease-injected pigs (table 2). These criteria, however, were not studied in the other trials, and it is impossible to conclude from this trial whether the increased hematocrit and hemoglobin values were due to urease toxicity or to a hemagglutinative effect. As pointed out previously (Kornegay et al., 1964), the Sigma urease type II powder was found to have a hemagglutination titer of over 100 per modified SU as compared to a zero titer for urease powder prepared by the authors.

In contrast to the previous trials, urease-injected pigs exhibited no tetany, but were quiet and lay very still, while control pigs were active. Rectal temperatures remained normal.

In agreement with results from Trial II, a
significant difference in urease activity was found in the serum following an intraperitoneal injection of urease (table 2). Serum potassium levels were significantly elevated with no change in serum sodium levels. Serum transaminase values presented in table 2 reveal no difference between the glutamic-oxaloacetic values for control and injected pigs. Glutamic-pyruvic values for urease-injected pigs were significantly lower than control values following the urease injection, which might indicate that the transaminase is being used up in detoxifying the ammonia produced. The control glutamic-pyruvic values were similar to those reported by Calloway et al. (1962); however, their glutamic-oxaloacetic values are approximately twice the values obtained in this study.

At post-mortem examination excess fluid was found in the peritoneal and pericardial cavities, the lungs were congested and hemorrhagic, and mucous membranes were hyperemic. These observations are similar to those reported by Handford (1961) for dogs, and by Shone (1962) for cattle suffering from urea toxicity.

Results of these trials demonstrate that urease is toxic to swine, and they confirm the finding that the poisonous effect of injected urease is due to the ammonia produced (Carnot et al., 1921; Handford, 1961; Kirk and Sumner, 1931; Tauber and Kleiner, 1931). The blood ammonia N levels observed in these trials in urease-injected pigs have been shown to be toxic in other species (Dinning et al., 1948; Gallup et al., 1953; Lewis, 1960; Repp et al., 1955).

The increased serum potassium levels found in the urease-injected pigs in this study agree with the work reported by Lewis (1961), in which elevated potassium levels were observed in two sheep which had 1 mole of ammonium chloride placed in the rumen. Lewis suggested that the elevated plasma potassium observed

### Table 2. Effect of an Intraperitoneal Injection of 75 SU of Urease Per Pound of Body Weight Upon Hematocrit and Hemoglobin, Serum Urease Activity, Electrolytes, and Transaminase Activity in Growing Pigs (Trial III)

<table>
<thead>
<tr>
<th>Item</th>
<th>0</th>
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<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>31.5±1.8*</td>
<td>31.8±1.9</td>
<td>32.2±1.9</td>
</tr>
<tr>
<td>75 units*</td>
<td>32.4±1.0</td>
<td>39.3±1.2*</td>
<td>47.4±1.0*</td>
</tr>
<tr>
<td>Hemoglobin, g./100 ml.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10.6±0.8</td>
<td>10.3±0.6</td>
<td>10.0±0.8</td>
</tr>
<tr>
<td>75 units</td>
<td>10.5±0.5</td>
<td>12.6±0.5</td>
<td>15.6±1.0</td>
</tr>
<tr>
<td>Urease activity, SU/ml x 1000</td>
<td>-0.1±0.4</td>
<td>0.1±0.3</td>
<td>0.3±0.4</td>
</tr>
<tr>
<td>Control</td>
<td>0.3±0.3</td>
<td>8.8±3.5*</td>
<td>24.6±10.6*</td>
</tr>
<tr>
<td>75 units</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium, mg./100 ml.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>27.9±0.9</td>
<td>25.0±1.6</td>
<td>25.4±1.5</td>
</tr>
<tr>
<td>75 units</td>
<td>27.4±1.2</td>
<td>30.6±1.1*</td>
<td>43.6±3.3*</td>
</tr>
<tr>
<td>Sodium, mg./100 ml.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>312±4</td>
<td>311±4</td>
<td>303±3</td>
</tr>
<tr>
<td>75 units</td>
<td>312±3</td>
<td>311±4</td>
<td>310±7</td>
</tr>
<tr>
<td>Transaminase, Sigma units/ml.</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Glutamic-oxaloacetic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>19.4±1.1</td>
<td>18.9±1.6</td>
<td>17.4±1.1</td>
</tr>
<tr>
<td>75 units</td>
<td>19.7±1.5</td>
<td>16.5±1.0</td>
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<tr>
<td>Glutamic-pyruvic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>25.1±1.1</td>
<td>26.1±1.5</td>
<td>21.2±1.1</td>
</tr>
<tr>
<td>75 units</td>
<td>23.1±1.4</td>
<td>16.3±0.5*</td>
<td>16.5±0.5*</td>
</tr>
</tbody>
</table>

* Sigma urease type II powder.
* Six pigs per treatment.
* Standard Error.
* Survival time after urease injection of six pigs was 5, 6, 7, 10, and 11 hr. respectively.
* Significantly (P<0.01) different from control.
* Significantly (P<0.05) different from control.
* One unit of transaminase activity is defined as that amount of enzyme which will cause a decrease in OD-340 of 0.001 per min. per cm. light path.
may have been due to a limitation of available energy, which results in a migration of intracellular potassium to extra-cellular fluid, or that movement of potassium may have been associated with acidosis.

As pointed out previously, the Sigma urease type II powder used in Trial III had high hemagglutinative activity; therefore, the effects of urease and hemagglutinin were confounded in this trial, making interpretation difficult and possibly accounting for the deviation in results observed as compared with Trials I and II.

Summary

Three trials were conducted to determine the effects of large intraperitoneal injections of jackbean urease upon growing swine and its relationship to ammonia toxicity. Plasma urea N levels were significantly decreased and plasma ammonia N levels were significantly increased following injections of urease (50, 75 and 100 modified SU/lb. BW). Serum urease activity and potassium levels were increased, and serum sodium levels were unchanged in pigs given a large dose of urease. The urine ammonia N concentration was increased and the urine urea N concentration was unchanged when pigs were injected with urease. There was an elevation of rectal temperature of treated pigs in Trials I and II with no change in Trial III. Pigs in Trials I and II showed tetany, while those in Trial III did not. Post-mortem examination revealed excess fluid in the peritoneal and pericardial cavities, congested and hemorrhagic lungs, and hyperemic mucous membranes. Effects of urease and hemagglutinin were confounded in Trial III, and this could explain the difference in results obtained as compared to Trials I and II.

Literature Cited