EFFECTS OF INTRAPERITONEAL INJECTIONS OF UREASE UPON PERFORMANCE AND HEMATOLOGY OF GROWING SWINE

I. IMMUNIZATION

II. TOXICITY

Thesis for the Degree of Ph. D.

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ABSTRACT

EFFECTS OF INTRAPERITONEAL INJECTIONS OF UREASE UPON PERFORMANCE AND HEMATOLOGY OF GROWING SWINE

I. IMMUNIZATION

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by Ervin T. Kornegay

Six trials were conducted to study the effects of intraperitoneal injections of urease upon the performance and hematology of growing pigs. The first three trials which included 81 pigs were designed to study the effects of urease immunization upon the following criteria: feed intake and gain, feed efficiency, serum antiurease activity, plasma urea N and ammonia N, urine urea N and ammonia N, intestinal urease activity and ammonia N levels. Urease prepared from jackbean meal was used in Trials I and III while Sigma urease type II powder was used in Trial II. Starting doses ranged from 0.1 to 10 Sumner units per pound of bodyweight with the size of subsequent doses determined by arithmetic progression. A corn-soybean ration fortified with minerals and vitamins was fed in all trials in the immunization study.

The pigs immunized with urease exhibited significantly greater serum antiurease activity than did nonimmunized pigs. In the first two trials, the low levels of urease appeared to be more effective in stimulating antibody production than the high levels of urease, while all levels of urease were equally effective in Trial III. In Trials II and III, the greatest serum antiurease activity occurred after about 28 days, while in Trial I the greatest activity occurred after 72 days.

Gain and feed efficiency of immunized pigs were not improved in Trials II and III; however, there was some improvement in Trial I at the 10 unit level. Plasma urea N levels were unchanged between treatment groups in all trials. In general, ammonia N levels of plasma were unchanged; however, the 0.5 unit treatment group (low) in Trial III had significantly lower plasma ammonia levels at 56 and 73 days on experiment. Intestinal sections plus contents (from the caudal end of the ileum) taken in Trials I and III revealed that the urease immunized pigs had less urease activity than the control pigs, with the difference between treatment and control groups significant in Trial III ($P < 0.05$). Sigma urease type II powder used in Trial II was found to have high hemagglutinative activity. Urease immunization appeared to have no effect on the utilization of fed urea by growing pigs. Higher levels of urea N and lower levels of ammonia N were observed in pigs fed rations with added urea than in pigs fed the control ration. Gain, feed intake, and feed efficiency were decreased at the 2.5 and 3.4 percent urea levels.

Three trials were conducted in the toxicity phase to study the effect of a large intraperitoneal injection of urease on growing pigs and its relationship to ammonia toxicity. Urease prepared from jackbean meal by the author was used in Trials I and II and Sigma urease type II powder was used in Trial III. Plasma urea N levels were significantly decreased, and plasma ammonia N levels were significantly increased following a large intraperitoneal injection of urease (50, 75 and 100 modified Sumner units per pound of bodyweight). All pigs receiving 75 and 100 unit levels died. Ammonia N levels in pigs which died ranged from 2.0 to 3.0 milligrams per 100 milliliters of plasma. Serum urease activity and potassium levels were increased, and serum protein, sodium and calcium levels were unchanged in pigs given a large dose of urease

(Trials II and III). Liver urease activity and ammonia N levels of the urease injected pigs were larger than for the control pigs though the differences were not statistically significant (Trial II). Urine ammonia N levels of treated pigs were larger than values for control pigs in Trials I and II while the opposite was true in Trial III. Urine urea N levels were decreased in Trial III and unchanged in Trials I and II. The electrophoretic components of serum protein in Trial III showed gamma globulin of urease injected pigs to be significantly greater than values for the control pigs. The other components of serum protein were not significantly different. Serum transaminase values determined in Trial III disclosed that glutamic-oxalacetic values were unchanged and that glutamic-pyruvic values were lower following a large urease injection. There was an elevation of rectal temperatures 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 which could explain the differences in results obtained in Trials I and II, and Trial III.

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I. IMMUNIZATION

II. TOXICITY

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DISSERTATION: Effects of Intraperitoneal Injections of Urease Upon the Performance and Hematology of Growing Swine

I. Immunization

II. Toxicity

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I. INTRODUCTION

Antimicrobial agents have been effective in improving the performance of animals under a variety of environmental conditions. Most investigators agree that these agents alter the microflora of the gastrointestinal tract. Some believe that a toxic material produced by the microflora is eliminated or its production is inhibited by low level antimicrobial agents in the diet. Frost et al. (1955), Jukes (1957) and Visek (1962) have reviewed this subject.

Ammonia has been suggested as one of the toxins, perhaps the most important one, whose production is inhibited by low levels of antimicrobial agents in the diet (Francois and Michel, 1955; Melnykawycz and Johansson, 1955; Sherlock, 1958; Dang and Visek, 1960; Visek, 1962). The gastrointestinal tract has been shown to be the major site of ammonia production in the animal (Dintzis and Hastings, 1953; Lawrence et al., 1958).

Studies with isotopically labeled urea and antibacterial agents have demonstrated that urea is hydrolyzed in the gastrointestinal tract by urease, the only enzyme known to break down urea to CO_2 and ammonia (Dintzis and Hastings, 1953; Kornberg et al., 1954a,b; Visek, 1962).

Urease has been reported to occur in over 200 species of bacteria as well as in higher plants and animals (Sumner and Somers, 1953). However, the exact origin of urease in the animal body is not agreed upon by all investigators. Most investigators believe that gastrointestinal urease is of bacterial origin while a few believe that tissues of higher

animals also produce this enzyme.

Parenteral injections of high levels of jackbean urease have been shown to be toxic to a number of laboratory animals (Carnot et al., 1921; Kirk and Sumner, 1931; Tauber and Kleiner, 1931; Handford, 1961; Dang and Visek, 1963). Death in urease poisoning, however, is believed to be caused by the ammonia released.

The antigenicity of urease was shown soon after its crystallization (Kirk and Sumner, 1931). Recently, Visek and coworkers have demonstrated improved growth performance of rats and chicks immunized with jackbean urease (Dang and Visek, 1960; Visek and Dang, 1960a,b; Harbers et al., 1962, 1963b).

Since no work with urease has been reported in swine, this study was initiated to determine: 1) the effects of jackbean urease immunization upon the performance and hematology of growing pigs; 2) the effects of a large intraperitoneal injection of jackbean urease on growing pigs and its relationship to ammonia toxicity.

II. REVIEW OF LITERATURE

A. Ammonia Production

Studies with liver disease, hepatectomy, and gastrointestinal tract removal have confirmed conclusions drawn from experiments with antibacterial agents that the gastrointestinal tract is the major site of ammonia production in the animal body.

Moore et al. (1946) reported accelerated growth in chicks (free of clinical disease) fed a purified diet containing succinylsulphathiazole and streptomycin. These workers postulated that gastrointestinal bacteria could have an inhibitory effect upon growth without causing clinical disease. They suggested further that these dietary supplements were inhibiting microflora which produced toxic substances or made vitamins unavailable. However, they pointed out that the possible systemic action of these antibacterial agents could not be overlooked.

Since these early observations, extensive literature on the use of antimicrobial agents has accumulated and many studies have been conducted in an attempt to elucidate the mechanism involved. A majority of investigators agree that these agents alter the microflora of the gastrointestinal tract. The removal or inhibition of a toxic material produced by the microflora is often mentioned as an explanation for the improved growth performance when antimicrobial agents are fed at low levels. Ammonia has been suggested as one of the most important toxins (Francois and Michel, 1955; Melnyknowycz and Johansson, 1955; Michel and Francois, 1955; Sherlock, 1958; Dang and Visek, 1960; Visek, 1962).

Nencki et al. in 1896 demonstrated that the portal blood ammonia concentration was much higher than that of the peripheral blood. Folin and Denis (1912) postulated that the higher portal blood ammonia concentration was the result of ammonia production by intestinal bacteria acting on nitrogenous substrates. This view was supported later by studies of Phear and Ruebner (1956) and by studies of the effects of antibiotics on ammonia production in the gut (Dintzis and Hastings, 1953; Silen et al., 1955).

Bessman (1959) in a review article states that there are three sources of ammonia in the organism: gut, kidney and muscle. In the gut, ammonia comes from the hydrolysis of glutamine which is formed from protein by hydrolytic enzymes and from the hydrolysis of urea by urease. The renal venous outflow contains more ammonia than the arterial inflow. This probably results from the back diffusion of ammonia from the tubules of the kidney during secretion of ammonia in the urine. Urinary ammonia does not come from blood ammonia, but from the hydrolysis of glutamine by glutaminase to ammonia and glutamic acid in the kidney. Other major sources of urinary ammonia are derived from the action of D- and L-amino acid oxidases on the corresponding amino acids. Increased amounts of ammonia appear in the venous blood following muscle contraction.

It is now established that the removal of ammonia from the blood is defective in the presence of liver disease (Singh et al., 1954) and that intestinal bacteriostasis diminishes the toxic symptoms of patients with impending hepatic failure. Falcon and Fisher (1959) showed that neomycin therapy reduced blood ammonia levels in hepatic coma. Lawrence et al. (1958) reported that the severity of the rise in blood

ammonia of a dog following hepatectomy was less when the animal was treated previously with neomycin.

Bollman and Mann (1930) and Lawrence et al. (1958) noted a progressive rise in blood ammonia after total hepatectomy in dogs and demonstrated that removal of the gastrointestinal tract prevented this rise to a large degree. These findings re-emphasize the importance of the gastrointestinal tract in the production of ammonia.

B. Urease Activity and Urea Hydrolysis

For many years, urea was considered an end product of amino acid metabolism in mammals and was thought to be metabolically inert in simple-stomached animals. It is now believed that urea hydrolysis in vivo is an enzymatic process and that urease is the only known enzyme to decompose urea to carbon dioxide and ammonia (Dintzis and Hastings, 1953; Kornberg et al., 1954a,b; Kornberg and Davies, 1955; Visek, 1962).

Luck (1924b) in his early studies of the presence of urease in the gastric mucosa of a number of animals suggested that urea was broken down to carbon dioxide and ammonia. Reports by Schoenheimer (1942) and Bloch (1946) using urea-N^{15} indicated that only a very small portion of the nitrogen of ingested urea was transformed to ammonia and protein nitrogen, while most of the labeled urea was excreted as such.

Recent studies, however, in several species of animals have revealed that isotopically labeled urea is hydrolyzed in the alimentary tract even when injected parenterally. Leifer et al. (1948) found that 21 percent of urea-C^{14} injected intraperitoneally into mice appeared in the expiratory air as $C^{14}O_2$ in 48 hours. Kornberg and Davies (1952) reported that cats injected intravenously with urea-C^{14} expired 1 to 2 percent of the urea as $C^{14}O_2$ in 4 to 5 hours. When the cats were injected

with urea-N^{15} subcutaneously, they found that approximately 5 percent of the urea was broken down after 40 hours.

Hastings et al. (1950) using urea-C^{14} confirmed that urea was broken down to carbon dioxide and ammonia in the animal body. Gastrectomized mice excreted urea carbon as carbon dioxide at about one-half the rate of normal mice. Removal of the stomach and intestinal tract practically eliminated the excretion of urea carbon as carbon dioxide.

Levenson et al. (1959) using germ-free and conventional rats compared the utilization of urea-C^{14} administered by subcutaneous injection and by stomach tube. The conventional rats expired 2 percent of the dosed urea-C^{14} as $C^{14}O_2$ in 6 hours. The germ-free rats expired only 1/100 as much in the same period of time. The pattern of urea metabolism was similar when urea was given either subcutaneously or by mouth. The small fraction of C^{14} expired by the germ-free rats indicated that some urea was being broken down. They pointed out, however, that urea is spontaneously hydrolyzed in vitro at pH 7 and 37°C. This would account for the breakdown of urea in the germ-free rats.

Studies of the rate of urea decomposition in cats (Wyatt and Kornberg, 1952; Kornberg et al., 1954b) showed that the factor must be enzymatic in nature since the observed rate of breakdown was 10,000 times faster than the rate of uncatalyzed spontaneous decomposition.

Dintzis and Hastings (1953) reported urease activity to be five times greater in the last 5 centimeters of the small intestine and in the colon than in the stomach. Feces were found to have a high ureolytic activity.

Studies by Liu et al. (1955) in pigs, Rose and Dekker (1956) in rats and Walser and Bodenlos (1959) in humans showed by using

isotopically labeled urea in the diet that a small but definite amount of the ingested urea could be found later incorporated into body protein. Rose and Dekker (1956) fed one treatment group of rats a diet in which the protein was made up of the essential amino acids only in the minimum required amounts. A second group of rats received a casein diet. Both diets contained 1.23 percent urea-N^{15}. The distribution of the isotope in the excreta and carcass protein indicated an extensive utilization of the urea nitrogen by the rats on the amino acid diet, but not by those on the casein diet. Cystine, glutamic acid and aspartic acid obtained from the former group had a high body concentration of the N^{15}. On the other hand, the dietary essential amino acid histidine had an extremely low level of N^{15}.

Visek et al. (1959) showed that the addition of 100 ppm. of either chlortetracycline, penicillin or arsanilic acid to a casein diet decreased in vivo hydrolysis of urea-C^{14} by rats and reduced in vitro ureolytic activity of gastrointestinal contents. A recent study by Harbers et al. (1963a) showed that barbituric acid and a combination of barbituric acid and chlortetracycline, but not chlortetracycline alone, fed to chicks were effective in lowering the ureolytic activity and ammonia level of the small intestine.

Dang and Visek (1963) reported that rats and mice immunized with jackbean urease metabolized less urea-C^{14} in vivo. Gastrointestinal urease activity of the immunized animals was reduced 60 percent from controls and the ammonia concentration of gastrointestinal contents was reduced 30 percent. Harbers et al. (1963b) showed that both jackbean urease immunization and increased vitamin A levels suppressed urease activity in the gastrointestinal tract.

C. Urease Occurrence in Nature and Origin in Animals

Musculus in 1876 was the first to report experiments with the enzyme, urease (Varner, 1960). He obtained urease by filtering bacteria on filter paper, drying the paper and using the dry paper, impregnated with an acid-base indicator, to test for urea. Miquel (1890) observed urease in many species of microorganisms. Urease has now been reported to occur in over 200 species of bacteria as well as in higher plants and animals (Sumner and Somers, 1953. The authors did not indicate whether the bacteria were aerobic or anaerobic). Huet and Aladame (1952) assayed over 200 anaerobic bacteria and found only four species which possessed urease. These and other workers (Gibbons and Doetsch, 1959) concluded that urease was sparse among anaerobic forms of bacteria.

The richest plant source known is the jackbean (Canavalia ensiformis, Canavali obtusifolia) which contains 0.15 percent urease (on a dry weight basis). This is approximately 16 times the urease content of the soybean (Mateer and Marshall, 1916). The richest known source of urease is Bacillus pasteurii which contains up to one percent of its dry weight as urease (Larson and Kallio, 1954). Urease has also been reported in the liver of two species of mollusks, in one species of worm, in a crustacean, in the horseshoe crab and in the larvae of the blowfly (Varner, 1960).

The presence of urease in gastric mucosa was first demonstrated by Luck (1924a) who found that suspensions of dog liver, when mixed with suspensions of dog gastric mucosa, liberated considerably more ammonia than either suspension alone, and that as a result the urea content of the mixture decreased to zero (Luck, 1924b). When urea solutions were incubated with the gastric mucosal suspension, stoichiometric amounts

of ammonia were formed. Luck, therefore, concluded that urease was present in the gastric mucosa.

Experiments in vitro have shown that gastric urease occurs in a large variety of animals. Urease is reported to occur in the stomach of men (Cardin, 1933; FitzGerald and Murphy, 1949; Glick, 1949), cats (Davies and Kornberg, 1950; FitzGerald and Murphy, 1950), chicks (Harbers et al., 1963b), mice (FitzGerald and Murphy, 1950; Davies and Kornberg, 1951), sheep, bullocks and goats (Luck, 1924b) and in two varieties of frogs (Glick et al., 1950; Korff et al., 1951). In contrast, the presence of urease in the stomach of rabbits, rats, and pigs has been disputed (Luck, 1924b; Linderstrom-Lang and Ohlsen, 1936; Weil, 1944; FitzGerald, 1946; FitzGerald and Murphy, 1950; Chao and Tarver, 1953) However, Cheosakul et al. (1958), Visek et al. (1959), Dang and Visek (1963) and Harbers et al. (1963b) have reported urease activity of the gastrointestinal contents of rats.

Much of the evidence that urea is hydrolyzed by urease in simple-stomached animals has been reviewed recently by Visek et al. (1959) and Visek (1962). The exact origin of urease in the animal body is not agreed upon by all workers. Visek (1962) points out that the preponderance of evidence favors Kornberg and Davies' (1955) conclusion that urease is not endogenously produced by tissues of warm blooded species.

Kornberg and Davies' (1955) conclusion was based mainly upon the inhibitory effect that antibiotics have on urease activity. Experiments by Dintzis and Hastings (1953), and Kornberg et al. (1954a,b) showed that in vivo urea hydrolysis may be completely suppressed by high dietary levels of penicillin, oxytetracycline, and sulfaguanidine.

Experiments with mice and cats showed that antibiotics indirectly affect urease activity by inhibiting the activity and growth of

intestinal microorganisms. Dintzis and Hastings (1953) with mice and Kornberg et al. (1954a) with cats showed that a mixture of penicillin, terramycin and sulfaguanidine (PTS) did not inhibit the urease activity of mucosal suspensions in vitro and that no urease inhibitor was formed in the tissue as a result of PTS treatment (Kornberg and Davies, 1955).

Also, studies with cats subjected to partial gastrectomy before and after treatment with PTS (Kornberg et al., 1954a) showed that PTS did not directly inhibit urease in vivo, but exerted its effect by inhibiting or eliminating urease-containing bacteria. It was also noted that after 3 days of treatment with PTS, the stomach contained urease despite a total absence of urea-splitting microorganisms, but that after 5 days of treatment both bacteria and urease activity had disappeared (Kornberg et al., 1954a). The authors interpreted this to mean that the urease was of bacterial origin, that it had been deposited within the mucosa at a site protected from inactivation by acid and pepsin, and that it was (after PTS treatment) broken down in the normal course of protein metabolism. The high dietary levels of PTS used by these workers reduced fecal bacterial counts from 10^6 to about 50 per milligram. However, fecal bacterial counts have not been consistently changed, either quantitatively or qualitatively, in birds and mammals fed low levels of antibacterial agents for growth stimulation (Braude et al., 1953; Jukes, 1957).

The conclusion that gastrointestinal urease is of bacterial origin is strongly favored by the work of Levenson et al. (1959) who obtained no evidence for enzymatic hydrolysis of urea in germ free rats.

Walser and Bodenlos (1959) using both N^{15} and C^{14} labeled urea to study urea metabolism in man concluded that in man, as in animals, a

considerable fraction of synthesized urea is continuously being degraded by intestinal bacteria. The following lines of evidence were presented in support of their conclusion: 1) the estimated rate of urea production exceeded the measured rate of urea excretion by approximately 20 percent; 2) the recovery of labeled urea in the urine following intravenous injections was incomplete; 3) ten percent of the excess N^{15} appeared in urinary ammonia and nonurea nitrogen; 4) a significant fraction of the labeled urea molecule in plasma, following N^{15} injection was found to be newly synthesized; 5) the recovery of labeled urea in the urine became nearly complete following oral ingestion of neomycin.

Conway et al. (1959), however, believe that gastric urease is not entirely of bacterial origin. They base their conclusion chiefly on the following points: 1) the urease in the gastric mucosa is contained within the surface epithelial cells, which contain no bacteria; 2) the amount of urease in the animal is relatively large and would require such a large bacterial population to supply it that a bacterial origin for such urease appears untenable; 3) the fetus has gastric urease (Cardin, 1933).

It is believed by Kornberg and Davies (1955) that gastric urease must be situated within the stomach wall at a site protected from attack by acid and pepsin.

D. Comparison of Urease from Various Sources

Kirk and Sumner (1932) in a study of soybean and jackbean urease reported that the two were immunologically identical and that they were equally toxic at high levels. Nikoloff (quoted in Varner, 1960) made an extensive comparison of cat gastric urease and plant urease and concluded that there are two ureases in the gastric mucosa, both

different from plant urease in electrophoretic and immunologic properties. Conway et al. (1959) stated: "Mouse gastric urease is found to have an acid optimum, and its pH activity curve shows that it has a greater tolerance of acid conditions than the urease of the jackbean or of the bacterium examined. This seems to indicate that gastric urease is not identical with vegetable or bacterial urease." Seneca et al. (1962) recently reported the molecular weight of bacterial urease to be 473,000 which is the same as reported by Sumner et al. (1938) for jackbean urease.

E. Isolation and Properties of Urease

Urease was the first enzyme to be isolated in crystalline form (Sumner, 1926). The procedure developed by Sumner (1926) which appears very simple is still in use today. However, Gorin et al. (1962) state that their first attempts to prepare crystalline urease by the Sumner procedure were unsatisfactory and it became apparent that rigid control of all experimental conditions was necessary to obtain reproducible results. They pointed out further that Sumner's method did not afford a good recovery of the enzymatic activity from the starting material, but that modifications effective in extracting more enzyme would also extract more inactive material which interfered with the subsequent purification. Handford (1961) states: "Attempts to prepare crystalline urease from Jack Bean meal by the acetone extraction method of Sumner (1926) were unsuccessful."

In Sumner's method, 100 grams of jackbean meal is stirred into 500 milliliters of 31.6 percent acetone and then filtered at 3° to 6°C. After a few hours, urease crystals form in the filtrate and are removed by centrifugation. Recrystallization is satisfactorily accomplished

from aqueous citrate-acetone as described by Dounce (1941).

Varner in a recent review (1960) summarizes the molecular properties, the specificity, and the kinetics of urease. The molecular weight of jackbean urease (Sumner et al., 1938) and bacterial urease (Seneca et al., 1962) was reported to be 473,000. Setlow (1952) reported that the molecular weight per active site, as determined by deuteron inactivation, is about 100,000. Hand (1939) concluded from diffusion rate studies that proteins of a weight as low as 17,000 carry urease activity. The study of jackbean urease in monolayers indicated that complete inactivation results from unfolding to form a monolayer (Langmuir and Schaefer, 1939). Setlow (1952) using dry heat inactivation studies confirmed that the molecule is not unfolded in the active state. The isoelectric point of urease was reported to be 5.0 to 5.1 (Sumner, 1951).

Sumner and Poland (1933) demonstrated that twice recrystallized urease gave a nitroprusside test. From this, they concluded that there were sulfhydryl groups present in the urease molecule. Others have confirmed this conclusion (Hellerman et al., 1943; Gorin et al., 1962). Hellerman et al. (1943) reported evidence for the existence of three types of mercapto groups of differing reactivity in urease. According to their study, a unit of 473,000 molecular weight contains: 1) 20 to 22 highly reactive groups which are not concerned with enzymatic activity; 2) a like number which are less reactive, but still able to combine with para-chloromercuribenzoate and which are essential for enzymatic action; 3) and a third group, possibly as many as 60 more groups, which react only after denaturation of the protein in concentrated guanidine hydrochloride solution. Gorin et al. (1962) using para-chloromercuribenzoate found 50 mercapto groups per molecule of enzyme, which compares fairly

well with the value 40 to 44 found by Hellerman et al. (1943). Their results, however, do not support the estimate of Hellerman et al. (1943) regarding the existence of some 60 groups of low reactivity besides the 40 to 44 groups.

The relative effectiveness of various metal ions as inhibitors of urease was shown to be: $Ag^{+} > Hg^{++} > Cu^{++} > Cd^{++} > Co^{++} > Ni^{++} > Mn^{++}$, with Pb^{++} and Fe^{++} unassigned but less than Cu^{++} (Shaw, 1954). This order correlates with the relative insolubility of the sulfides of these metals. The various "activators" or protectors of urease (proteins, amino acids, gum arabic) function by binding heavy metals, thereby protecting the urease sulfhydryl groups (Varner, 1960).

There are at present no data which allow any definite conclusions about the molecular weight or the number of active sites per molecule of urease. One group of data suggests a molecular weight of 100,000 per active site, while another group of data suggests that the molecular weight per active site is about 20,000 (Varner, 1960).

Sumner (1951) stated: "Urease is absolutely specific. Hundreds of compounds have been tested and have been shown not to be hydrolyzed by urease; among these were various substituted ureas and related compounds."

The effects of temperature, pH and substrate concentration on the rate of urease-catalyzed hydrolysis of urea are complex and interrelated (Varner, 1960). Wall and Laidler (1953) showed that the rate of urea hydrolysis increased with increasing substrate concentration, until at very high urea concentrations, the rate actually decreased. This high concentration effect may result from an inhibition of the reaction by ammonium ions or from an inhibition by urea. In THAM[1] sulfate buffer the pH activity curve of jackbean urease showed a sharp optimum at pH 8.0

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[1] Trishydroxymethylaminomethane

(Wall and Laidler, 1953). Sumner (1951) had previously reported the pH optimum to vary from 6.4 to 7.6 as a function of buffer ions and substrate concentration.

F. Physiological Role of Urease

Numerous workers have speculated about the physiological role of gastric urease. Mathews (1920) postulated that urease was involved in hydrochloric acid secretion by the stomach. Others have supported this view, suggesting that ammonium ions, derived from urea by the action of urease, lead to the formation of hydrogen ions (Cardin, 1933; Mann and Mann, 1939; Glick , 1949). On the contrary, however, isolated gastric mucosa of whole stomachs from frogs, toads, mice, rats, cats, dogs and skunks can secrete acid in the absence of added urea or ammonia (Davies and Kornberg, 1951; Kornberg and Davies, 1955). It has also been shown that the secretion of hydrochloric acid by mouse stomachs and frog gastric mucosa is not increased by the addition of either urea or ammonium chloride to the media (Davenport and Jensen, 1949; Davies and Kornberg, 1951). This and other evidence led Kornberg and Davies (1955) in a review of gastric urease research to state: "The evidence thus appears conclusive that gastric urease plays no role in the mechanism of formation of hydrochloric acid by the stomach."

It has been frequently suggested that gastric urease plays a role in protecting the mucosa from attack by acid and pepsin (Luck, 1924a; FitzGerald, 1946; Glick et al., 1950; Conway, 1953). Conway (1953) points out that urea therapy of peptic ulcers has been shown to be a valuable therapeutic procedure, and particularly so in the treatment of ulcer cases with dyspepsia resistant to standard medical treatment. Even though the ingestion of large amounts of urea has been shown to be

beneficial in treating ulcer cases (FitzGerald and Murphy, 1950), it appears that gastric urease is not essential to the welfare of the stomach because normal frogs, rats, cats and pigs have been found with no detectable trace of this enzyme (Kornberg and Davies, 1955). Also, it has been shown that the gastric urease activity in cats is completely abolished by treatment with an antibacterial mixture (Kornberg et al., 1954a) and that the ability of the cats to secrete acid was not changed.

Kornberg and Davies (1955) concluded their review by stating that gastric urease is of bacterial origin and plays no essential role in gastric physiology. On the contrary, Conway (1953) stated that gastric urease in the human subject has a functional significance in neutralizing gastric acidity and protecting the mucosa.

There is as yet no firmly established function of urease in plants (Varner, 1960). In the cotyledons of citrullus, urease concentration changes during growth, showing an initial rise followed by an abrupt drop to a low value (Williams and Sharma, 1954). Urease and arginase concentration changes are almost parallel during germination in the soybean (Varner, 1960), suggesting that urease is involved in arginine metabolism. It has been presumed that urease allows bacteria to utilize the urea of animal wastes as a source of ammonia (Seneca et al., 1962).

Lyubimor (1956) reported that living, urea splitting bacteria do not secrete urease into the surrounding medium, and that in Micrococcus ureae and Proteus vulgaris most of the urease was bound within the bacterial cells and was liberated only after death and decomposition of the cells.

G. Urease and Ammonia Toxicity

Parenteral injections of urease have been shown to be toxic to a number of laboratory animals. Carnot et al. (1921) found soybean urease highly toxic to dogs. Tauber and Kleiner (1931) reported that jackbean urease was toxic when injected subcutaneously into mice or intravenously into rabbits. They obtained a MLD of 90 Sumner units per kilogram of body weight for mice. The blood urea was quickly and completely transformed into ammonia and carbon dioxide. They suggested that ammonia was the toxic agent since the symptoms closely resembled those exhibited in ammonia poisoning. A large amount of urease was found in the blood one hour after the injections.

Kirk and Sumner, the same year (1931), showed that single injections of recrystallized jackbean urease, either subcutaneously, intraperitoneally, or intravenously were toxic for the rabbit at a level of 27.5 Sumner units per kilogram of body weight. They also showed that a dose of approximately 25 units given intraperitoneally was toxic for a guinea pig. They confirmed other findings that the poisonous effect of injected urease was due to the ammonia produced.

Handford (1961) produced death in dogs from intravenous injections of 13.5 modified Sumner units per kilogram of body weight. Plasma ammonia and glutamine were elevated while blood urea was decreased. He pointed out that the use of urease afforded a unique method for studying ammonia metabolism in vivo, since, within limits, a self-perpetuating, cyclic release of ammonia is achieved.

Recently, Dang and Visek (1963) reported the results of single parenteral injections of jackbean urease in nonimmunized animals. The acute LD_{50} in Sumner units (10 micrograms of protein per unit) of

urease per kilogram of body weight were: mice, intraperitoneal (IP) or subcutaeous (SQ), 50 to 60, intravenous (IV), 25; rats, IP, 48 to 50. IV, 20; rabbits, IP, 25, IV, 6; and guinea pigs, IP, 35. The serum of moribund animals had toxic levels of ammonia, distorted electrolyte levels and depressed urea concentrations.

Ammonia toxicity has also been reported to be the cause of death in ruminant urea poisoning. (Dinning et al, 1948; Gallup et al., 1953; Pierson and Aanes, 1959; Lewis, 1960). Dinning et al. (1948) believed that approximately 4 milligrams per 100 milliliters was the lethal level of blood ammonia in cattle and sheep while Gallup et al (1953) reported death at 8 milligrams per 100 milliliters. Death may occur from a few minutes to several hours following onset of symptoms. A number of symptoms of urea toxicity (ammonia) have been reported: incoordination, staggering, dullness, frothy salivation, polyuria, labored breathing, and violent muscular spasms (Dinning et al., 1948; Gallup et al., 1953; Pierson and Aanes, 1959). Autopsy of animals has failed to show any characteristic gross tissue changes (Gallup et al., 1953; Pierson and Aanes, 1959); however, a number of general conditions have been observed: congested and edematous lungs, fatty degeneration of liver and kidney, and hyperemia of the mucous membranes of the digestive tract.

The deleterious effects of ammonia have been a matter of interest to many investigators in both plant and animal research. When administered or absorbed at a slow rate, vast quantities of ammonia may be tolerated by most living organisms because of their highly efficient detoxifying mechanism in which urea, glutamine and asparagine are formed (Warren, 1962).

In most biological fluids ammonia exists in two forms, ionized and nonionized; the relative proportions of each are determined primarily by

the pH of the fluid. Since toxicity depends on the ammonia which enters the organism, and hence the cell, it is of particular importance that cell membranes are relatively impermeable to the ionized form, whereas the nonionized ammonia passes tissue barriers with ease (Milne et al., 1958).

Several ammonium compounds have been used to produce ammonia toxicity in both ruminants and nonruminants (Greenstein et al., 1956; Handford, 1959; Lewis, 1960, 1961; Warren 1962). Following the administration of ammonium chloride to sheep, Lewis (1961) reported: decreased pH of the urine and blood; increased ammonia, sodium and potassium in the urine; decreased sodium and chloride and increased potassium in blood plasma; increased urea in the urine; and increased ammonia and urea in the blood.

Although the toxicity of ammonia to the central nervous system is well known, there is little or no factual information on its specificity of action. It has been postulated that excess ammonia may disturb the Krebs cycle by using alpha-ketoglutaric acid, thereby blocking the synthesis of subsequent members of the cycle (Bessman et al., 1954; McDermott, 1957). Since the metabolism of the central nervous system is more dependent upon aerobic glycolysis than the rest of the organism (McDermott, 1957), any derangement in the Krebs cycle might seriously alter the glycolytic cycle in the brain. Results of Handford (1959), however, showed no correlation between the blood level of ammonia and alpha-ketoglutaric acid. These results are in agreement with the clinical observations of Dawson et al. (1957), but contrary to studies of du Ruisseau et al. (1957) and Summerskill et al. (1957) which showed that blood alpha-ketoglutaric acid concentration was significantly decreased.

Although the liver plays a major role in ammonia detoxification in both normal and abnormal states, the arteriovenous cerebral and peripheral ammonia difference noted in clinical states of ammonia toxicity also implies a continuous removal of ammonia by tissues other than the liver (Lawrence et al., 1958).

Duda and Handler (1958) and Krebs et al. (1949) have shown that glutamine synthesis is the primary mechanism involved in the clearance of ammonia from animal tissue. Handford (1961) obtained increased plasma glutamine levels in dogs given toxic levels of urease intravenously. Apparently the synthesis of urea via the arginine-ornithine cycle is completely independent of the synthesis of glutamine (Handford, 1959). It has been postulated that blood ammonia levels may not reflect cerebral intracellular concentration, because of the activity of a system that rapidly binds ammonia by the formation of glutamine from glutamic acid which is synthesized at the expense of alpha-ketoglutaric acid.

Studies of the fate of ammonia-N^{15} given by carotid infusion to cats demonstrated that the major mechanism for ammonia removal in the brain is via glutamine formation, whereas in liver it is urea formation (Berl et al., 1962).

Arginine, other members of the Krebs-Henseleit cycle, and related compounds have been used with varying degrees of success to protect against ammonia intoxication (Krebs et al., 1949; Greenstein et al., 1956; Najarian and Harper, 1956; Handford, 1959; Wright and Horwitt, 1962; Lowestein et al., 1963).

Erecinska and Worcel (1963) recently reported that glutamate reversed the inhibitory effect of ammonia on respiration of rat-liver mitochondria by preventing amination of alpha-ketoglutarate. Succinate also abolished the inhibition, but unlike the action of glutamate,

succinate allowed reductive amination of alpha-ketoglutarate to proceed at a high rate with no depression in oxygen uptake.

Although the evidence is not conclusive, it appears that ammonia interferes with aerobic glycolysis by using alpha-ketoglutaric acid, thereby retarding reactions in the Krebs cycle. Handford (1959) suggested that studies of tissue changes in addition to blood changes are in the required direction to resolve the problem.

H. Urease Immunization

Kirk and Sumner (1931) using crystalline jackbean urease to study immunologic reactions were probably the first to clearly demonstrate that the serum of rabbits immunized with crystalline urease contained antibodies which inhibited the hydrolysis of urea by urease *in vitro* and *in vivo*. They gave rabbits gradually increasing doses of jackbean urease intraperitoneally over a period of 60 days. At first, the injections were given every 8 days, but during the last 30 days, injections were given every 2 or 3 days. The initial injection contained 2.2 Sumner units per kilogram of body weight, while the final injection contained about 220 units per kilogram of body weight. Rabbits immunized with urease were found to withstand 100 times the amount of urease found to be fatal to normal rabbits and to show no rise of blood ammonia. Urease was injected either intraperitoneally or intravenously. They found that the most rapid immunization in rabbits was obtained by giving intraperitoneal injections of a urease-antiurease mixture. Serum from immune rabbits was shown to confer passive immunity to normal rabbits and guinea pigs. It was also shown that rabbits severely poisoned by toxic doses of urease could be restored to normal by intravenous injections of antiurease.

Little work was done with urease immunization until Dang and Visek (1960), Visek and Dang (1960a,b), and Harbers et al. (1962, 1963b) reported increased growth rate and improved feed efficiency of rats and chicks immunized with jackbean urease. Dang and Visek (1960) injected rats intraperitoneally and chicks subcutaneously thrice weekly with recrystallized urease for a 4-week period. In rats, the starting dose was 10 units per kilogram of body weight, progressing to 25 units per kilogram of body weight. The chicks received about the same level of urease. The controls in each case received an equal volume of 0.85 percent NaCl. In both rats and chicks there were no differences in body weight during the 0 to 4 week injection period. But during the 4 to 8 week period, the immunized chicks gained significantly faster ($P < 0.01$) and were more efficient in converting feed to weight gain. In two successive experiments immunized rats grew slightly faster and showed a significant improvement in feed efficiency. Antibodies to urease were demonstrated in sera of immunized rats by in vivo methods with urea-C^{14} and by in vitro tests. However, they were unable to detect the presence of antibodies in sera of the immunized chicks. The urea splitting activity of the gastrointestinal contents of immunized animals averaged 40 percent less than that of controls.

Visek (1962) postulated that the improved performance of rats and chicks immunized to jackbean urease is due to a decrease in ureolytic activity of the gastrointestinal tract. This reduction in urea breakdown, caused by an antiurease-urease reaction, results in a decreased amount of ammonia which the body must detoxify, and thereby results in a saving of energy to the animal. The energy saved then contributes to growth. This hypothesis is based upon the following scheme of urea metabolism. Urea

in the gastrointestinal tract is hydrolyzed into carbon dioxide and ammonia by urease. The lungs exhale the carbon dioxide while ammonia must be detoxified by other methods which require energy. From the gastrointestinal tract, the ammonia may go to the liver where it is formed into urea, or it may react with glutamic acid to form glutamine in the peripheral tissue. The major portion of the urea is removed through the kidneys, while a portion returns to the gastrointestinal tract. Energy is required in forming urea and glutamine. Two moles of ATP are required per mole of ammonia via the urea cycle and one mole of ATP per mole of ammonia via the glutamine route.

III. EXPERIMENTAL PROCEDURES

A. Immunization

1. General

The objective of this experiment was to study the effect of urease immunization upon the performance and hematology of growing pigs. Three trials utilizing 81 growing pigs were conducted in this investigation. Trial I was started June 4, 1962 and was terminated September 10, 1962. Trial II was conducted during the period from February 7, 1963 to April 7, 1963. Trial III, the concluding study, was conducted from May 10, 1963 to July 22, 1963.

Four levels of urease were used in Trial I to determine the level or levels of urease which would be effective. Due to a limited supply of urease, only three pigs were used at the high urease level, whereas the other levels had six pigs each. Urease used in Trial I was prepared from jackbean meal by the author. The method of preparation will be described later.

Trial II, with ten pigs per treatment and three levels of urease, was conducted to resolve the level of urease that would produce a significant response. A commercial urease powder (Sigma type II) of high urease activity was used in Trial II. A later study of this urease showed that it contained high hemagglutinative activity as well as high urease activity. Effects of urease in Trial II were, therefore, confounded with the effects of the hemagglutinin. A third trial with three levels of urease was conducted to determine the urease level which would be effective.

To avoid hemagglutinative effects, urease prepared by the same method as in Trial I was used in Trial III.

Yorkshire and Hampshire-Yorkshire crossbred pigs of both sexes were used in these trials. All pigs were randomly allotted with sex and weight balanced. The pigs were maintained in concrete-floored pens and had free access to water and feed at all times. Feed and growth data were collected at two-week intervals and, in some cases, one-week intervals. Composition of the basal ration which was used in all three trials is shown in Table 1.

Blood samples were obtained from the anterior vena cava of all animals by the technique described by Carle and Dewhirst (1942). Blood (14 milliliters) taken at each sampling period was divided equally for plasma and serum. A combination of sodium fluoride and ethylenediaminetetraacetate (EDTA) was used as an anticoagulant for plasma samples. Sodium fluoride was used especially to inactivate enzymes. An additional 2 milliliters of blood was placed in a heparinized vial when hematocrit and hemoglobin values were determined. All tubes were tightly corked except when they were being sampled. Serum samples were "rimmed" in the tube and left at room temperature for one to two hours. Separation of both serum and plasma from cells was completed in an International centrifuge, size 2, model V, at 2000 x g for 20 minutes. Serum was removed, placed in vials and frozen for later determinations of serum antiurease and antihemagglutinative (Trial II only) activity.

At the time of slaughter, three-inch intestinal sections plus contents were taken from the cranial end of the duodenum and from the caudal end of the ileum. The sections with contents in place were tied off with strings and then removed by cutting on the outside of the strings. With

TABLE 1

IMMUNIZATION STUDY. COMPOSITION OF RATIONS[a]

Ingredients	Basal	1.25% Urea	2.50% Urea	3.40% Urea
	Percent	Percent	Percent	Percent
Corn	75.30	80.95	87.10	91.60
Soybean meal	20.00	13.00	5.50	-
Alfalfa meal	2.50	2.50	2.50	2.50
Dicalcium phosphate	0.80	0.90	1.20	1.30
Limestone	0.60	0.60	0.40	0.40
Trace mineral salt[b]	0.50	0.50	0.50	0.50
B vitamin concentrate[c]	0.10	0.10	0.10	0.10
B_{12} supplement[d]	0.15	0.15	0.15	0.15
A and D mixture[e]	0.05	0.05	0.05	0.05
Urea	-	1.25	2.50	3.40

[a]All rations contained 17 percent crude protein, by calculation and analysis, and 0.50 percent calcium and 0.47 percent phosphorus by calculation.

[b]Contained 0.01 percent cobalt, 0.005 percent copper, 0.007 percent iodine, 0.15 percent iron, 1.2 percent manganese, 0.8 percent zinc (high) and 97 percent salt.

[c]Supplied 2 grams riboflavin, 4 grams d-patothenic acid, 9 grams niacin, and 90 grams choline chloride per pound of concentrate.

[d]Contained 6 milligrams of vitamin B_{12} per pound of supplement.

[e]Contained 3,632,000 IU of vitamin A and 800,000 IU of vitamin D per pound of mixture.

strings left in place, the sections with contents were frozen until determinations of ammonia and ureolytic activity were made.

The data were treated statistically by the analysis of variance (Snedecor, 1956). Treatment means were compared by the multiple range test of Duncan (1955).

2. Trial I

Twenty-one Yorkshire and crossbred pigs with an average weight of 39 pounds were injected intraperitoneally with the following levels of twice recrystallized urease[1]; 0 (saline), 0.1, 1 and 10 modified Sumner units (SU) per pound of body weight. There were 6 pigs per treatment with the exception of the 10 unit treatment in which only 3 pigs were

[1]Urease was prepared from jackbean meal by the author.

used. All pigs except those in the 10 unit treatment were injected thrice weekly for 4 weeks. Pigs in the 10 unit treatment received only six injections. Size of subsequent injections was determined by arithmetic progression.

$$a, a+d, a+2d, a+3d \ldots a+(n-1)d$$

where: a is the first injection with values of 0, 0.1, 1 and 10; d is the common increase with values of 0, 0.1, 1 and 10; n is the number of the injection with values from 1 to 6 for 10 unit treatment and 1 to 12 for other treatments. Weekly weights were recorded in the early part of the trial and these weights were used in determining the dose size.

Blood samples were taken at 0, 8, 15, 22, 36, 50, 64, 69, 72, 83 and 97 days for ammonia, urea and antiurease activity determinations. Only 15, 36, 64, 72 and 97-day values will be reported as they are representative of samples gathered on the other dates. On the first and fifteenth day of the trial, additional blood samples were taken from three pigs in each treatment at 1 and 6 hours (1 and 4 hours on the fifteenth day) after the urease injection to study plasma ammonia and urea behavior. On the fifteenth day, urine collections were taken 24 hours before and after the urease injection. The same three pigs in each treatment were used for urine collections. Pigs were removed thrice daily from the metabolism crates and allowed to eat and drink. Urine was collected in large bottles containing 10 milliliters of 25 percent H_2SO_4.

One-half of the pigs on each treatment were given an additional injection of urease after 62 days on experiment (35 days after the twelfth injection) to study anamnestic response. Intestinal sections plus contents were taken from the pigs at the time of slaughter.

3. Trial II

Thirty crossbred pigs with an average weight of 66 pounds were randomly divided by weight and sex into three equal groups. They were injected intraperitoneally with the following levels of urease[2]; 0 (saline), 0.1 and 1 modified SU per pound of body weight. Urease dose was based upon weekly pig weights and was increased by arithmetic progression as described in Trial I. The urease injection schedule was modified after five urease injections because of an apparent severe effect of the urease injections on growth and feed consumption. Injections were discontinued after the sixth injection.

Investigation of the commercial urease powder showed it to have high hemagglutinative activity. This will be discussed in detail in the RESULTS AND DISCUSSION section.

At the end of 47 days, each treatment group was randomly divided into two equal groups. One-half of the pigs on each treatment received a ration containing 1.25 percent urea (Table 1). The urea ration was made by replacing part of the soybean meal with urea and the total nitrogen was adjusted to that of the basal ration. At the end of 16 days, the urea was increased to 2.5 percent by replacing more of the soybean meal (Table 1), and the total nitrogen level was kept the same as the basal ration. After two weeks on 2.5 percent urea, all soybean meal was removed and urea was added to balance total nitrogen. This ration contained 3.4 percent urea.

Blood samples were taken at 14, 29, 47 and 77 days for urea, ammonia, anti-urease activity and antihemagglutinative activity determinations. Hematocrit and hemoglobin values were determined at 14 and 29 days.

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[2]Sigma type II urease powder was used.

4. Trial III

Thirty Yorkshire and crossbred pigs with an average weight of 31 pounds were injected intraperitoneally with the following levels of twice recrystallized urease[3], 0 (saline), 0.5 and 5 modified SU per pound of body weight. There were 10 pigs per treatment. Urease dose was based upon weekly pig weights and was increased by arithmetic progression as described in Trial I. Injections were made on the following days: 2, 4, 6, 8, 11, 14, 18 and 25. Blood samples were taken at 28, 56 and 73 days for urea, ammonia and antiurease determinations. At the end of 56 days, each treatment was randomly divided into two equal groups. One-half of the pigs on each treatment received a ration containing 3.4 percent urea (Table 1) for the remainder of the trial. Intestinal sections plus contents were taken at the end of the trial.

5. Urease

a. Preparation. Urease was extracted from finely ground defatted jackbean meal[4] using the method of Sumner (1926), and Kirk and Sumner (1934). It was recrystallized twice using Dounce's procedure (1941). The extraction mixture was prepared by mixing 160 milliliters of reagent grade acetone and 340 milliliters of cold deionized water containing 10^{-3} M EDTA. The use of cold water kept the mixture below 28°C. The 32 percent acetone-water mixture was added to 100 grams of jackbean meal in a 1 liter beaker. The mixture was stirred for 5 minutes and then filtered through Eaton-Dikeman No. 541 filter paper into a 500 milliliter

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[3]Urease was prepared from jackbean meal by the author.

[4]Jackbean meal used in Trial I was purchased from Sigma Chemical Co., St. Louis, Mo. and from General Biochemicals, Chagrin Falls, Ohio for Trial III.

Erlenmeyer flask which was iced after approximately 150 milliliters of filtrate had been collected. The total amount of filtrate was 350 to 400 milliliters. The filtrate was then put into large glass containers which were kept in the cold room at 4°C. After at least 24 hours in the cold room, a white sediment was present in the bottom of the containers. The upper part of the liquid was decanted and left for additional crystallization to occur. Up to four "crops" of crystals were obtained from the filtrate over a period of about five weeks. The remaining liquid containing the precipitate was centrifuged at 2000 x g in the cold room for 50 minutes (International centrifuge, size 2, Model V). The clear centrifuge was decanted and the tubes containing residue were allowed to drain in the cold room until the smell of acetone was no longer evident. However, the residue was not allowed to dry out. The residue was dissolved in 6 milliliters of EDTA water and centrifuged at 20,000 x g and 4°C for 30 minutes (Lourdes Beta-Fuge, Model A). The supernatant contained the urease, and the precipitate contained the impurities.

To the crude urease solution was added 0.05 volumes of 0.5 M citrate buffer [5], pH 6.0; and after thorough mixing, ice cold acetone was added slowly with constant stirring to make a 25 percent acetone solution. The mixture was then allowed to stand for at least one week in the cold room. Crystals were separated by centrifuging at 2,000 x g and 4°C for 1 hour. The crystals were then dissolved in EDTA water and centrifuged at 20,000 x g and 4°C for 30 minutes. The supernatant contained the urease. The same procedure was used for the second recrystallization as was used for the first recrystallization. Crystals from the second

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[5]Citrate buffer 0.5 M, pH 6.0 - Approximately 5 milliliters of 0.5 M citric acid was added to 95 milliliters of 0.5 M sodium citrate. The pH was adjusted to 6.0 with 0.5 M citric acid. Deionized water was used. The buffer was stored in the cold room in a bottle with a nonmetal cap.

recrystallization were dissolved for injection in a 0.85 percent NaCl solution.

Although this method of urease preparation seems rather simple, the results were often quite variable and disappointing. As pointed out in the literature review, urease is rapidly inactivated by ions of heavy metals and is sensitive to temperature and pH. Deionized distilled water with 10^{-3}M EDTA was used in all steps of preparation. Vigorous mixing was avoided. All stages of preparation, except initial extraction, were carried out in the cold room at 4°C. As previously mentioned, additional "crops" of crystals were obtained by allowing the centrifugate to stand in the cold room. The additional "crops" proved worthwhile as they yielded as much activity as did the first "crop".

b. Enzyme activity. One unit of urease activity has been defined by Sumner and Graham (1925) as that amount of enzyme which will liberate 1 milligram of ammonia nitrogen from a urea-phosphate solution[6] pH 7.0 in 5 minutes at 20°C. This is now known as a Sumner unit (SU). A modified SU was used in this experiment so that the assay could be conducted in a water bath (27 ± 0.5°C) at room temperature. All other conditions of the definition were the same. Urease activity was determined by measuring the amount of ammonia liberated by mixing Nessler's reagent[7] directly with the acidified reaction mixture (Sumner, 1951).

A 1.0 milliliter aliquot of the diluted urease solution (usually a 1:1000 dilution of urease solution with phosphate buffer, pH 7.0) to be

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[6]Urea phosphate buffer - Three grams of urea, 6.8 grams of Na_2HPO_4 and 2.8 grams of KH_2PO_4 were dissolved in deionized water and adjusted to 100 milliliters with deionized water.

[7]Nessler's reagent - Fifteen milliliters of deionized water and 15 milliliters of Nessler's stock reagent (Folin & Wu) were mixed. With constant stirring, the mixture was added to 70 milliliters of standardized 10 percent NaOH. The reagent was prepared fresh for each assay.

tested was placed into a 100 milliliter volumetic flask which was immersed in a water bath at 27 ± 0.5°C. The 3.0 percent phosphate buffer was also kept in the water bath. At the start of the assay period, 1.0 milliliter of urea phosphate buffer was added with rapid mixing. The reaction was allowed to proceed with mixing for exactly 5 minutes. It was then stopped by quickly adding 1.0 milliliter of 1 N H_2SO_4 and mixing. About 80 milliliters of deionized water were added and 5 milliliters of Nessler's reagent were then blown into the flask and mixed. The solution was then diluted to the mark with deionized water. A blank was made in which only the urease was omitted. The optical density at 480 millimicrons was then determined in a Bausch and Lomb Spectronic 20 and compared to the optical density readings produced by an ammonia standard.

c. Hemagglutinative activity. Hemagglutinative activity was based upon the highest dilution of urease which would agglutinate porcine erythrocytes. A 0.2 milliliter quantity of urease solution was serially diluted and tested with an equal volume of a 2 percent suspension of erythrocytes.[8] Tubes were mixed and incubated at room temperature overnight. A positive pattern consisted of a thin layer of uniformly agglutinated cells covering the bottom of the tube. A negative pattern consisted of a red, compact button of red blood cells in the center of the bottom of the tube. The hemagglutination titer was designated as the highest dilution of urease solution in which a positive pattern was observed.

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[8] Heparinized erythrocytes from the control pigs were washed with 10 volumes of 0.85 percent NaCl at room temperature 4 times and then the cell concentration was adjusted to 2.0 percent with 0.85 percent NaCl.

6. Biological Determinations

a. Ammonia N. Plasma ammonia N, urine ammonia N, and ammonia N as a product from urease action on urea, were determined by the microdiffusion method (Conway, 1957). The Öbrink modified Conway units were used (Öbrink, 1955). Öbrink's modification of the Conway microdiffusion unit avoids the use of "greasy" top fixatives by having an extra chamber (closing chamber) which is half-filled with the same solution as used in the outer diffusion chamber (middle chamber) to liberate the ammonia. The absorbing fluid is contained in a center or inner chamber.

Plasma or urine ammonia N was determined as follows. One-half milliliter of plasma or 0.25 milliliter of a 1:50 dilution of urine was placed in the middle chamber of the unit. The center chamber contained 1.0 milliliter of 0.5 percent boric acid indicator[9]. One milliliter of 45 percent potassium carbonate[10] containing 0.025 percent NPX Tergitol was then placed in the middle chamber on the opposite side from the plasma (or urine) so that the two did not mix until the top was in place. Then approximately 1.5 milliliters of the same potassium carbonate solution was placed in the closing chamber and the lid was put in place. The plasma (or urine) and potassium carbonate were then mixed thoroughly and the units were incubated at 40°C for 1.0 hour. (Incubation time should be uniform with all samples.) Units were removed from the warm room and the tops were removed carefully so as not to drop potassium

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[9]Boric acid indicator, 0.5 percent - Five grams of boric acid were dissolved completely in 200 milliliters of ethanol and 700 milliliters of deionized water. Ten milliliters of a mixed methyl red-bromcresol green indicator (Methyl red, 0.066 grams, and bromcresol green, 0.033 grams, were dissolved in 70.0 milliliters of ethanol and then adjusted to 100 milliliters with deionized water) was added to the boric acid solution and brought to the desired red color with weak NaOH. Then 0.025 percent NPX Tergitol (Union Carbide Chemical Co.) was added and the volume was adjusted to 1000 milliliters with deionized water.

[10]Potassium carbonate was boiled for 15 minutes to free the solution of ammonia.

carbonate into the center chamber. Contents of the center chamber (now green) were then titrated with approximately 0.002 N H_2SO_4 from a microburette until the first permanent pink color appeared. The concentration of unknowns was calculated from ammonia standards which were analyzed at the same time. All determinations were performed in duplicate and a reagent blank was also used. Determinations of plasma ammonia were made within 2 to 3 hours after the samples were taken.

b. Serum antiurease activity. Determination of antibody production was based upon the ability of the immune serum to inhibit the power of urease to hydrolyze urea (Kirk and Sumner, 1931). This is called antiurease activity. The method is given below.

All reactions were carried out in the Öbrink modified Conway unit. One milliliter of 1.0 percent boric acid indicator[11] was placed in the center chamber and 0.25 milliliter of serum was placed in the middle chamber. One-half milliliter of urease-phosphate buffer solution (1.0 modified SU per milliliter) was then placed in the middle chamber. The top was put in place (without potassium carbonate) and the serum and urease were thoroughly mixed and the unit was placed in the warm room at 40°C for 40 minutes. After incubation, 0.5 milliliter of 3.0 percent urea-phosphate buffer was added quickly to the serum-urease mixture and thoroughly mixed. This reaction was carried out at room temperature (24 to 25°C). Exactly 5 minutes later, 1.0 milliliter of 45 percent potassium carbonate was carefully and quickly added to the mixture. The top was then sealed with 1.5 milliliters of potassium carbonate solution and the samples were mixed and placed in the warm room for 1.0 hour.

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[11]One percent boric acid indicator was prepared the same as 0.5 percent boric indicator except 1.0 percent boric acid was used instead of 0.5 percent.

Ammonia produced was determined by titrating the contents of the center chamber with standardized H_2SO_4, which was about 0.02 N. Controls were carried out on all samples in order to determine the amount of ammonia produced by the action of urease upon the serum urea. Phosphate buffer was added to the controls instead of urea-phosphate buffer.

Control values (serum + urease + buffer) were substracted from urease activity values (serum + urease + urea). The difference between the original urease activity (urease + urea) and the activity after incubation with serum represented the antiurease activity. Kirk and Sumner (1931) defined one unit of antiurease activity as that amount of antibody which will inhibit one unit of urease activity.

All samples were determined in duplicate. A preliminary test showed that a urea blank and a urease blank were not necessary.

c. Preparation of intestinal sections. Intestinal sections were prepared for determinations of ammonia and ureolytic activity as follows. Frozen tissues were placed in the cold room (4°C) 15 to 30 minutes before needed. Ends of the section including strings were cut off and the remainder of the section was cut into small pieces and placed in a semi-micro container. A minimum amount of deionized water was added and the sample was homogenized in the cold room for 1 minute at high speed on a two-speed Waring Blendor, Model No. PB-5. 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 x g for 50 minutes in the cold room. The supernatant was separated by filtering through two layers of cheese cloth. Only the supernatant was saved for assay, as Visek et al. (1959) indicated no difference per volume in ureolytic activity between the entire homogenate or its supernatant.

A 10 milliliter aliquot of the supernatant was taken for dry matter determination at 100°C for 15 hours. Ureolytic activity and ammonia were determined as rapidly as possible after thawing of the tissues.

d. Intestinal ammonia and urease activity. One milliliter of intestinal supernatant was placed in the middle chamber of the Öbrink modified Conway unit which contained 1.0 milliliter of 0.5 percent boric acid indicator in the center chamber. Then 0.5 milliliter of 3.0 percent urea-phosphate buffer was added and mixed with the intestinal supernatant. The reaction was allowed to proceed in the warm room (40°C) for 40 minutes. One milliliter of 45 percent potassium carbonate was then added carefully and quickly to the mixture. The top was then sealed with 1.5 milliliters of potassium carbonate solution and the sample was mixed with the potassium carbonate and placed in the warm room for 1 hour. Ammonia produced was determined by titrating the contents of the center chamber with standardized H_2SO_4, which was about 0.002 N. 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. Urease activity was expressed as modified SU per gram of supernatant dry matter. Ammonia was expressed as ammonia N per gram of supernatant dry matter. All samples were carried out in duplicate.

e. Serum antihemagglutinative activity. 1) Serial dilution of serum. Serum antihemagglutinative activity was based upon the highest dilution of serum which would inhibit hemagglutination. A 0.2 milliliter serum sample was serially diluted and incubated with an equal volume of a 1:500 dilution of Sigma type II urease (5 grams in 20 milliliters of H_2O) for 1 hour at room temperature (24 to 25°C). Then 0.2 milliliter of a 2.0 percent suspension of erythrocytes was added, mixed and incubated at room

temperature overnight. The tubes were read in the same manner as for the hemagglutinative activity test. The serum titer of antihemagglutinative activity was the highest dilution of serum in which hemagglutination was completely inhibited.

2) Serial dilution of urease. Serum antihemagglutinative activity was based upon the lowest dilution of urease in which agglutination was inhibited. In this test, a 0.2 milliliter urease sample (Sigma type II, 5 grams in 20 milliliters of H_2O) was serially diluted and incubated with an equal volume of a 1:5 dilution of serum at room temperature. One hour later, 0.2 milliliter of a 2.0 percent suspension of erythrocytes was placed in the tubes and mixed. These were incubated at room temperature overnight and read in the same manner as for the hemagglutinative activity test. The serum titer of antihemagglutinative activity in this test is the lowest dilution of the urease in which there is complete inhibition of hemagglutination.

f. Hematocrit, hemoglobin and urea N. The hematocrit values were determined by the procedure outlined by McGovern et al. (1955). Hemoglobin was determined by the cyanmethemoglobin method described by Crosby et al. (1954). Brown's para-dimethylaminobenzaldehyde method (1959) was used for urea N. A 1:50 dilution of urine was used for urea N.

B. Toxicity

1. General

The objectives of this experiment were to study the effect of a large intraperitoneal injection of urease on growing pigs and its relationship to ammonia toxicity. Twenty crossbred pigs weighing 20 to 30 pounds were used in three trials. Two pigs, a control and a treatment, were used in Trial I to determine if a single large injection of 100

modified SU per pound of body weight was toxic to the pig, and if the blood urea and ammonia level were changed. In Trial II with two pigs per treatment, three levels of urease, 0 (saline), 50 and 100 modified SU per pound of body weight, were given intraperitoneally in a single dose. Trial III consisted of two treatments, 0 (saline) and 75 modified SU per pound of body weight, with six pigs per treatment. Twice recrystallized urease prepared from jackbean meal by the author (Part A) was used in Trials I and II, while Sigma urease powder, type II, was used in Trial III.

All pigs were placed in metal metabolism crates approximately 20 hours before they were injected and were left in the crates after they were injected. The pigs in all trials were fasted during the entire experimental period, but were allowed access to water thrice daily. Urine was collected, in large bottles containing 10 milliliters of 25 percent H_2SO_4 or HCl, before and after the injection of urease.

Blood samples were taken before the pigs were placed in the crates, before the urease was injected, and at specific intervals thereafter. Frequency of blood sampling was greater in Trials I and II than in Trial III. Plasma and serum were obtained as outlined in Part A. Sterile tubes, corks and vials were used for serum to avoid any possible bacterial contamination which might influence the urease activity.

The pigs were weighed at the beginning and at the end of each trial. Rectal temperatures were recorded at the time of blood sampling. Hemoglobin, hematocrit, ammonia and urea were determined as described in Part A. A 1:50 dilution of urine was used for urea and ammonia determinations. Liver samples taken from all pigs in Trial II were stored and prepared in the same way as intestinal sections.

Liver ammonia and urease activity determinations were the same as for intestinal sections except that 0.5 milliliter of liver supernatant was used, and the reaction was allowed to proceed at room temperature for 5 minutes. The same method, using 0.25 milliliter of serum was used in determining urease activity. Pigs which died were autopsied.

The data were tested by the analysis of variance and the t-test (Snedecor, 1956). Treatment means were compared by Duncan's (1955) multiple range test.

2. Biological Determinations

a. Glucose. The Nelson-Somogyi microtechnique was used for blood glucose (American Association Clinical Chemists, 1953). A $Ba(OH)_2$ supernatant of whole blood was prepared by mixing 0.2 milliliter of whole blood, 1 milliliter of 0.3 N $Ba(OH)_2$ and 1 milliliter of 5 percent $ZnSO_4$. The mixture was centrifuged for 10 minutes at 2,000 x g. One milliliter of alkaline copper reagent and 0.5 milliliter of the supernatant were placed in a Folin sugar tube and mixed. A marble was placed on top of the tube and it was autoclaved for 5 minutes at 115°C. After cooling for 1 minute in water at room temperature, 1 milliliter of arsenomolybdate reagent was added, mixed and the solution was diluted to the 10 milliliter mark with water. Optical density was measured at 540 millimicrons using a Spectronic 20. Concentration of sugar in the unknowns was calculated from glucose standards carried out simultaneously. All determinations were carried out in duplicate and within 1 hour after the blood samples were taken.

b. Serum protein. The serum protein was determined according to the method first described by Waddell (1956). Five lambda of serum was diluted to 5 milliliters (1:1000) with 0.85 percent NaCl. A reading at

wavelengths of 215 millimicrons and 225 millimicrons was made on a Beckman Model DU spectrophotometer. The absorbance at 225 millimicrons was substracted from that at 215 millimicrons. This difference multiplied by 14.4 gave the protein concentration in the serum expressed in grams per 100 milliliters.

c. Electrophoresis. The serum protein fractions were separated on a Spinco, Model R, paper electrophoresis system (Spinco Technical Bulletin 6050A) at room temperature. The relative intensities of the separated proteins were determined by scanning with a Spinco Model RB Analytrol equipped with two 500 millimicron filters and a B-5 cam.

d. Serum sodium and potassium. Sodium and potassium determinations were made with a Beckman DU spectrophotometer equipped with a flame attachment using an oxygen-acetylene burner. A 0.1 milliliter serum sample was diluted to 10 milliliters (1:100) with deionized water. Both sodium and potassium determinations were made from the same dilution of serum. The standard solution contained sodium:potassium in the ratio of 1:1. Sodium was read at approximately 770 millimicrons and potassium was read at approximately 590 millimicrons. Standard curves were run with every set of samples and were determined in the range of 10 to 40 milligrams of potassium per 100 milliliters and 100 to 500 milligrams of sodium per 100 milliliters.

e. Total nitrogen. A modification of the semi-micro Kjeldahl procedure outlined in the Official Methods of the Association of Official Agricultural Chemists (1960) was used to determine total nitrogen in urine samples. American Instrument Company's semi-micro Kjeldahl equipment was used. To a 0.25 milliliter urine sample in the digestion flask was added 1.9 grams of K_2SO_4, 2 milliliters of concentrated H_2SO_4 and

1 milliliter of 10 percent $CuSO_4$. A small glass bead was added, and the sample was digested for at least 2 hours after the solution had cleared. Then samples were cooled slightly (about 5 to 10 minutes) and 10 milliliters of deionized water were added carefully. The solution was mixed and cooled to room temperature. The flask was connected to the distillation apparatus and 10 milliliters of 40 percent NaOH was added after the distillate receiver, which contained 10 milliliters of 2 percent boric acid and 2 drops of 0.1 percent bromcresol green indicator, was in place. Steam was passed through the sample for 7 minutes with the tip of the condenser submersed in the boric acid indicator and 1 minute with the tip of the condenser above the surface of the indicator. The distillate was titrated with standardized 0.05 N HCL from the blue color back to the original yellow-green color. A blank determination was run.

f. Serum calcium. Methods of Mori (1959) and Appleton et al. (1959) were combined and modified by C. L. Zutaut (unpublished data) for the determination of serum calcium. Into a 10 milliliter beaker containing approximately 7 milliliters of 0.5 M KOH, 0.25 milliliter of serum was introduced. Two drops of 0.1 percent thymolpthalein in ethanol and a small amount of calcein[12] indicator from the tip of a micro spatula were added to the beaker. (The amount of indicator to be used can be determined by the individual analyst.) The solution was swirled to dissolve the indicators. The solution of diluted serum, which had a green fluorescence, was titrated with CDTA[13] in a Sargent Spectro-Electro Titrator, Model SE using manual control until the green color changed to a light

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[12]Calcein - fluoresceinbismethyleneiminodiacetic acid

[13]CDTA solution - Approximately 39 grams of 1,2-diaminocyclohexane-N, N,N',N'-tetraacetic acid (CDTA) were dissolved in 1 liter of deionized water. This was the stock CDTA solution and was diluted 100 times for the working solution.

purple at the end point. A standard calcium solution containing 10 milligrams of calcium per 100 milliliters and a blank were treated the same way as the specimen.

g. Serum glutamic-oxalacetic and glutamic-pyruvic transaminase. Determination of serum glutamic-oxalacetic transaminase (SGO-T) and glutamic-pyruvic transaminase (SGP-T) activity was made according to the procedure described in Sigma Technical Bulletin No. 410 (12-61). The Sigma 410-OP combination kit was used as a source of reagents.

IV. RESULTS AND DISCUSSION

A. Immunization

1. Trial I

A summary of feed consumption, average daily gain and feed efficiency data obtained in this trial is presented in Table 2. Pigs in each treatment were group fed; therefore, a statistical analysis could not be made on feed consumption and feed efficiency data. In general, feed consumption was greatest for the 0.1 and 10 unit treatment groups. The 10 unit treatment group appeared to be more efficient than the other groups at all periods except 79-98 days. Average daily gain was significantly ($P < 0.05$) greater in the 10 unit treatment group than in the control or the 1 unit group at 0-58, 0-79, 0-98 and 30-58 days. Note that gain was also greatest in the 10 unit treatment group, though not significant, at 0-30 days. Individual average daily gains, sex, age, initial weights and final weights are given in Appendix Table 1.

The mean values for plasma urea N and ammonia N and serum antiurease activity are shown in Table 3. Plasma urea N and ammonia N levels between treatments were not statistically different. Urea N values tended to increase in all groups with age and/or weight; whereas, ammonia N values appeared to decrease. Individual values for urea N and ammonia N are given in Appendix Tables 2 and 3, respectively.

Serum antiurease activity of all treatment groups was significantly ($P < 0.01$) greater than the control at 64 and 72 days (Table 3). Antiurease activity was present at 15 days, though not statistically

TABLE 2

TRIAL I. FEED CONSUMPTION, AVERAGE DAILY GAIN AND FEED EFFICIENCY OF UREASE IMMUNIZED AND NONIMMUNIZED GROWING PIGS[a b]

Time, days	0-30	30-58	58-79	79-98	0-58	0-79	0-98
Av. daily feed intake, lb.							
Control	2.65	3.68	4.29	5.12	3.22	3.50	3.82
0.1 Unit	3.00	3.87	4.46	5.51	3.55	3.70	4.10
1 Unit	2.97	3.74	4.29	5.42	3.40	3.64	3.94
10 Unit	2.83	4.23	4.34	6.46	3.67	3.78	4.30
Av. daily gain, lb.							
Control	1.18	1.56	1.65	1.40	1.36	1.46	1.43
	(0.10)[c]	(0.03)	(0.08)	(0.06)	(0.06)	(0.06)	(0.05)
0.1 Unit	1.34	1.55	1.69	1.48	1.44	1.51	1.50
	(0.06)	(0.06)	(0.05)	(0.09	(0.05)	(0.04)	(0.04)
1 Unit	1.27	1.48	1.59	1.36	1.37	1.43	1.42
	(0.07)	(0.08)	(0.10)	(0.15)	(0.05)	(0.04)	(0.06)
10 Unit	1.42	1.77[e]	1.82	1.60	1.59[d]	1.66[d]	1.64[d]
	(0.04)	(0.08)	(0.09)	(0.13)	(0.05)	(0.05)	(0.06)
Feed per lb. gain, lb.[f]							
Control	2.26	2.33	2.59	3.63	2.36	2.43	2.66
0.1 Unit	2.24	2.40	2.63	3.72	2.42	2.48	2.72
1 Unit	2.33	2.40	2.69	3.95	2.48	2.54	2.77
10 Unit	2.00	2.14	2.37	4.00	2.25	2.29	2.61

[a] Jackbean urease prepared by the author.

[b] Six pigs per treatment except 10 unit treatment which had only three pigs. Average initial weight, 39 pounds.

[c] Standard error of the mean in parentheses under mean.

[d] Significantly larger than the least two treatment means ($P < 0.05$).

[e] Significantly " " " other treatment means ($P < 0.05$).

[f] Pigs group fed.

significant because of the large within-group variation, especially in the 0.1 unit group. At 36 days, serum antiurease activity of the 0.1 unit group and 1 unit group was significantly larger than the control group. Although the serum antiurease activity of the 10 unit group was larger than the control group, the difference was not significant at 36 days. As evidenced in Table 3, the maximum urease activity recorded for all groups occurred at 72 days. Note in Appendix Table 4 that this was true for the pigs which received the additional urease injection at 64 days, as well as for those which received only saline at that time.

TABLE 3

TRIAL I. PLASMA UREA N AND AMMONIA N LEVELS, AND SERUM ANTIUREASE ACTIVITY OF UREASE IMMUNIZED AND NONIMMUNIZED GROWING PIGS[ab]

Time, days	0	15	36	64	72[c]	97[c]
Urea N, mg./100 ml.						
Control	13.4±1.0[d]	10.6±0.9	17.4±0.7	27.3±1.3	24.3±1.2	18.0±0.9
0.1 Unit	14.7±0.9	12.0±1.3	18.5±1.2	24.0±1.2	22.2±1.9	17.1±0.7
1 Unit	12.3±0.8	10.2±0.8	18.5±1.1	26.7±1.3	22.8±1.0	17.1±1.7
10 Unit	9.6±1.6	10.8±1.7	15.7±1.9	23.4±3.0	26.8±2.6	18.2±2.2
Ammonia N, mcg./100 ml.						
Control	638±46	856±81	780±41	604±32	467±30	577±14
0.1 Unit	694±91	758±46	899±65	541±19	460±35	491±19
1 Unit	690±87	836±43	921±51	557±19	434±11	515±32
10 Unit	750±24	683±110	1004±110	589±21	473±45	533±15
Antiurease activity, unit/ml. x 10^3						
Control	0±18	-1±14	3±11	0±9	-7±13	-4±9
0.1 Unit	1±13	44±32	70±4[e]	158±26[ee]	195±19[ee]	25±25
1 Unit	0±15	61±18	97±25[ee]	173±18[ee]	246±24[ee]	62±20
10 Unit	-5±4	44±10	53±20	131±8[ee]	244±50[ee]	57±57

[a]Jackbean urease prepared by the author.

[b]Six pigs per treatment except 10 unit treatment which had only three pigs. Average initial weight, 39 pounds.

[c]One-half of the pigs on each treatment given an additional injection after 64 days on experiment.

[d]Standard error of the mean.

[e]Significantly greater than the least treatment mean ($P < 0.05$); [ee]($P < 0.01$).

Although all pigs had an increase in antiurease activity following either urease or saline injection at 64 days, there appeared to be a greater increase of activity in pigs on the 1 and 10 unit treatment groups which received urease. Pigs on the 10 unit treatment had the greatest response to the additional urease injection. Serum antiurease activity in treated pigs at 97 days was about the same as at 15 days, and was not significantly different from the control pigs. It is evident from this data that all levels of urease injected in this trial were effective in stimulating antibody production with some evidence of the 1 unit level being more effective than the 0.1 or 10 unit levels.

As pointed out in the EXPERIMENTAL PROCEDURES, the measurement of antibody production in these trials was based upon the inhibition of urease by serum. Marucci and Mayer (1954) in quantitative studies on the inhibition of crystalline urease by rabbit antiurease reported that the inhibition of the enzymatic activity of urease by a specific antibody is only partial. They observed that mixtures of urease and antiurease in the equivalence zone displayed about 70 percent of the activity of the enzyme alone, or 30 percent inhibition. Mixtures in the extreme antibody excess region showed approximately 80 percent inhibition. Their data did not support Kirk and Sumners' (1931) idea that decrease of the enzymatic activity of urease when flocculated by antibodies was due simply to the aggregation. Marucci and Mayer (1954) explained the inhibition of urease activity in terms of steric hindrance on a molecular level - the more antibody molecules combined with a molecule of urease, the greater the steric interference.

Plasma urea N and ammonia N values following the first and sixth urease injection (15th day) are presented in Table 4 and Appendix Table 5. The urea N level of pigs in the 10 unit treatment group was significantly ($P < 0.01$) less than other groups at 1 and 6 hours after the first injection of urease, and the ammonia N level was significantly ($P < 0.01$) greater at the same time. Urea N and ammonia N values between groups following the sixth urease injection were not different. Although pigs receiving the 10 unit level of urease did not show symptoms of urease toxicity following the first urease injection, the urea N and ammonia N data do suggest that slightly more ammonia was being produced than could be adequately metabolized by the body. At 15 days, antibody production was probably great enough to tie up most of the urease injected, so that plasma levels of ammonia were not increased.

TABLE 4

TRIAL I. EFFECT OF INITIAL AND SECONDARY INJECTIONS OF UREASE UPON PLASMA UREA N AND AMMONIA N LEVELS OF GROWING PIGS[a b]

	1st Day of Trial[c]			15th Day of Trial[c]		
Time, hours	0	1	6	0	1	4
Urea N, mg./100 ml.						
Control	14.0±1.6[d]	14.1±0.6	13.1±0.6	10.2±1.6	11.2±2.1	11.0±2.3
0.1 Unit	13.1±0.1	13.5±0.5	12.4±0.4	11.2±2.4	10.7±3.3	10.1±3.0
1 Unit	13.3±1.3	13.4±1.0	12.6±0.5	10.4±0.6	10.3±0.7	10.6±1.5
10 Unit	9.6±1.6	10.5±1.0[f]	9.4±0.5[f]	10.8±1.7	11.8±1.5	11.5±1.6
Ammonia N, mcg./100 ml.						
Control	681±63	525±12	553±57	877±102	729±98	510±33
0.1 Unit	884±76[e]	665±81	501±54	663±27	902±88	413±15
1 Unit	591±21	590±47	539±26	816±89	735±77	671±140
10 Unit	750±24	960±56[f]	854±62[f]	683±111	716±38	574±25

[a] Jackbean urease prepared by the author.

[b] Three pigs per treatment.

[c] Blood samples taken and urease injection made at zero hour, then blood samples taken at 1 and 6 (or 4) hours.

[d] Standard error of the mean.

[e] Significantly greater than the least two treatment means ($P < 0.05$).

[f] Significantly different from the other treatment means ($P < 0.01$).

Urinary data collected in this trial 24 hours before and after the sixth urease injection (15th day) are shown in Table 5. Urine flow was significantly different between treatment groups following the urease injection. Urine urea N levels of the 1 and 10 unit groups were significantly ($P < 0.05$) larger than urine urea N levels of the other two groups during the post injection period. Urine ammonia N levels of the 0.1 unit group were increased and were slightly greater than for the control group after the injection. Most of the ammonia N and urea N difference between the groups is accounted for mainly by the difference in urine flow, as the concentration of urea N and ammonia N per unit of urine was not greatly different between the groups. It would appear from this data that the urease injected pigs had a greater urea N and ammonia N excretion rate than the control pigs, which would suggest that urea other than

TABLE 5

TRIAL I. EFFECT OF A SECONDARY INJECTION OF UREASE ON URINE FLOW, UREA N, AND AMMONIA N OF UREASE SENSITIZED GROWING PIGS [abc]

Treatment	Control	0.1 Unit	1 Unit	10 Unit
Size of urease injection SU/pound BW	0	0.6	6	60
Urine flow, ml./hr.				
Pre-injection	33.1±8.8[d]	29.6±4.3	39.6±1.8	32.7±6.9
Post-injection	13.2±1.1	23.1±2.3[ee]	29.1±1.3[ee]	18.4±0.8[e]
Urine urea N, mg./hr.				
Pre-injection	293±54	367±37	293±18	368±30
Post-injection	188±17	309±18	352±50[e]	392±79[e]
Change	-105±42	-58±45	59±31[e]	23±51
Urine Ammonia N, mg./hr.				
Pre-injection	16.7±2.5	16.1±2.8	17.0±3.2	16.5±1.5
Post-injection	12.9±2.2	23.5±4.1	18.0±1.4	17.2±0.4
Change	-4.2±2.4	7.4±5.5[e]	1.0±1.6	0.7±1.1

[a]Jackbean urease prepared by the author.
[b]Three pigs per treatment.
[c]All pigs placed in metabolism cages 24 hours before and after the sixth urease (15th day of trial) injection.
[d]Standard error of the mean.
[e]Significantly greater than least treatment mean ($P < 0.05$); [ee]($P < 0.01$).

that found in the blood plasma was hydrolyzed. Blood plasma urea N and ammonia N values taken at the same time were not different from the controls and do not support the urinary data. It is pointed out that although urine means are different, the standard error is large and the differences are just significant at the 5 percent level. Individual values are given in Appendix Table 6.

Analysis of urease activity and ammonia N levels of intestinal sections plus contents indicates no significant difference between groups (Table 6). However, it does appear that urease activity of the ileum sections from pigs in the 1 and 10 unit groups was decreased. Note in Table 6 and Appendix Table 7 that the within treatment variation is

TABLE 6

TRIAL I. UREASE ACTIVITY AND AMMONIA N LEVELS OF INTESTINAL SECTIONS PLUS CONTENTS FROM UREASE IMMUNIZED AND NONIMMUNIZED PIGS [abc]

	Urease Activity, units/gm. DM x 10^3		Ammonia N, mcg./gm. DM	
	S-1[d]	S-2[d]	S-1	S-2
Control	25±13[e]	174±91	450±86	697±79
0.1 Unit	11±7	216±71	582±109	770±74
1 Unit	16±6	60±28	530±85	572±138
10 Unit	15±4	49±34	442±56	694±108

[a]Jackbean urease prepared by the author.
[b]Pigs slaughtered at the end of experiment, 98 days.
[c]Six pigs in control and 0.1 unit, 5 pigs in 1 unit and 3 pigs in 10 unit.
[d]S-1 from the duodenum and S-2 from the caudal end of the ileum.
[e]Standard error of the mean.

rather large. Urease activity of the ileum sections was more than five times larger than the urease activity of the duodenum sections. Dintzis and Hastings (1953) reported that there was a five fold greater urease activity in the last 5 centimeters of the small intestine and in the colon than in the stomach. Dang and Visek (1960, 1963) and Harbers et al. (1963b) observed 15-60 percent less urease activity of the gastrointestinal tract of urease immunized rats as compared to nonimmunized rats. A 30 percent decrease in the ammonia concentration was observed by Dang and Visek (1963).

2. Trial II

In Table 7 are shown feed consumption, average daily gain, and feed efficiency data obtained in this trial. Individual average daily gains, sex, age, initial weights, and final weights are given in Appendix Table 8. Feed intake during the first 22 days of the trial was less in both treatment groups with the 1 unit treatment group having the more depressed appetite. Average daily gain during this same period was significantly ($P < 0.01$) decreased in the 1 unit group. Feed efficiency

TABLE 7

TRIAL II. FEED CONSUMPTION, AVERAGE DAILY GAIN AND FEED EFFICIENCY OF UREASE IMMUNIZED AND NONIMMUNIZED GROWING PIGS [ab]

Time, days	0-22	22-47	47-89[c]	0-47	0-89
Av. daily feed intake, lb.[d]					
Control	4.37	5.50	6.97	4.97	5.92
0.1 Unit	3.53	5.40	6.84	4.52	5.62
1 Unit	2.58	5.28	6.91	4.02	5.38
Av. daily gain, lb.					
Control	1.20	1.56	1.76	1.39	1.56
	(0.11)	(0.07)	(0.08)	(0.08)	(0.07)
0.1 Unit	1.10	1.56	1.69	1.34	1.51
	(0.04)	(0.09)	(0.10)	(0.06)	(0.06)
1 Unit	0.63[f]	1.66	1.75	1.18	1.44
	(0.13)	(0.10)	(0.09)	(0.07)	(0.06)
Feed per lb. gain, lb.					
Control	3.65	3.54	3.96	3.58	3.79
0.1 Unit	3.22	3.45	4.04	3.36	3.72
1 Unit	4.08	3.19	3.94	3.42	3.72

[a]Sigma urease type II powder.
[b]Ten pigs per treatment with initial weight of 66 pounds.
[c]During this period, one-half of the pigs in each group fed rations with added urea. See Table 9 for treatments.
[d]Pigs in each treatment group fed.
[e]Standard error of the mean in parentheses under the treatment mean.
[f]Significantly less than other two treatment means ($P < 0.01$).

was not greatly different between the groups although the 1 unit group appeared less efficient than the other two groups. As mentioned in the EXPERIMENTAL PROCEDURES section, the reduced feed intake and poor gain were probably due to a hemagglutinin factor present in the Sigma urease powder, as gain and feed intake returned to normal when urease injections were discontinued. Feed intake, gain and feed efficiency were about the same for all groups during the remainder of the trial. Though not significantly different, gain and efficiency of the 1 unit group were greater during the 22-47 day period than that of the other two groups.

The mean values for plasma urea N and ammonia N and serum anti-urease activity are given in Table 8. Urea N and ammonia N levels were

TABLE 8

TRIAL II. PLASMA UREA N AND AMMONIA N LEVELS AND SERUM ANTIUREASE ACTIVITY OF UREASE IMMUNIZED AND NONIMMUNIZED GROWING PIGS [ab]

Time, days	14	29	47	77[c]
Urea N, mg./100 ml.				
Control	15.3±0.9[d]	13.8±0.7	17.1±1.0	18.8±1.0
0.1 Unit	14.0±0.7	14.9±0.8	17.5±0.7	18.2±1.5
1 Unit	15.1±1.3	15.1±1.2	18.0±1.2	21.0±1.3
Ammonia N, mcg./100 ml.				
Control	583±28	541±31	378±31	419±8
0.1 Unit	518±20	648±34	395±27	473±7
1 Unit	609±30	617±42	365±16	441±12
Antiurease activity, units/ml.x 10^3				
Control	14±9	-13±7	4±7	2±6
0.1 Unit	184±11[f]	244±16[e]	222±15[ee]	141±21[e]
1 Unit	145±27[f]	204±12[f]	154±16[f]	81±13[f]

[a]Sigma urease type II powder.

[b]Ten pigs per treatment with initial weight of 66 pounds.

[c]During this period, one-half of the pigs in each group fed rations with added urea. See Table 9 for treatments.

[d]Standard error of the mean.

[e]Significantly larger than other two treatment means ($P < 0.05$); [ee]($P < 0.01$).

[f]Significantly larger than least treatment mean ($P < 0.01$).

not different between treatments. This is in agreement with results obtained in Trial I. As observed in Trial I, there was also an increase of urea N and a decrease of ammonia N with weight and/or age in this trial. Also, urea N and ammonia N values in this trial were of the same magnitude as those reported in Trial I. Serum antiurease activity of the treatment groups was significantly ($P < 0.01$) greater than that of the control group after the fourteenth day of the trial. At 29, 47 and 77 days, serum antiurease activity of the 0.1 unit treatment group was significantly greater than that of the 1 unit group and the control group. The 1 unit group had significantly larger serum antiurease activity than the control group during the same time. Thus, it appears in

this trial that the 0.1 unit level of urease was more effective in stimulating production of antibodies than the 1 unit level. However, stress from the hemagglutinin factor may have interfered with antibody production in the 1 unit group. In contrast to Trial I, the maximum antiurease activity in this trial was reached around the 29th day. It should be noted that pigs in Trial II received fewer urease injections than pigs in Trial I.

Investigation of the Sigma urease type II powder showed it to have a hemagglutination titer of over 100 per modified SU as compared to a zero titer for urease prepared by the author. Sumner and Howell (1936a, b) identified Concanavalin A, one of four crystallizable globulins of the jackbean, as a hemagglutinin. These authors reported that Concanavalin A agglutinated washed horse, dog, cat, rabbit, guinea pig, and rat erythrocytes, but had no action upon well-washed human and cow erythrocytes and little or no action upon goat, sheep and pig erythrocytes. They also observed that Concanavalin A produced precipitation with all sera tested. It was observed that pig serum was precipitated by Sigma urease. Recrystallization of Sigma urease powder failed to reduce the hemagglutinative activity.

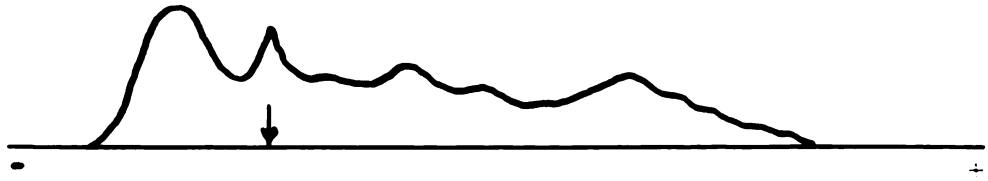
The paper electrophoretic pattern of Sigma urease and urease prepared by the author is shown in Figure 1. Note that the Sigma urease contained at least four fractions, while urease prepared by the author contained mainly one fraction, perhaps two. A comparison of urease activity per unit of crude protein revealed that the author's urease contained 57 times more urease activity per unit of crude protein than Sigma urease (57 and 1 modified SU/mg. crude protein, respectively).

Serum from normal (control) pigs was found to inhibit the agglutination of pig erythrocytes by Sigma urease. About 4 milliliters of

serum was required to neutralize 1 milliliter of Sigma urease solution containing 100 modified SU. Hirst (1948) in rabbits, and Ginsberg and Horsfall (1949) in humans, guinea pigs, rabbits and mice reported components of normal serum which inhibit agglutination of erythrocytes by certain virus. A cross reaction probably occurs here. The Sigma urease hemagglutinin is probably immunologically similar to some other antigen which had previously elicited antibody production. No difference could be detected in antihemagglutinative activity between control and treated pigs. Also, only one band was observed on Ochterlong plates between Sigma urease and immune serum from pigs injected with Sigma urease. These results suggest that the hemagglutinin factor in Sigma urease is probably a hapten or incomplete antigen. A hapten cannot elicit antibody production, but can combine with antibodies.

From data shown in Table 9, average daily gain of pigs receiving 2.5 and 3.4 percent urea in their ration was significantly decreased,

A. Urease prepared by the author.



B. Sigma urease type II powder.

FIGURE 1. ELECTROPHORETIC PATTERN OF SIGMA UREASE TYPE II AND UREASE PREPARED BY THE AUTHOR

TABLE 9

TRIAL II. FEED CONSUMPTION, AVERAGE DAILY GAIN AND FEED EFFICIENCY OF UREASE IMMUNIZED AND NONIMMUNIZED GROWING PIGS FED UREA TO SUPPLY PART OF THE PROTEIN REQUIREMENT [ab]

Urease, units	0	0.1	1	Average
1.25 Percent urea ration fed 47-63 day				
Av. daily feed intake, lb. [c]				
Control	6.78	6.55	6.51	6.61
Urea	6.48	6.39	6.65	6.50
Av. daily gain, lb.				
Control	1.83	1.84	1.92	1.86
	(0.10)[d]	(0.20)	(0.13)	(0.07)
Urea	1.76	1.56	1.80	1.71
	(0.12)	(0.14)	(0.15)	(0.07)
Feed per lb. gain, lb.				
Control	3.71	3.56	3.41	3.56
Urea	3.67	4.09	3.69	3.81
2.50 Percent urea ration fed 63-77 day				
Av. daily feed intake, lb.				
Control	7.33	6.93	7.51	7.26
Urea	7.11	7.47	6.87	7.15
Av. daily gain, lb.				
Control	1.98	1.71	1.93	1.88[e]
	(0.16)	(0.16)	(0.11)	(0.07)
Urea	1.79	1.96	1.60	1.78
	(0.15)	(0.16)	(0.14)	(0.08)
Feed per lb. gain, lb.				
Control	3.69	4.04	3.90	3.87
Urea	3.98	3.82	4.29	4.02
3.40 Percent urea ration fed 77-89 day				
Av. daily feed intake, lb.				
Control	7.30	6.72	7.86	7.29
Urea	6.98	7.13	6.15	6.76
Av. daily gain, lb.				
Control	1.78[f]	1.68	1.82[f]	1.76[ee]
	(0.08)	(0.19)	(0.19)	(0.07)
Urea	1.33	1.35	1.38	1.36
	(0.14)	(0.15)	(0.18)	(0.08)
Feed per lb. gain, lb.				
Control	4.09	3.99	4.33	4.14
Urea	5.24	5.28	4.45	4.98

[a]Sigma urease type II powder.

[b]Five pigs per treatment. Each initial treatment group divided into two groups after 47 days on experiment. Average weight, 126 pounds.

[c]Pigs in each treatment group fed.

[d]Standard error of mean in parentheses under mean.

[e]Significantly greater than urea group ($P < 0.05$); [ee] ($P < 0.01$).

[f]Significantly " " least three treatment mean ($P < 0.05$).

while 1.25 percent urea in rations did not significantly decrease gain. Feed consumption and feed efficiency were only slightly decreased at the 1.25 and 2.5 percent urea levels, but were decreased more at the 3.4 percent urea level. Urease immunization appeared to have no effect on the performance of the pigs fed urea. Appendix Table 12 contains the individual average daily gain values.

Hanson and Ferrin (1955) observed that 1.5 percent urea added to rations containing 10.5 percent crude protein could be fed to 35-pound pigs without toxic effects. When pigs weighed 125 pounds, the urea was reduced to one percent. The addition of urea did not affect the acceptability of the ration; although no beneficial effects were observed. Hays et al. (1957) reported that the harmful level of urea for growing pigs appeared to be between 0.62 and 0.76 percent of the total ration, provided the preformed protein level was adequate. Initial weight of pigs in these experiments was 23 to 30 pounds and pigs were fed to 100 pounds. Results of feeding urea in Trial II indicate that growing pigs over 100 pounds can be fed rations containing up to 2.5 percent urea without severe growth depression and decreased feed intake if the crude protein level is about 17 percent.

Plasma urea N and ammonia N values of pigs fed rations with urea added are shown in Table 10 and Appendix Table 13. The plasma urea N difference between the urea and no urea groups was not significant at the 2.5 percent urea level, but was significant ($P < 0.01$) at the 3.4 percent urea level. Some of the individual means differed significantly, however, at the 2.5 percent urea level. Pigs on all three levels of urease with 3.4 percent added urea had significantly larger plasma urea N values than the no urea groups. The plasma urea N level of the 1 unit

TABLE 10

TRIAL II. PLASMA UREA N AND AMMONIA N LEVELS OF UREASE IMMUNIZED AND NONIMMUNIZED GROWING PIGS FED UREA TO SUPPLY PART OF THE PROTEIN REQUIREMENT [ab]

Urease, units	0	0.1	1	Average
Plasma urea N, mg./100 ml.				
2.5 Percent urea ration[c]				
Control	15.8±1.2[d]	16.8±1.8	22.2±1.4[ee]	18.3±1.1
Urea	21.8±1.4[e]	19.6±1.2	19.8±3.4[g]	20.4±1.2
3.4 Percent urea ration[c]				
Control	16.9±1.5	16.8±2.2	23.4±2.2[e]	19.0±1.4
Urea	28.4±2.3[ee]	26.1±1.7[ee]	25.6±3.9[ee]	26.7±1.5[h]
Plasma ammonia N, mcg./100 ml.				
2.5 Percent urea ration				
Control	442±23	493±26[ff]	477±15[e]	471±13
Urea	396±20	454±17[g]	405±9	418±11[h]
3.4 Percent urea ration				
Control	450±17[ee]	540±13[ff]	550±21[ff]	513±15
Urea	348±32	514±27[f]	380±18	414±25[h]

[a]Sigma urease type II powder.
[b]Five pigs per treatment.
[c]Blood samples taken at the end of the period.
[d]Standard error of the mean in parentheses under mean.
[e]Significantly larger than the least two treatment means ($P < 0.05$); [ee]($P < 0.01$).
[f]Significantly larger than the least three treatment means ($P < 0.05$);[ff]($P < 0.01$).
[g]Significantly larger than the treatment mean ($P < 0.05$).
[h]Significantly different from the no urea group ($P < 0.01$).

treatment group with no urea added was significantly larger than 0.1 unit group and controls. Ammonia N levels of pigs receiving rations with urea added at the 2.5 and 3.4 percent levels were significantly less than those of pigs without urea added. Blood urea N levels have been reported to increase in rats and swine when urea is added to the ration (Finlayson and Baumann, 1956a, b; Rumsfeld, 1956; Hays et al., 1957); however, the author is not aware of any reports in the literature of the ammonia N levels of swine fed urea. These ammonia levels are contrary to what the

author would have predicted but are in agreement with ammonia levels obtained in another urea study by the author (unpublished data).

3. Trial III

Feed consumption, average daily gain and feed efficiency data collected in this trial are presented in Table 11. Average daily feed intake and gain were less for the 5 unit treatment group during the 0 to 28 day period than for the other two groups. Feed efficiency was similar for all treatment groups during the entire trial. Feed intake and gain between groups during the 28 to 56 day period were not different. During the 56 to 73 day period when one-half of the pigs were fed rations with 3.4 percent urea added, feed intake was less in the 5 unit treatment group. Note in Table 13 that this decreased feed intake of the 5 unit group was due to pigs which were fed the ration with added urea.

The depression of feed intake and daily gain of the 5 unit group during the first week of the trial accounted for most of the reduction in feed intake and gain during the 0 to 28 day period. This apparent affect of the urease injections on feed intake and gain at the 5 unit level may have resulted from elevated ammonia levels in these animals, thereby causing a stress which depressed appetite and reduced daily gains. After about a week, the pigs would be producing antibodies and could neutralize the urease injected, thus removing the stress from ammonia. Although ammonia was not determined before and after the first urease injection in this trial, ten SU per pound of body weight in Trial I did significantly raise plasma ammonia after the initial urease injection. Feed intake and daily gain, however, were not affected in Trial I. Also pigs in Trial I were ten pounds heavier than pigs in this trial. Individual average daily gains, sex, age, initial weights and final weights are shown in Appendix Table 15.

TABLE 11

TRIAL III. FEED CONSUMPTION, AVERAGE DAILY GAIN AND FEED EFFICIENCY OF UREASE IMMUNIZED AND NONIMMUNIZED GROWING PIGS [ab]

Time, days	0-28	28-56	56-73[c]	0-56
Av. daily feed intake, lb.[d]				
Control	3.06	4.42	5.65	3.74
0.5 Unit	3.17	4.35	6.08	3.74
5 Unit	2.75	4.42	5.18	3.58
Av. daily gain, lb.				
Control	1.23 (0.04)[e]	1.50 (0.05)	1.39 (0.13)	1.36 (0.04)
0.5 Unit	1.33[f] (0.05)	1.51 (0.05)	1.26 (0.12)	1.43 (0.04)
5 Unit	1.15 (0.05)	1.46 (0.07)	1.25 (0.20)	1.31 (0.05)
Feed per lb. of gain, lb.				
Control	2.49	2.95	4.05	2.74
0.5 Unit	2.39	2.98	4.20	2.69
5 Unit	2.38	3.04	4.14	2.74

[a]Jackbean urease prepared by the author.
[b]Ten pigs in each treatment with initial weight of 31 pounds.
[c]One-half of the pigs in each group fed a ration with 3.4 percent urea added from 56-73 days.
[d]Pigs in each treatment group fed.
[e]Standard error of the mean in parentheses under the treatment mean.
[f]Significantly greater than the least treatment mean ($P < 0.05$).

Gain and feed efficiency data obtained in Trials II and III do not support the general hypothesis suggested by Visek and coworkers (Dang and Visek, 1960; Visek and Dang, 1960a; Harbers et al., 1962, 1963b) of increased growth rate and improved feed efficiency of animals immunized with jackbean urease. However, gain was significantly ($P < 0.05$) increased and feed efficiency was improved in Trial I at the 10 unit level.

Plasma urea N and ammonia N means are shown in Table 12 with the individual values shown in Appendix Tables 16 and 17, respectively. As observed in Trials I and II, plasma urea N levels were not significantly different between treatment groups and were of the same magnitude as reported in the previous two trials. Ammonia levels of the 0.5 unit group

TABLE 12

TRIAL III. PLASMA UREA N AND AMMONIA N LEVELS AND SERUM ANTIUREASE ACTIVITY OF UREASE IMMUNIZED AND NONIMMUNIZED GROWING PIGS [ab]

Time, days	28	56	73[c]
Urea N, mg./100 ml.			
Control	13.9±0.8[d]	18.4±1.2	25.3±2.5
0.5 Unit	15.0±0.7	17.8±1.1	24.7±2.5
5 Unit	13.2±0.6	17.9±1.2	19.4±1.9
Ammonia N, mcg./100 ml.			
Control	695±27	710±32[e]	484±18[ee]
0.5 Unit	715±20	605±16	392±18
5 Unit	688±17	712±38[e]	466±21[e]
Antiurease Activity, unit/ml. x 10^3			
Control	1±9	1±10	1±4
0.5 Unit	90±8[ee]	47±9[ee]	38±6[ee]
5 Unit	84±14[ee]	67±13[ee]	35±4[ee]

[a]Jackbean urease prepared by the author.
[b]Ten pigs in each treatment with an initial weight of 31 pounds.
[c]One-half of the pigs in each group fed a ration with 3.4 percent urea added from 56 to 73 days.
[d]Standard error of the mean.
[e]Significantly greater than the least treatment mean ($P < 0.05$); [ee]($P < 0.01$).

at 56 and 73 days were significantly less than those of the control and 5 unit groups. These results are in contrast to no difference observed in Trials I and II. As noted in the previous two trials, there was an increase of urea N and a decrease of ammonia N with weight and/or age in this trial.

Both 0.5 and 5 unit levels were equally effective in stimulating antibody production (Table 12) with both treatment groups having significantly greater ($P < 0.01$) serum antiurease activity than the controls. These findings are in general agreement with results from Trial I; however, in Trial II, the lower level (0.1 unit) had the highest serum antiurease activity. As pointed out in Trial II, the reduced antibody production of the 1 unit treatment group may have resulted from the hemagglutinative

stress. Antiurease activity was greater at 28 days than at 56 or 73 days. This agrees with data reported in Trial II where the greatest serum antiurease activity was found at 29 days and is in contrast to results obtained in Trial I where the greatest antiurease activity occurred after 72 days. Fewer urease injections were given in Trials II and III than in Trial I. Serum antiurease levels in this trial were about one-half as large as obtained in the two previous trials. Note that pigs used in this trial were younger than pigs used in Trials I and II. Appendix Table 18 contains the individual values.

A summary of results obtained during the period when one-half of the pigs in each treatment were fed 3.4 percent urea rations is summarized in Table 13 and Appendix Table 19. Feed consumption, average daily gain and feed efficiency of pigs fed rations with added urea were decreased. Performance of pigs fed 3.4 percent urea was affected more in this trial than in Trial II. Pigs in this trial were approximately 50 pounds lighter than pigs in Trial II when high levels of urea (2.5 and 3.4 percent) were added to the ration. It appears from data obtained in Trial II and in a urea study (unpublished data) that 150 to 200 pound pigs can utilize or tolerate higher urea levels than 100 to 150 pound pigs.

Plasma urea N levels of pigs fed rations with added urea were significantly ($P < 0.01$) higher than those of the control pigs. The plasma ammonia N levels of pigs fed rations with added urea were less than the levels of control pigs, but only approached statistical significance. These urea N and ammonia N levels are comparable to results obtained in Trial II.

Urease activity of intestinal sections plus contents from urease immunized pigs was significantly less than the urease activity of intestinal sections plus contents from control pigs (Table 14). Ammonia N

TABLE 13

TRIAL III. FEED CONSUMPTION, AVERAGE DAILY GAIN, FEED EFFICIENCY, PLASMA UREA N AND PLASMA AMMONIA N LEVELS OF UREASE IMMUNIZED AND NON-IMMUNIZED PIGS FED 3.4 PERCENT UREA TO SUPPLY PART OF THE CRUDE PROTEIN REQUIREMENT [ab]

Urease, units	0	0.5	5	Average
Av. daily feed intake, lb.				
Control	5.81	5.62	5.97	5.80
Urea	5.51	5.25	4.40	5.05
Av. daily gain, lb.				
Control	1.69	1.56	1.76	1.68
	(0.05)[d]	(0.09)	(0.07)	(0.04)
Urea	1.09[ee]	1.02[ee]	0.74[ee]	0.95[ee]
	(0.15)	(0.09)	(0.21)	(0.09)
Feed per lb. of gain, lb.				
Control	3.42	3.60	3.38	3.46
Urea	5.03	5.01	5.94	5.26
Urea N, mg./100 ml.				
Control	18.9	17.6	15.8	17.4
	(1.7)	(1.7)	(1.2)	(0.9)
Urea	31.6[f]	30.5[f]	23.0[g]	28.3[ee]
	(2.3)	(1.8)	(2.8)	(1.4)
Ammonia N, mcg./100 ml.				
Control	490	455	498[h]	482
	(27)	(24)	(28)	(15)
Urea	478	419	433	443
	(27)	(21)	(27)	(17)

[a]Jackbean urease prepared by the author.

[b]Five pigs in each treatment, except the 0.5 unit group without urea which had only four pigs. Each initial treatment group divided into two groups after 56 days on experiment.

[c]Pigs in each treatment group fed.

[d]Standard error of the mean shown in parentheses under mean.

[e]Significantly different from control treatment mean ($P < 0.05$); [ee]($P < 0.01$).

[f]Significantly greater than least four treatment means ($P < 0.05$).

[g]Significantly " " " two treatment means ($P < 0.05$).

[h]Significantly " " " treatment mean ($P < 0.05$).

concentration of the intestinal sections plus contents was not significantly different between groups. Intestinal urease activity and ammonia N levels were lower and there was less variation within treatment groups

in this trial than in Trial I. These results are in agreement with reports by Dang and Visek (1960, 1963) and Harbers et al. (1963b) who observed less gastrointestinal urease activity of immunized rats as compared to nonimmunized rats.

TABLE 14

TRIAL III. UREASE ACTIVITY AND AMMONIA N LEVELS OF INTESTINAL SECTIONS PLUS CONTENTS FROM UREASE IMMUNIZED AND NONIMMUNIZED PIGS [abc]

Treatment	Urease Activity units/gm. DM x 10^3	Ammonia N mcg./gm. DM
Control	33.7±4.3[d]	182±36
0.5 Unit	25.1±5.5[e]	154±17
5 Unit	16.5±3.3[e]	172±36

[a]Jackbean urease prepared by the author.
[b]Six pigs from each treatment slaughtered at the end of experiment, 73 days. Intestinal sections plus contents taken from the caudal end of the ileum.
[c]One-half of the pigs in each group fed a ration containing 3.4 percent urea from 56 to 73 days.
[d]Standard error of the mean.
[e]Significantly less urease activity in immunized pigs than in control pigs ($P<0.05$).

B. Toxicity

1. Trial I

Results obtained in this trial are contained in Appendix Table 21 with plasma ammonia and urea values presented graphically in Figure 2. The plasma ammonia level was significantly greater in the pig given urease at the level of 100 SU per pound of body weight, reaching a maximum of 3.5 milligrams of ammonia N per 100 milliliters 9 hours after urease injection. A significant decrease of plasma urea followed the rise in plasma ammonia.

There was an increase of the urine ammonia level of the pig injected with urease whereas the level decreased in the control pig. The urine

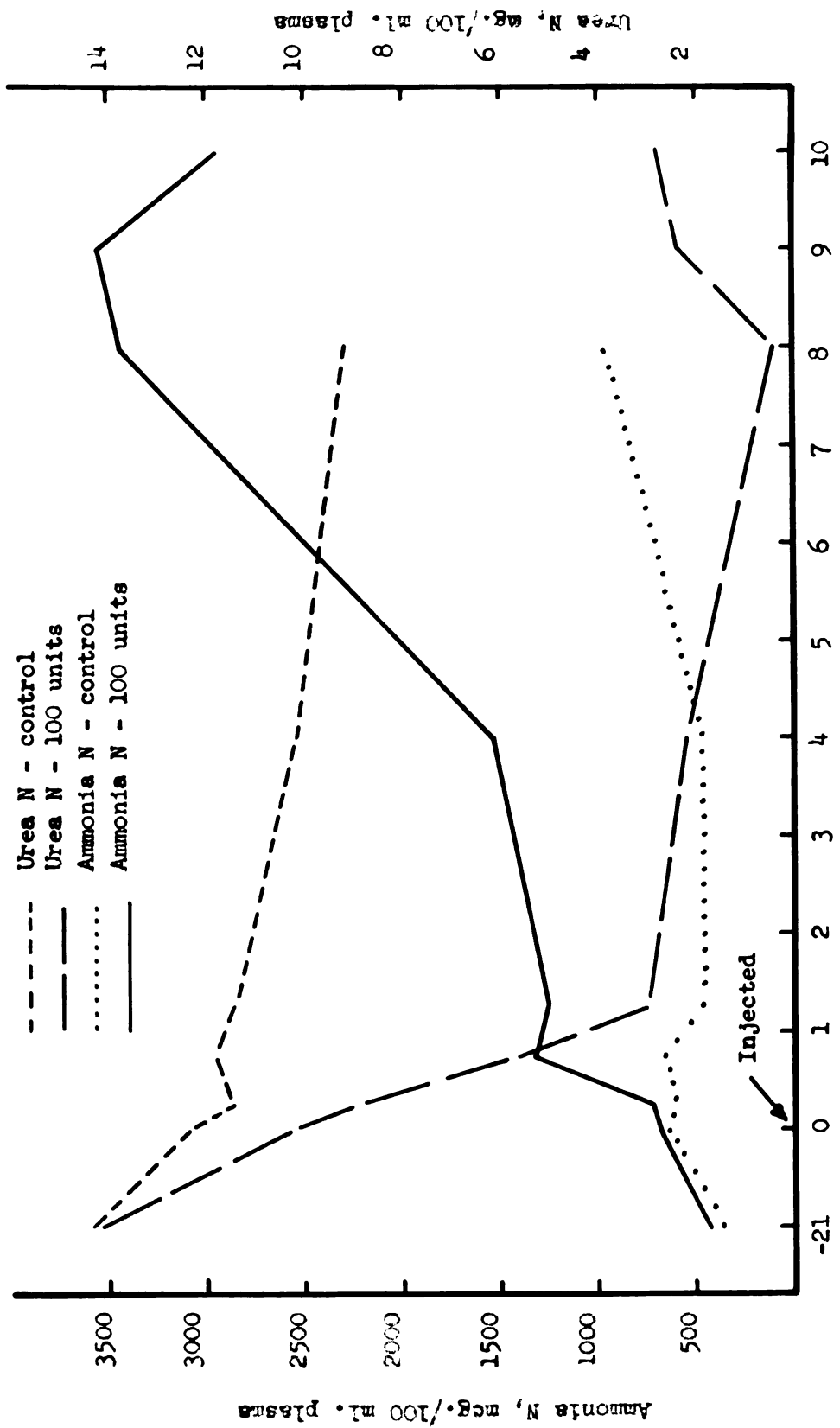


FIGURE 2. TRIAL I. EFFECT OF AN INTRAPERITONEAL INJECTION OF 100 SU OF UREASE PER POUND OF BODY WEIGHT ON PLASMA UREA AND AMMONIA OF A GROWING PIG

urea level was lower in both pigs during the post-injection phase, but there was no difference between the two pigs.

The urease injected pig went into convulsions with severe twitching and tetanic spasms of the sketal muscles after 8 hours. This continued for 2 hours at which time the pig died. A rise in rectal temperature was recorded reaching 43°C, 9 hours after the injection.

2. Trial II

A summary of results obtained in this trial is presented in Figure 3 and in Tables 15 and 16. Plasma ammonia rose significantly following the injection of either 50 or 100 SU of urease per pound of body weight (Figure 3). The maximum level in milligrams per 100 milliliters of plasma was 1.7 and 3.0 for the 50 and 100 unit groups, respectively. As seen in Figure 3, plasma urea levels were significantly lower following urease injection. Note that the urea level of all three groups was lower after 22 hours of fasting. Urea levels of control and urease injected pigs began increasing about 7 hours after injection. This initial decrease in plasma urea after about 20 hours of fasting and then the rise about 10 hours later has been observed by the author in other swine studies (unpublished data). Chance et al. (1962) in swine and Henneman et al. (1962) in man have made a similar observation. Individual plasma ammonia and urea data is contained in Appendix Table 22.

From data shown in Table 15, urease activity was found in serum after an intraperitoneal injection of urease. Although the amount found in the treated animals was large compared to the controls, the differences were not statistically significant due to the large individual variation of urease injected pigs (Appendix Table 23). Serum urease activity was greater 6 hours after the urease was injected than 10 hours after the

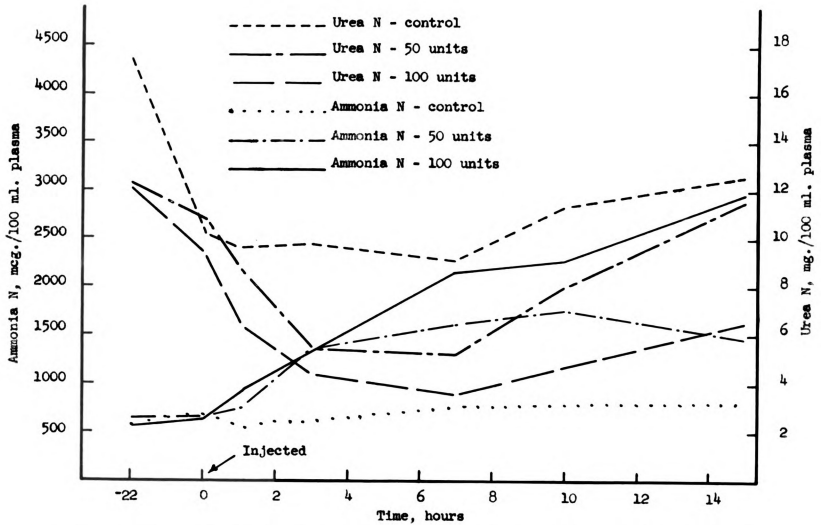


FIGURE 3. TRIAL II. EFFECT OF INTRAPERITONEAL INJECTIONS OF 50 AND 100 SU OF UREASE PER POUND OF BODY WEIGHT ON PLASMA UREA AND AMMONIA OF GROWING PIGS

TABLE 15

TRIAL II. SERUM UREASE ACTIVITY. PROTEIN, POTASSIUM AND SODIUM OF GROWING PIGS INJECTED INTRAPERITONEALLY WITH 50 AND 100 SU OF UREASE PER POUND OF BODY WEIGHT [ab]

Time, hours	0	6	10
Urease activity, units/ml. x 10^3			
Control	0.1±0.1[c]	-0.1±0.1	0.1±0.1
50 Units[d]	0.1±0.2	37.9±12.5	7.8±3.0
100 Units[e]	0.3±0.2	83.8±67.6	33.6±27.1
Protein, gm./100 ml.			
Control	5.7±0.2	5.6±0.2	5.3±0.1
50 Units	5.5±0.1	5.9±0.3	5.6±0.1
100 Units	5.3±0.1	5.4±0.5	5.3±0.1
Potassium, mg./100 ml.			
Control	25.1±3.1	23.5±3.6	23.8±2.9
50 Units	21.6±1.0	28.3±0.2[f]	27.4±0.1[f]
100 Units	24.8±2.4	28.6±3.6[f]	25.7±0.5[f]
Sodium, mg./100 ml.			
Control	326±2	332±1	298±2
50 Units	325±10	325±0	313±16
100 Units	319±4	312±19	321±8

[a] Jackbean urease prepared by the author.
[b] Two pigs per treatment.
[c] Standard error of mean.
[d] One pig died 25 hours after injection.
[e] Both pigs died: one after 15 hours and one after 20 hours.
[f] Significant increase ($P < 0.05$) after urease injection.

injection. This finding agrees with work reported by Kirk and Sumner (1931) who found urease in the blood after intraperitoneal injections.

Serum protein and sodium values of pigs injected with urease were not statistically different from saline controls. The serum protein values are in line with values reported by Miller et al. (1961). Serum potassium was significantly increased in pigs receiving urease injection.

Liver urease activity and ammonia level of urease injected pigs were larger (Table 16) than values for control pigs though not statistically significant.

TABLE 16

TRIAL II. 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 [ab]

Treatment	Control	50 Units[c]	100 Units[d]
Liver urease activity units/gm. DM x 10^3	2.7±5.5[e]	7.2±7.2	144.5±111.7
Liver ammonia N mcg./gm. DM	883±50	1228±164	1511±32
Urine flow, ml./hr.[f]			
pre-injection	8.6±2.3	7.3±0.2	6.6±1.0
post-injection	6.4±1.0	11.7±1.1[g]	12.4±1.5[g]
Urine urea N, mg./hr.			
pre-injection	90.3±16.1	85.9±7.1	79.2±16.7
post-injection	42.7±3.9	39.3±5.7	41.4±4.2
change	47.6±19.9	46.6±12.8	37.8±20.9
Urine ammonia N, mg./hr.			
pre-injection	6.7±1.2	4.2±2.4	5.3±0.2
post-injection	5.7±1.2	8.3±3.5	6.3±1.2
change	-1.0±0.0	4.1±1.1[g]	1.0±1.2

[a] Jackbean urease prepared by the author
[b] Two pigs per treatment.
[c] One pig died 25 hours after injection.
[d] Both pigs died: one after 15 hours and one after 20 hours.
[e] Standard error of mean.
[f] Twenty-two hour pre-injection collection period and 18 hour post-injection collection period.
[g] Significantly greater than least mean ($P < 0.05$).

From data shown in Table 16 and Appendix Table 24, it appears that urine urea N was not affected by these levels of urease injection. However, urine ammonia and urine flow were significantly increased following urease injection. Rector et al. (1959) in dogs and Lewis (1961) in sheep reported increased ammonia in the urine following administration of ammonium chloride. In an earlier study, Lewis et al. (1957) reported that the liver conversion of ammonia to urea was complete up to about 80 milligrams of ammonia N per 100 milliliters of ruminal fluid. It thus appears

that the increased ammonia N in the urine results from the liver's inability to convert all ammonia produced to urea. Another strong possibility, in urease toxicity, is that the urea may be broken down after leaving the liver.

Both pigs receiving the 100 unit level of urease died - one after 15 hours and the other after 20 hours. Only one pig on the 50 unit level died - 25 hours after urease injection. Pigs injected with 100 units of urease showed intermittent tetany starting about 4 hours after the injection. Tetany was more severe in pig 87-8 than in pig 78-4. The rectal temperature of pig 78-4 remained normal while that of pig 87-8 was elevated as tetany became more intense, reaching a level of 44°C at death. Pig 78-7, which died on the 50 unit level, appeared to have no tetany, was very limp, and had a subnormal temperature, dropping to 36.1°C before death.

3. Trial III

As indicated in Figure 4, and Appendix Table 25, plasma ammonia was significantly elevated and urea was significantly decreased following the injection of 75 SU of Sigma urease type II powder per pound of body weight compared to pigs receiving a saline injection. The maximum ammonia level was 2.5 milligrams of ammonia N per 100 milliliters of plasma. All pigs injected with urease died. The ammonia and urea results in this trial are similar to those reported for Trials I and II are in agreement with work reported for other species. Kirk and Sumner (1931) reported an elevated ammonia N level (5.0 mg./100 ml. blood) in a rabbit injected intraperitoneally with 22 SU per kilogram of body weight. Ten and forty SU of urease injected intravenously into rabbits were reported by Tauber and Kleiner (1931) to transform all blood urea into ammonia 15

minutes after the injection. They reported ammonia N values of 8-18.5 milligrams per 100 milliliters of blood. Recently, Handford (1961) showed that 13.5 SU per kilogram of body weight injected intravenously into dogs caused a highly significant elevation of plasma and blood ammonia N and a decline in blood urea N. Plasma ammonia N values of 7-8 milligrams per 100 milliliters were reported by this worker for dogs given a urease injection.

Dinning et al. (1948) and Gallup et al. (1953) studying the toxic effects of a large dose of urea given to reminants suggested that death

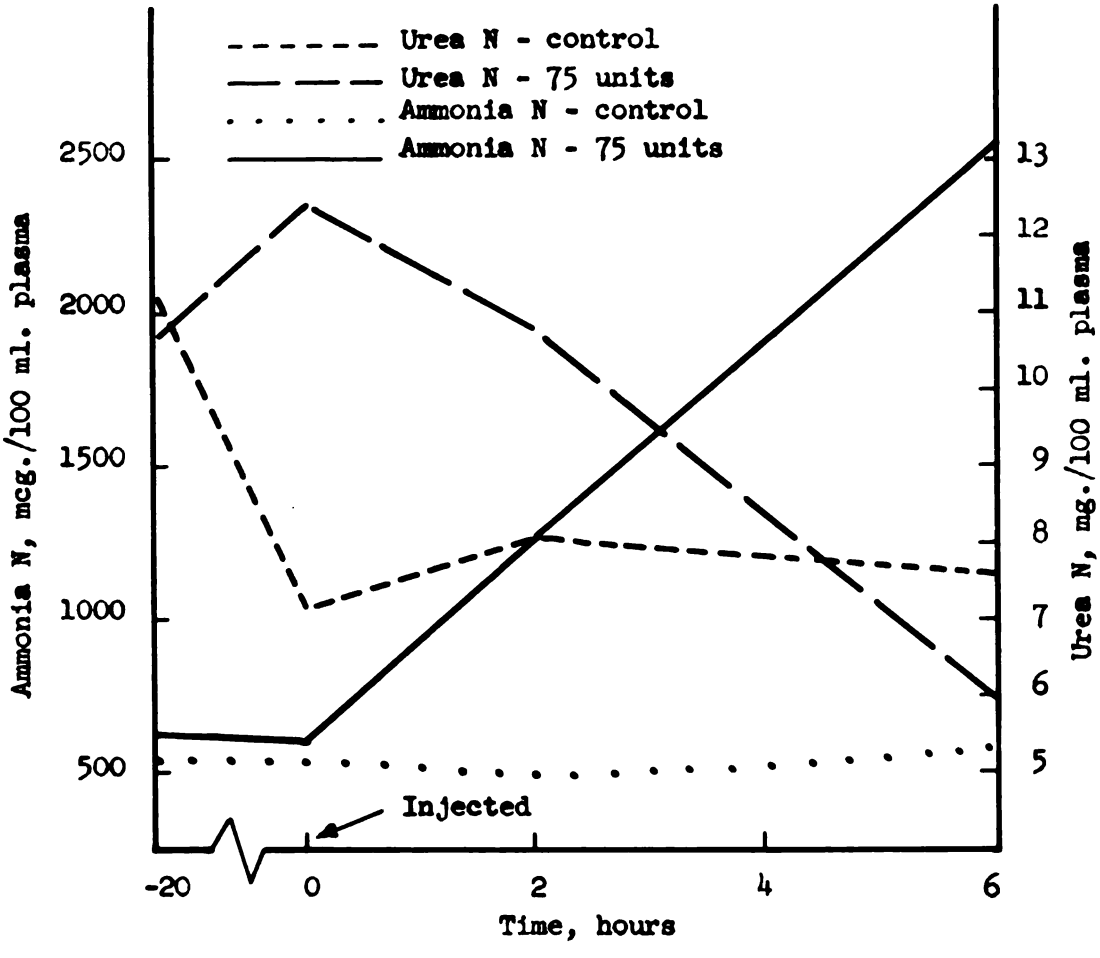


FIGURE 4. TRIAL III. EFFECT OF AN INTRAPERITONEAL INJECTION OF 75 SU OF UREASE PER POUND OF BODY WEIGHT ON PLASMA UREA AND AMMONIA OF GROWING PIGS

in urea poisoning was due to high blood ammonia levels. They reported that 4-8 milligrams of ammonia N per 100 milliliters of blood was fatal. In contrast, Lewis (1960) demonstrated that sheep showed symptoms of ammonia toxicity when the blood level of ammonia N reached about 0.5 milligrams per 100 milliliters. Repp et al. (1955) reported that the critical blood level of ammonia N in lambs was about 1 milligram per 100 milliliters, whereas normal values were about 0.145. Normal values of blood ammonia N for cattle and sheep were reported by Dinning et al. (1948) to be from 0.3 to 1.0 milligrams per 100 milliliters. From data collected in the three trials reported here, plasma ammonia N levels of 2 to 4 milligrams per 100 milliliters were obtained in pigs which died. Normal values ranged from 0.4 to 1.2 milligrams per 100 milliliters of plasma with an average of 0.58.

The lack of agreement between normal and toxic levels of blood ammonia could be explained by the following factors: 1) species difference; 2) type of diet that animal receives (Warren and Newton, 1959); 3) time interval between feeding and blood sampling (Clarke et al., 1959); 4) time interval between blood sampling and analysis (Delorme et al., 1958); 5) method of handling the blood samples (Conn, 1962; Miller and Rice, 1962); and 6) method of analysis.

Analysis of hematocrit and hemoglobin data indicates a significant difference between control and urease injected pigs (Table 17 and Appendix Table 26). 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 the hemagglutinative effect. As discussed previously, the Sigma urease powder was found to have high hemagglutinative activity. This hemagglutinin factor may have influenced other criteria which were measured.

TABLE 17

TRIAL III. EFFECT OF AN INTRAPERITONEAL INJECTION OF 75 SU OF UREASE PER POUND OF BODY WEIGHT UPON HEMATOCRIT, HEMOGLOBIN, BLOOD GLUCOSE AND RECTAL TEMPERATURE OF GROWING PIGS [ab]

Time, hours	-20	0	2	6
Hematocrit, percent				
Control	29.2±1.4[c]	31.5±1.8	31.8±2.0	32.2±1.9
75 Units[d]	32.1±0.6	32.4±1.0	39.3±1.2[ee]	47.4±1.1[ee]
Hemoglobin, gm./100 ml.				
Control	9.9±0.5	10.6±0.8	10.3±0.6	10.0±0.8
75 Units	9.7±0.4	10.5±0.5	12.6±0.5[e]	15.6±1.0[ee]
Glucose, mg./100 ml.				
Control	79.0±3.8	60.1±1.9	68.2±1.9	73.9±5.9
75 Units	71.7±3.9	51.9±3.3	56.9±6.3	40.5±4.8[ee]
Temperature, °C				
Control	40.1±0.2	39.6±0.1	- - -	39.6±0.1
75 Units	39.9±0.1	39.6±0.1	- - -	39.9±0.4

[a]Sigma urease type II powder.
[b]Six pigs per treatment.
[c]Standard error of mean.
[d]Survival time after injection: 5, 6, 7, 10, 10, and 11 hours.
[e]Significantly different from control ($P < 0.05$); [ee]($P < 0.01$).

Glucose was significantly decreased 6 hours after the urease was injected. Winitz et al. (1956) demonstrated an initial increase and then a decrease in blood ammonia from LD_{99} dose of amino acids given to rats (toxicity believed to be due to ammonia). They also pointed out that death in many instances was accompanied by a severe drop in the blood sugar level. As seen in Table 17, there was a slight rise in blood glucose 2 hours after the injection and then a sharp drop at 6 hours. However, the initial rise noted in this trial is believed to be due to fasting rather than to urease because a similar change was obtained in the controls. The decreased glucose level may have resulted from the use of glucose to produce intermediates in the urea cycle and glutamic acid -

these intermediates and glutamic acid needed in the detoxification of ammonia. Individual glucose values are shown in Appendix Table 27.

Handford (1958) reported that in dogs suffering from ammonia intoxication, pyruvic acid and glucose levels were elevated. Although pyruvic acid was not measured directly in this trial, there was evidence from the transaminase determination that serum from urease injected pigs probably contained a much larger amount of pyruvic acid than serum from control pigs. This evidence is based upon the observation that a greater amount of DPNH was used or destroyed in equilibrating the reaction mixture when serum from urease injected pigs was used than when serum from control pigs was used.

In agreement with results from Trial II, urease activity was found in the serum following an intraperitoneal injection of urease (Table 18). The difference was statistically significant. Urease activity was greater at 6 hours than at 2 hours after the injection. Individual values are shown in Appendix Table 28.

Statistical analysis showed a significant elevation of serum potassium 2 hours after the urease injection and a highly significant elevation 6 hours after the injection. These results agree with results obtained in Trial II. Lewis (1961) reported increased plasma potassium levels in two sheep which had one mole of ammonium chloride placed in the rumen. He suggested that the elevated plasma potassium may have been due to a limitation of available energy which results in a migration of intracellular potassium to extracellular fluid. He also pointed out that the movement of potassium may be associated with acidosis. Analysis of serum sodium and calcium values indicates no significant change. Individual values are given in Appendix Tables 28 and 29.

TABLE 18

TRIAL III. EFFECT OF AN INTRAPERITONEAL INJECTION OF 75 SU OF UREASE PER POUND OF BODY WEIGHT UPON SERUM UREASE ACTIVITY, ELECTROLYTES, AND TRANSAMINASE ACTIVITY IN GROWING PIGS [ab]

Time, hours	0	2	6
Urease activity, SU/ml. x 10^3			
Control	-0.1±0.4	0.1±0.3	0.3±0.4
75 Units	0.3±0.3	8.8±3.5[d]	24.6±10.6[d]
Potassium, mg./100 ml.			
Control	27.9±0.9	25.0±1.6	25.4±1.5
75 Units	27.4±1.2	30.6±1.1[d]	43.6±3.3[dd]
Sodium, mg./100 ml.			
Control	312±4	311±4	303±3
75 Units	312±3	311±4	310±7
Calcium, mg./100 ml.			
Control	11.8±0.4	11.1±0.1	11.0±0.6
75 Units	11.7±0.5	11.4±0.4	10.2±0.2
Transaminase, Sigma units/ml.[c]			
Glutamic-Oxalacetic			
Control	19.4±1.1	18.9±1.6	17.4±1.1
75 Units	19.7±1.5	16.5±1.0	19.8±4.1
Glutamic-Pyruvic			
Control	25.1±1.1	26.1±1.5	21.2±1.1
75 Units	23.1±1.4	16.3±0.5[dd]	16.5±0.9[dd]

[a]Sigma urease type II powder.
[b]Six pigs per treatment.
[c]One unit of transaminase activity defined as that amount of enzyme which will cause a decrease in OD_{340} of 0.001 per minute and per centimeter light path.
[d]Significantly different from control ($P < 0.05$); [dd]($P < 0.01$).

Serum transaminase values are given in Table 18 and Appendix Table 10. The normal glutamic-pyruvic values are similar to ones reported by Calloway et al. (1962); however, their glutamic-oxalacetic values are approximately twice the values obtained in this study. Glutamic-pyruvic values for urease injected pigs were significantly lower than control values following the injection. Glutamic-oxalacetic values were not different.

From data shown in Table 19 and Appendix Table 31, gamma globulin was the only fraction of serum protein which showed a significant difference between control and urease injected pigs. Percent gamma globulin was elevated following urease injection. The large difference observed between gamma globulin of the two groups at zero hour is related to the age difference between the two groups. Age and gamma globulin were significantly correlated ($r = 0.68$) at zero hour. Miller et al. (1961) reported that percent gamma globulin increased from 9.1 in 49-day-old pigs to 14.4 in 63-day-old pigs.

Urinary data is summarized in Table 20 and individual values are given in Appendix Table 32. Urine flow, total N, urea N, and ammonia N

TABLE 19

TRIAL III. EFFECT OF AN INTRAPERITONEAL INJECTION OF 75 SU OF UREASE PER POUND OF BODY WEIGHT UPON ELECTROPHORETIC PATTERNS OF SERUM [ab]

Time, hours	0	2	6
Gamma globulin, percent			
Control (54)[c]	8.7±1.1	8.5±1.6	9.2±1.5
75 Units (61)	13.2±2.2	14.1±2.3	14.5±1.4[d]
Beta globulin, percent			
Control (54)	13.5±0.4	15.1±0.7	15.2±0.8
75 Units (61)	14.5±0.4	15.3±1.1	15.8±0.5
Alpha globulin, percent			
Control (54)	19.3±1.2	19.1±0.9	19.7±0.5
75 Units (61)	17.1±1.3	14.4±1.2	18.5±1.1
Albumin, percent			
Control (54)	58.4±1.1	57.2±1.5	55.9±1.9
75 Units (61)	55.2±2.9	53.2±3.2	51.1±1.7

[a]Sigma urease type II powder.
[b]Six pigs per treatment.
[c]Average age of pigs in days in parentheses.
[d]Significantly greater than control ($P < 0.05$).

TABLE 20

TRIAL III. URINE FLOW, TOTAL N, UREA N, AMMONIA N AND DIFFERENCE[a] N OF GROWING PIGS GIVEN HIGH UREASE INJECTIONS[b]

Period	Pre-injection	Post-injection
Urine flow, ml./hr.		
Control	9.93±1.43	20.05±2.76
75 Units[d]	12.18±2.81	5.10±0.59[cc]
Total N, mg./hr.		
Control	98.5±5.2	108.3±9.1
75 Units	75.1±13.9	40.2±8.3[cc]
Urea N, mg./hr.		
Control	77.6±6.0	80.0±4.6
75 Units	60.3±11.0	23.6±5.7[cc]
Ammonia N, mg./hr.		
Control	6.8±1.4	11.6±3.0
75 Units	6.4±1.5	2.8±0.5[c]
Difference N, mg./hr.		
Control	14.1±1.9	16.7±2.2
75 Units	8.4±2.7	13.8±2.8

[a]Difference N is total N minus urea and ammonia N.
[b]Six pigs per treatment.
[c]Significantly less than control ($P < 0.05$); [cc]($P < 0.01$).
[d]Survival time after injection: 5, 6, 7, 10, 10 and 11 hours.

values from urease injected pigs were all significantly less than values from the control pigs. These results are in contrast to results from Trials I and II and those reported by Lewis (1961). The effect of urease on urine flow, urea N and ammonia N in this trial are confounded with the effects of the hemagglutinin. Urease injected pigs in this trial survived a much shorter time than did pigs in the previous two trials and their symptoms were somewhat different.

In contrast to the previous trials, urease injected pigs exhibited no tetany, but lay very still while control pigs were moving around. Their rectal temperatures remained normal. They did show some frothing as pigs in the previous trial had shown. 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 post-mortem observations are similar to ones reported by Handford (1961) for dogs given a crude filtered suspension of jackbean meal and by Shone (1962) for cattle suffering from urea toxicity.

V. SUMMARY

Six trials were conducted to study the effects of intraperitoneal injections of urease upon the performance and hematology of growing pigs. The first three trials consisting of 81 pigs were designed to study the effects of urease immunization upon the following criteria: feed intake and gain, feed efficiency, serum antiurease activity, plasma urea N and ammonia N, urine urea N and ammonia N, intestinal urease activity and ammonia N levels. Urease prepared from jackbean meal was used in Trials I and III, while Sigma urease type II powder was used in Trial II. Starting doses ranged from 0.1 to 10 Sumner units per pound of body-weight with the size of subsequent doses determined by arithmetic progression. A corn-soybean ration fortified with minerals and vitamins was fed in all trials in the immunization study.

The pigs immunized with urease exhibited significantly greater serum antiurease activity than did nonimmunized pigs. In the first two trials, the low levels of urease appeared to be more effective in stimulating antibody production than the high levels of urease, while all levels of urease were equally effective in Trial III. In Trials II and III, the greatest serum antiurease activity occurred after about 28 days, while in Trial I the greatest activity occurred after 72 days. Gain and feed efficiency of immunized pigs were not improved in Trials II and III; however, there was some improvement in Trial I at the 10 unit level. Plasma urea N levels were unchanged between treatment groups in all trials. In general, ammonia N levels of plasma were unchanged; however, the 0.5 unit treatment group (low) in Trial III had

significantly lower plasma ammonia levels at 56 and 73 days on experiment. Intestinal sections plus contents (from the caudal end of the ileum) taken in Trials I and III revealed that the urease immunized pigs had less urease activity than the control pigs, with the difference between treatment and control groups significant in Trial III ($P < 0.05$). Sigma urease type II powder used in Trial II was found to have high hemagglutinative activity. Urease immunization appeared to have no effect on the utilization of fed urea by growing pigs. Higher levels of urea N and lower levels of ammonia N were observed in pigs fed rations with added urea than in pigs fed the control ration. Gain, feed intake, and feed efficiency were decreased at the 2.5 and 3.4 percent urea levels.

Three trials were conducted in the toxicity phase to study the effect of a large intraperitoneal injection of urease on growing pigs and its relationship to ammonia toxicity. Urease prepared from jackbean meal by the author was used in Trials I and II and Sigma urease type II powder was used in Trial III. Plasma urea N levels were significantly decreased, and plasma ammonia N levels were significantly increased following a large intraperitoneal injection of urease (50, 75 and 100 modified Sumner units per pound of bodyweight). All pigs receiving 75 and 100 unit levels died. Ammonia N levels in pigs which died ranged from 2.0 to 3.0 milligrams per 100 milliliters of plasma. Serum urease activity and potassium levels were increased, and serum protein, sodium and calcium levels were unchanged in pigs given a large dose of urease (Trials II and III). Liver urease activity and ammonia N levels of the urease injected pigs were larger than for the control pigs though the differences were not statistically significant (Trial II). Urine ammonia N levels of treatment pigs were larger than values

for control pigs in Trials I and II while the opposite was true in Trial III. Urine urea N levels were decreased in Trial III and unchanged in Trials I and II. The electrophoretic components of serum protein in Trial III showed gamma globulin of urease injected pigs to be significantly greater than values for the control pigs. The other components of serum protein were not significantly different. Serum transaminase values determined in Trial III disclosed that glutamic-oxalacetic values were unchanged and that glutamic-pyruvic values were lower following a large urease injection. There was an elevation of rectal temperatures 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. Postmortem 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 which could explain the differences in results obtained in Trials I and II, and Trial III.

VI. CONCLUSIONS

The results obtained in three trials conducted in the immunization phase indicate that under these experimental conditions, urease immunization has little effect upon the growth rate and feed efficiency of growing swine. However, urease immunization was effective in stimulating antibody production as measured by antiurease activity, and was effective in reducing intestinal urease activity. Significant antiurease activity was present in the serum of immunized pigs after 14 days on experiment. In general, all levels of urease injected were equally effective in producing antiurease activity with some evidence of partial inhibition of antibody production at the higher urease levels. Although the various levels of urease injected appeared equally effective in producing antiurease activity, the reduction in intestinal urease activity was greatest at the high urease levels. Plasma ammonia N levels of immunized pigs were not different from the control pigs in the first two trials, but there was a trend toward lower plasma ammonia N levels in one group of the immunized pigs in the third trial. Plasma urea N levels were unchanged except when urea rations were fed. Urease immunization appeared to have little effect upon the utilization of fed urea by the growing pig. Feed intake, gain and feed efficiency were decreased when pigs were fed rations with 2.5 and 3.4 percent urea. Plasma urea N levels were increased and ammonia N levels were decreased in pigs fed urea rations.

Three trials conducted in the toxicity phase demonstrated that large intraperitoneal injections of urease produce ammonia toxicity and death

in the pig. Plasma urea N levels were decreased and plasma ammonia N, serum urease activity and serum potassium levels were increased in pigs given a large dose of urease intraperitoneally. There were no changes in serum sodium and calcium levels. Electrophoretic components of serum protein were unchanged with the exception of gamma globulin which was slightly increased following the urease injection. Liver samples from urease injected pigs had greater urease activity and ammonia N concentration than control pigs. Urine ammonia N levels were greater in treatment pigs than in control pigs in the first two trials where urease was prepared by the author. Urine urea N levels were unchanged in these two trials. Urine ammonia N and urea N levels were decreased in Trial III where Sigma urease type II powder was injected. The Sigma urease was found to have high hemagglutinative activity. Survival time of urease injected pigs was less when the Sigma urease was used, even though ammonia N levels were somewhat lower. Pigs in the first two trials exhibited more characteristic symptoms of ammonia toxicity: tetany, excess fluid in body cavities, congested lungs and hyperemic mucous membranes. Effects of urease and hemagglutinin were confounded in Trial III. The toxic level of urease for the nonimmunized growing pig is between 10 and 50 modified SU per pound of bodyweight as 10 units were not toxic in the immunization phase and 50 units were toxic in the toxicity phase.

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APPENDIX TABLE 1

TRIAL I. AGE, SEX, INITIAL WEIGHT, FINAL WEIGHT, AND AVERAGE DAILY GAIN OF GROWING PIGS IN IMMUNIZATION STUDY (POUNDS)[a]

Pig No.	Sex	Age Days	Initial Wt., lb.	Final Wt., lb.	Time, days 0-30	0-58	0-79	0-98	30-58	58-79	79-98
					Control						
10-3	M	60	44	191	1.27	1.41	1.51	1.50	1.57	1.76	1.47
10-4	M	60	41	188	1.33	1.45	1.54	1.50	1.57	1.80	1.32
151-10	F	66	35	172	1.17	1.36	1.34	1.40	1.57	1.28	1.63
39-3	F	61	37	173	1.17	1.31	1.39	1.38	1.46	1.62	1.36
39-5	F	61	40	161	0.70	1.09	1.24	1.24	1.50	1.66	1.21
155-9	F	60	36	192	1.43	1.55	1.62	1.59	1.68	1.80	1.47
Av.			(39)	(180)	(1.18)	(1.36)	(1.46)	(1.43)	(1.56)	(1.65)	(1.40)
SE					0.10	0.06	0.06	0.05	0.03	0.08	0.06
					0.1 Unit						
2-11	M	77	43	190	1.13	1.40	1.46	1.50	1.68	1.62	1.68
151-8	M	66	38	204	1.57	1.66	1.72	1.69	1.74	1.90	1.58
200-3	F		36	176	1.27	1.34	1.44	1.42	1.42	1.71	1.36
10-5	F	60	39	191	1.43	1.50	1.52	1.55	1.57	1.57	1.68
10-6	F	60	35	170	1.30	1.34	1.44	1.38	1.40	1.71	1.10
151-9	F	66	42	187	1.33	1.41	1.48	1.48	1.50	1.66	1.47
Av.			(39)	(186)	(1.34)	(1.44)	(1.51)	(1.50)	(1.55)	(1.69)	(1.48)
SE					0.06	0.05	0.04	0.04	0.06	0.05	0.09

[a] Jackbean urease prepared by the author.

APPENDIX TABLE 1 (CONTINUED)

TRIAL I. AGE, SEX, INITIAL WEIGHT, FINAL WEIGHT, AND AVERAGE DAILY GAIN OF GROWING PIGS IN IMMUNIZATION STUDY (POUNDS)[a]

Pig No.	Sex	Age Days	Initial Wt., lb.	Final Wt., lb.	Time, days 0-30	0-58	0-79	0-98	30-58	58-79	79-98
					1 Unit						
3-9	M	69	40	183	1.33	1.41	1.48	1.46	1.50	1.66	1.36
3-10	M	69	41	194	1.30	1.53	1.58	1.56	1.78	1.71	1.47
10-2	F	60	42	177	1.30	1.22	1.28	1.38	1.14	1.42	1.78
39-4	F	61	36	156	1.40	1.40	1.35	1.22	1.40	1.24	0.68
155-7	F	60	38	-	0.93	1.21	1.41	-	1.50	1.95	-
155-8	F	60	35	180	1.33	1.43	1.47	1.48	1.54	1.57	1.52
Av.			(39)	(178)	(1.27)	(1.37)	(1.43)	(1.42)	(1.48)	(1.59)	(1.36)
SE					0.07	0.05	0.04	0.06	0.08	0.10	0.15
					10 Unit						
39-8	M	61	42	198	1.50	1.62	1.65	1.59	1.74	1.71	1.36
150-6	F	67	40	196	1.37	1.50	1.57	1.59	1.64	1.76	1.68
150-10	M	67	41	213	1.40	1.66	1.75	1.76	1.92	2.00	1.78
Av.			(41)	202	(1.42)	(1.59)[b]	(1.66)[b]	(1.64)[b]	(1.77)[b]	(1.82)	(1.60)
SE					0.04	0.05	0.05	0.06	0.08	0.09	0.13

[a] Jackbean urease prepared by the author.
[b] Significantly greater than the least two treatment means ($P < 0.05$).
[c] Significantly greater than the other treatment means ($P < 0.05$).

APPENDIX TABLE 2

TRIAL I. PLASMA UREA N LEVELS IN IMMUNIZATION STUDY (mg./100 ml)[a]

Pig No.	Time, days 0	15	36	64	72	97
			Control			
10-3	14.5	13.0	19.0	29.2	23.6	19.2
10-4	11.0	7.3	16.6	29.4	24.4	16.6
151-10	16.6	10.2	15.1	30.7	29.6	21.7
39-3	14.0	11.8	17.3	22.0	22.3	18.4
39-5	14.4	12.4	16.2	27.5	24.3	16.1
155-9	9.8	8.7	19.9	25.0	21.6	15.7
Av.	(13.4)	(10.6)	(17.4)	(27.3)	(24.3)	(18.0)
SE	1.0	0.9	0.7	1.3	1.2	0.9
			0.1 Unit			
2-11[b]	13.0	15.8	14.4	22.5	15.5	18.6
151-8	13.4	10.0	21.6	25.0	24.4	18.6
200-3	12.9	7.9	19.7	20.4	22.0	14.9
10-5	14.2	11.8	20.1	28.6	27.5	17.6
10-6[b]	15.6	10.6	15.7	20.0	18.2	17.7
151-9[b]	18.9	15.6	19.7	27.8	25.5	15.4
Av.	(14.7)	(12.0)	(18.5)	(24.0)	(22.2)	(17.1)
SE	0.9	1.3	1.2	1.2	1.9	0.7
			1 Unit			
3-9[b]	12.0	10.4	19.5	29.5	26.0	21.8
3-10	11.9	9.5	20.7	27.0	25.7	22.6
10-2[b]	15.9	11.4	18.6	22.0	21.5	14.0
39-4	12.5	13.4	20.1	25.4	19.3	16.7
155-7[b]	10.8	8.9	18.3	27.6	22.7	11.9
155-8	10.8	7.6	13.5	21.8	20.9	15.6
Av.	(12.3)	(10.2)	(18.5)	(26.7)	(22.8)	(17.1)
SE	0.8	0.8	1.1	1.3	1.0	1.7
			10 Unit			
39-8[b]	12.8	14.1	18.3	29.1	30.0	22.2
150-6[b]	8.1	9.9	16.8	18.9	22.1	14.7
150-10	7.8	8.5	12.1	22.2	28.5	17.7
Av.	(9.6)	(10.8)	(15.7)	(23.4)	(26.8)	(18.2)
SE	1.6	1.7	1.9	3.0	2.6	2.2

[a]Jackbean urease prepared by the author.

[b]One-half of the pigs on each treatment were given an additional injection of urease after 64 days on experiment.

APPENDIX TABLE 3

TRIAL I. PLASMA AMMONIA N LEVELS IN IMMUNIZATION STUDY (mcg./100 ml)[a]

Pig No.	Time, days 0	15	36	64	72	97
			Control			
10-3	556	970	715	512	402	535
10-4	760	672	700	571	365	596
151-10	728	988	833	538	529	545
39-3	506	567	905	674	561	591
39-5	734	860	664	620	476	631
155-9	546	1078	865	706	466	566
Av.	(638)	(856)	(780)	(604)	(467)	(577)
SE	46	81	41	32	30	14
			0.1 Unit			
2-11[b]	996	612	829	550	386	520
151-8	918	672	777	545	598	540
200-3	738	706	782	592	481	515
10-5	494	816	1171	566	492	409
10-6[b]	484	834	1017	538	360	494
151-9[b]	536	910	818	452	444	470
Av.	(694)	(758)	(899)	(541)	(460)	(491)
SE	91	46	65	19	35	19
			1 Unit			
3-9[b]	630	984	767	544	444	556
3-10	556	682	1110	538	413	520
10-2[b]	586	784	1013	566	439	454
39-4	506	886	844	646	428	631
155-7[b]	772	798	869	522	476	404
155-8	091	884	920	528	402	525
Av.	(690)	(836)	(921)	(557)	(434)	(515)
SE	87	43	51	19	11	32
			10 Unit			
39-8[b]	708	568	787	630	386	530
150-6[b]	750	904	1074	576	540	510
150-10	792	576	1150	561	492	560
Av.	(750)	(683)	(1004)	(589)	(473)	(533)
SE	24	110	110	21	45	15

[a]Jackbean urease prepared by the author.

[b]One-half of the pigs on each treatment were given an additional injection of urease after 64 days on experiment.

APPENDIX TABLE 4

TRIAL I. SERUM ANTIUREASE ACTIVITY IN IMMUNIZATION STUDY (units/ml. x 10^3)[a]

Pig No.	Time, days 0	15	36	64	72	97
			Control			
10-3	6	34	10	11	38	-20
10-4	-66	-8	-14	8	-50	-26
151-10	22	-8	-31	-6	-16	-20
39-3	28	-31	48	-44	0	0
39-5	46	-40	8	22	16	34
155-9	-40	45	-2	7	-30	10
Av.	(0)	(-1)	(3)	(0)	(-7)	(-4)
SE	18	14	11	9	13	9
			0.1 Unit			
2-11[b]	-8	-4	74	230	186	68
151-8	-14	67	62	208	218	66
200-3	-42	159	91	174	262	82
10-5	14	115	70	136	216	-4
10-6[b]	50	-40	82	113	139	-76
151-9[b]	4	-14	40	89	150	15
Av.	(1)	(44)	(70)[c]	(158)[cc]	(195)[cc]	(25)
SE	13	32	4	26	19	25
			1 Unit			
3-9[b]	-2	35	146	196	261	62
3-10	20	70	28	138	253	42
10-2[b]	-72	133	190	242	352	154
39-4	14	29	56	154	216	10
155-7[b]	27	16	89	122	185	34
155-8	14	81	74	183	209	70
Av.	(0)	(61)	(97)[cc]	(173)[cc]	(246)[cc]	(62)
SE	15	18	25	18	24	20
			10 Unit			
39-8[b]	-9	24	65	137	294	120
150-6[b]	1	50	80	141	295	86
150-10	3	57	13	115	145	35
Av.	(-5)	(44)	(53)	(131)[cc]	(244)[cc]	(57)
SE	4	10	20	8	50	57

[a]Jackbean urease prepared by the author.

[b]One-half of the pigs on each treatment were given an additional injection of urease after 64 days on experiment. All other pigs injected with saline.

[c]Significantly greater than the least treatment mean ($P < 0.05$); [cc]($P < 0.01$).

APPENDIX TABLE 5

TRIAL I. PLASMA UREA N AND AMMONIA N LEVELS OF GROWING PIGS FOLLOWING INITIAL AND SECONDARY INJECTIONS OF UREASE[a]

Pig No.	Urea N, mg./100 ml.						Ammonia N, mcg./100 ml.					
	1st Day[b]			15th Day			1st Day			15th Day		
	0 hr.	1 hr.	6 hr.	0 hr.	1 hr.	4 hr.	0 hr.	1 hr.	6 hr.	0 hr.	1 hr.	4 hr.
						Control						
10-3	14.5	15.0	14.3	13.0	15.3	15.5	556	546	620	970	914	522
10-4	11.0	14.3	12.2	7.3	8.5	8.3	760	524	598	672	690	446
151-10	16.6	13.0	12.9	10.2	9.7	9.2	728	504	440	988	582	562
Av.	(14.0)	(14.1)	(13.1)	(10.2)	(11.2)	(11.0)	(681)	(525)	(553)	(877)	(729)	(510)
SE	1.6	0.6	0.6	1.6	2.1	2.3	63	12	57	102	98	33
						0.1 Unit						
2-11	13.0	14.6	13.0	15.8	16.2	15.9	996	824	610	612	1064	434
151-8	13.4	13.2	11.7	10.0	18.5	8.1	918	610	444	672	760	384
200-3	12.9	12.8	12.4	7.9	7.3	6.2	738	560	450	706	882	422
Av.	(13.1)	(13.5)	(12.4)	(11.2)	(10.7)	(10.1)	(884)[c]	(665)	(501)	(663)	(902)	(413)
SE	0.1	0.5	0.4	2.4	3.3	3.0	76	81	54	27	88	15

[a] Jackbean urease prepared by the author.

[b] Blood samples were taken at zero hour, then urease was injected and blood samples were taken at 1 and 6 (or 4) hours.

[c] Significantly greater than the least two treatment means ($P < 0.05$).

APPENDIX TABLE 5 (CONTINUED)

TRIAL I. PLASMA UREA N AND AMMONIA N LEVELS OF GROWING PIGS FOLLOWING INITIAL AND SECONDARY INJECTIONS OF UREASE [a]

Pig No.	Urea N, mg./100 ml.						Ammonia N, mcg./100 ml.					
	1st Day[b]			15th Day			1st Day			15th Day		
	0 hr.	1 hr.	6 hr.	0 hr.	1 hr.	4 hr.	0 hr.	1 hr.	6 hr.	0 hr.	1 hr.	4 hr.
						1 Unit						
3-9	12.0	11.9	11.7	10.4	11.0	11.5	630	502	490	984	686	948
3-10	11.9	13.2	13.1	9.5	8.9	7.7	556	668	524	682	886	488
10-2	15.9	15.2	13.1	11.4	10.9	12.6	586	600	504	784	632	576
Av.	(13.3)	(13.4)	(12.6)	(10.4)	(10.3)	(10.6)	(591)	(590)	(539)	(816)	(735)	(671)
SE	1.3	1.0	0.5	0.6	0.7	1.5	21	47	26	89	77	140
						10 Unit						
39-8	12.8	12.3	-	14.1	14.5	14.6	708	848	944	568	776	612
150-6	8.1	10.3	8.6	9.9	9.4	9.5	750	1016	734	904	642	528
150-10	7.8	9.0	10.2	8.5	11.5	10.5	792	1016	884	576	739	582
Av.	(9.6)	(10.5)[d]	(9.4)[d]	(10.8)	(11.8)	(11.5)	(750)	(960)[d]	(854)[d]	(683)	(716)	(574)
SE	1.6	1.0	0.5	1.7	1.5	1.6	24	56	62	111	38	25

[a]Jackbean urease prepared by the author.

[b]Blood samples were taken at zero hour, then urease was injected and blood samples were taken at 1 and 6 (or 4) hours.

[c]Significantly greater than the least two treatment means ($P<0.05$).

[d]Significantly different from the other treatment means ($P<0.01$).

APPENDIX TABLE 6

TRIAL I. URINE FLOW, UREA N AND AMMONIA N OF GROWING PIGS GIVEN A SECONDARY INJECTION OF UREASE IN IMMUNIZATION STUDY [a][b]

Pig No.	Urine flow, ml./hr. Pre injection	Urine flow, ml./hr. Post injection	Urea N, mg./hr. Pre injection	Urea N, mg./hr. Post injection	Urea N, mg./hr. Change	Ammonia N, mg./hr. Pre injection	Ammonia N, mg./hr. Post injection	Ammonia N, mg./hr. Change
				Control				
10-3	42.3	11.9	305	168	-137	15.7	8.7	-7.0
10-4	41.7	16.5	379	223	-156	21.5	16.3	-6.2
151-10	15.4	11.1	196	174	-22	13.0	13.7	0.7
Av.	(33.1)	(13.2)	(293)	(188)	(-105)	(16.7)	(12.9)	(-4.2)
SE	8.8	1.1	54	17	42	2.5	2.2	2.4
				0.1 Unit				
2-11	37.1	23.4	356	330	-26	14.1	31.6	17.5
151-8	29.6	20.0	435	274	-161	12.5	18.7	6.2
200-3	22.1	27.9	310	324	14	21.7	20.1	-1.6
Av.	(29.6)	(27.1)	(367)	(309)	(-58)	(16.1)	(23.5)[c]	(7.4)[c]
SE	4.3	2.3	37	18	45	2.8	4.1	5.5
				1 Unit				
3-9	43.1	27.9	326	435	109	22.0	20.1	-1.9
3-10	38.8	27.7	264	263	-1	17.3	18.6	1.3
10-2	36.9	31.7	288	358	70	11.7	15.4	3.7
Av.	(39.6)	(29.1)	(293)	(352)[c]	(59)[d]	(17.0)	(18.0)	(1.0)
SE	1.8	1.3	18	50	31	3.2	1.4	1.6
				10 Unit				
39-8	46.3	17.3	393	516	123	17.7	17.2	-0.5
150-6	27.7	18.1	319	265	-54	13.5	16.4	2.9
150-10	24.2	20.0	293	294	1	18.4	17.9	-0.5
Av.	(32.7)	(18.4)	(368)	(392)[c]	(23)	(16.5)	(17.2)	(0.7)
SE	6.9	0.8	30	79	51	1.5	0.4	1.1

[a]Jackbean urease prepared by the author.

[b]All pigs placed in metabolism cages 24 hours before and after the sixth urease (15th day of trial) injection.

[c]Significantly greater than least treatment mean ($P < 0.05$).

APPENDIX TABLE 7

TRIAL I. UREASE ACTIVITY AND AMMONIA N LEVELS OF INTESTINAL SECTIONS PLUS CONTENTS FROM PIGS IN IMMUNIZATION STUDY [ab]

Pig No.	Urease Activity units/gm. DM x 10^3 S-1[c]	S-2[c]	Ammonia N mcg./gm. DM S-1	S-2
		Control		
10-3	4	174	260	729
10-4	5	587	134	1014
39-3	45	12	562	632
39-5	78	232	693	666
151-10	-10	5	489	728
155-9	24	34	566	414
Av.	(25)	(174)	(450)	(697)
SE	13	91	86	79
		0.1 Unit		
2-11	29	284	492	862
10-5	1	150	378	528
10-6	-14	16	564	563
151-8	29	278	1115	958
200-3	13	498	470	891
Av.	(11)	(216)	(582)	(770)
SE	7	71	109	74
		1 Unit		
3-9	22	83	630	1108
3-10	5	-7	270	354
10-2	34	95	778	488
39-4	15	132	498	388
155-8	3	-4	473	520
Av.	(16)	(60)	(530)	(572)
SE	6	28	85	138
		10 Unit		
39-8	23	113	376	643
150-6	14	-4	398	537
150-10	8	38	554	902
Av.	(15)	(49)	(442)	(694)
SE	4	34	56	108

[a] Jackbean urease prepared by the author.
[b] Pigs slaughtered at the end of experiment, 98 days.
[c] S-1 from the duodenum and S-2 from the lower end of the ileum.

APPENDIX TABLE 8

TRIAL II. AGE, SEX, INITIAL WEIGHT, FINAL WEIGHT, AND AVERAGE DAILY GAIN OF GROWING PIGS IN IMMUNIZATION STUDY (POUNDS)[a]

Pig No.	Sex	Age Days	Initial Wt., lb.	Final Wt., lb.	Time, days 0-22	22-47	47-89[b]	0-47	0-89
					Control				
93-7	M	87	59	224	1.55	1.84	2.02	1.70	1.85
94-7	M	84	63	187	0.73	1.44	1.71	1.11	1.39
94-9	F	84	70	207	1.18	1.60	1.69	1.40	1.54
95-3	F	84	65	179	1.18	1.40	1.26	1.30	1.28
96-3	F	83	69	197	1.23	1.12	1.74	1.17	1.44
96-6	F	83	65	222	1.64	1.88	1.76	1.77	1.76
96-8	M	83	64	207	1.41	1.64	1.69	1.53	1.61
97-6	M	81	70	239	1.55	1.68	2.21	1.62	1.90
100-1	F	81	72	207	0.55	1.60	1.98	1.11	1.52
101-6	M	79	56	174	0.95	1.36	1.50	1.17	1.33
Av.			(65)	(204)	(1.20)	(1.56)	(1.76)	(1.39)	(1.56)
SE					0.11	0.07	0.08	0.08	0.07
					0.1 Unit				
93-1	F	87	76	231	1.27	1.84	1.93	1.57	1.74
94-4	M	84	56	195	1.23	1.28	1.90	1.26	1.56
95-4	F	84	66	180	1.09	1.56	1.21	1.34	1.28
96-1	F	83	72	196	1.18	1.16	1.64	1.17	1.39
96-9	M	83	65	194	1.05	1.44	1.67	1.26	1.45
97-2	F	81	63	209	0.95	1.44	2.12	1.21	1.64
98-3	M	81	66	226	1.27	2.12	1.88	1.72	1.80
99-3	F	82	60	192	1.09	1.44	1.71	1.28	1.48
100-4	M	81	64	203	0.91	1.80	1.76	1.38	1.56
101-7	M	79	67	172	0.91	1.56	1.10	1.26	1.18
Av.			(66)	(200)	(1.10)	(1.56)	(1.69)	(1.34)	(1.51)
SE					0.04	0.09	0.10	0.06	0.06

[a]Sigma urease type II powder.

[b]One-half of pigs in each group were fed rations with added urea during this period. See Table 9 for treatments.

APPENDIX TABLE 8 (CONTINUED)

TRIAL II. AGE, SEX, INITIAL WEIGHT, FINAL WEIGHT, AND AVERAGE DAILY GAIN OF GROWING PIGS IN IMMUNIZATION STUDY (POUNDS)[a]

Pig No.	Sex	Age Days	Initial Wt., lb.	Final Wt., lb.	Time, days				
					0-22	22-47	47-89[b]	0-47	0-89
					1 Unit				
93-3	F	87	71	203	0.32	1.84	1.88	1.13	1.48
95-1	F	84	71	177	0.45	1.48	1.40	1.00	1.19
95-7	M	84	61	202	0.91	1.80	1.81	1.38	1.47
96-2	F	83	65	186	0.59	1.56	1.64	1.11	1.36
96-7	M	83	65	204	0.91	1.24	2.10	1.09	1.56
96-11	M	83	58	162	0.50	1.08	1.57	0.81	1.17
97-3	F	81	71	215	1.05	2.04	1.67	1.57	1.62
98-5	M	81	70	227	1.09	1.80	2.10	1.47	1.76
100-5	M	81	58	189	-0.23	2.04	2.02	0.98	1.47
101-1	F	79	69	183	0.73	1.68	1.33	1.23	1.28
Av.			(66)	(195)	(0.63)[c]	(1.66)	(1.75)	(1.18)	(1.44)
SE					0.13	0.10	0.09	0.07	0.06

[a]Sigma urease type II powder.

[b]One-half of pigs in each group were fed rations with added urea during this period. See Table 9 for treatments.

[c]Significantly less than other two treatment means ($P < 0.01$).

APPENDIX TABLE 9

TRIAL II. PLASMA UREA N LEVELS IN IMMUNIZATION STUDY (mg./100 ml.)[a]

Pig No.	Time, days 14	29	47	77[b]
		Control		
93-7[b]	17.4	14.7	23.4	24.7
94-7	10.7	11.0	14.3	16.4
94-9[b]	16.2	14.8	16.9	20.9
95-3[b]	13.2	15.2	16.8	17.1
96-3	17.6	10.9	12.9	13.7
96-6[b]	14.3	12.9	16.5	21.9
96-8	15.4	11.9	14.5	13.9
97-6	19.1	14.1	20.6	20.2
100-1	11.4	13.6	15.5	14.9
101-6[b]	17.4	15.8	19.5	24.5
Av.	(15.3)	(13.8)	(17.1)	(18.8)
SE	0.9	0.7	1.0	1.0
		0.1 Unit		
93-1	14.1	12.7	18.8	15.8
94-4	18.5	19.8	17.3	16.1
95-4[b]	12.8	15.2	19.7	17.7
96-1[b]	14.5	12.3	15.9	16.0
96-9[b]	13.9	12.7	13.6	20.2
97-2	10.6	13.1	14.6	20.2
98-3[b]	15.6	15.5	19.6	21.9
99-3	11.8	14.8	19.2	20.8
100-4[b]	12.1	17.4	19.7	22.3
100-7	15.8	15.9	16.4	11.1
Av.	(14.0)	(14.9)	(17.5)	(18.2)
SE	0.7	0.8	0.7	1.5
		1 Unit		
93-3	12.6	18.2	17.9	23.8
95-1[b]	11.3	8.4	14.0	22.5
95-7	17.3	19.7	23.5	26.4
96-2[b]	12.9	13.6	16.4	12.4
96-7	17.3	15.1	16.8	19.2
96-11	11.2	11.1	12.3	18.5
97-3[b]	13.4	15.4	20.0	13.8
98-5	16.0	20.7	17.9	23.2
100-5[b]	24.7	15.8	24.4	31.5
101-1[b]	14.2	13.1	16.9	18.8
Av.	(15.1)	(15.1)	(18.0)	(21.0)
SE	1.3	1.2	1.2	1.3

[a]Sigma urease type II powder.

[b]One-half of pigs in each treatment were fed rations containing urea. See Table 9 for treatments.

APPENDIX TABLE 10

TRIAL II. PLASMA AMMONIA N LEVELS IN IMMUNIZATION STUDY (mcg./100 ml.)[a]

Pig No.	Time, days 14	29	47	77[b]
		Control		
93-7[b]	700	457	357	415
94-7	658	713	393	462
94-9[b]	557	531	357	368
95-3[b]	647	457	399	391
96-3	479	628	327	509
96-6[b]	454	468	298	344
96-8	567	451	637	401
97-6	510	480	387	377
100-1	562	674	321	462
101-6[b]	695	554	304	462
Av.	(583)	(541)	(378)	(419)
SE	28	31	31	8
		0.1 Unit		
93-1	447	526	363	471
94-4	605	686	578	519
95-4[b]	516	446	411	448
96-1[b]	437	685	327	391
96-9[b]	613	708	447	486
97-2	490	776	339	462
98-3[b]	469	668	357	476
99-3	516	776	285	434
100-4[b]	515	668	363	467
101-7	572	543	483	580
Av.	(518)	(648)	(395)	(473)
SE	20	34	27	7
		1 Unit		
93-3	479	417	351	420
95-1[b]	768	686	321	410
95-7	551	371	357	436
96-2[b]	536	560	315	415
96-7	582	605	452	486
96-11	696	663	411	509
97-3[b]	649	634	339	415
98-5	701	743	333	486
100-5[b]	582	708	447	415
101-1[b]	526	782	321	368
Av.	(609)	(617)	(365)	(441)
SE	30	42	16	12

[a]Sigma urease type II powder.

[b]One-half of pigs in each treatment were fed rations containing urea. See Table 9 for treatments.

APPENDIX TABLE 11

TRIAL II. SERUM ANTIUREASE ACTIVITY IN IMMUNIZATION STUDY (units/ml. x 10^3)[a]

Pig No.	Time, days 14	29	47	77
		Control		
93-7[b]	34	-27	0	-3
94-7	-35	-27	-24	-2
94-9[b]	-1	-27	35	24
95-3[b]	28	18	-28	14
96-3	28	-41	33	-13
96-6[b]	7	1	-13	-25
96-8	55	10	22	-5
97-6	28	0	-7	32
100-1[b]	28	-42	16	-19
101-6	-29	7	8	14
Av.	(14)	(-13)	(4)	(2)
SE	9	7	7	6
		0.1 Unit		
93-1	158	308	317	244
94-4[b]	193	196	239	165
95-4[b]	210	207	142	193
96-1[b]	221	174	194	176
96-9[b]	195	304	205	195
97-2	197	290	237	86
98-3[b]	206	310	257	153
99-3	148	247	236	79
100-4[b]	206	187	202	77
101-7	107	212	191	43
Av.	(184)[c]	(244)[d]	(222)[dd]	(141)[d]
SE	11	16	15	21
		1 Unit		
93-3	267	258	109	115
95-1[b]	51	270	179	79
95-7	197	177	136	138
96-2[b]	179	207	115	53
96-7	99	187	115	51
96-11	45	170	113	59
97-3[b]	163	205	162	144
98-5	20	154	261	57
100-5[b]	251	217	144	93
101-1[b]	177	200	210	18
Av.	(145)[c]	(204)[c]	(154)[c]	(81)[c]
SE	27	12	16	13

[a]Sigma urease type II powder.

[b]One-half of pigs in each treatment were fed rations containing urea. See Table 9 for treatments.

[c]Significantly greater than the least treatment mean ($P < 0.01$).

[d]Significantly " " " other two treatment means ($P < 0.05$); [dd]($P < 0.01$).

APPENDIX TABLE 12

TRIAL II. AVERAGE DAILY GAIN OF IMMUNIZED AND NONIMMUNIZED GROWING PIGS FED UREA TO SUPPLY PART OF THE CRUDE PROTEIN REQUIREMENTS (POUNDS)[a]

Urea					Control				
Pig No.	Time, days				Pig No.	Time, days			
	47-63	63-77	77-89	47-89		47-63	63-77	77-89	47-89
					Control				
93-7	2.13	2.14	1.75	2.02	94-7	1.81	1.71	1.58	1.71
94-9	1.81	1.86	1.33	1.69	96-3	1.69	1.64	1.92	1.74
95-3	1.38	1.29	1.08	1.26	96-8	1.56	1.93	1.58	1.69
96-6	1.75	2.00	1.50	1.76	97-6	2.13	2.57	1.92	2.21
101-6	1.75	1.64	1.00	1.50	100-1	1.94	2.07	1.92	1.98
Av.	(1.76)	(1.79)	(1.33)	(1.65)	Av.	(1.83)	(1.98)	(1.78)[c]	(1.87)
SE	0.12	0.15	0.14	0.13	SE	0.10	0.16	0.08	0.10
					0.1 Unit				
95-4	1.19	1.50	0.92	1.21	93-1	2.13	1.79	1.83	1.93
96-1	1.69	1.79	1.42	1.64	94-4	2.06	1.64	2.00	1.90
96-9	1.25	2.43	1.33	1.67	97-2	2.25	2.14	1.92	2.12
98-3	1.88	1.93	1.83	1.88	99-3	1.56	1.86	1.75	1.71
100-4	1.81	2.14	1.25	1.76	101-7	1.19	1.14	0.92	1.10
Av.	(1.56)	(1.96)	(1.35)	(1.63)	Av.	(1.84)	(1.71)	(1.68)	(1.75)
SE	0.14	0.16	0.15	0.11	SE	0.20	0.16	0.19	0.18
					1 Unit				
95-1	1.56	1.57	1.00	1.40	93-3	2.13	2.14	1.25	1.88
96-2	1.81	1.29	1.83	1.64	95-7	1.88	1.71	1.83	1.81
97-3	2.13	1.43	1.33	1.67	96-7	2.00	1.93	2.42	2.10
100-5	2.13	2.14	1.75	2.02	96-11	1.44	1.64	1.67	1.57
101-1	1.38	1.57	1.00	1.33	98-5	2.13	2.21	1.92	2.10
Av.	(1.80)	(1.60)	(1.38)	(1.61)	Av.	(1.92)	(1.93)	(1.82)[c]	(1.89)
SE	0.15	0.14	0.18	0.12	SE	0.13	0.11	0.19	0.10

[a]Sigma urease type II powder.
[b]Urea level: 47-63,1.25: 63-77,2.5: 77-89.3.4: 47-99, over urea level; Period and urea level,respectively.
[c]Significantly greater than least three treatment means ($P < 0.05$).

APPENDIX TABLE 13

TRIAL II. PLASMA UREA N LEVELS OF IMMUNIZED AND NONIMMUNIZED GROWING PIGS FED UREA TO SUPPLY PART OF THE CRUDE PROTEIN REQUIREMENTS (mg./100 ml.)[a]

Urea[b]			Control		
Pig	Time, days		Pig	Time, days	
No.	63-77	77-89	No.	63-77	77-89
		Control			
93-7	24.7	27.2	94-7	16.4	18.6
94-9	20.9	30.0	96-3	13.7	16.2
95-3	17.1	26.9	96-8	13.9	12.9
96-6	21.9	22.1	97-6	20.2	21.7
101-6	24.5	36.0	100-1	14.9	15.0
Av.	(21.8)[c]	(28.4)[cc]	Av.	(15.8)	(16.9)
SE	1.4	2.3	SE	1.2	1.5
		0.1 Unit			
95-4	17.7	23.9	93-1	15.8	14.1
96-1	16.0	20.9	94-4	16.1	21.1
96-9	20.2	27.2	97-2	20.2	18.1
98-3	21.9	30.6	99-3	20.8	21.3
100-4	22.3	28.0	101-7	11.1	9.5
Av.	(19.6)	(26.1)[cc]	Av.	(16.8)	(16.8)
SE	1.2	1.7	SE	1.8	2.2
		1 Unit			
95-1	22.5	28.2	93-3	23.8	29.2
96-2	12.4	30.1	95-7	26.4	28.0
97-3	13.8	20.9	96-7	19.2	20.5
100-5	31.5	35.8	96-11	18.5	17.5
101-1	18.8	13.2	98-5	23.2	21.8
Av.	(19.8)[d]	(25.6)[cc]	Av.	(22.2)[cc]	(23.4)[c]
SE	3.4	3.9	SE	1.4	2.2

[a]Sigma urease type II powder.

[b]Urea levels: 63-77,2.5 and 77-89,3.4; period and urea level, respectively. Blood samples were taken at the end of the period.

[c]Significantly larger than the least two treatment means ($P < 0.05$); [cc]($P < 0.01$).

[d]Significantly " " " " treatment mean ($P < 0.05$).

APPENDIX TABLE 14

TRIAL II. AMMONIA N LEVELS OF IMMUNIZED AND NONIMMUNIZED GROWING PIGS FED UREA TO SUPPLY PART OF THE CRUDE PROTEIN REQUIREMENT (mcg./100 ml.)[a]

Urea[b]			Content		
Pig No.	Time, days 63-77	77-89	Pig No.	Time, days 63-77	77-89
		Control			
93-7	415	338	94-7	462	462
94-9	368	393	96-3	509	492
95-3	391	427	96-8	401	406
96-6	344	201	97-6	377	415
101-6	462	380	100-1	462	474
Av.	(396)	(348)	**Av.**	(442)	(450)[cc]
SE	20	32	**SE**	23	17
		0.1 Unit			
95-4	448	487	93-1	471	556
96-1	391	551	94-4	519	564
96-9	486	573	97-2	462	504
98-3	476	534	99-3	434	513
100-4	467	423	101-7	580	564
Av.	(454)[e]	(514)[d]	Av.	(493)[d]	(540)[dd]
SE	17	27	SE	26	13
		1 Unit			
95-1	410	385	93-3	420	534
96-2	415	385	95-7	486	479
97-3	415	329	96-7	486	594
100-5	415	363	96-11	509	594
101-1	368	440	98-5	486	547
Av.	(405)	(380)	Av.	(477)[c]	(550)[dd]
SE	9	18	SE	15	21

[a]Sigma urease type II powder.

[b]Urea levels: 63-77,2.5 and 77-89,3.4; period and urea level, respectively. Blood samples were taken at the end of the period.

[c]Significantly larger than the least two treatment means ($P < 0.05$);[cc]($P < 0.01$).

[d]Significantly larger than the least three treatment means ($P < 0.05$);[dd]($P < 0.01$).

[e]Significantly larger than the least treatment mean ($P < 0.05$).

APPENDIX TABLE 15

TRIAL III. SEX, INITIAL WEIGHT, FINAL WEIGHT, AND AVERAGE DAILY GAIN OF GROWING PIGS IN IMMUNIZATION STUDY[a]

Pig No.	Sex	Age Days	Initial Wt., lb.	Final Wt., lb.	Time, days 0-28	28-56	0-56	56-73[b]
				Control				
1-4[b]	F	52	26	109	1.18	1.25	1.21	0.88
2-2[b]	M	52	32	110	0.96	1.32	1.14	0.82
4-2	M	49	35	147	1.36	1.60	1.48	1.71
4-7[b]	F	49	31	129	1.32	1.64	1.48	0.88
11-3	M	44	27	128	1.11	1.43	1.27	1.76
56-3[b]	M	52	33	128	1.11	1.50	1.30	1.29
57-4	M	51	27	129	1.21	1.53	1.38	1.47
57-6	F	51	34	142	1.36	1.43	1.39	1.76
59-4[b]	M	49	30	145	1.39	1.75	1.57	1.59
89-2	F	50	34	142	1.29	1.59	1.39	1.76
Av.			(31)		(1.23)	(1.50)	(1.36)	(1.39)
SE					0.04	0.05	0.04	0.13
				0.5 Unit				
1-5	F	52	34	129	1.32	1.32	1.32	1.24
4-4	F	49	33	152	1.50	1.68	1.59	1.76
4-5[b]	F	49	30	128	1.43	1.53	1.48	0.88
6-1[b]	M	48	26	118	1.36	1.39	1.38	0.88
11-1[b]	M	44	32	124	1.39	1.32	1.36	0.94
11-4	M	44	32	137	1.32	1.50	1.41	1.53
56-1[b]	M	52	31	128	1.18	1.60	1.39	1.00
57-2[b]	M	51	35	152	1.57	1.75	1.66	1.41
57-5	M	51	27	c	1.04	c	c	c
59-7	F	49	27	129	1.14	1.43	1.30	1.71
Av.			(31)		(1.33)[d]	(1.51)	(1.43)	(1.26)
SE					0.05	0.05	0.04	0.12
				5 Unit				
3-2[b]	M	51	33	113	1.14	1.39	1.27	0.52
4-6	F	49	34	134	1.32	1.29	1.30	1.59
5-7[b]	F	48	28	85	0.86	1.10	0.98	0.12
11-2	M	44	28	136	1.29	1.50	1.39	1.76
11-5[b]	M	44	26	110	1.25	1.36	1.30	0.65
56-2[b]	M	52	35	127	1.18	1.36	1.27	1.23
57-1	M	51	30	137	1.25	1.57	1.41	1.65
57-3	M	51	31	145	1.14	1.71	1.43	2.00
59-10	F	49	30	126	0.86	1.46	1.16	1.82
89-4[b]	F	50	34	140	1.25	1.82	1.54	1.18
Av.			(31)		(1.15)	(1.46)	(1.31)	(1.25)
SE					0.05	0.07	0.05	0.20

[a]Jackbean urease prepared by the author.

[b]One-half of the pigs in each group were fed a ration with 3.4 percent urea added form 56 to 73 days.

[c]Pig died after 39 days; autopsy showed stomach ulcer.

[d]Significantly greater than the least treatment mean ($P < 0.05$).

APPENDIX TABLE 16

TRIAL III. PLASMA UREA N LEVELS IN IMMUNIZATION STUDY (mg./100 ml.)[a]

Pig No.	Time, days 28	56	73[b]
	Control		
1-4[b]	10.9	11.4	25.6
2-2[b]	15.3	16.6	36.5
4-2	14.7	21.8	19.7
4-7[b]	11.3	16.1	26.7
11-3	11.2	18.2	20.5
56-3[b]	13.6	20.2	36.0
57-4	15.0	20.2	18.3
57-6	19.0	24.4	23.5
59-4[b]	16.1	19.9	33.4
89-2	12.3	15.7	12.4
Av.	(13.9)	(18.4)	(25.3)
SE	0.8	1.2	2.5
	0.5 Unit		
1-5	13.7	13.0	13.0
4-4	19.2	23.6	20.8
4-5[b]	17.8	18.3	26.7
6-1[b]	16.2	12.4	30.0
11-1[b]	14.5	18.6	27.9
11-4	16.2	22.8	19.5
56-1[b]	14.9	19.7	30.8
57-2[b]	12.3	15.3	37.0
57-5	11.9	d	d
59-7	13.5	16.8	16.9
Av.	(15.0)	(17.8)	(24.7)
SE	0.7	1.1	2.5
	5 Unit		
3-2[b]	12.5	16.1	15.7
4-6	14.0	11.9	15.1
5-7[b]	16.3	16.8	17.0
11-2	12.7	21.8	19.8
11-5[b]	14.7	23.9	24.7
56-2[b]	13.7	19.5	28.1
57-1	11.2	14.1	12.3
57-3	12.0	16.3	15.5
59-10	10.4	16.9	16.5
89-4[b]	14.7	22.0	29.5
Av.	(13.2)	(17.9)	(19.4)
SE	0.6	1.2	1.9

[a]Jackbean urease prepared by the author.

[b]One-half of the pigs in each group were fed a ration with 3.4 percent urea added from 56-73 days.

[c]Pigs in each treatment were group fed.

[d]Pig died 39 days; autopsy showed stomach ulcer.

APPENDIX TABLE 17

TRIAL III. PLASMA AMMONIA N LEVELS IN IMMUNIZATION STUDY (mcg./100 ml.)[a]

Pig No.	Time, days 28	56	73[b]
	Control		
1-4[b]	772	629	442
2-2[b]	683	708	405
4-2	785	650	465
4-7[b]	535	752	535
11-3	637	672	418
56-3[b]	767	730	543
57-4	716	885	582
57-6	625	699	497
59-4[b]	800	841	465
89-2	627	531	489
Av.	(695)	(710)[e]	(484)[ee]
SE	27	32	18
	0.5 Unit		
1-5	692	557	414
4-4	674	553	414
4-5[b]	782	628	395
6-1[b]	660	651	446
11-1[b]	800	624	349
11-4	655	553	489
56-1[b]	730	677	525
57-2[b]	637	566	382
57-5	813	d	d
59-7	702	637	502
Av.	(715)	(605)	(392)
SE	20	16	18
	5 Unit		
3-2[b]	698	752	424
4-6	674	713	418
5-7[b]	749	841	535
11-2	730	747	535
11-5[b]	553	632	382
56-2[b]	651	562	428
57-1	754	890	535
57-3	698	752	558
59-10	716	575	446
89-4[b]	656	659	395
Av.	(688)	(712)[e]	(466)[e]
SE	17	38	21

[a]Jackbean urease prepared by the author.

[b]One-half of the pigs in each group were fed a ration with 3.4 percent urea added from 56 to 73 days.

[c]Pigs in each treatment were group fed.

[d]Died 39 days; autopsy showed stomach ulcer.

[e]Significantly greater than the least treatment mean ($P<0.05$); [ee]($P<0.01$).

APPENDIX TABLE 18

TRIAL III. SERUM ANTIUREASE ACTIVITY IN IMMUNIZATION STUDY (units/ml. x 10^3)[a]

Pig No.	Time, days 28	56	73[b]
	Control		
1-4[b]	-11	-28	1
2-2[b]	-26	19	3
4-2	37	53	0
4-7[b]	24	-19	-10
11-3	-43	2	-11
56-3[b]	26	-25	25
57-4	-12	4	13
57-6	-22	53	2
59-4[b]	38	-30	0
89-2	4	-23	-15
Av.	(1)	(1)	(1)
SE	9	10	4
	0.5 Unit		
1-5	88	29	60
4-4	81	87	20
4-5[b]	123	57	60
6-1[b]	114	47	46
11-1[b]	84	75	14
11-4	85	21	39
56-1[b]	25	6	29
57-2[b]	90	58	46
57-5	106	-	-
59-7	101	42	24
Av.	(90)[e]	(47)[e]	(38)[e]
SE	8	9	6
	5 Unit		
3-2[b]	117	26	29
4-6	131	60	40
5-7[b]	105	163	25
11-2[b]	88	84	41
11-5	89	39	22
56-2[b]	43	93	50
57-1	120	23	15
57-3	104	47	34
59-10	43	66	57
89-4[b]	-4	70	35
Av.	(84)[e]	(67)[e]	(35)[e]
SE	14	13	4

[a]Jackbean urease prepared by the author.

[b]One-half of the pigs in each group were fed a ration with 3.4 percent urea added from 56 to 73 days.

[c]Pigs in each treatment were group fed.

[d]Died 39 days; autopsy showed stomach ulcer.

[e]Significantly greater than the least treatment mean ($P < 0.01$).

APPENDIX TABLE 19

TRIAL III. AVERAGE DAILY GAIN, PLASMA UREA N AND AMMONIA N LEVELS OF IMMUNIZED AND NONIMMUNIZED PIGS FED 3.4 PERCENT UREA TO SUPPLY PART OF THE CRUDE PROTEIN REQUIREMENTS[ab]

Urea				Control			
Pig No.	Av. da. gain, lb.	Urea N mg./100 ml.	Ammonia N mcg./100 ml.	Pig No.	Av. da. gain, lb.	Urea N mg./100 ml.	Ammonia N mcg./100 ml.
				Control			
1-4	0.88	25.6	442	4-2	1.71	19.7	465
2-2	0.82	36.5	405	11-3	1.76	20.5	418
4-7	0.88	26.7	535	57-4	1.47	18.3	582
56-3	1.29	36.0	543	57-6	1.76	23.5	497
59-4	1.59	33.4	465	89-2	1.76	12.4	489
Av.	(1.09)	(31.6)[e]	(478)	Av.	(1.69)[d]	(18.9)	(490)
SE	0.15	2.3	27	SE	0.05	1.7	27
				0.5 Unit			
4-5	0.88	26.7	395	1-5	1.24	13.0	414
6-1	0.88	30.0	446	4-4	1.76	20.8	414
11-1	0.94	27.9	349	11-4	1.53	19.5	489
56-1	1.00	30.8	525	59-7	1.71	16.9	502
57-2	1.41	37.0	382	-	-	-	-
Av.	(1.02)	(30.5)[e]	(419)	Av.	(1.56)[d]	(17.6)	(455)
SE	0.09	1.8	21	SE	0.09	1.7	24

[a] Jackbean urease prepared by the author.
[b] Blood samples taken at the end of the experiment. Pigs fed urea rations for 17 days (56 to 73 days).
[c] Significantly greater than the least treatment mean ($P < 0.05$).
[d] Significantly " " " " three treatment means ($P < 0.01$).
[e] Significantly " " " " four treatment means ($P < 0.01$).

APPENDIX TABLE 19 (CONTINUED)

TRIAL III. AVERAGE DAILY GAIN, PLASMA UREA N AND AMMONIA N LEVELS OF IMMUNIZED AND NONIMMUNIZED PIGS FED 3.4 PERCENT UREA TO SUPPLY PART OF THE CRUDE PROTEIN REQUIREMENTS [a] [b]

Urea				Control			
Pig No.	Av. da. gain, lb.	Urea N mg./100 ml.	Ammonia N mcg./100 ml.	Pig No.	Av. da. gain, lb.	Urea N mg./100 ml.	Ammonia N mcg./100 ml.
			5 Unit				
3-2	0.52	15.7	424	4-6	1.59	15.1	418
5-7	0.12	17.0	535	11-2	1.76	19.8	535
11-5	0.65	24.7	382	57-1	1.65	12.3	535
56-2	1.23	28.1	428	57-3	2.00	15.5	558
89-4	1.18	29.5	395	59-10	1.82	16.5	446
Av.	(0.74)	(23.0)	(433)	Av.	(1.76)[d]	(15.8)	(498)[c]
SE	0.21	2.8	27	SE	0.07	1.2	28

[a] Jackbean urease prepared by the author.
[b] Blood samples taken at the end of the experiment. Pigs fed urea rations for 17 days (56 to 73 day).
[c] Significantly greater than the least treatment mean ($P < 0.05$).
[d] Significantly " " " " three treatment means ($P < 0.01$).
[e] Significantly " " " " four treatment means ($P < 0.01$).

APPENDIX TABLE 20

TRIAL III. UREASE ACTIVITY AND AMMONIA N LEVELS OF INTESTINAL SECTIONS PLUS CONTENTS FROM PIGS IN IMMUNIZATION STUDY [ab]

Pig No.	Urease activity units/gm. DM x 10^3	Ammonia N mcg./gm. DM
	Control	
1-4[c]	34.7	258
4-2	28.1	83
11-3	53.8	310
56-3[c]	24.2	151
59-4[c]	30.2	106
89-2	31.2	186
Av.	(33.7)	(182)
SE	4.3	36
	0.5 Unit	
4-4	27.8	187
4-5[c]	37.5	188
11-1[c]	28.4	113
11-4	4.7	128
57-2[c]	38.9	200
59-7	13.2	107
Av.	(25.1)[d]	(154)
SE	5.5	17
	5 Unit	
3-2[c]	8.4	135
4-6	16.9	191
11-2	12.9	146
56-2[c]	19.0	129
59-10	15.3	90
89-4[c]	26.3	340
Av.	(16.5)[d]	(172)
SE	3.3	36

[a] Jackbean urease prepared by the author.

[b] Pigs slaughtered at the end of experiment, 73 days. Intestinal sections plus contents were taken from the lower end of the ileum.

[c] These pigs received rations containing 3.4 percent urea from 56 to 73 days.

[d] Immunized pigs had significantly ($P<0.05$) less urease activity than control pigs.

APPENDIX TABLE 21

TRIAL I. PLASMA AND URINE UREA N AND AMMONIA N LEVELS IN TOXICITY STUDY [ab]

	Plasma Urea N mg./100 ml.		Plasma Ammonia N mg./100 ml.	
Time	Control	100 Unit	Control	100 Unit
-21	14.4	14.1	368	422
0	12.4	10.3	658	675
15 min.	11.5	8.9	614	720
45 "	11.9	5.7	658	1317
75 "	11.5	3.0	456	1255
4 hr.	10.2	2.2	482	1519
8 "	9.2	0.5	992	3433
9 "	c	2.4	c	3554
10 "	c	2.9	c	2949
Av.	(11.6)	(5.6)[d]	(604)	(1760)[dd]

	Urine Urea N mg./hr.			Urine Ammonia N mg./hr.		
	Pre injection[e]	Post injection[e]	Change	Pre injection	Post injection	Change
Control	102	67	-35	5.6	1.5	-4.1
100 Unit	155	102	-53	3.1	4.8	1.7

[a] Jackbean urease prepared by the author.

[b] Two pigs: 69-10, control and 64-5, 100 units; weighed 30 and 35 pounds, respectively. Pigs were fasted during entire trial.

[c] Sample not taken.

[d] Significantly different from control ($P < 0.05$); [dd] ($P < 0.01$).

[e] Twenty hour pre-injection and eight hour post-injection collection period.

APPENDIX TABLE 22

TRIAL II. PLASMA UREA AND AMMONIA N LEVELS IN TOXICITY STUDY [ab]

Pig No.	Time, hours -22	0	1	3	7	10	15
Urea N, mg./100 ml.							
				Control			
87-5	14.8	9.3	9.0	10.3	9.5	11.8	13.0
78-6	16.5	11.2	10.2	9.4	9.7	10.8	12.2
Av.	(15.6)	(10.2)	(9.6)[c]	(9.8)[d]	(9.1)[d]	(11.3)[c]	(12.6)[c]
SE	0.9	1.0	0.6	0.5	0.1	0.5	0.4
				50 Units			
87-2	11.6	9.4	8.4	5.4	5.1	6.7	10.9
78-7	13.0	12.4	9.0	5.6	5.6	9.4	12.4
Av.	(12.3)	(10.9)	(8.7)	(5.5)	(5.2)	(8.0)	(11.6)
SE	0.7	1.5	0.3	0.1	0.3	1.4	0.8
				100 Unit			
78-4	14.4	9.2	5.2	3.4	2.6	3.8	4.7
87-8	9.8	9.6	7.4	5.1	4.6	5.8	8.4
Av.	(12.1)	(9.4)	(6.3)	(4.2)	(3.6)	(4.8)	(6.5)
SE	2.3	0.2	1.1	1.0	1.0	1.0	1.9
Ammonia N, mcg./100 ml.							
				Control			
87-5	566	615	566	604	599	1176	750
78-6	593	744	539	604	917	1133	884
Av.	(580)	(680)	(552)	(604)	(758)	(1154)	(817)
SE	14	64	14	0	159	21	67
				50 Unit			
87-2	577	593	712	1213	1278	1726	1197
78-7	653	728	809	1537	1952	1753	1731
Av.	(615)	(660)	(760)	(1375)[c]	(1615)	(1739)	(1464)
SE	38	67	49	162	337	14	267
				100 Unit			
78-4	545	631	1321	1564	2217	2589	4660
87-8	626	647	512	1172	2114	2001	1327
Av.	(585)	(639)	(916)	(1368)[c]	(2165)[c]	(2295)[c]	(2993)
SE	41	8	406	196	51	294	1665

[a] Jackbean urease prepared by the author.

[b] Pigs 78-7, 78-4 and 87-8 died 25, 15, and 20 hours, respectively, after the injection.

[c] Significantly greater than least treatment mean ($P < 0.05$).

[d] Significantly " " " two treatment means ($P < 0.05$).

APPENDIX TABLE 23

TRIAL II. SERUM UREASE ACTIVITY, POTASSIUM, SODIUM AND PROTEIN IN TOXICITY STUDY [a,b]

Pig No.	Control 0 hr.	Control 6 hr.	Control 10 hr.	Pig No.	50 Unit 0 hr.	50 Unit 6 hr.	50 Unit 10 hr.	Pig No.	100 Unit 0 hr.	100 Unit 6 hr.	100 Unit 10 hr.
	Urease activity, unit/ml. x 10^3										
87-5	0.1	0.0	0.6	87-2	-0.1	25.4	4.8	78-4	0.1	151.4	60.8
78-6	0.0	-0.1	-0.4	78-7	0.4	50.3	10.8	87-8	0.5	16.3	6.3
Av.	(0.1)	(-0.1)	(0.1)	Av.	(0.1)	(37.9)	(7.8)	Av.	(0.3)	(83.8)	(33.6)
SE	0.1	0.1	0.1	SE	0.2	12.5	3.0	SE	0.2	67.6	27.1
	Potassium, mg./100 ml.										
87-5	22.0	19.9	20.9	87-2	20.7	23.5	27.5	78-4	27.2	33.6	25.1
78-6	28.3	27.0	26.8	78-7	22.6	28.2	27.3	87-8	22.4	22.6	26.2
Av.	(25.1)	(23.5)	(23.8)	Av.	(21.6)	(28.3)[c]	(27.4)[c]	Av.	(24.8)	(28.6)[c]	(25.7)[c]
SE	3.1	3.6	2.9	SE	1.0	0.2	0.1	SE	2.4	3.6	0.5
	Sodium, mg./100 ml.										
87-5	324	332	297	87-2	335	325	297	78-4	316	331	329
78-6	328	333	300	78-7	315	325	330	87-8	323	294	313
Av.	(326)	(332)	(298)	Av.	(325)	(325)	(313)	Av.	(319)	(312)	(321)
SE	2	1	2	SE	10	0	16	SE	4	19	8
	Total serum protein, gm./100 ml.										
87-5	5.5	5.4	5.2	87-2	5.4	5.6	5.1	78-4	5.2	5.3	5.2
78-6	6.0	5.9	5.5	78-7	5.5	6.2	6.2	87-8	5.5	5.5	5.4
Av.	(5.7)	(5.6)	(5.3)	Av.	(5.5)	(5.9)	(5.6)	Av.	(5.3)	(5.4)	(5.3)
SE	0.2	0.2	0.1	SE	0.1	0.3	0.5	SE	0.1	0.1	0.1

[a] Jackbean urease prepared by author.
[b] Pigs 78-7, 78-4 and 87-8 died 25, 15 and 20 hours, respectively, after the injection.
[c] Significant increase ($P < 0.05$) after urease injection.

APPENDIX TABLE 24

TRIAL II. UREASE ACTIVITY AND AMMONIA N LEVEL OF LIVER; AND URINARY FLOW, UREA N, AND AMMONIA N LEVEL IN TOXICITY STUDY [a][b][c]

Pig No.	Urease activity units/gm. DM x 10^3	Ammonia N mg./gm. DM	Urine flow, mg./hr.		Urine Urea N, mg./hr.			Urine Ammonia N, mg./hr.		
			Pre injection	Post injection	Pre injection	Post injection	Change	Pre injection	Post injection	Change
Control										
87-5	-2.7	797	11.0	5.4	106.4	38.9	67.5	5.5	4.5	-1.0
78-5	8.2	970	6.3	7.4	74.3	46.6	27.7	7.8	6.8	-1.0
Av.	(2.7)	(883)	(8.6)	(6.4)	(90.3)	(42.7)	(47.6)	(6.7)	(5.7)	(-1.0)
SE	5.5	50	2.3	1.0	16.1	3.9	19.9	1.2	1.2	0.0
50 Unit										
87-2	0.0	1064	7.1	10.6	78.8	45.0	33.8	1.8	4.8	3.0
98-7	14.5	1392	7.5	12.8	93.1	33.7	59.4	6.6	11.8	5.2
Av.	(7.2)	(1228)	(7.3)	(11.7)	(85.9)	(39.3)	(46.6)	(4.2)	(8.3)	(4.1)[d]
SE	7.2	164	0.2	1.1	7.1	5.7	12.8	2.4	3.5	1.1
100 Unit										
78-4	256.1	1479	7.6	10.9	96.0	37.2	58.8	5.4	7.6	2.2
87-8	33.0	1543	5.7	13.9	62.5	45.6	16.9	5.1	4.9	-0.2
Av.	(144.5)	(1511)	(6.6)	(12.4)	(79.2)	(41.4)	(37.8)	(5.3)	(6.3)	(1.0)
SE	111.7	32	1.0	1.5	16.7	4.2	20.9	0.2	1.2	1.2

[a] Jackbean urease prepared by the author.
[b] Twenty-two hour pre-injection and eighteen-hour post-injection collection periods.
[c] Pigs 78-7, 78-4 and 87-8 died 25, 15 and 20 hours, respectively, after the injection.
[d] Significantly greater than least treatment mean ($P < 0.05$).

APPENDIX TABLE 25

TRIAL III. UREA N AND AMMONIA N LEVELS IN TOXICITY STUDY [ab]

Control					75 Unit[c]				
Pig No.	-20 hr.	0 hr.	2 hr.	6 hr.	Pig No.	-20 hr.	0 hr.	2 hr.	6 hr.
Urea N, mg./100 ml.									
20-1	9.7	5.5	6.5	6.4	16-10	10.0	6.2	5.4	3.1
20-3	13.0	7.1	8.6	8.0	97-5	8.8	8.8	9.0	7.4
21-7	11.4	4.7	5.9	5.7	101-2	10.0	12.8	12.1	6.3
20-7	12.9	12.8	13.2	11.8	18-4	9.4	15.0	11.2	7.4
16-2	8.8	6.9	7.9	7.2	16-8	8.2	12.4	11.9	0.0
21-4	12.6	6.4	6.7	6.7	19-2	17.3	19.0	15.2	-
Av.	(11.4)	(7.2)	(8.1)	(7.6)	Av.	(19.6)	(12.4)[d]	(10.8)	(6.0)
SE	0.7	1.2	1.1	0.9	SE	4.7	1.8	1.4	1.4
Ammonia N, mcg./100 ml.									
20-1	639	500	500	649	16-10	562	472	1006	1556
20-3	680	585	511	719	97-5	572	557	961	2746
21-7	577	631	580	523	101-2	644	642	1432	5365
20-7	407	523	460	587	18-4	706	699	1148	2407
16-2	500	534	477	577	16-8	577	636	1352	672
21-4	541	528	466	471	19-2	624	556	1835	-
Av.	(557)	(550)	(499)	(588)	Av.	(614)	(594)	(1289)[dd]	(2549)[d]
SE	40	20	18	36	SE	23	33	143	791

[a]Pigs fasted during entire trial.

[b]Sigma urease type II powder.

[c]Survival time after injection: 16-10,11; 97-5,10; 101-2,7; 18-4,10; 16-8,6; and 19-2,5; pig numbers and hours, respectively.

[d]Significantly different from control ($P < 0.05$); [dd]($P < 0.01$).

APPENDIX TABLE 26

TRIAL III. HEMATOCRIT AND HEMOGLOBIN LEVELS IN TOXICITY STUDY [ab]

Control					75 Unit[c]				
Pig No.	-20 hr.	0 hr.	2 hr.	6 hr.	Pig No.	-20 hr.	0 hr.	2 hr.	6 hr.
Hematocrit, Percent									
20-1	25.2	26.0	25.3	27.2	16-10	33.8	33.0	38.5	47.3
20-3	28.0	31.5	31.4	31.8	97-5	32.2	33.3	39.5	47.5
21-7	28.5	32.5	34.2	33.8	101-2	34.0	32.8	40.8	46.5
20-7	27.0	26.4	27.2	26.8	18-4	31.1	32.6	40.5	44.5
16-2	33.4	36.3	37.8	37.8	16-8	33.8	34.9	42.3	51.0
21-4	33.0	36.0	34.9	35.9	19-2	27.7	27.8	34.0	-
Av.	(29.2)	(31.5)	(31.8)	(32.2)	Av.	(32.1)	(32.4)	(39.3)[dd]	(47.4)[dd]
SE	1.4	1.8	2.0	1.9	SE	0.6	1.0	1.2	1.1
Hemoglobin, gm./100 ml.									
20-1	7.7	12.6	11.7	8.1	16-10	10.3	11.7	12.1	18.7
20-3	7.9	9.4	9.1	10.1	97-5	10.3	11.0	13.2	13.6
21-7	9.7	10.5	10.3	10.6	101-2	10.5	10.6	13.0	16.5
20-7	7.7	7.3	8.1	7.5	18-4	9.0	9.8	12.3	13.6
16-2	9.9	12.1	11.9	12.1	16-8	10.3	11.4	14.1	15.7
21-4	10.3	11.9	10.8	11.7	19-2	7.9	8.3	10.6	-
Av.	(8.9)	(10.6)	(10.3)	(10.0)	Av.	(9.7)	(10.5)	(12.6)[d]	(15.6)[dd]
SE	0.5	0.8	0.6	0.8	SE	0.4	0.5	0.5	1.0

[a]Pigs fasted during entire trial.

[b]Sigma urease type II powder.

[c]Survival time after injection: 16-10,11; 97-5,10; 101-2,7; 18-4,10; 16-8,6; and 19-2,5; pig numbers and hours, respectively.

[d]Significantly different from control ($P < 0.05$); [dd]($P < 0.01$).

APPENDIX TABLE 27

TRIAL III. BLOOD GLUCOSE AND TEMPERATURE IN TOXICITY STUDY [ab]

Control					75 Unit[c]				
Pig No.	-20 hr.	0 hr.	2 hr.	6 hr.	Pig No.	-20 hr.	0 hr.	2 hr.	6 hr.
Glucose, mg./100 ml.									
20-1	92.2	59.4	71.0	99.7	16-10	71.0	59.7	79.2	41.9
20-3	86.2	68.7	71.7	79.7	97-5	67.7	60.4	61.8	40.0
21-7	76.9	58.5	71.7	68.3	101-2	71.7	49.9	44.3	52.5
20-7	75.3	56.2	62.4	70.0	18-4	56.2	42.9	46.9	44.6
16-2	65.0	56.4	62.1	58.2	16-8	81.2	42.4	52.5	23.4
21-4	78.3	61.1	70.3	67.7	19-2	82.2	55.8	-	-
Av.	(79.0)	(60.1)	(68.2)	(73.9)	Av.	(71.7)	(51.9)	(56.9)	(40.5)[d]
SE	3.8	1.9	1.9	5.9	SE	3.9	3.3	6.3	4.8
Temperature, °C									
20-1	40.1	40.2		39.8	16-10	40.3	39.8		40.3
20-3	40.6	39.6		39.7	97-5	39.6	39.3		39.2
21-7	40.2	39.3		39.8	101-2	40.1	40.2		41.2
20-7	39.2	39.5		39.6	18-4	40.2	39.3		39.3
16-2	39.7	39.5		39.3	16-8	39.5	39.4		39.6
21-4	40.3	39.4		39.5	19-2	39.9	39.7		-
Av.	(40.1)	(39.6)		(39.6)	Av.	(39.9)	(39.6)		(39.9)
SE	0.2	0.1		0.1	SE	0.1	0.1		0.4

[a]Pigs fasted during the entire trial.

[b]Sigma urease type II powder.

[c]Survival time after injection: 16-10,11; 97-5,10; 101-2,7; 18-4,10; 16-8,6; and 19-2,5; pig numbers and hours, respectively.

[d]Significantly different from control ($P < 0.01$).

APPENDIX TABLE 28

TRIAL III. SERUM UREASE ACTIVITY AND POTASSIUM LEVEL IN TOXICITY STUDY[a]

Control				75 Unit[b]			
Pig No.	0 hr.	2 hr.	6 hr.	Pig No.	0 hr.	2 hr.	6 hr.
Urease activity, SU/ml. x 10^3							
20-1	0.0	-0.2	0.9	16-10	0.7	3.2	13.3
20-3	-0.2	0.9	-0.2	97-5	-0.9	5.0	12.3
21-7	0.5	0.5	0.9	101-2	0.7	25.3	27.1
20-7	0.2	-	-0.7	18-4	0.0	4.1	5.6
16-2	-1.8	-0.9	1.7	16-8	0.9	10.6	64.7
21-4	0.5	0.0	-0.5	19-2	0.2	4.7	-
Av.	(-0.1)	(0.1)	(0.3)	Av.	(0.3)	(8.8)	(24.6)[c]
SE	0.4	0.3	0.4	SE	0.3	3.5	10.6
Potassium, mg./100 ml.							
20-1	26.7	20.8	20.6	16-10	24.0	29.1	37.2
20-3	29.2	22.8	28.0	97-5	26.0	29.0	40.2
21-7	27.9	21.5	21.2	101-2	25.7	28.6	56.2
20-7	25.0	29.4	25.7	18-4	26.9	30.1	40.1
16-2	31.5	29.0	28.2	16-8	31.4	31.1	44.3
21-4	26.9	26.5	28.5	19-2	30.3	35.5	-
Av.	(27.9)	(25.0)	(25.4)	Av.	(27.4)	(30.6)[c]	(43.6)[cc]
SE	0.9	1.6	1.5	SE	1.2	1.1	3.3

[a]Sigma urease type II powder.

[b]Survival time after injection: 16-10,11; 97-5,10; 101-2,7; 18-4,10; 16-8,6; and 19-2,5; pig numbers and hours, respectively.

[c]Significantly different from control ($P < 0.05$);[cc]($P < 0.01$).

APPENDIX TABLE 29

TRIAL III. SODIUM AND CALCIUM LEVELS IN TOXICITY STUDY[a]

Control				75 Unit[b]			
Pig No.	0 hr.	2 hr.	6 hr.	Pig No.	0 hr.	2 hr.	6 hr.
Sodium, mg./100 ml.							
20-1	317	312	298	16-10	3.6	300	298
20-3	304	324	294	97-5	320	303	309
21-7	301	300	306	101-2	317	309	299
20-7	306	311	309	18-4	306	307	309
16-2	318	321	310	16-8	302	321	336
21-4	326	300	301	19-2	311	324	-
Av.	(312)	(311)	(303)	Av.	(312)	(311)	(310)
SE	4	4	3	SE	3	4	7
Calcium, mg./100 ml.							
20-1	12.5	11.1	9.7	16-10	11.3	12.9	10.0
20-3	11.9	11.4	10.4	97-5	11.4	11.9	9.8
21-7	11.1	10.8	13.9	101-2	13.6	11.6	-
20-7	13.3	11.2	10.0	18-4	10.9	10.1	10.0
16-2	11.1	11.5	11.2	16-8	10.4	10.8	11.0
21-4	11.1	10.8	10.7	19-2	12.7	11.1	-
Av.	(11.8)	(11.1)	(11.0)	Av.	(11.7)	(11.4)	(10.2)
SE	0.4	0.1	0.6	SE	0.5	0.4	0.2

[a]Sigma urease type II powder.

[b]Survival time after injection: 16-10,11; 97-5,10; 101-2,7; 18-4,10; 16-8,6; and 19-2,5; pig numbers and hours, respectively.

APPENDIX TABLE 30

TRIAL III. SERUM GLUTAMIC-PYRUVIC AND GLUTAMIC-OXALACETIC TRANSAMINASE IN TOXICITY STUDY [a]

Control				75 Unit[b]			
Pig No.	0 hr.	2 hr.	6 hr.	Pig No.	0 hr.	2 hr.	6 hr.
Serum glutamic-pyruvic transaminase activity, unit/ml.							
20-1	24.1	24.9	18.6	16-10	16.4	13.9	17.5
20-3	23.4	27.4	23.5	97-5	25.4	16.9	18.8
21-7	22.4	20.7	19.8	101-2	24.2	16.7	16.6
20-7	23.9	24.2	20.1	18-4	23.0	15.8	13.1
16-2	28.5	29.0	24.8	16-8	24.2	17.6	16.6
21-4	28.7	30.4	24.8	19-2	25.2	17.1	-
Av.	(25.1)	(26.1)	(21.2)	Av.	(23.1)	(16.3)[c]	(16.5)[c]
SE	1.1	1.5	1.1	SE	1.4	0.5	0.9
Serum glutamic-oxalacetic transaminase activity, unit/ml.							
20-1	18.4	20.1	16.1	16-10	15.0	12.7	10.5
20-3	23.3	26.0	20.0	97-5	18.6	14.0	18.9
21-7	17.7	18.6	20.0	101-2	24.6	18.8	35.0
20-7	16.0	14.0	15.4	18-4	17.7	17.4	18.9
16-2	22.1	18.2	17.7	16-8	24.0	17.9	15.8
21-4	19.2	16.5	14.5	19-2	18.5	17.4	-
Av.	(19.4)	(18.9)	(17.4)	Av.	(19.7)	(16.5)	(19.8)
SE	1.1	1.6	1.1	SE	1.5	1.0	4.1

[a]Sigma urease type II powder.

[b]Survival time after injection: 16-10,11; 97-5,10; 101-2,7; 18-4,10; 16-8,6; and 19-2,5; pig numbers and hours, respectively.

[c]Significantly different from control ($P < 0.01$).

APPENDIX TABLE 31

TRIAL III. SERUM ELECTROPHORETIC PATTERN IN TOXICITY STUDY [a]

Control					75 Unit				
Pig No.	Age, days	0 hr.	2 hr.	6 hr.	Pig No.	Age, days	0 hr.	2 hr.	6 hr.
Gamma Globulin, percent									
20-1	54	8.0	8.0	8.5	16-10	65	15.6	15.5	14.8
20-3	54	6.1	4.1	5.3	97-5	60	6.9	8.1	10.6
21-7	48	9.1	5.8	7.0	101-2	58	16.5	15.1	18.8
20-7	54	6.9	8.8	10.9	18-4	61	16.0	14.6	13.1
16-2	65	13.9	15.2	15.4	16-8	65	18.4	23.2	15.4
21-4	48	8.3	9.3	8.3	19-2	56	5.9	8.0	-
Av.	(54)	(8.7)	(8.5)	(9.2)	Av.	(61)	(13.2)	(14.1)	(14.5)[b]
SE		1.1	1.6	1.5	SE		2.2	2.3	1.4
Beta Globulin, percent									
20-1		13.3	14.9	16.2	16-10		14.4	17.5	15.5
20-3		15.2	15.7	14.3	97-5		13.8	13.5	14.6
21-7		13.2	13.5	12.2	101-2		14.1	17.8	17.1
20-7		13.9	16.7	17.8	18-4		15.3	17.9	14.9
16-2		12.2	17.4	16.4	16-8		13.2	12.5	16.8
21-4		13.5	12.8	14.3	19-2		16.2	12.7	-
Av.		(13.5)	(15.1)	(15.2)	Av.		(14.5)	(15.3)	(15.8)
SE		0.4	0.7	0.8	SE		0.4	1.1	0.5

[a] Sigma urease type II powder.
[b] Significantly greater than control ($P < 0.05$).

APPENDIX TABLE 31 (CONTINUED)

TRIAL III. SERUM ELECTROPHORETIC PATTERN IN TOXICITY STUDY[a]

Control				75 Unit[b]			
Pig No.	0 hr.	2 hr.	6 hr.	Pig No.	0 hr.	2 hr.	6 hr.
Alpha Globulin, percent							
20-1	16.8	18.3	19.2	16-10	17.8	14.6	16.8
20-3	24.2	19.1	18.8	97-5	11.5	15.1	17.9
21-7	20.7	22.1	21.7	101-2	15.7	14.7	16.0
20-7	17.8	17.5	20.2	18-4	20.8	20.3	21.7
16-2	16.5	16.7	18.3	16-8	17.5	21.4	20.3
21-4	19.8	20.9	20.2	19-2	19.1	18.5	-
Av.	(19.3)	(19.1)	(19.7)	Av.	(17.1)	(14.4)	(18.5)
SE	1.2	0.9	0.5	SE	1.3	1.2	1.1
Albumin, percent							
20-1	61.9	58.9	56.2	16-10	52.2	52.4	52.9
20-3	54.5	61.2	51.7	97-5	67.8	63.2	56.9
21-7	57.0	58.7	59.1	101-2	53.7	52.4	48.1
20-7	61.4	57.0	51.2	18-4	47.9	47.2	50.3
16-2	57.4	50.8	50.0	16-8	50.9	42.9	47.6
21-4	58.3	57.0	57.1	19-2	58.8	61.3	-
Av.	(58.4)	(57.2)	(55.9)	Av.	(55.2)	(53.2)	(51.1)
SE	1.1	1.5	1.9	SE	2.9	3.2	1.7

[a]Sigma urease type II powder.

APPENDIX TABLE 32

TRIAL III. URINE FLOW, TOTAL N, UREA N, AMMONIA N AND DIFFERENCE N IN TOXICITY STUDY[ab]

Pig No.	Urine Flow		Total N		Urea N		Ammonia N		Difference N	
	Pre injection	Post injection	Pre injection	Post injection	Pre injection	Post injection	Pre injection	Post injection	Pre injection	Post injection
	ml./hr.		mg./hr.		mg./hr.		mg./hr.		mg./hr.	
Control										
20-1	8.86	23.3	91.3	79.5	75.4	66.2	3.8	2.7	12.1	10.6
20-3	12.15	24.50	111.5	125.0	96.5	94.2	6.7	12.2	8.3	18.6
21-7	8.76	15.60	108.9	124.0	73.6	85.1	13.2	21.2	22.1	17.7
20-7	5.21	9.45	82.9	84.5	60.7	68.7	7.2	5.2	15.0	10.6
16-2	15.50	20.15	85.1	105.0	65.6	77.2	3.6	9.6	15.9	18.2
21-4	9.15	19.30	111.2	132.0	93.6	88.4	6.1	18.8	11.5	24.8
Av.	(9.93)	(20.05)	(98.5)	(108.3)	(77.6)	(80.0)	(6.8)	(11.6)	(14.1)	(16.7)
SE	1.43	2.76	5.2	9.1	6.0	4.6	1.4	3.0	1.9	2.2
75 Unit[c]										
16-10	9.31	6.21	94.5	50.3	66.9	23.4	9.9	4.2	17.7	22.7
97-5	14.50	7.50	66.0	34.2	59.7	20.7	2.6	1.3	3.7	12.2
101-2	14.00	4.24	135.4	64.7	110.2	43.3	10.4	3.9	14.8	17.5
18-4	5.35	4.67	54.6	23.4	44.4	13.0	2.7	2.0	7.5	8.4
16-8	23.00	4.00	54.8	11.3	46.1	5.8	8.1	1.6	0.6	3.9
19-2	5.55	4.00	45.3	57.0	34.7	35.4	4.4	3.7	6.2	17.9
Av.	(12.18)	(5.10)[dd]	(75.1)	(40.2)[dd]	(60.3)	(23.6)[dd]	(6.4)	(2.8)[d]	(8.4)	(13.8)
SE	2.21	0.59	13.9	8.3	11.0	5.7	1.5	0.5	2.7	2.8

[a]Difference N is total N minus urea and ammonia N.

[b]Sigma urease type II powder.

[c]Survival time after injection: 16-10,11; 97-5,10; 101-2,7; 18-4,10; 16-8,6; and 19-2,5; pig numbers and hours, respectively.

[d]Significantly less than control ($P < 0.05$);[dd]($P < 0.01$).