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An Orally Administered Microcapsule System for Treating Chronic Renal Failure Patients

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ABSTRACT

Ingestible adsorbents for the removal of uremic metabolites are being investigated as adjunctive therapy in the treatment of chronic uremia. In particular, a microcapsule product containing urease and zirconium phosphate (UZP) has been investigated for removing urea. A dog model, simulating chronic uremia, was developed to investigate: (1) the concentration of various nitrogenous metabolites (urea, creatinine, and uric acid) in the GI tract, (2) flux rates of H_2O and various nitrogenous metabolites in the GI tract, and (3) the efficacy of the microcapsule product. The results of these perfusion studies suggest that urea and creatinine can be removed from the GI tract via ingestible adsorbents. In addition, the model may be useful in investigating suspect uremic toxins, e.g., guanidinosuccinic acid (GSA). The reduction of blood urea nitrogen levels in the dog model when the animal was fed the microcapsule product was limited by the capacity of the zirconium phosphate to bind ammonium ion. Preliminary clinical studies with the microcapsule product indicate that it may be of potential adjunctive therapy in patients suffering from chronic renal failure.

Index Entries: Animal model, of chronic renal failure; adsorbents, in uremia; microcapsules, for the treatment of chronic renal failure; GI tract, and microcapsules; nitrogenous metabolites, and microcapsules; flux rate, and microcapsules; uremia, treatment with microcapsules.

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INTRODUCTION

Early studies by Pendleton and West (*1*) in 1932 demonstrated that urea moved from the blood into the small intestine in uremic dogs. In 1946, Twiss and Kolff (*2*) showed in uremic patients that urea could be removed when an isotonic solution was perfused through an isolated intestinal loop. Since these early studies had demonstrated the presence of urea and its removal from the gastrointestinal tract, Yatzidis (*3*) in 1964 investigated the use of activated charcoal for the removal of uremic wastes from the intestinal tract. In addition to Yatzidis' studies with charcoal, other investigations with charcoal have been unable to confirm any significant reduction in serum levels of urea, creatinine, uric acid, or guanidines (*4,5*) via this approach.

At the same time that Yatzidis proposed the use of charcoal for removal of nitrogenous wastes from the intestinal tract, he also proposed using charcoal in a hemoperfusion microapparatus as an effective artificial kidney. However, clinical studies performed with the Yatzidis charcoal artificial kidney showed an adverse affect on platelets and the release of embolizing particles (*6,7*).

To overcome these platelet and emboli problems, Chang's group (*8*) proposed the general idea of "artificial cells," i.e., microcapsules containing adsorbents. Since the charcoal particles were now encased within blood compatible membranes, they could not leave the microcapsules to cause embolism, and platelets did not now come in direct contact with the charcoal. In their early clinical hemoperfusion studies, Chang et al. (*9*) showed that after 2 h hemoperfusion over 300 g of albumin-coated activated charcoal, creatinine and uric acid clearances were comparable to or superior to the standard hemodialyzers of that time. However, in these studies, only a small fraction of blood urea was removed.

Chang (*10*) had earlier demonstrated in experimental animals that microencapsulated urease in a hemoperfusion unit could convert blood urea to ammonia. Chang and Loa (*11*) then extended the concept by administering microencapsulated urease and ammonia adsorbents to the rat intestinal tract. The ammonia adsorbents used in these studies were zirconium phosphate or an ion exchange resin (Dowex 50W-X12). Their studies indicated that a combination of microencapsulated urease and an ammonia adsorbent lowered systemic blood urea levels to 60.6 ± 5.0% of the control levels within 4 hours.

The use of microencapsulated adsorbents for removal of nitrogenous materials from the gastrointestinal tract was continued by Sparks et al. (*12*) and Gardner et al. (*13*). In addition, other unencapsulated adsorbents, e.g., oxystarch, were also being investigated for binding urea (*14*). Excellent reviews on the potential of adsorbents for clinical applications and in uremia therapy are available (*15,16*).

It is the purpose of this paper to describe the uremic dog model and to discuss some of the experiments conducted with this model and its

potential role in the evaluation of adsorbents. It is the data obtained with this dog model that led to the capsule feeding studies conducted in dogs and patients using the combination microcapsule system (urease and zirconium phosphate) (*17,18*).

METHODS

Production of Uremic Dog Model

Uremic dogs were produced by a modification of Bricker's technique in a two-step process (*19*). Following ligation (5/6th of the arterial branches) of the left kidney, a period of 2 wk or more elapsed before the right kidney was removed. During this intervening period the dog was referred to as "control" dog. When the right kidney was removed, the dog was now referred to as an "azotemic" or uremic dog. The blood urea nitrogen (BUN) level, following removal of the right kidney, dictated the amount of protein and water intake the dog received. Protein intake varied from 0.5 to 5.0 g/kg body weight, depending on the degree of uremia desired for experimental purposes. Blood urea nitrogen levels following stabilization generally ranged from 60 to 100 mg%. The dogs were stabilized by feeding a commercial grade of Purina Dog Lab Meal. Dextrose or starch was added to the diet of some of the dogs to maintain their presurgery weight. This addition was necessary because the extremely low-protein diets of some of the dogs (0.5 g protein/kg body weight) did not supply enough calories to maintain the dog's weight. The caloric intake of control and uremic dogs was maintained at 70 kcal/kg body weight/dog. Water intake in stabilized uremic dogs averaged between 600 and 800 mL/dog/d, depending upon the dog's weight and BUN level.

Gut Aspiration Studies

One of the questions that needed resolution was to determine and compare the concentrations of urea, creatinine, and uric acid in the intestinal tract of control and uremic dogs. To accomplish this goal, a percutaneous catheter system was designed. The catheter system was constructed of materials that are generally acknowledged to be biocompatible (Fig. 1). The catheter was inserted at the same time as the right kidney was removed.

Dogs having a duodenal catheter were aspirated 4 h post-feeding while those possessing a jejunal catheter were aspirated 4.5–5 h post-feeding. As soon as 25 mL of gut fluid was obtained from the dog, the sample was capped and placed on an ice bath. Following aspiration, a blood sample was taken from the dog. The gut sample was then centrifuged to remove any solid debris. The gut supernatant and serum samples were then frozen until assayed for urea, creatinine, and uric acid using standard autoanalyzer assays for these substances.

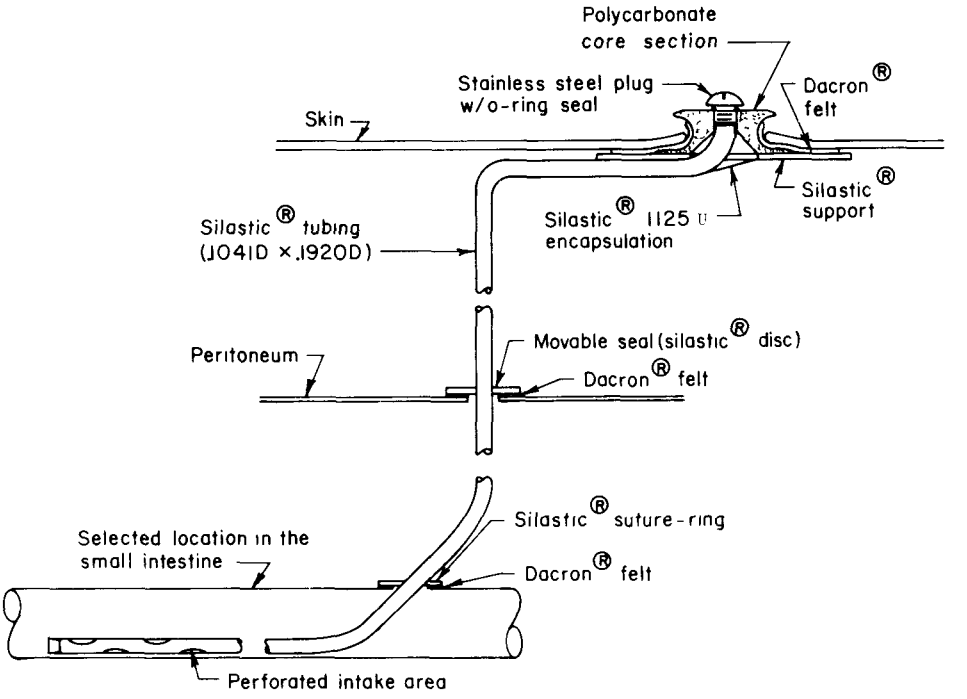

Fig. 1. Schematic of percutaneous catheter used for chronic implantation.

Flux Rate Studies

Water

In addition to the studies designed to measure the concentrations of urea, creatinine, and uric acid in the intestinal tract of control and uremic dogs, information on the net rate of water movement and the flux rate of these substances in the intestinal tract was needed. Data obtained from these studies would help assess the feasibility of using gastrointestinal adsorbents for the removal of these nitrogenous waste products. An advanced model of the original percutaneous catheter (Fig. 1) was designed for conducting the triple lumen perfusion studies. In essence, this consisted of three silicone rubber tubes, i.e, an infusion tube plus two more tubes attached to each other, but with a fixed distance (20 cm) between their openings. Triple-lumen perfusion studies were performed on control, "control," and azotemic dogs. All perfusions were performed at 37°C and on dogs that had been fasted for 24 h.

In the triple-lumen perfusion studies, a test solution, containing polyethylene glycol (PEG-4000) and isotonic saline (0.15*M* NaCl), was infused at 10 mL/min through the infusion catheter. The initial 30–70 min perfusion period was designed for equilibration of salt and water.

Following the equilibration period, the perfusion solution was continuously infused at 10 mL/min for at least 60 min. Perfusate samples

were collected continuously (1 mL/min) and simultaneously at points 15 and 35 cm distal to the infusion site. Samples were pooled (i.e., 0–10 min, 10–20 min, etc) and analyzed for PEG concentration by Hyden's method (*20*). The calculations used in determining the net rate of water movement are similar to those presented by Cooper et al. (*21*).

Urea, Creatinine, and Uric Acid

These studies were similar to the above with the exception that radiolabeled materials were used to determine the net flux rate of urea, creatinine, and uric acid. Two hours before the start of the perfusion study, 20 μCi of ^{14}C-creatinine or uric acid, or 25 μCi of ^{14}C-urea was injected intravenously. Blood samples were obtained at 30 min into the equilibration period, followed by samples 60 and 100 min later. Blood samples were clotted and 0.2 mL of serum was placed in Bray's solution and counted using standard liquid scintillation counting techniques. The blood concentration of the nitrogenous metabolite under study was also determined.

Gut fluid samples, collected during the test period, were analyzed for PEG by Hyden's method (*20*). In addition, 0.5 mL of the gut fluid was placed in 10 mL of Bray's solution for liquid scintillation counting. The calculations of the net rate of water movement were again based upon those used by Cooper et al. (*21*). However, to calculate the net flux rate of the nitrogen metabolite under study, the following equation was employed:

$$M = Q_L U_d - Q_E U_p$$

where M = the net flux rate of the nitrogenous metabolite expressed in mg/min entering the test segment

Q_L and Q_E = the flow rates in mL/min leaving and entering the test segment, respectively

U_d and U_p = the gut concentrations of the nitrogenous metabolite (mg/mL) at the distal and proximal sites, respectively.

Guanidinosuccinic Acid (GSA) Studies

Samples of serum and urine were obtained from three chronically stabilized azotemic dogs and six chronic uremic patients at The Ohio State University Hospital, Columbus, Ohio. Urine samples were collected over a 24-h period while blood was taken at the end of the 24-h urine-collection period.

The Sakaguchi reagents used in the detection of GSA consisted of 0.02% 8-hydroxyquinoline in 3*N* NaOH and 0.70% *N*-bromosuccinimide. Dowex 1W-X8 (chloride form) resin (50-100 and 200-400 mesh) was converted to the acetate form by first washing with 0.1*N* NaOH and subsequently washing with 3*M* sodium acetate. Prior to using the resin, it was

washed once with 1*N* acetic acid and equilibrated with 0.1*N* acetic acid. The Dowex 50W-X8 resin (Na^+ form, 200-400 mesh) was washed with 3*M* sodium acetate, followed by washing with 1*N* acetic acid, and equilibrated with 0.25*N* acetic acid.

Samples of urine or serum were applied at a rate of 1 mL/min to a 30 × 0.7 cm glass column packed to a height of 25 cm with Dowex 1W-X8 resin (acetate form, 200-400 mesh for urine, 50-100 mesh for serum). The column was then washed free of nonspecific, Sakaguchi positive material using 0.1*N* acetic acid (Elution I). The GSA was then eluted (Elution II) for detection and measurement with 0.25*N* acetic acid using a flow rate of 1 mL/min. As a consequence of the limited amount of serum which could be applied to the column at one time, a second column was employed to concentrate GSA and thus improve resolution in the microgram range. The 0.25*N* acetic acid eluate, containing GSA from the Dowex 1W-X8 column, was directed through a column (1 mL Tuberculin syringe barrel) of Dowex 50W-X8 resin previously equilibrated with 0.25*N* acetic acid. The GSA retained on the Dowex 50W-X8 resin was then rapidly eluted using 0.25*N* acetic acid (adjusted to pH 4.5) with 4*N* NaOH. Elution in this manner produced a sharp peak that permitted measurement of very small amounts (3 μg) of GSA. Measurement of GSA from serum or urine was accomplished using a Technicon Autoanalyzer with flow cell colorimeter and logarithmic recorder.

Microcapsules

An organic phase separation process was used to prepare the combination microcapsule system used in the dog feeding studies (*17*) and patient studies (*18*). Cellulose acetate butyrate (CAB) was initially dispersed in toluene followed by dissolution in chloroform. The final CAB concentration equaled 2.5%. To this stirred polymer solution was added an aqueous core solution containing the urease–silica adduct and zirconium phosphate. Preparation of the urease–silica adduct has previously been reported (*22*). The final zirconium phosphate concentration in the core solution equaled 25%. The CAB was then precipitated around the aqueous core by slowly adding a miscible nonsolvent (toluene). Following complete precipitation, the capsule wall was hardened by a series of decantations and the addition of petroleum ether. Residual solvent was removed by placing the capsules on aluminum foil and drying in an airflow hood for 24 h. The crenated capsules were then placed in a sealed container and stored at 4°C until administered.

In three dog feeding studies (*17*), 90 g (dehydrated) of the combination capsule were placed in the dog's diet each day. The feeding studies were conducted over 5, 4, and 2 d, respectively. In the patient studies (*18*), patients received 50 g (dehydrated) of the combination capsule the first day and 100 g on days 2 through 7. Each day's dose was consumed four times daily, immediately before meals. The capsules were adminis-

tered to the patients in the form of a milkshake-like slurry in cool water (1:4 capsule to water ratio).

RESULTS

Gut Aspiration Studies

A comparison of the serum concentration of each of the metabolites studied in relation to the gut concentration is shown in Table 1. It can be seen from Table 1 that (1) the gut urea nitrogen concentration in uremic dogs is approximately 0.7 that of the serum urea concentration, (2) serum uric acid levels do not rise in uremic dogs when compared with control dog values, and (3) both uric acid and creatinine concentrations in control dogs are approximately doubled in value between the duodenal and jujunal segments. However, uric acid and creatinine values remain essentially unchanged in uremic dogs when comparing the same segments.

Flux Rate Studies

The net rate of water movement in control plus "control" and azotemic dogs is shown in Fig. 2. It can be seen in Fig. 2 that there is a general increase in water absorption in both dog groups, i.e., control plus "control" vs azotemic, as one moves down the intestinal tract. In fact, water absorption has doubled for control plus "control" dogs between the duodenum and ileum while the increase for azotemic dogs for the same intestinal segments is 50% higher. In addition, there is a definite increase in water absorption between the duodenal and jejunal regions in the control plus "control" group while there is essentially no difference in water absorption between these two intestinal regions in azotemic dogs.

TABLE 1
Comparison of Serum and Gut Concentration of Various Nitrogenous Metabolites in Two Segments of the Intestinal Tract

	Duodenum		Jejunum	
	Control	Uremic	Control	Uremic
Serum urea nitrogen, mg%	20	100	20	100
Gut urea nitrogen, mg%	14	66	19	86
Serum uric acid, mg%	0.5	0.5	0.5	0.5
Gut uric acid, mg%	1.7	1.7	3.8	1.9
Serum creatinine, mg%	1.0	6.0	1.0	6.0
Gut creatinine, mg%	3.5	3.7	6.3	2.8

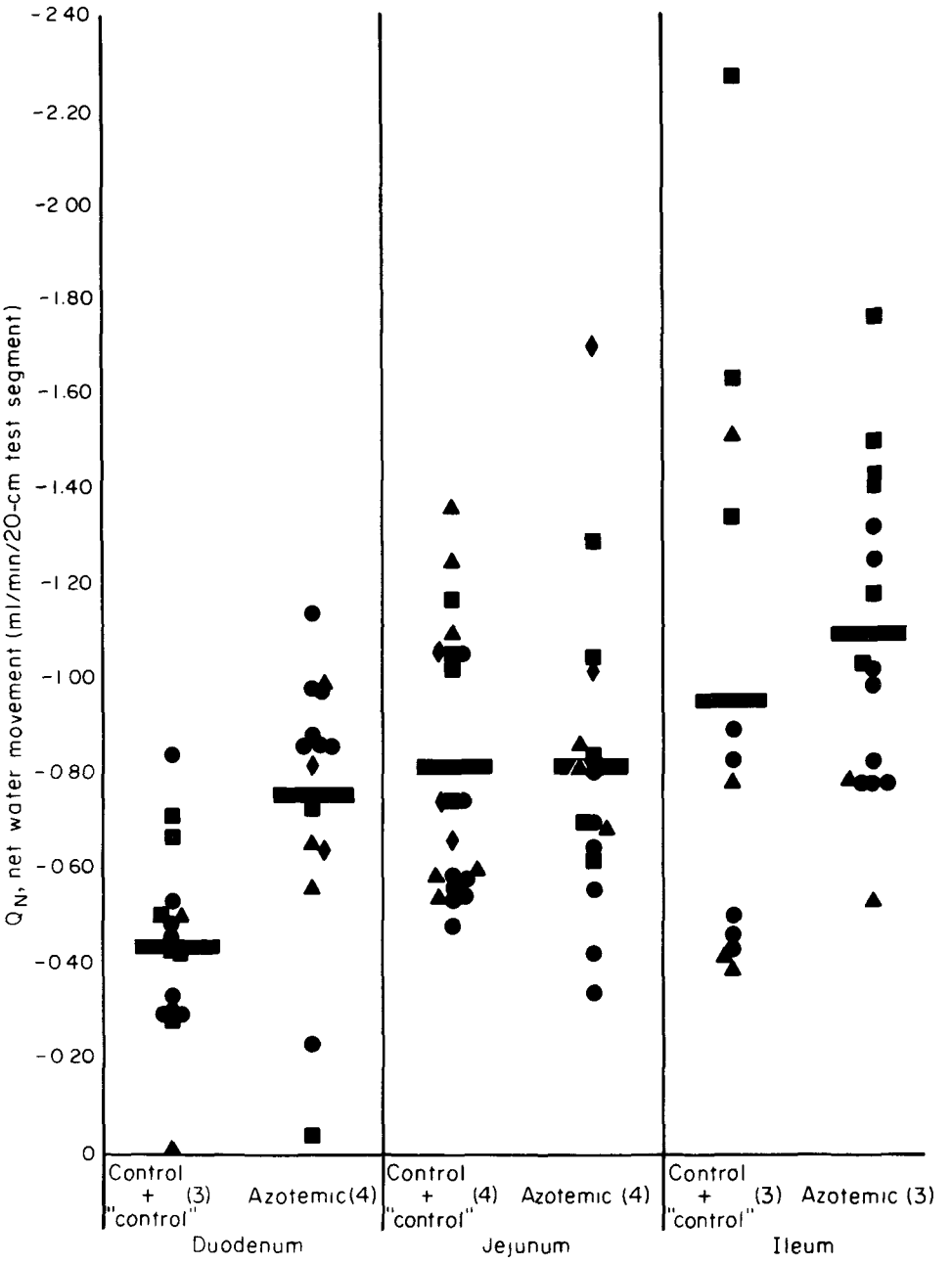

Fig. 2. Net rate of water movement in control plus "control" dogs and azotemic dogs. Different symbols represent individual perfusions of the same dog. The number of dogs examined are in parentheses while the horizontal bar represents average net water movement.

Figures 3 and 4 show the results of the triple lumen perfusion studies involving the flux rates of urea and creatinine. The linear relationship between blood urea and its flux rate is what one would expect if urea is passively transported. The correlation between gut creatinine flux rate and serum creatinine concentrations is not as good as that seen for urea (Fig. 4). Thus, there is a less favorable transfer of creatinine to the intestinal lumen as compared with urea.

No correlation was observed between serum uric acid concentrations and gut uric acid flux rates. We believe this occurs because the dog (unless of a Dalmation breed) possesses the enzyme uricase, whereas man does not. Thus, the dog does not obtain elevated serum uric acid levels during azotemia.

GSA Studies

The guanidinosuccinnic acid results on the three stabilized uremic dogs and six uremic patients are shown in Table 2. The GSA levels in the serum and urine of uremic patients agrees with those values previously reported by Stein et al. (23). To our knowledge, this is the first time GSA has been shown to be present in a chronically stabilized uremic dog. Thus, the possibility of using this dog model for delineating the role of GSA in the overall uremic syndrome now exists.

DISCUSSION

The gut aspiration and triple-lumen perfusion studies performed on control, "control," and stabilized azotemic dogs has demonstrated that a chronic indwelling percutaneous catheter can be placed and maintained in a dog over a prolonged period of time. The advantages of a chronic indwelling catheter are that the catheter is precisely fixed at a defined location in the intestinal tract, no anesthetic is necessary for performing the intestinal perfusion and no Thiry-Villa must be performed. Thus, the dog's physiologic processes in the gastrointestinal tract should be normalized after accommodating the catheter for a short period of time. Obviously, a perfusion study is a steady-state condition and does not represent necessarily the "true" situation.

It would appear that urea is definitely one compound that can be removed via an intestinal adsorbent. This is based upon the gut aspiration studies that indicated the concentration of urea in the intestinal tract was roughly 0.7 that of the serum urea concentration. In addition, as a result of the triple-lumen perfusion studies, we now know that large quantities of urea are capable of moving into the intestinal tract, depending upon the degree of azotemia. If one assumes an average length of 10 ft for the human small intestine, and that similar urea flux rates were obtained, a total of 33 g urea/d could be excreted into the small intestine of an azotemic person who had a blood urea nitrogen value of 80 mg%. Thus,

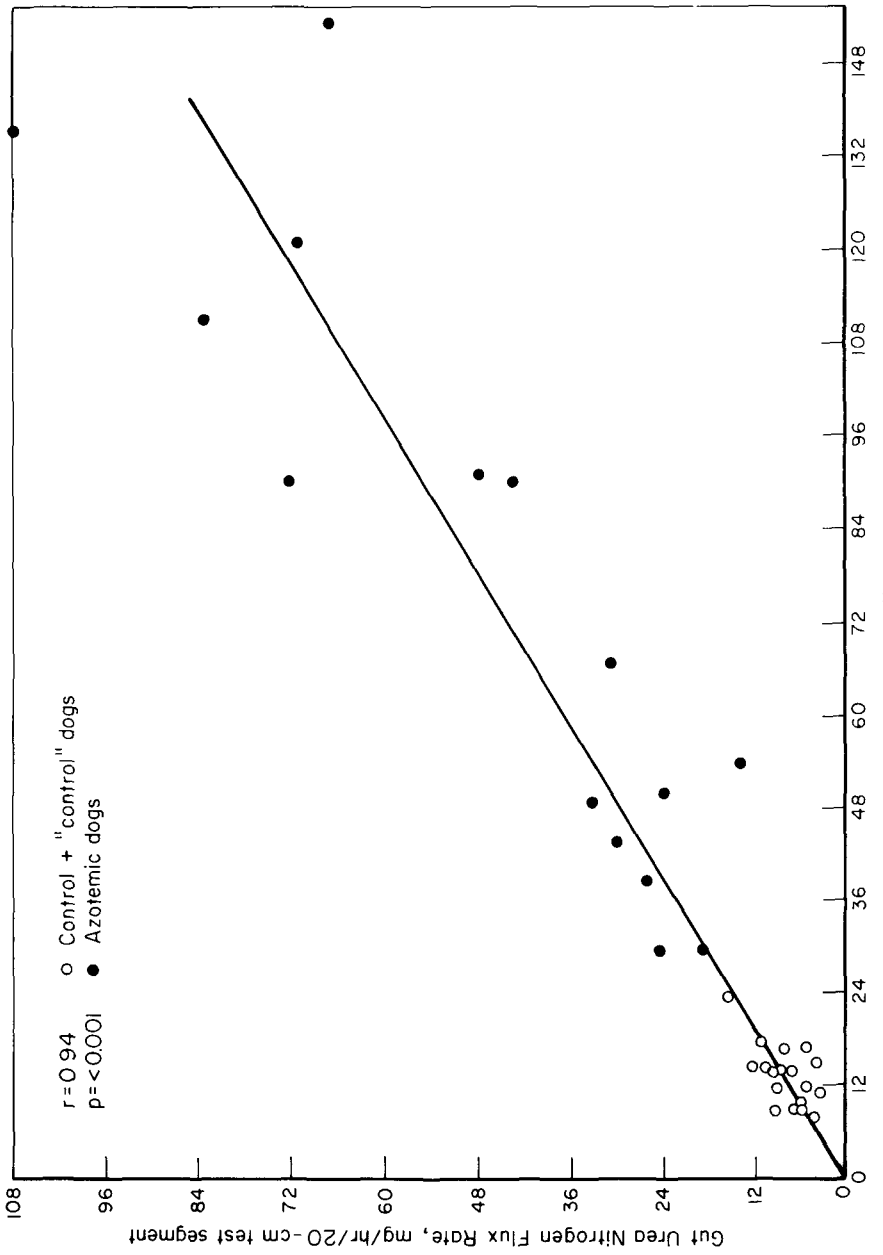

Fig. 3. Relationship between blood urea nitrogen levels and gut urea nitrogen flux rate.

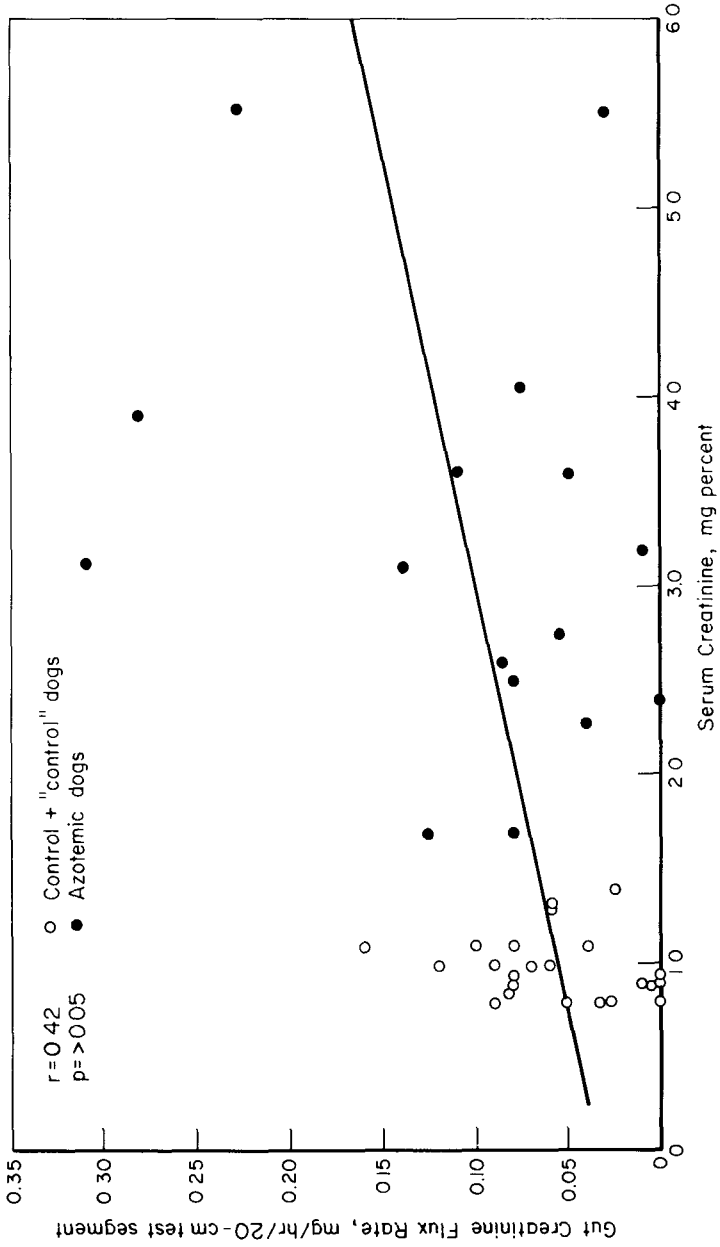

Fig. 4. Relationship between serum creatinine levels and gut creatinine flux rates.

TABLE 2
GSA Values in Uremic Patients and Stabilized Uremic Dogs

Name	Serum, GSA mg/100 mL	Urine, GSA mg/100 mL
J. P.	0.60	4.43
P. H.	1.04	2.40
H. R.	0.68	8.21
J. L.	0.21	3.72
S. L.	0.32	1.73
W. C.	0.46	0.35
Dog 39	<0.03	0.14
Dog 40	<0.03	0.15
Dog 43	0.03	0.28

large amounts of urea are available for binding or conversion by an enzyme in the small intestinal region.

Creatinine and uric acid removal via gastrointestinal adsorbents may prove more difficult. The reasons for this conclusion are: (1) a less favorable or undefined flux rate was observed; (2) a lower concentration of these metabolites exists in the gastrointestinal tract; (3) uric acid and its movement into the intestinal tract may be regulated by other than passive processes, i.e., facilitated diffusion (*24*); and (4) the relatively short period of time that an adsorbent system will be in the small intestinal tract.

In our studies, creatinine does move across the intestinal tract, but the correlation between the serum concentration and flux rate bordered on being significant. If this is true, then some other mechanism must be regulating its intestinal permeability, other than a passive process. However, the dog serum creatinine levels were relatively low (5–6 mg%) as compared to serum creatinine concentrations seen in uremic patients (15–25 mg%).

With a serum creatinine concentration of 5.0 mg%, the total creatinine delivered across a dog's small intestinal tract [average of 13 ft (*25*)] would approximate 72 mg/d under the conditions of these experiments. Thus, a dog (18-kg body weight) with an assumed equilibrated pool of 5 mg% (500 mg total) would excrete approximately 14% of its pool daily into the small intestinal tract.

Uric acid flux rates were nullified in these studies by the low serum levels of uric acid. This happened, we believe, because the dogs used possess the enzyme uricase. The presence of this enzyme would reduce the serum uric acid concentration, even with reduced kidney function. Thus, our perfusion studies indicated no correlation between serum uric acid levels and gut uric acid flux rates.

Preliminary results on the efficacy of the combination capsule system fed orally to stabilized azotemic dogs, and reported elsewhere, indicate the capsule system functioned as intended (*17*). Three separate feeding studies, covering 5, 4, and 2 d, respectively, indicated the blood urea nitrogen level as well as urinary urea output decreased. Glomerular filtration rate, as measured by endogenous creatinine clearance, was relatively constant during these feeding studies. Thus, the present combination capsule can remove up to 10 g of urea from the intestinal tract if 300 g of crenated capsules are administered.

In the limited clinical studies conducted to date, the microcapsules containing urease and zirconium phosphate have decreased blood urea nitrogen levels by 20% when 100 g of the capsules were ingested each day over a 7-d period (*18*). In addition, fecal nitrogen output increased 0.66 g/d. The theoretical nitrogen binding capacity is 1.2–1.4 g N/100 g capsules/d. Higher fecal nitrogen outputs could probably have been achieved if the oral supplementation of calcium salts had been administered separately, i.e., the calcium salts were administered with the capsules.

This microcapsule system may also be beneficial in other respects since it (1) increased serum bicarbonate levels and (2) decreased serum magnesium and potassium levels. The addition of bicarbonate ion to the body pool may be of importance in correcting the acidotic condition seen in most uremic patients. The reduction of elevated levels of either potassium or magnesium would be desirable as well.

The principal side effects associated with the ingestion of 100 g of the capsules/d were a sensation of fullness and satiety for a short period of time following ingestion. Some of the reported and potentially troublesome problems seen in these preliminary studies, e.g., decreased serum Ca^{2+}, increased serum phosphate and increased urinary sodium, can be minimized in future studies. As Kjellstrand et al. (*18*) stated, "the potential of this system is probably not for replacing dialysis, but to delay the onset of dialysis, to decrease the time or frequency of dialysis or to reduce body chemistry oscillations in order to prevent some of the side effects of dialysis."

SUMMARY

A uremic dog model and percutaneous catheter system have been developed for studying the feasibility of using ingestible adsorbents for removal of nitrogenous metabolites from the intestinal tract. Based upon the perfusion studies, it appears that urea is definitely one compound that may be removed via an ingestible adsorbent. The development of a combination capsule system (urease and zirconium phosphate) appears to hold promise as adjunctive therapy in uremic patients by lowering blood urea nitrogen levels.

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