Studies on the stability of vitamin A in the stomach and small intestine

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EDITORIAL SYNOPSIS The instability of vitamin A alcohol relative to vitamin A acetate has been studied *in vivo* and *in vitro*.

Vitamin A alcohol is more unstable than vitamin A acetate in human gastric and duodenal juice and in rats' stomachs and intestines and is more marked at lower pH levels and in rats' stomachs.

Alpha tocopherol improved the absorption curve of vitamin A alcohol in man by protecting it in gastric and duodenal juice. This protective effect could not be demonstrated in the rat.

An advantage of the vitamin A absorption test is that differential intestinal absorption can be tested by the use of vitamin A alcohol and vitamin A acetate. Vitamin A acetate is poorly absorbed in all types of malabsorption, whereas vitamin A alcohol is well absorbed in malabsorption due to maldigestion, *i.e.*, when pancreatic juice is deficient in function and emulsification. The pattern of absorption in over 100 cases on which these absorption tests were carried out in the past five years (Fitzgerald, Fennelly, and Hingerty, 1961, 1962) is as follows:—

VITAMIN A ABSORPTION
(25,000 i.u. orally)

	<i>Vitamin A Acetate</i>	<i>Vitamin A Alcohol</i>
Normal	Normal	Normal
Coeliac disease	Reduced	Reduced
Pancreatic malabsorption	Reduced	Normal

The normal person shows a rise in serum vitamin A concentration to over 800 i.u.%. Occasionally, however, absorption curves after the ingestion of vitamin A alcohol are poor in relation to those of vitamin A acetate. This paradoxical situation is probably due to the greater instability of vitamin A alcohol.

Hopkins (1906) noted rapid destruction of biological activity of fatty substances when they are heated and exposed to air and later McCollum, Simmonds, Becker, and Shipley (1922) used the

instability of vitamin A as a means of identification. A number of authors noted the instability of vitamin A in studies of liver content in subjects after dosage of vitamin A with and without tocopherol. Tocopherol increased the liver storage of vitamin A and carotene. Vitamin A alcohol is unstable, particularly if exposed to oxygen and HCl (Davies and Moore, 1941; Harris, Kaley, and Hickman, 1944; Hickman, Harris, and Woodside, 1942; Moore, 1957).

Our experiments have dealt with the instability under varying conditions of vitamin A in human gastric and duodenal juice and in rats' stomachs and intestines.

MATERIALS AND METHODS

EXPERIMENT I Studies of stability *in vitro* were carried out by the incubation of both preparations (25,000 i.u.) separately with human gastric and duodenal juice (10 ml.) of varying pH at 37°C. Breakdown was followed at two, four, eight, and 24 hours by estimating vitamin A, using a modification of the method of Kimble (1939), in which 0.5 ml. of the mixture was extracted with 5 ml. absolute alcohol and 5 ml. petroleum ether; 0.5 ml. of the petroleum ether layer was dried in a stream of nitrogen and dissolved in 0.5 ml. chloroform, then 2 ml. antimony trichloride solution was added and the blue colour read immediately in a Unicam spectrophotometer at 620 mμ. Tocopherol, when added, was prepared as a solution of alpha-tocopherol acetate, 1 ml. containing 50 mg.

EXPERIMENT II Wistar strain rats weighing 200 to 250 g. were used for experiments *in vivo*. They were kept fasting for 20 to 24 hours. The stomachs of the anaesthetized animals were isolated and also 2 cm. of small intestine,

just distal to the pylorus. The circulation was not deliberately cut off though some damage was unavoidable. Then 0.3 ml. and 0.1 ml. of the vitamin A solution containing 25,000 i.u. and 8,333 i.u. were injected into the stomach and intestine respectively. Tocopherol acetate in amounts from 2.5 to 5 mg. was added to the vitamin A preparation to estimate protective action. Two hours later the stomach and intestine were excised and extracted with 5 ml. absolute alcohol and 50 ml. petroleum ether. At the same time 0.1 ml. of the original vitamin A preparation used was similarly extracted. Vitamin A was estimated as described in the first experiment.

EXPERIMENT III Over the past six years over 100 vitamin A absorption tests have been carried out in studies of the absorptive capacity of various patients. As already pointed out, anomalous curves were found in five of these in whom we checked the effect of administering alpha-tocopherol with vitamin A alcohol in a repeat of the test, leaving an interval of some days between tests.

RESULTS

Table I shows the breakdown of vitamin A acetate and alcohol respectively in gastric and duodenal juice at varying pH. Vitamin A acetate appears to be quite stable when incubated with duodenal juice when the pH is over 3.8. In one case the duodenal juice was 3.8 pH.; in this case much more breakdown occurred. At lower pH levels the breakdown was even more marked. One reading 20% and 9% appears so much out of line as to be a probable experimental error so it is excluded from the mean figures. Vitamin A alcohol is more unstable at all pH levels, particularly at the more acid levels. Breakdown is fairly marked in the first four hours but most of it occurs between four and 24 hours.

Table II shows quite clearly the protective effect of alpha-tocopherol on vitamin A alcohol. Protection is given at all pH levels. The amount remaining after two, four, and 24 hours' incubation with gastric juice compares well with that of vitamin A acetate remaining after similar intervals. The long-term protective effect of tocopherol in duodenal juice is also evident.

The results of experiments *in vivo* set out in Table III confirm the instability of vitamin A alcohol in

TABLE II
PROTECTION GIVEN TO VITAMIN A ALCOHOL BY
TOCOPHEROL IN HUMAN GASTRIC AND
DUODENAL JUICE

Juice	2 Hours ¹	4 Hours	24 Hours
Gastric (6 tests) (pH 1.45 to 2.1)	100	100	78.3
Duodenal (6 tests) (pH 3.8 to 7.0)	89	77	61

¹Percentage of initial concentration remaining at two, four, and 24 hours.

both rats' stomachs and intestines, probably more in the former. Vitamin A acetate is relatively much more stable. Efforts to demonstrate vitamin A alcohol protection by tocopherol during experiments *in vivo* on rats' stomachs and intestines were not successful and increased breakdown of the vitamin was seen in many experiments.

Table IV shows the improvement in the vitamin A alcohol absorption in man after the test had been repeated with administration of alpha tocopherol to prevent breakdown. It appears that 100 to 200 mg. is the optimum dose of tocopherol.

TABLE III
BREAKDOWN OF VITAMIN A ACETATE AND
VITAMIN A ALCOHOL IN RATS' STOMACHS AND
INTESTINES AND EFFECT OF ADDED TOCOPHEROL

Vitamin A Acetate (9 Rats)		Vitamin A Alcohol (17 Rats)		Vitamin A Alcohol (19 Rats)+Tocopherol	
Stomach	Intestine	Stomach	Intestine	Stomach	Intestine
99	93	64	78	44	53

¹Percentage of initial concentration remaining after two hours.

DISCUSSION

Vitamin A is broken down in two sites in the alimentary tract, the stomach and the intestine, but is more marked in the stomach than in the intestine (Tables I and III). The instability is due to oxidation of the vitamin in the stomach and small intestine. The increased breakdown with increased acidity is well

TABLE I
BREAKDOWN OF VITAMIN A ACETATE AND ALCOHOL IN HUMAN GASTRIC AND DUODENAL
JUICE DURING INCUBATION AT 37° C.

Preparation	Juice	2 Hours ¹	4 Hours	8 Hours	24 Hours
Vitamin A acetate	Gastric (5) ² pH 1.45 to 2.1	71	66.5	—	33.5
Vitamin A acetate	Duodenal (5) pH 3.8 to 7.0	100	92.5	96	85.4
Vitamin A alcohol	Gastric (12) pH 1.45 to 2.1	72	52	41	18
Vitamin A alcohol	Duodenal (12) pH 3.8 to 7.0	—	74	63	24

¹Percentage of initial vitamin A concentration remaining at various intervals.

²No. of tests in brackets.

TABLE IV
POST-ABSORPTION LEVELS IN FIVE SUBJECTS
SHOWING PARADOXICAL CURVES AND
EFFECT OF TOCOPHEROL

No.	Description	Acetate (i.u./100 ml. serum)	Alcohol	Tocopherol plus Vitamin A Alcohol
1	Normal subject	1,000	755	1,200 (100 mg.) ¹
2	Normal subject	—	320	731 (100 mg.) 566 (500 mg.) 509 (1,000 mg.)
3	Mucoviscidosis	500	300	500 (100 mg.)
4	Chronic uraemia	1,600	1,000	1,000 (100 mg.)
5	Chronic uraemia (1957)	3,300	1,300	1,750 (200 mg.) 2,275 (200 mg.)
	(1959)	2,200	1,100	2,300 (200 mg.)

¹Tocopherol was given on the evening before and on the morning of the test in the amounts stated in brackets.

shown in Table I. In these experiments vitamin A acetate was comparatively stable. Tocopherol provides good protection during experiments *in vitro* in gastric and duodenal juice and apparently *in vivo* in human subjects (Table IV).

If we consider the possible degree of breakdown of vitamin A alcohol it is surprising that in almost all normal human subjects equal absorption curves are seen after the ingestion of vitamin A acetate and vitamin A alcohol. The excessive breakdown of vitamin A alcohol seen in a few cases would suggest the use of the more stable acetate as a measure of absorptive capacity. The lesser stability of the vitamin A alcohol under certain conditions might obscure that difference in absorption between acetate and alcohol seen in patients with pancreatogenous steatorrhoea. The demonstration that serum levels of a patient are lower after vitamin A acetate than after the alcohol should always suggest pancreatic maldigestion. The opposite type of curve (where the

alcohol is very poorly absorbed) might suggest that it has been broken down, unless the ester figure is also low, when a malabsorption defect would be the more reasonable explanation.

The apparent absence of a significant effect of tocopherol in protecting the vitamin A alcohol in the rat may have been due to an excess of tocopherol, or possibly to interference by the tocopherol in the procedure for determination of vitamin A.

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