bly reflects increased motor activity by the cyprids.

Changes in rate of oxygen uptake by barnacle cyprids reflect the energy cost of active transport of copper ions across excretory membranes of the cells of the intestinal epithelium. Earlier histochemical studies (1) suggested that copper ion is normally excreted by this route.

The progressive decrease of oxygen uptake as larger amounts of copper are added to the environment correlates as well with reduction of motor activity. Examination of a few individuals at each concentration revealed that duration of periods of swimming activity decreased progressively as more copper was added, except at very low concentrations. By 100 p.p.m. observable activity stopped immediately after immersion and the cyprid shell remained tightly closed.

Summary. Oxygen uptake by planktonic cyprids of *Balanus amphitrite niveus* was elevated by addition of 0.5, 1, and 5 mg of copper ion per liter of sea water. Concentrations from 10 to 500 mg/l progressively depress oxygen absorption. Analysis of individual patterns relates increased oxygen consumption to increased motor activity and energy cost of active transport.

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Effect of Urease Immunity upon Rats with Controlled Vitamin A Intakes* (28384)

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It has been reported that ammonia production due to bacteria in the gastrointestinal tract is depressed in animals fed levels of antibacterial agents known to stimulate growth(1,2). Of the various ammonia producing systems present in the gastrointestinal tract, urea hydrolysis is one which has been inhibited by antibiotics(2,3,4,5), sulfonamides(6), certain organic arsenicals(2), cyclic urea derivatives(7,8) and immunity to crystalline jackbean urease(9). Stimulation of growth concurrently with suppressed gastrointestinal ureolytic activity has been observed in both avian and mammalian species(2,8).

Present evidence indicates that animals immunized with urease excrete antiurease in the feces and urine(10). Presumably the antibody enters the gastrointestinal lumen where it cross-reacts with urease(s) which is predominately of bacterial origin. Such immunity has suppressed *in vivo* C^{14} urea breakdown and has yielded antisera which depressed urease activity in homogenates of gastrointestinal contents from non-immunized animals(2). Unpublished data from this laboratory show that ammonia concentration is lower in the gastrointestinal tracts of immunized animals than in non-immunized controls.

Recently Guerrant(11) demonstrated increased growth in rats fed a diet containing limited amounts of Vit. A and supplemented with antibiotics. A similar approach has been used in these studies to investigate this response of rats to immunization with jackbean urease under conditions where dietary antibiotics have produced an increase in growth. Reported herein are 2 experiments with rats immunized with crystalline jackbean urease and fed the USP Vit. A Assay

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Diet supplemented with controlled amounts of Vit. A. Data are presented upon growth, serum antiurease activity, liver Vit. A storage, gastrointestinal ammonia concentration and gastrointestinal urease activity.

Materials and methods. Urease extracted from finely ground jackbean meal† by the method of Sumner (12) was recrystallized at least 3 times using Dounce's procedure(13). Double distilled water from an all-glass apparatus was used in preparation of the enzyme and of all other solutions. The crystalline enzyme was stored in 0.85% NaCl at –4°C. Urease activity was determined using a urea-phosphate buffer as substrate(12). Kjeldahl determinations of protein nitrogen were found to range from 1.5 to 1.7 μg of nitrogen per unit‡ of urease activity. Appropriate dilutions for injections were prepared weekly using 0.85% NaCl. These were standardized and stored at 4°C without preservatives. Control animals were injected with equal volumes of 0.85% NaCl prepared as above but lacking urease.

Two successive experiments, each lasting 8 weeks, were conducted with a total of 100 weanling male Sprague-Dawley rats having an initial weight of 45-70 g. They were housed in individual cages and provided with food and water *ad libitum*. Food intakes and body weights were recorded weekly. The animals were depleted of Vit. A by feeding the USP Vit. A Assay Diet during the first 4 weeks. During this period one-half of the animals were actively immunized by single weekly injections of crystalline jackbean urease. Dosages per animal per week for the first 4 weeks, respectively, were 10, 15, 20, and 25 units per kg of body weight administered subcutaneously. Control animals received equal volumes of sterile 0.85% NaCl. During the 28 days following the 4 week depletion and immunization period, all rats were continued on the above diet and received daily dosages of either 2.2 or 22.0 USP units of Vit. A acetate in oil *via* oral catheter. At the end of the 28 day post-immunization period the animals were fasted for 16 hours and sacrificed. Livers were analyzed for Vit. A by the method of Ames *et al.*(14). Gastrointestinal tracts plus contents were removed following sacrifice of the animals in Experiment II and placed in plastic vials which were immediately immersed in a snow of dry ice. The specimens were stored at –10°C and analyzed for ammonia and ureolytic activity as previously described(8). Serum antiurease titers were obtained by the method of Visek *et al.*(10).

Results. There were no differences between immunized and control animals during the immunization period with respect to growth or growth per unit of feed consumed (Table I). However, during the repletion or post-immunization period, growth of the immunized animals was greater than control. When the data from both experiments were averaged, this increase was 31% for animals receiving 2.2 I.U of Vit. A and 17% for those receiving 22 I.U. These growth differences were statistically significant ($P<0.05$) for Exp. I and the combined experiments, but not for Exp. II alone. During the same period the immunized animals gained an average of 40% more per unit of feed consumed ($P<0.05$). Increasing the intake of Vit. A also increased growth ($P<0.05$) and improved efficiency of feed utilization.

Gastrointestinal ammonia concentration and urea splitting activity are presented in Table II. Either immunizing the animals or increasing the level of daily Vit. A supplementation resulted in reduced ammonia concentrations in the small and large intestines. Ammonia in the small intestine of immunized animals was lower than that of the controls by an average of 1284 and 113 μg per g wet weight on the lower and higher vitamin levels, respectively ($P<0.05$). The reduction in the large intestine was 802 and 86 μg on the respective vitamin levels. Increasing the dietary Vit. A level reduced ammonia concentration in the small intestine by an average of 1539 μg in control rats and by 368 μg in those which were immunized ($P<0.01$). In the large intestine a similar reduction of 1583 and 867 μg was found in the 2 respective groups ($P<0.05$).

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‡ A unit of urease activity is that quantity of enzyme which will liberate one mg of ammonia nitrogen in 5 minutes in a urea-phosphate buffer at pH 7.0 at 20°C.

TABLE I. Growth of Weanling Rats Immunized to Jackbean Urease While Undergoing Vit. A Depletion Followed by Repletion. (No. of animals in parentheses.)

Control	Depletion (0-4 wk)	Repletion (4-8 wk) 2.2 I.U. daily	Repletion (4-8 wk) 22.0 I.U. daily
	Exp I		
Wt gain, g			
Control (30)	124.3 ± 8.4*	23.3 ± 4.5	42.9 ± 18.1‡
Immunized (30)	126.9 ± 14.1	42.6 ± 11.5†	50.9 ± 10.5†‡
Gain g/feed g			
Control	.29 ± .05	.20 ± .01	.27 ± .09‡
Immunized	.29 ± .03	.27 ± .02†	.38 ± .10†‡
	Exp II		
Wt gain, g			
Control (20)	82.3 ± 3.8	41.0 ± 13.3	74.6 ± 9.1§
Immunized (20)	84.7 ± 3.2	46.1 ± 8.7	87.6 ± 8.7§
Gain g/feed g			
Control	.32 ± .01	.13 ± .02	.18 ± .02§
Immunized	.32 ± .01	.18 ± .02†	.25 ± .04†§

* Mean ± standard error.
† Immunization vs. control significant at $P<.05$.
‡ Higher vs. lower vit A supplementation significant at $P<.05$.
§ Higher vs. lower vit A supplementation significant at $P<.01$.

Intestinal ureolytic activity is expressed as μg of urea hydrolyzed per g of wet weight. Suppression of urease activity by either immunization or increase in Vit. A intake was similar to that observed with intestinal ammonia concentrations. The variation in urease activity within treatments was greater than that observed for ammonia. Immunization reduced ureolytic activity in the small intestine of animals receiving 2.2 and 22.0 I.U. Vit. A daily by 906 and 1250 μg, respectively, while in the large intestine this reduction was 3506 and 2189 μg, respectively. Increasing Vit. A intake reduced ureolytic activity in the small intestine by an average of 2218 μg in control animals and 2562 μg in those immunized ($P<0.05$). This reduction in the large intestine was 4382 and 2965 μg for control and immunized animals, respectively.

Antibody titers in the immunized animals were detected in serum dilutions of $10^{-3.7}$ while those in controls ranged from $10^{-1.0}$ to $10^{-1.7}$ (Table III). The control animals had levels of circulating antiurease usually found in non-immunized animals and human subjects(9,15). Antibody titers were similar in all immunized groups.

Liver Vit. A storage was not influenced by immunization at either level of vitamin intake (Table IV). Histological examinations of sections of jejunum and trachea revealed no differences consistent with Vit. A intake or immunization to jackbean urease.

Discussion. Actively immunizing rats with

TABLE II. Ammonia Concentration and Urea Hydrolysis in Gastrointestinal Contents Following Immunization with Jackbean Urease and Vit A Supplementation.

	Vitamin A intake daily: 2.2 I.U. Small intestine	2.2 I.U. Large intestine	22.0 I.U. Small intestine	22.0 I.U. Large intestine
Ammonia, μg/g wet wt				
Control	4582 ± 932*	5299 ± 823	3043 ± 380§	3716 ± 155‡
Immunized	3298 ± 150†	4497 ± 1440	2930 ± 358†§	3630 ± 355‡
Urea hydrolysis, μg/g wet wt				
Control	6173 ± 932	8810 ± 1311	3955 ± 851‡	4428 ± 1256
Immunized	5267 ± 987	5204 ± 1403	2705 ± 519‡	2239 ± 998

* Mean ± standard error.
† Immunization vs. control significant $P<.05$.
‡ Higher vs. lower vit A supplementation significant $P<.05$.
§ Higher vs. lower vit A supplementation significant $P<.01$.

TABLE III. Serum Antiurease Titers by Hemagglutination* of Control and Immune Animals on Limited Vit A Intake.

Group	Range of dilutions with sensitized RBC†	Range of dilutions with unsensitized RBC
Control		
2.2 I.U. vit A	$10^{-1.0}$–$10^{-1.7}$	$10^{-1.0}$–$10^{-1.7}$
22.0 I.U. vit A	$10^{-1.0}$–$10^{-1.7}$	$10^{-1.0}$–$10^{-1.7}$
Immunized		
2.2 I.U. vit A	$10^{-3.0}$–$10^{-3.7}$	$10^{-1.0}$–$10^{-1.7}$
22.0 I.U. vit A	$10^{-2.7}$–$10^{-3.7}$	$10^{-1.0}$–$10^{-1.7}$

* Dilutions of $10^{-1.0}$ to $10^{-1.3}$ were considered negative and $10^{-1.7}$ equivocal.
† Human type O erythrocytes.

jackbean urease during Vit. A depletion produced no gross untoward effects. Greater growth and efficiency of feed conversion were noted following the fourth week in the immunized group. This latent period has been previously observed in animals fed adequate levels of Vit. A(9). The level of Vit. A, however, altered growth response in that increasing the daily intake from 2.2 to 22.0 I.U. decreased by approximately 50% the difference in growth between control groups and those immunized. Preliminary data suggest that immunization does not improve growth on this dietary regimen when Vit. A supplementation exceeds the daily requirement. A similar effect in rats has been found with antibiotics(11). Liver Vit. A values were unaltered by immunization, further suggesting that urease antibody had a sparing action on the vitamin. A sparing effect on Vit. A has also been observed with dietary antibiotics in rats(11) and chicks(16).

Enzyme activity and ammonia concentration in the gastrointestinal tract were influenced by both urease immunization and Vit. A level. Immunized animals had less free ammonia and less ureolytic activity in the small and large intestine. This observation corroborates previous evidence that urease antibody must enter the intestinal lumen, cross-react with bacterial urease(s) and remain active from its site of secretion until eliminated from the gastrointestinal tract. Antibodies to urease(10) and to other toxins (17) have been observed in feces and urine of immunized subjects. The ability of these antibodies to maintain immunological competence under the conditions encountered is indicated.

TABLE IV. Liver Vit. A in Rats Following Vit. A Supplementation for 28 Days.

Vit A daily	2.2 I.U.	22.0 I.U.
	I.U. vit A/g wet wt	
Control	1.4 ± 1.2*	14.8 ± 7.1
Immunized	1.3 ± 1.1	13.0 ± 3.2

* Standard error of mean.

Of interest is the observation that increasing the dietary Vit. A decreased gastrointestinal ammonia concentration and urease activity. The influence was greater in control than in immunized animals. Suppression of urea hydrolysis has also been found in animals fed various antibacterial agents and in animals reared under germ-free conditions(2, 4,5,18). The presence of circulating antiurease, however, did not produce the characteristically thin mucosa observed with antibiotic supplementation(19) or germ-free conditions(20).

Summary. Two successive experiments, each lasting 8 weeks, were conducted with a total of 100 weanling male rats. Animals actively immunized with crystalline jackbean urease and given either 2.2 or 22.0 I.U of Vit. A daily following a 4-week depletion period grew faster and were more efficient in feed utilization. Both immunizing with urease and increasing the dietary Vit. A level reduced ammonia production and ureolytic activity in the gastrointestinal tract. The evidence indicates that depression of ammonia production and urease activity in the gastrointestinal tract is beneficial to animals fed limited levels of Vit. A.

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Quantitative Relation of EDTA to Availability of Zinc for Turkey Poults.* (28385)

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Addition of disodium ethylenediaminetetraacetic acid (EDTA) to a diet containing isolated soybean protein has been shown to increase the availability of zinc for turkey poults(1). Scott and Zeigler(2) showed that EDTA would also improve zinc availability for chickens and that 300 mg of EDTA per kg of diet was more effective than 150 mg/kg. Lease *et al.*(3) found that zinc in sesame seed meal could be made more available to chicks by the addition of EDTA to the diet.

The mechanism by which EDTA improves the availability of zinc is not known. The present experiments were conducted to measure the relative effectiveness of EDTA and zinc as dietary supplements to a zinc-deficient diet for turkey poults.

Experimental. Broad Breasted Bronze turkey poults were fed a practical stock diet for 5 days before being divided into groups of 10 birds each and fed the experimental diets. The basal diet (Table I) contained isolated soybean protein and was supplemented with zinc oxide or EDTA as indicated in Table I. The zinc content of a similar basal ration was 25.5 mg/kg(1). The birds were housed in electrically heated cages which were coated with an epoxy resin to reduce the amount of zinc available in the environment. The experiments were continued for 17 days and the poults were weighed twice weekly and their legs were scored for perosis.

Results and discussion. When poults were fed the basal diet alone growth was very poor and a mild degree of perosis was observed. As zinc was added to the diet, growth was improved in proportion to the amount of zinc added to the level of 30 mg/kg. In Experiment 3, 50 and 200 mg/kg of zinc were also added to observe the effect of higher levels on the severity of perosis. Perosis increased as the level of zinc was increased to about 15 to 30 mg/kg and then decreased as higher levels of zinc were added, probably because at very low zinc levels growth was not sufficient to allow the expression of this deficiency symptom. It was only when reasonably good growth could be obtained that zinc became limiting for bone formation.

EDTA was added in varying levels, both to the basal diet alone and to the basal diet containing 7 and 10 mg/kg of added zinc.

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