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THE ABSENCE OF GASTRIC UREASE IN GERM-FREE ANIMALS

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SUMMARY

1. Measurements were made of the urease (urea amidohydrolase, EC 3.5.1.5) content of the gastric mucosae of conventional, germ-free, and fetal animals to test whether gastric urease arises from micro-organisms or is constitutive.

2. Homogenates of gastric mucosae of conventional sheep, dogs, cats, rats, guinea pigs, and chickens; of germ-free rats, guinea pigs, chickens, dogs, cats, and pigs; and of fetal dogs, cats, rats, sheep, and humans were prepared and analyzed for urease activity.

3. There was no urease in the gastric mucosae of the germ-free or fetal animals, although enzymic activity was usually found in the same tissue from their conventional adult counterparts, which had a wide range of enzymatic activity.

INTRODUCTION

The enzyme urease (urea amidohydrolase, EC 3.5.1.5) has been found in the gastric mucosa of many animals. However, there is a controversy as to whether it is a constitutive[1] enzyme or whether it owes its presence in the stomach to contamination by bacterial flora[2–6]. Studies in which a cat was fed with antibiotics (penicillin, oxytetracycline, terramycin, sulfaguanidine)[2–5,7] for 5 days showed that, under these conditions, there was a complete absence of urease activity. The objection has been raised, however, that these agents may have been inhibiting[8] or destroying the enzyme. Although experiments in which the treatment of mucosal suspensions *in vitro* with the above-mentioned antibiotics showed no effect on urease activity, the possibility still existed that there might be an inhibition of a possible biosynthesis of urease by the mucosa. In an attempt to resolve these problems and to provide evidence which would permit a clear decision to be made as to the role of bacterial contamination, a series of experiments were performed on gastric mucosae from both conventional animals and their germ-free counterparts. Germ-free animals were (a) those which were born and raised under bacteriologically sterile conditions and (b) fetuses obtained by Caesarian section from conventional animals.

METHODS

Sources of tissue

Germ-free animals. Chicks and guinea pigs were obtained from the colony at Bethesda. Germ-free rats were obtained from the LOBUND Institute and kindly provided by Dr. JAMES REYNIERS, then at Notre Dame University. The animals were lightly anesthetized with ether and killed by a blow on the head. The stomachs were removed at once and the contents tested for acidity. After gentle and thorough washing with distilled water to remove the contents, the stomachs were used immediately or quickly frozen and stored for later use.

Frozen stomachs of germ-free dogs, cats, pigs, and rats were generously supplied by Dr. RICHARD C. GRIESEMER of the Department of Veterinary Pathology of Ohio State University.

Fetal animals. Tissues from the following animals were used: human, dog, cat, rat, and sheep. In the case of the sheep, samples were taken from all four stomachs, homogenized, and used immediately. Human fetal stomachs were generously supplied by Dr. R. G. WILLIAMS of the Department of Anatomy, University of Pennsylvania.

Conventional animals. Mucosa was stripped from the stomachs of the dog, cat, the fourth stomach of the sheep, and the proventriculus of the chicken. The whole of the stomachs from rats and guinea pigs were used because of their small size. All tissue was frozen as soon as possible after excision, if it was not immediately prepared for analysis.

Method of preparation

Portions of whole stomach or of mucosa were rapidly homogenized with ice-cold water or phosphate buffer (Na_2HPO_4/KH_2PO_4, 0.36 M, pH 6) in an ice-cooled Virtis homogenizer. After centrifugation, the supernatant was collected for analysis. Alternatively, the tissue was ground in a sterile mortar and pestle with Merck's reagent grade sea sand which had been thoroughly acid-washed, freed of acid, and sterilized.

Method of analysis

The procedure of CONWAY[9] was used at 25° for measurement of urease activity. In addition to controls at zero time, incubations of homogenates with urea were carried out for 20 and 60 min. In all cases, the diffusion time, after addition of saturated K_2CO_3 solution, was 2 h.

RESULTS

The stomachs of all the germ-free chickens, guinea pigs and rats had contents with a pH well below 3. The mucosae were carefully examined by eye and observed to be free of gastritis, erosions or ulcerations.

The results in Table I show that there was no gastric urease either in those animals born and raised under sterile conditions or in those having no opportunity to acquire bacterial flora (fetuses). One human fetal stomach, however, which had deliberately been left exposed overnight at room temperature had a small but definite urease activity.

The gastric mucosae of conventional animals, on the other hand, may or may

TABLE I

ASSAY OF GASTRIC MUCOSA OF GERM-FREE AND FETAL ANIMALS FOR UREASE ACTIVITY

The mucosa was homogenized with water and the homogenate incubated with 4.6 mM urea for 20 and 60 min in a classical Conway unit. The reaction was stopped with saturated K_2CO_3 solution and the liberated ammonia allowed to diffuse into 1 ml of 5 mM HCl. (Diffusion time was 2 h). Excess HCl was titrated with 50 mM $Ba(OH)_2$. Urease activity is expressed as μmoles urea hydrolyzed per mg dry wt. of tissue per h. Zero activity is equivalent to <0.010 μmole urea hydrolyzed per mg dry wt. of tissue per h.

Germ-free			*Fetal*		
Species	*Number of animals*	*Activity*	*Species*	*Number of animals*	*Activity*
Chicken	4	<0.010	Dog	1	<0.010
	4*	<0.010	Cat	5	<0.010
Guinea pig	3	<0.010	Rat	23	<0.010
Rat	12	<0.010	Sheep	2	<0.010
Dog	2	<0.010	Human	5	<0.010 in 4
Pig	2	<0.010			trace (0.012)
Cat	2	<0.010			in 1**

* Penicillin added to diet.
** Stomach deliberately left exposed to air at room temperature overnight.

not contain urease (Table II). Enzyme activity was demonstrated in only 1 of 19 guinea pigs. Neither contamination of the diet with rat feces nor substitution of soybean meal for the normal diet had any effect on this result. None of the chickens tested

TABLE II

ASSAY OF GASTRIC MUCOSA OF CONVENTIONAL ANIMALS FOR UREASE ACTIVITY

Urease activity is expressed as μmoles urea hydrolyzed per mg dry wt. of tissue per h. Zero activity is equivalent to <0.010 μmole urea hydrolyzed per mg dry wt. of tissue per h.

Species	*Number of animals*	*Activity*
Chicken	3	<0.010
Chick	4 (control)	<0.010
	4*	<0.010
Guinea pig	11 (control)	0.042§
	4**	<0.010
	4***	<0.010
Rat	7	0.018§§
		<0.010§§§
Dog	4	0.410†
Cat	3	0.230††
Sheep	2	<0.010

* Penicillin in diet.
** Diet contaminated with rat feces.
*** Soy bean diet.
§ 1 of 11 animals. The rest had <0.010.
§§ 2 of 7 animals had urease.
§§§ 5 of 7 animals had no urease.
† 2 of 4 animals had urease.
†† All animals tested had urease.

showed the presence of the enzyme. On the other hand, in the stomachs of 2 of 7 rats there was a small activity. In dogs, of the 4 animals used, one had moderate activity and one was very high in the enzyme. All of 3 cat stomachs tested contained large amounts of urease.

Alternative methods of preparation of the homogenate appeared to have no effect on the results. There was no destruction of any urease present if the whole stomach was frozen and thawed, although similar treatment of homogenates resulted in some loss of activity. In these experiments, however, all homogenates were prepared and used at once, either from the fresh or the frozen tissue.

DISCUSSION

The results of these studies demonstrate that there is no urease activity in gastric tissue obtained from animals which have not been contaminated by bacteria. In all of the germ-free and fetal animals tested (Table I), no trace of this enzyme could be found. On the other hand, young or adult animals grown under normal conditions yielded gastric mucosae in which urease activity was occasionally demonstrated (Table II). All but the fetal animals were making hydrochloric acid. Therefore, one could not equate an absence of urease with an achlorhydric stomach[6].

These findings parallel those in which animals were treated with antibiotics. The objection that the enzyme was being inhibited *in vivo* could be answered by the results of *in vitro* experiments in which it was demonstrated that a mixture of penicillin, oxytetracycline, and sulfaguanidine had no effect on urease activity, whether in a homogenate or in a jack-bean meal preparation[6]. Any possibility that the synthesis of urease by the animal itself was being blocked by these antibiotics is most unlikely in view of the results obtained with germ-free animals.

It is of interest to consider the results obtained by various workers on the effects of oxytetracycline. In an earlier report LIEBER AND LEFÈVRE[8] stated that the action of oxytetracycline was the pharmacodynamic one of inhibition of the urease. However, later, the same authors[10] advanced the view that the effect was antibiotic. BELDING AND KERN[11] found that although oxytetracycline inhibited jack-bean urease *in vitro*, it did not behave unequivocally in the same manner with all cat gastric mucosae tested. In addition, these authors, in their studies with cat fetuses, could not confirm the work of CARDIN[12] who claimed to have found gastric urease in fetal cats, dogs, and humans. Our results with human fetal stomach tissue do not agree with Cardin's findings on human fetuses. Similarly, we do not confirm the demonstration of urease in the stomach of the fetal sheep as reported by CONWAY and collaborators[13]. Our measurements were made on absolutely fresh mucosa obtained immediately after the fetus had been removed by Caesarean section.

That urease occurs at all in gastric mucosal tissue is dependent on the presence of specific urease-containing bacterial strains[14–16]. RAHMAN AND DECKER[17] confirmed this assumption in their analyses of ruminal mucosae. In this connection it is interesting to note that if there was a time delay between the isolation of the fetal mucosa and the assay, urease activity appeared as the mucosa became contaminated (Table I).

Further strong evidence that gastric urease is bacterial in origin has been provided by the convincing studies of LEVENSON *et al.*[18] on the metabolism of urea in the conventional and the germ-free rat. These workers found no significant carbon

dioxide production in germ-free animals given radioactive urea either by injection or by gastric intubation. The trace amount of urea hydrolyzed was accounted for by the well-known non-enzymatic conversion of urea to ammonia[16]. WARREN AND NEWTON[19] in their report on portal and peripheral blood ammonia concentrations in germ-free and conventional guinea pigs provide added substantiation for the role of bacteria in the gut, as do the studies of DUCLUZEAU *et al.*[20] on the hydrolysis of urea by treatment of germ-free rats with ureolytic and non-ureolytic strains of bacteria.

The suggestion[1] that gastric urease plays a necessary part in a local defense mechanism against the acid secretions and therefore is important as a constitutive enzyme is disproved by these results and by the finding that the mucosae of pigs have never been found to contain urease. Germ-free animals secreted acid and did not have gastric urease. Their mucosae were quite normal and without visible evidence of gastric erosion.

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