Effect of Certain B Vitamin Deficiencies on Gastric Secretion in the Rat. (19060)

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Evidence has been accumulating for a number of years that nutritional deficiencies may impair the process of gastric secretion (1,2). Pellagra has been associated with a high incidence of achlorhydria. Goldsmith and associates(3) have observed recently the depression (absence in one) of hydrochloric acid secretion in two patients while on a pellagragenic diet with return of function when the diet was supplemented with nicotinic acid. In animal work, decreased acid secretion in vitamin deficient dogs has been observed; marked increases followed supplementation with a B complex preparation(4) or with yeast(5). Dyer and Roe(6) found that thiamine deficiency decreased acid secretion only when their rats were moribund, whereas Shay and associates(7) reported that this deficiency increased secretion.

The following experiments were conducted to obtain further information regarding the influence of certain specific B vitamin deficiencies on the secretory process.

Methods. Male rats of the Sprague-Dawley strain were received from the National Institutes of Health breeding colony at weaning (weight, 40-55 g; age, 21-27 days) and littermates were distributed equally to form groups of similar mean weight. The animals were housed individually in wire mesh bottom metal cages and had continuous access to tap water. Diet was supplied *ad libitum* to all except the pair-fed animals which were given their allotment of food each day before noon. Lack of water or food, or feeding the pair-fed animals late in the day, caused erratic results. With only a few exceptions, both *ad libitum* and pair-fed animals were used as controls to the deficient animals in each individual experiment. Satisfactory deficiencies developed in all experimental groups during our standard 3-week feeding period. Animals were considered deficient when they exhibited poor or no growth which could be definitely stimulated by a supplement of the particular vitamin which was absent from the diet. Evidence of early polyneuritis was present in the thiamine deficient animals at the end of the 3-week feeding period. Since nicotinic acid is synthesized in adequate amounts by the rat unless the dietary tryptophan level is relatively low, the low protein basal diet I (Table I)(8), less nicotinic acid, was used to produce a nicotinic acid deficiency. The corn-containing basal diet II

TABLE I. Basal Diets.

	I (%)	II (%)	III (%)
Casein "vitamin-free"	9	9	20
Cystine	.15	.15	—
Gelatin	3	—	—
Corn meal (unenriched)	—	40	—
Sucrose	80	43	72
Wesson oil	3	3	3
Salts O & M(9)	4	4	4
Vitamin mixture*	1	1	1

* Containing the following in mg/g: thiamine .4, riboflavin .6, pyridoxine .5, Ca pantothenate 4, choline chloride 200, inositol 20, nicotinic acid 4, 2-methylnaphthoquinone .2, biotin .02, and folic acid .2, in corn starch (Argo).

Vit A, D, and E as supplements: A and D 2000 and 400 U.S.P. units twice weekly. E 3 mg tocopherol once weekly.

1. Ruffin, Julian M., and Dick, MacDonald, *Ann. Int. Med.*, 1939, v12, 1940.

2. Williams, Ray D., Mason, Harold L., Wilder, Russell M., and Smith, Benjamin F., *Arch. Int. Med.*, 1940, v66, 785.

3. Goldsmith, Grace A., Personal communication.

4. Cowgill, George R., and Gilman, Alfred, *Arch. Int. Med.*, 1934, v53, 58.

5. Webster, D. R., and Armour, J. C., PROC. SOC. EXP. BIOL. AND MED., 1933, v30, 1297.

6. Dyer, Helen M., and Roe, Joseph H., *Am. J. Digest. Dis.*, 1941, v8, 329.

7. Shay, Harry, Komarov, S. A., Gruenstein, Margot, and Fels, S. S., *Gastroenterology*, 1946, v6, 199.

8. Hundley, James M., *J. Biol. Chem.*, 1949, v181, 1.

9. Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1917, v32, 369.

(Table I), less nicotinic acid, was fed as another means of producing nicotinic acid deficiency. Basal diet I, less either thiamine or riboflavin, was used to produce the corresponding deficiencies with this type of diet. The complete vitamin mixture was included in the control diets. Deficiencies of thiamine, riboflavin, pyridoxine, and pantothenic acid were produced by feeding the higher protein basal diet III (Table I) from which the corresponding individual vitamins had been omitted. Control animals received the same basal diet containing the complete vitamin mixture.

As a further test of the effect of individual vitamin deficiencies, acute reversal experiments were done. Deficiencies of thiamine, riboflavin, pyridoxine, and pantothenic were produced by feeding basal diet III less the corresponding individual vitamin. Sixteen hours before pylorus ligation, food was withdrawn and half of each group was given by stomach tube an aqueous solution or suspension of the vitamin which had been omitted from the diet, while the other half was given a similar quantity of water. The accumulated gastric juice following pylorus ligation was used to study the effect of the various deficiencies. Our procedure was slightly modified from the method of Shay *et al.*(10). Food was removed from the cage at 5 p.m. so that the stomach would be empty the following morning at about 9 a.m. when the pylorus was ligated. Intraperitoneal sodium pentobarbital anesthesia (30 mg/kg), supplemented with ether when necessary, was used. Intra-abdominal trauma was minimized by gentle handling. The pylorus was manipulated with a small glass hook and ligated using care to avoid blood vessels. The abdomen was closed in two layers with the skin edges closely approximated so that covering the wound to prevent the animal from ingesting blood and serum was not necessary. The animal was returned to its cage, without water and still without food. At the end of our standard 6-hour secretory period,* timed from the operation, the animal was sacrificed with chloroform, care being taken to clamp off the esophagus as soon as possible to avoid loss of juice. The stomach was removed, the gastric juice expressed into a graduated centrifuge tube, and the stomach wall inspected. An animal was discarded for any one of the following reasons: failure to awaken from anesthesia within 1 hour, hemorrhage, perforation of an ulcer either into the peritoneal or the pleural cavity, excessive solids (over 0.5 cc) in the stomach contents, or grossly bloody juice.

After the volume had been measured the juice was centrifuged and aliquots of the clear supernatant were used for the determination of the concentration of free and total acid (dimethylaminoazobenzene and phenolphthalein, respectively) and pepsin (milk clotting method of Saemundsson)(12). Previously, two methods of comparing volume or rate of secretion have been used. In some types of work it has been possible to use animals of approximately the same size and merely compare the total volume secreted in a standard period of time(13). In other work, volume of secretion has been related to body size by the expression cc/100 g body wt/hr(10). This method is satisfactory, particularly if the size range of the animals is not excessive, and accordingly we have included in the tables figures for cc/100 g body weight (per 6 hrs). In addition, we have expressed volume of secretion as cc/100 sq cm body surface† (per 6 hrs). Using the latter method, we have obtained more constant figures for animals over a very wide weight range(15).

Results. Since the day to day variation for

10. Shay, Harry, Komarov, S. A., Fels, Samuel S., Meranze, David, Gruenstein, Margot, and Siplet, Herman, *Gastroenterology,* 1945, v5, 43.

* After about 6 hours, volume of juice increases at a much slower rate and acute forestomach ulcers begin to appear(11).

11. Pauls, Frances, Wick, Arne N. and MacKay, Eaton M., *Gastroenterology,* 1947, v8, 774.

12. Saemundsson, Johann, *Acta med. Scand. Supp.,* 208, 1948.

13. Madden, Robert J., Ramsburg, Helen H., and Hundley, James M., *Gastroenterology,* 1951, v18, 119.

† Calculated according to the formula of Diack (14) as surface area $= kW^{2/3}$ using $k = 7.5$.

14. Diack, Samuel L., *J. Nutr.,* 1930, v3, 289.

15. Hawk, Edgar A., and Hundley, James M., *J. Nutr.,* 1951 in press.

TABLE II. Volume, Acid Concentration, Pepsin Concentration, and Pepsin Output for Control and Deficient Animals.

Deficiency	Basal diets	No. rats	Wt	Volume		Acid concentration*		Pepsin†	
				cc/100 g	cc/100 cm²	Free	Total	mg/cc	mg/100 cm²
Control *ad lib.*	I	17	77	6.5 ± .2‡	3.7 ± .1‡	106 ± 2‡	123 ± 2‡	1 ± .03‡	3.6 ± .1‡
" pr. fed	I	24	58	7.3 ± .1	3.6 ± .1	97 ± 2	117 ± 2	1 ± .04	3.5 ± .1
Nicotinic acid	I	24	50	7.8 ± .2	3.8 ± .1	106 ± 1	124 ± 2	.9 ± .03	3.5 ± .1
Thiamine	I	17	45	4.8 ± .2	2.3 ± .1	90 ± 4	113 ± 4	1.1 ± .05	2.6 ± .1
Riboflavin	I	18	55	5.9 ± .3	3 ± .1	103 ± 2	127 ± 2	1 ± .02	3.1 ± .2
Control	II	9	103	5.9	3.6	100	116	.8	3.1
Nicotinic acid	II	9	72	6.3	3.5	103	123	.9	3.1
Control *ad lib.*	III	53	108	6.3 ± .1	4 ± .1	100 ± 2	119 ± 1	.9 ± .02	3.4 ± .1
" pr. fed	III	56	71	6.7 ± .1	3.7 ± .1	101 ± 1	125 ± 1	.9 ± .02	3.2 ± .1
Thiamine	III	27	60	5.4 ± .2	2.8 ± .1	86 ± 4	109 ± 3	1.1 ± .03	3.2 ± .1
Riboflavin	III	27	58	6.7 ± .2	3.4 ± .1	105 ± 3	124 ± 2	.9 ± .02	3.1 ± .1
Pyridoxine	III	21	66	5.5 ± .2	2.9 ± .1	81 ± 2	112 ± 2	1.5 ± .1	4.2 ± .2
Pantothenic acid	III	22	70	6.1 ± .2	3.3 ± .1	107 ± 1	129 ± 1	.9 ± .02	3 ± .1

* Milliequivalents/liter. † As activity equivalent to crystalline pepsin (Armour & Co.). ‡ Probable error of the mean.

the various groups was not significant, the data for all animals in similar experimental groups have been combined in Table II.

Nicotinic acid deficiency did not reduce the volume of secretion. The figures for cc/100 sq cm are almost identical for deficient and control groups. If expressed as cc/100 g, the deficient animals secreted more than did the controls.

Both thiamine and pyridoxine deficiencies sharply reduced the volume of secretion below that of the control animals. The differences between the performances of deficient and control animals were apparent whether expressed as volume per unit of body weight or per unit of surface area and were statistically highly significant.‡

Riboflavin and pantothenic acid deficiencies were accompanied by moderate depressions in volume of secretion when this was expressed as cc/100 sq cm. The differences were of borderline statistical significance. When volume was expressed as cc/100 g, the differences were less definite or even not present as in the case of the riboflavin deficient animals on basal diet III.

In the acute reversal experiments (Table III) there was an insignificant rise in the volume of secretion following supplementation with the missing vitamin in the thiamine and pantothenic acid deficient animals and no change in the riboflavin deficient group. However, in the pyridoxine deficient group, there was a marked rise in volume of secretion in the supplemented animals.

Pepsin concentrations in the nicotinic acid, riboflavin, and pantothenic acid deficient groups were approximately equal to the control values (Table II). In both thiamine deficient groups, pepsin concentration was very slightly elevated but total output was moderately depressed in the basal diet I animals and was very near the same as that for the controls in the basal diet III animals. However, in the case of the pyridoxine deficient animals both pepsin concentration and total output were definitely above the con-

‡ Statistical significance of differences was determined according to the test procedure described in *Chemistry of Food and Nutrition,* Henry C. Sherman, 7th ed., The Macmillan Co., New York, 1946.

TABLE III. Volume, Acid Concentration, Pepsin Concentration, and Pepsin Output in Deficient Animals After a Single Administration of the Missing Vitamin.

Deficiency	Supplement	No. of rats	Vol, cc/100 cm²	Free acid*	Pepsin,† mg/cc	Pepsin, mg/100 cm²
Thiamine	Water	16	2.9 ± .1‡	91 ± 5‡	.9 ± .02‡	2.7 ± .1‡
	100 μg thiamine	16	3.2 ± .1	88 ± 4	.9 ± .03	3 ± .1
Riboflavin	Water	15	3.8 ± .1	103 ± 2	.9 ± .03§	3.1 ± .1
	100 μg riboflavin	16	3.9 ± .1	112 ± 2	.9 ± .03§	3.3 ± .1
Pyridoxine	Water	16	3 ± .1	90 ± 2	1.2 ± .03	3.4 ± .1
	100 μg pyridoxine	15	4.3 ± .1	116 ± 2	.9 ± .03	4.2 ± .2
Panto. acid	Water	22	3.5 ± .1	110 ± 2	.8 ± .02‖	2.7 ± .1
	1 mg Ca panto.	23	3.9 ± .1	109 ± 1	.8 ± .02‖	3.1 ± .1

* Milliequivalents/liter.
† As activity equivalent to crystalline pepsin (Armour & Co.).
‡ Probable error of the mean.
§ 8 determinations. ‖ 15 determinations.

TABLE IV. Effect of Addition of Vitamin B_{12} and/or Aureomycin to Control Diet (Basal III).

Addition	No. of rats	wt	Vol, cc/100 cm²	Free acid concentration*	Pepsin,† mg/cc
Control	10	114	3.9	106	.8
Vit. B_{12} (5 μg %)	9	125	3.9	96	.8
Aureomycin (20 mg %)	9	133	3.9	105	.8
Vit. B_{12} (5 μg %) and aureomycin (20 mg %)	10	136	3.9	101	.7

* Milliequivalents/liter.
† As activity equivalent to crystalline pepsin (Armour & Co.).

trol levels. In the acute reversal experiments, pepsin concentration fell with pyridoxine supplementation but the total amount secreted actually rose, tending to follow the increase in volume of secretion. The concentration of free and total acid varied but little in any of the groups, although the values for the thiamine and pyridoxine deficient animals were slightly below control levels (Table II).

Except in the thiamine and riboflavin deficient animals, the stomachs showed no gross changes other than an occasional acute forestomach ulcer which did not appear to be related to the nutritional status of the animal. In many of the thiamine deficient animals, we noted the presence in the glandular portion of the stomach of the acute hemorrhagic ulcers previously described by Shay *et al.*(7). Grossly similar but fewer and less severe lesions were seen in the stomachs of the riboflavin deficient animals.

Since none of the diets used in the experiments reported above contained added vit B_{12}, and in view of the current interest in antibiotics, additional groups of animals were fed basal diet III modified by the addition of either crystalline aureomycin, 20 mg %§; vit. B_{12}, 5 μg %; or both. These animals were slightly larger than the controls at the end of the standard feeding period, as shown in Table IV, but changes in volume of secretion, acid and pepsin concentration, and total output of pepsin were very small and definitely not significant.

Discussion. The nicotinamide-containing DPN or coenzyme I has been strongly suspected as a participant in the acid secretory process(16). Or inability to obtain evidence of impaired acid secretion in nicotinic acid deficiency does not rule out an important role for DPN in the process of secretion since the coenzyme level in the particular cells concerned may have been maintained above the level necessary for normal function.

Our results with thiamine deficient animals differ from those of Shay *et al.*(7) who reported increased secretory rates in this deficiency. However, this difference may be explained as resulting from the very different

§ Kindly supplied by Drs. T. H. Jukes and E. L. R. Stokstad of Lederle Laboratories.

16. Patterson, W. B., and Stetten, DeWitt Jr., *Science*, 1949, v109, 256.

technics employed. Whereas our animals were started from weaning and maintained for 3 weeks on the deficient diet, those of Shay *et al.* were larger when started on a deficient diet and were maintained until considerable weight losses had occurred. Furthermore, we employed only pylorus ligation with collection of total gastric contents 6 hours later, whereas the other workers used pylorus ligation plus a fistula for juice collection at intervals over a longer period of time, with fluids being given as required. A third difference was that our animals were out of anesthesia shortly after the operation while those of Shay *et al.* were kept anesthetized during much of the secretory period. Our results also differ from those of Dyer and Roe(6) who found that thiamine deficiency decreased the secretory rate only when their animals were moribund. While our animals were definitely deficient, they were not moribund. In view of the marked circulatory disturbances frequently observed in thiamine deficiency, it seems possible that the observed depression in secretion may be at least partially explained on the basis of circulatory depression and thus may not be entirely a direct biochemical effect on the secretory mechanism.

The sharp depression in volume of secretion in the presence of pyridoxine deficiency is evidence that this substance plays a previously unsuspected role in the secretory process. Even better evidence for the involvement of this vitamin is the observation that secretion can be returned to a high level by means of a small supplement of pyridoxine alone given about 16 hours before the start of the secretory test. The rapidity of this effect and the fact that the test was conducted with no possibility of further access to food increases the probability of a specific role for pyridoxine in gastric secretion. The reason for the increased pepsin concentration and output in the pyridoxine deficient animals is not apparent at this time. The small depressions in volume produced by the riboflavin and pantothenic acid deficiencies and the slight changes observed in the acute reversal experiments do not constitute impressive evidence implicating these vitamins in the secretory process.

The correlation of both free and total acid concentrations of the juice with volume secreted is compatible with the accepted theory that acid is secreted by the parietal cells as a constant mixture. The small reductions in acid concentration observed in a few of our experiments could be explained on the basis of a relatively greater reduction in secretion of the acid component of the juice as compared with secretion of non-acid components and with salivary contamination.

Although vit. B_{12} was not added to the diets routinely, it is obvious from the results obtained when this substance was included that our experiments were not complicated by an unrecognized deficiency of vit. B_{12}.

It may be noted that, although the vitamin deficiencies produced were quite severe, achlorhydria such as has been reported in human deficiency diseases was not seen in any of the animals. This difference may be explained by the fact that our experiments were conducted in young animals over a short period of time and multiple vitamin deficiencies were not present.

Summary. 1. The influence of various individual B vitamin deficiencies on gastric secretion has been investigated in the pylorus ligated rat. Pyridoxine deficiency sharply reduced acid secretion. This effect was completely reversed by pyridoxine given only 16 hours before the start of the secretory test. Thiamine deficiency markedly depressed acid secretion. Nicotinic acid deficiency did not depress secretion, while riboflavin and pantothenic acid deficiencies produced only small depressions. 2. Neither aureomycin nor vit. B_{12} had any effect on gastric secretion when added to the complete basal diet, although each increased weight gain slightly. 3. Pepsin secretion was not markedly depressed in any of the deficiencies studied, with the possible exception of thiamine. Pyridoxine deficiency increased both concentration and total output of pepsin.

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