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Effect of Urease Injection on Body Weights of Growing Rats and Chicks.* (26046)

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Antibacterial agents, fed in trace amounts, enhance growth of birds and animals under a variety of environmental conditions(1-4). Some investigators have postulated that these agents suppress toxins produced by gastrointestinal bacteria. Ammonia has been suggested as one of the toxins whose production is inhibited(5-8). We have recently reported that addition of 100 ppm of 3 antimicrobial agents to a casein diet significantly decreased *in vivo* hydrolysis of C-14 urea by rats and reduced production of urease by gastrointestinal bacteria(9). Our preliminary investigations have shown growth stimulation in rats and chicks immunized with jackbean urease (10, 11). Urease stimulates production of antiurease in rabbits which on passive transfer to normal rabbits produces a drop in blood ammonia(12). In the experiments herein reported the effects of jackbean urease injections on growth of rats and chickens fed soybean diets of suboptimal nutrient content are described.

Materials and methods. Urease was crystallized from jackbean meal and recrystallized twice from ethyl alcohol by the method of Kirk and Sumner(12). Enzymic activity was determined and purity of the preparations was checked using paper chromatography. The enzyme dissolved in 0.85% NaCl was injected intraperitoneally into rats and subcutaneously into chickens. Injections were made during the first 4 weeks of each experiment which lasted 8 weeks. In the rat experiments, injections were made every other day whereas in the chick experiment, injections were made on the same 3 successive days each week for the first 4 weeks. Control subjects received 0.85% NaCl in equal volume to that received by those which were immunized. In rats, the starting doses were 10 units† per kg of body weight, progressing to 25 units per kg body weight. Each rat received a total of 30 units of urease. The chicks received initially doses of 0.1 unit per injection and progressed to a dose of 10 units

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† One unit of urease activity is that amount of urease required to liberate one mg of ammonia N in 5 min. in a phosphate buffer at pH 7.0 at 20°C.

TABLE I. Body Weights of Male Sprague-Dawley Rats and Cockerels Immunized with Jack-bean Urease. (No./group shown in parentheses.)§

	0-4 wk	4-8 wk	0-8 wk
Chicks			
Wt gain, g			
Control (20)	405 ±11.7 *	777 ±35.8	1182 ±38.2
Exp. (19)	405 ± 9.8	905 ±26.6 ‡	1310 ±26.0 ‡
Avg feed/g gain			
Control (20)	2.02 ± .25	2.42 ± .19	2.28 ± .17
Exp. (19)	1.90 ± .29	2.27 ± .05	2.16 ± .15
Rats—Exp. I			
Wt gain, g			
Control (10)	151.2 ± 6.0	97.0 ± 4.9	248.2 ± 7.1
Exp. (20)	154.0 ± 4.2	107.8 ± 3.9	261.8 ± 6.9
Feed/g gain			
Control (10)	2.73 ± .07	5.37 ± .29	3.72 ± .06
Exp. (20)	2.69 ± .04	4.71 ± .12†	3.51 ± .05†
Rats—Exp. II			
Wt gain, g			
Control (10)	151.4 ± 4.4	101.9 ± 2.8	253.3 ± 5.6
Exp. (20)	150.1 ± 2.7	110.1 ± 2.8	260.2 ± 4.7
Feed/g gain			
Control (10)	2.63 ± .04	5.01 ± .13	3.58 ± .04
Exp. (20)	2.57 ± .03	4.59 ± .09†	3.41 ± .03†

* Stand. error of mean. † Statistically significant at 5% level. ‡ Statistically significant at 1% level. § Avg initial wt, g, for control and experimental groups respectively were: Chicks 38.3 ± 1.0 and 38.2 ± 0.6. Rats—Exp. I 39.2 ± 1.3 and 40.6 ± 0.4; Exp. II 56.9 ± 1.2 and 57.1 ± 0.8.

per injection in the fourth week. Each bird received a total of 52.5 units of urease. All experiments were conducted in a temperature controlled laboratory. The rats were individually housed in wire bottom cages and the chicks were maintained in batteries in groups of 10. Feed and water were provided *ad libitum*. Feed intakes and body weights were determined weekly. The basal diet for chicks was compounded as follows: solvent extracted soybean meal (50% protein) 40.0; sucrose 41.8; fat (Crisco) 8; cellulose (Alphacel) 4.0; salt mixture (U.S.P. XIV) 4.0; and vitamin mixture in sucrose 2.2. The vitamin mixture per 100 lb. of diet contained Vit. A acetate, 900,000 units; Vit. D (Viosterol) 100,000 units; dl-alpha tocopherol (250 I.U./g) 5 g; ascorbic acid 45 g; inositol 5 g; choline chloride, 75 g; menadione 2.25 g; para-amino benzoic acid 5 g; niacin 5 g; riboflavin 1 g; pyridoxine-HCl 1 g; thiamine-HCl 1 g; calcium pantothenate 3 g; biotin 20 mg; folic acid 90 mg; and Vit. B_{12} 1.35 mg. For the rat experiments the soybean oil meal of the diet was decreased to 31.4% and sucrose was increased accordingly. Two experiments with weanling male Sprague-Dawley rats having an initial weight of 40-50 g were conducted. Chicks used for the study were day-old cockerels of the Vantress-Arbor Acre cross.

Results. Chicks receiving urease injections gained an average of 1310 g in 8 weeks as compared to controls which gained 1182 g (Table I). This difference of 128 g in favor of the immunized birds is statistically significant ($P < 0.05$) and was the result of increased growth during the 4-8 week period since weight gains during the 0-4 week period were the same. Efficiency of growth expressed as g of feed per g of gain was in favor of the immunized birds during the entire experiment, being 2.0 *vs.* 1.90 during 0-4 weeks and 2.42 *vs.* 2.27 during 4-8 weeks. As an average for the entire 8 week experiment, the immunized birds required 2.28 g of feed per g of gain and controls required 2.16 g. Contrary to the results with rats, as described below, we were unable to demonstrate to our satisfaction the presence of antibodies in sera of the immunized chickens. This is not suf-

ficient evidence, however, to prove that antibodies to urease were not responsible for the growth effects observed(13-15).

In 2 successive experiments with a total of 60 rats, a combined test of significance demonstrated at a 1% level of probability that rats receiving urease injections require less feed per g of weight gained. In each experiment the feed required per g of weight gain was about 10% less for urease-treated rats than for control rats when calculated over the 4-8 week period (4.71 g per g of gain *vs.* 5.37 g per g of gain in Exp. I and 4.59 *vs.* 5.01 in Exp. II). In each case this difference is statistically significant at the 5% level. The increase in efficiency of food conversion to weight gain in the last four weeks of both experiments was responsible for overall differences in efficiency of food conversion for the entire 8 week experimental period (3.51 g of feed per g of gain *versus* 3.71 in Exp. I and 3.41 *versus* 3.58 in Exp. II). These differences are significant at the 5% level. Average gains in body weight were in favor of the immunized rats. In Exp. I average gain during the 4-8 week or post injection period was 97.0 g for control animals and 107.8 g for experimental animals. Corresponding values for Exp. II were 101.9 and 110.1. There was appreciable variation from rat to rat and none of the weight gain differences were statistically significant. In both experiments with rats the difference in average weight gains between control and treated animals for 0-4 weeks is rather small compared to its standard error. Since the rats had initially been distributed between control and experimental groups so as to make average initial weights nearly identical, it was thought that this effect might represent a close dependence between weight gain and initial weight. Analysis of covariance, however, demonstrated dependence on initial weight to be negligible and the adjusted estimates differed only trivially from those not adjusted. The figures shown in Table I are not adjusted for initial weight. No differences were observed in gain or feed required per gram of gain in the 0-4 week or injection periods.

At sacrifice no gross differences were observed between the organs of the immunized rats and their controls. Weights of adrenals, liver, spleen, and kidneys were not different between control rats and immunized rats. Sera collected from immunized rats were found to contain as much as 2.5 units of antiurease‡ per ml. The antisera protected normal rats against fatal doses of urease and inhibited the ureolytic activity of a phosphate buffer extract of gastrointestinal contents from these animals. Before sacrifice 4 randomly selected animals of the control group and 8 of the immunized group were studied for *in vivo* C-14 urea metabolism after having food and water withheld for 16 hours. In 4 hours after injection of C-14 urea, immunized animals expired an average of 2.58% of the radioactivity as $C^{14}O_2$ and control animals expired 4.31%. The urea splitting activity of the gastrointestinal contents of immunized animals averaged 40% less than that of controls. These differences were statistically significant ($P<0.05$).

Discussion. In all of the above experiments growth effects were not observed during the 0-4 week or injection period suggesting that antibody production had not reached a sufficient level. Experiments with rabbits in our laboratory indicate that easily detectable antiurease titers in the sera following a weekly schedule of urease injections do not occur until the third to fourth weeks. The time lapse before growth effects are evident suggests that antibodies are necessary to produce the results observed. The immunized rats of Exp. II had less urea splitting activity in their gastrointestinal contents as measured by 2 methods. Based on these and previous findings, it is reasonable to postulate that growth effects of dietary antibiotics and urease immunity would be additive. Antibodies to urease suppress the enzyme which is formed and antimicrobial agents decrease its synthesis(9). Our experiments now in progress with chicks fed soybean diets support this hypothesis.

Summary. Experiments with 40-day-old chicks and 60 weanling Sprague Dawley rats were conducted. The experimental subjects were immunized with jackbean urease injected

‡ A unit of antiurease is that amount of antibody which will inhibit one unit of urease activity.

during the first 4 weeks of experiments lasting 8 weeks. During the 4-8 week or post injection period, the immunized chicks gained 905 g *vs.* 777 g for their controls and were more efficient in converting feed to weight gain. In 2 successive experiments immunized rats grew slightly faster and showed a difference in feed efficiency which was statistically significant. In both chicks and rats there were no differences in body weight during the 0-4 week injection period. Antibodies to urease were demonstrable in sera of immunized rats by *in vivo* methods with C-14 urea and by *in vitro* tests. Soybean diets were fed.

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Haptoglobin Hemoglobin Metabolism in Rabbits Studied with I^{131} and Fe^{59} Labelled Complexes.* (26047)

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Several studies(1-3) attempting to trace the fate of haptoglobin-hemoglobin (HpHb) in man have pointed to the complexity of the problem. They have shown that the complex disappears from the blood either in a linear or exponential manner with a half life of about 4 hours, and it has been suggested that the complex may be removed *in toto* by the reticuloendothelial system(1). Studies in man have been restricted to short periods of observation because of the small amounts of hemoglobin that can be injected safely. The present experiments with I^{131}-labelled haptoglobin-hemoglobin (I^{131}HpHb) and haptoglobin Fe^{59} labelled hemoglobin (HpHbFe^{59}) complexes were undertaken in rabbits to follow the metabolism of the complex over longer periods of time. Attempts were also made to evaluate more directly the role of the reticuloendothelial system in the metabolism of the complex.

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Materials and methods. Concentration of haptoglobin in serum was determined by starch zone electrophoresis on blocks, 45 cm x 33 cm, using potato starch and barbital buffer pH 8.6 μ 0.05 at 400 volts for 15-18 hours(4). Increasing amounts of hemoglobin were added to 0.1 ml aliquots of serum, and the mixtures were introduced into 3 parallel rows of 15 slits. After electrophoresis, control sera containing hemoglobin in 3-fold excess of the combining capacity demonstrated