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New York Academy of Medicine	February 5, 1947
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SOUTHERN	
Tulane University	February 6, 1947

15766

Dietary Production of Gastric Ulcers in Rats and Prevention by Tocopherol Administration.

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A previous paper[1] from this laboratory reported that α-tocopherol prevented stomach ulcers in rats receiving minimal amounts of vitamin A. The possible explanation advanced to explain this action was based upon the well-known "sparing" of vitamin A by tocopherol, *in vivo*.[2]

Further experiments were designed to test this explanation and also to determine whether tocopherols would be beneficial in preventing stomach ulcers caused by dietary restrictions other than vitamin A.

In a preliminary experiment 40 weanling male rats were placed on a diet (USP XII, with olive oil), low in vitamins A and E, for 2 weeks. At this time supplementation was begun of all rats with 300 units of vitamin A-acetate daily. Half of the rats were also given 0.5 mg of d,α-tocopherol daily. After 7 weeks the animals were killed and examined for gastric lesions. Fifty per cent of the rats on the high-A, low-E diet had one or more ulcers in the fore-stomach, while none of the rats receiving vitamin E had lesions.

In a second experiment 170 male weanling rats were completely depleted of vitamin A in the usual manner. The diets used were based upon the USP vitamin A-test diet, but with variable amounts and kinds of fat. These variations are indicated in Table I. At the time of depletion 3 or 4 rats from each group

* Communication No. 108.

[1] Jensen, J. L., *Science*, 1946, **103**, 586.

[2] Hickman, K. C. D., Kaley, M. W., and Harris, P. L., *J. Biol. Chem.*, 1944, **152**, 303; Harris, P. L., Kaley, M. W., and Hickman, K. C. D., *J. Biol. Chem.*, 1944, **152**, 313; Hickman, K. C. D., Kaley, M. W., and Harris, P. L., *J. Biol. Chem.*, 1944, **152**, 321.

TABLE I.

Effect of Tocopherol and Dietary Fat on Stomach Lesions of Rats After Cure of Vitamin A Deficiency with High Doses of Vitamin A (10 Rats per Group).

Group	Diet—Fat	Avg depletion time (days)	Supplement after depletion: Units Vit. A per day	Supplement after depletion: Tocopherol per day	Avg wt gain 48 days	Incidence of gastric ulcers, %	Avg severity of ulcers graded 1-10
I	5% olive oil	62	30		96.4	50	4.6
I_A	" " "	62	30	.5 mg d,α	94.0	0	—
I_B	" " "	62	2		56.9	60	5.1
I_C	" " "	62	2	" " " "	63.1	0	—
I_D	" " "	62	2	" " phosphate inj.	59.8	50	4.3
II	10% " "	65	30		97.3	0	—
II_A	" " "	65	30	.5 mg d,α	100.7	0	—
III	5% stripped corn oil	60	30		99.1	30	3.7
III_A	" " " "	60	30	" " " "	101.9	0	—
IV	10% " " "	61	30		98.4	20	2.0
IV_A	" " " "	61	30	" " " "	100.2	0	—
V	Fat-low	51	30		62.5	80	6.1
V_A	" "	51	30	" " " "	57.8	80	5.7
V_B	" " + 100 mg sesame oil	51	30		77.1	30	3.9
V_C	" " " " " " "	51	30	" " " "	83.8	10	2.3

were examined, but no gross lesions were present. It is evident that vitamin A-deficiency *per se*, does not produce ulcers. The depleted animals were placed on a daily supplement of 2 or 30 units of vitamin A-ester concentrate, dissolved in olive oil, with or without 0.5 mg α-tocopherol. Half of the rats on the low-fat diet were supplemented daily with 100 mg of sesame oil to supply adequate linoleic acid.

Table I shows the data on gain in body weight and incidence and severity of rumen lesions after a 7-week experimental period.

It is highly significant that vitamin A even in high doses did not prevent ulcer development. This indicates that the action of tocopherol cannot be explained simply a sparing of vitamin A in the gastrointestinal tract. However, the fact that injections of tocopheryl phosphate did not prevent the lesions is presumptive evidence that the tocopherol effect is localized in the stomach. It is interesting to reflect at this time that in all vitamin A bioassays which have been conducted without providing adequate vitamin E to the animals during the depletion or curative periods, 50% or more of the animals used probably had gaping lesions in their stomach. These gastric ulcers certainly were an important factor in causing some of the disturbingly wide variations in growth response observed in vitamin A bioassays.

The influence of fat in the diet is interesting. The lesions occurred on the 5% level of olive oil or stripped (E-free) corn oil; but on the 10% oil level the lesions were absent or reduced in number even without tocopherol supplements. On the low-fat diet a very high incidence and severity of lesions was noted and tocopherol at the levels used had no beneficial effects.

Since fat in the diet is a significant factor in the prevention of rumen lesions, it was of interest to examine the stomachs of rats deficient only in essential fat acids. The fat-low diet used in the preceding experiment contained 56% corn starch which probably supplied about 0.6% fat as a constituent.[3] Thus the diet contained about 0.3 to 0.4% fat which furnished at least 10 mg linoleic acid daily to each animal.[4]

In the present experiment, weanling rats were placed on a fat-free diet of the following percentage composition: Casein (Labco) 20, sucrose 76, and salt mixture, USP No. 2, 4. B-complex vitamins were added to the casein to furnish 10 γ of thiamin, riboflavin, and pyridoxine, 25 γ calcium pantothenate, 1 mg choline chloride, and 0.1 mg *i*-inositol per gram of ration. Fifty units of vitamin A-acetate and 5 units of vitamin D were given daily in a propylene glycol solution.

After 7 weeks depletion the rats were divided into groups of 6 each and placed on experiment with and without 20 mg of ethyl linoleate also with and without 0.5 mg tocopherol daily. After 6 weeks the stomachs of the rats were examined for lesions. The results are shown in Table II. Hyperplasia of the juncture (or ridge) separating the glandular portion from the rumen, usually precedes or accompanies hyperplastic ulcers in the rumen. The incidence of such juncture hyperplasia has been indicated, as well as the incidence of ulcers.

The data in Table II show that rats deficient in essential fatty acids do not have stomach lesions. However, half of the rats receiving 20 mg of linoleate showed typical ulcers. Juncture hyperplasia was marked in this group. Either α- or γ-tocopherol prevented the lesions.

It was of further interest to determine whether ulcers produced by dietary restrictions other than vitamin A or essential fatty acids would also be prevented by tocopherol.

Male albino weanling rats were placed upon a pyridoxine-deficient ration for 6 weeks. The percentage composition of the ration was: Casein (Labco) 20, sucrose 71, salt mixture, USP No. 2, 4, lard 4, and cod liver oil 1. B-complex vitamins were added to the casein to furnish 10 γ thiamin and riboflavin, 25 γ calcium pantothenate, 1 mg choline chloride, and 50 γ of vitamin K per gram ration.

[3] Taylor, T. C., and Nelson, J. M., *J. Am. Chem. Soc.*, 1920, **42**, 1726.

[4] Taylor, T. C., and Lehrman, L., *J. Am. Chem. Soc.*, 1926, **48**, 1739.

TABLE II.
Effect of Tocopherol and Methyl Linoleate on Development of Gastric Lesions in Rats Deficient in Essential Fatty Acids.*

Trial	Daily supplement: Methyl linoleate (mg)	Daily supplement: d,α-tocopherol (mg)	Incidence of gastric lesions: Ulcers (%)	Incidence of gastric lesions: Juncture hyperplasia (%)
I	0	0	0	0
	0	0.5	0	17
	20.0	0	50	67
	20.0	0.5	0	0
	20.0	0.5†	0	0
II	0	0	0	33
	20.0	0	0	83
	20.0	0.5	0	17

* 6 animals per group.
† γ-tocopherol.

TABLE III.
Effect of Tocopherol in Preventing Development of Rumen Lesions in Rats Recovering from Pyridoxine Deficiency.

Pyridoxine (γ daily)	Mg α-tocopherol daily	No. of rats	Avg wt gain in 6 wk (g ± S.D.)	Incidence of rumen lesions (%)
0	0	7	4.3	0
	1	7	7.1	0
1.0	0	4	51.5 ± 6.3	50
	1	4	53.5 ± 4.9	0
2.0	0	7	71.4 ± 7.6	57
	1	7	82.7 ± 8.3	0
4.0	0	7	91.3 ± 5.0	29
	1	7	105.8 ± 4.8	0
8.0	0	6	124.0 ± 7.7	0
	1	5	120.0 ± 6.0	0

After 6 weeks depletion, the rats were fed various levels of pyridoxine with and without tocopherol. Pyridoxine (free-base) was prepared from synthetic pyridoxine-hydrochloride (Merck) and given in propylene glycol solution from a calibrated dropper.

The resulting gains in body weight during the subsequent 6 weeks and the incidence of rumen lesions at this time are summarized in Table III. These data indicate that pyridoxine deficiency in itself does not result in rumen lesions. However, when suboptimal doses of pyridoxine are fed, lesions do occur but are preventable by α-tocopherol. Higher doses of pyridoxine maintain normal stomachs in the rats even without tocopherol.

Discussion. It is evident that gastric lesions can be induced by a variety of dietary deficiencies when these are combined with low intakes of vitamin E. Severe tocopherol deficiency uncomplicated by deficiencies of other essentials does not induce ulcers. Hundreds of vitamin E-deficient female rats with evidence of resorption gestations have been free of stomach lesions. Furthermore, uncomplicated deficiencies of vitamin A, or of pyridoxine, or of essential fatty acids do not induce ulcers. Ulcers are formed only in rats which have stopped gaining weight as a result of any of these deficiencies and which then have been given the missing factor but without supplying tocopherols at the same time. Weight gain is resumed when vitamin A, pyridoxine, or linoleate, respectively, is supplied and it is during this period that stomach lesions appear unless sufficient tocopherol is present in the gastrointestinal tract. Tocopherols injected as the tocopheryl phosphate during the experimental period fail to prevent ulcer formation. Consequently,

the evidence points to a mechanistic explanation for lesion formation. Functionally, the epithelium of the forestomach of the rat becomes abnormal due to deficiencies of either vitamin A, or pyridoxine, or essential fatty acids. Anatomically there are no lesions because of the gradual adaptation of the organism as a whole to a low level of metabolic activity: food intake decreases, weight gain ceases, and probably gastric secretion of pepsin and HCl is diminished. However, administration of the missing essential factor induces food intake and resumption of weight gain. Unless adequate tocopherol is also administered the functionally abnormal epithelium of the forestomach cannot cope with the increased acid-pepsin medium with which it is in contact and as a result lesions are produced.[5] Whether tocopherols prevent the erosion of the stomach wall by specifically inhibiting enzyme activity or more fundamentally by increasing epithelial resistance perhaps by inducing greater mucous secretion or improving blood circulation in the mucosa[6] is not yet known.

The role of dietary fat in the etiology and treatment of gastric lesions is also undetermined. In our experiments an increase of fat (olive oil or vitamin E-free corn oil) from 5 to 10% in the diet reduced the incidence and severity of ulcers formed. Li and Freeman[7] also found a higher incidence of peptic ulcers in dogs on a low-fat (lard) than on a high-fat diet. However, Matzner and coworkers[8] reported their highest incidence and most severe stomach lesions in rats maintained on relatively high fat (butter or lard) intakes.

It is emphasized that no attempt should be made at this time to draw a parallel between these gastric ulcers in rats and those in humans. Much more must be learned concerning the etiology and pathology of the lesions in the 2 species before generalizations regarding treatment with fat or tocopherols can be made. The level of protein intake is also important and data showing a protein-tocopherol interrelationship as measured by ulcer production and cure is being prepared for publication.

Conclusions. 1. Ulcers in the forestomach were induced in rats: (a) Recovering from vitamin A-deficiency with 2, 30, or 300 units vit. A daily. (b) Recovering from a vit. B_6 deficiency with suboptimal amounts (1, 2, or 4 γ daily) of pyridoxine. (c) Recovering from an essential fatty acid deficiency with 20 mg of linoleate daily.

2. Deficiencies of these 3 essentials did not, in themselves, result in lesions. The ulcers occurred only during the curative period in which the specific nutrient was fed.

3. α-Tocopherol fed daily to the rats during the cure of the specific deficiency gave complete protection. γ-Tocopherol orally was also effective, but α-tocopheryl phosphate injected was not.

4. The amount of fat in the diet was related to the incidence of stomach ulcers in the vitamin A experiments. Ten per cent gave good protection. Five per cent allowed moderately severe lesions, which were preventable by tocopherol. Fat-low diets gave very severe lesions not preventable by the level of tocopherol fed, 0.5 mg daily.

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[5] Matzner, M. J., Windmer, C., Sobel, A. E., and Polayes, S. H., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 243.

[6] Bachrach, W. H., Grossman, M. I., and Ivy, A. C., *Gastroenterology*, 1946, **6**, 563.

[7] Li, T., and Freeman, S., *Gastroenterology*, 1946, **6**, 140.

[8] Matzner, M. J., Windmer, C., and Sobel, A. E., *Am. J. Dig. Dis.*, 1938, **5**, 1.