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Serum studies in man after administration of vitamin A acetate and vitamin A alcohol

I In normal subjects

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In 1934, Chesney and McCoord reported poor absorption of vitamin A in coeliac disease. Many papers have since been published on the use of vitamin A absorption tests as an index of small intestinal function. The absorption of fat-soluble vitamins is related to the absorption and transport of fats, since both are insoluble in water and are soluble in the same solvents (Clausen, 1943; Sobel, 1952). Vitamin A is absorbed in the upper small intestine as vitamin A alcohol, followed by reconversion to an ester before it reaches the circulation. The three main factors for its normal absorption are: 1, bile salts, 2, intestinal motility, and 3, pancreatic secretion (Gray, Morgareidge, and Cawley, 1940; Clausen, 1943). These factors are also necessary for the proper absorption of a neutral fat. After administration of a large dose of vitamin A orally both lymphatic and serum levels of vitamin A are raised, the greater in the lymphatic circulation (Eden and Sellers, 1949). Studies on a patient with a diverted thoracic duct have shown that vitamin A appears in increased amounts in thoracic duct lymph after oral administration of a large dose (Drummond, Bell, and Palmer, 1935). If post-absorptive serum curves are to be used as a test of intestinal absorption in disease it is important that the factors which affect the curves in normal controls should be understood. Hillman and Becker (1957) suggested that the vitamin A absorption test was of limited value, a conclusion principally based on differences between repeat tests carried out on individuals and groups under varying conditions and relying on a four-hour sample to test for absorption and using amounts from 50,000 to 150,000 units as a test dose of vitamin A.

It has long been known that many factors may affect vitamin A absorption curves. It is generally accepted that in patients with either normal or abnormal absorption, aqueous dispersions of vitamin A produce (*a*) higher liver storage levels, (*b*) higher serum levels, and (*c*) lower faecal excretion than do oily preparations (Lewis, Bodansky, Birmingham, and Cohlan, 1947; Kramer, Sobel, and Gottfried, 1947; May and Lowe, 1948; Barnes, Wollaeger, and Mason, 1950). That the relation of peak to pre-test levels of serum vitamin A should be used as an index of intestinal absorption has been suggested by Mendelhoff (1954), and he considered that a rise to four times the fasting level indicated normal absorption. The importance of taking a meal during the test was demonstrated in Mendelhoff's paper, and later by Hillman and Becker (1957), who found that fatty meals gave the sharpest rise in the serum curve.

In this paper we propose to discuss the effect of these factors and show the effect of diet and exercise on post-absorptive curves of normal controls.

MATERIAL AND METHODS

Double vitamin A absorption tests were carried out using either the vitamin A acetate or vitamin A alcohol, with an interval of at least 48 hours between the two tests. The vitamin A preparation was dissolved in arachis oil (1 ml. containing 250,000 i.u.) and was sealed in nitrogen in a glass ampoule. No emulsifiers were used as these may produce normal absorption curves even in malabsorptive states (Fox, 1949).

Normal subjects were studied, resting without a fatty meal and with a fatty meal, and ambulant without a fatty meal and with a fatty meal. These controls were residents, medical students, and other medical personnel, and some patients under investigation for conditions not related to the alimentary tract. Their ages ranged from 20 to 60 years, the majority being men between 20 and 30 years of age. There was no modification in their diet before the tests and no extra vitamin A was given before the test. Tests were carried out on 27 subjects (18 men and nine women).

The subjects were allowed only fluids on the morning of the test. At zero time, usually 9.0 a.m., 5 ml. blood was withdrawn into a dry tube. The subject was then given 250,000 i.u. of the vitamin A preparation in 200 ml. milk. One to two hours later some subjects were given a mixed meal containing 50 g. fat. Blood was withdrawn at three and five hours from zero. Serum vitamin A and serum

carotene were estimated simultaneously by the Carr-Price technique as modified by Kimble (1939). Values were corrected for carotene by the factor (0·4) of Moore (1957), who also described the chemical part of the test.

RESULTS

In Table I and Fig. 1 are shown the results of estimation of vitamin A post-absorption peaks and also the fasting carotene levels in 31 tests carried out on normal subjects. Fasting serum vitamin A levels varied from 83 to 333 i.u.% and the mean peak figure for vitamin A acetate absorption was 1,231 i.u.%, with a range of 465 to 1,803 i.u.%; for vitamin A alcohol the mean peak was 1,428 i.u.% with a range of 875 to 2,592 i.u.%. The medians were 1,205 i.u. % and 1,140 i.u.% respectively. All, except subjects 9 and 10, had a fatty meal during the test. Fasting serum carotene levels in our series varied from 80 to 320 μg.% in over 30 normal controls, many of whom are not included in this series as they were not studied with both vitamin preparations.

Subject No. 1 had three tests carried out in the space of 10 days, the highest figure resulting from the third test. Subject No. 3 was nauseated during the test which may explain the low figures. The absorption curve does not appear to be related to the fasting vitamin A level. In 30 tests carried out using three, five, and eight-hour samples the peak was at three hours in 15, at five hours in 14, and at eight hours in one, this last probably being due to a delay in giving the subject a meal after the test dose. Furthermore, in 30 tests carried out on abnormal subjects the same time relation was noted as regards peak figures. From this we can conclude that in 96·7% of cases samples at three and five hours should detect peak figures.

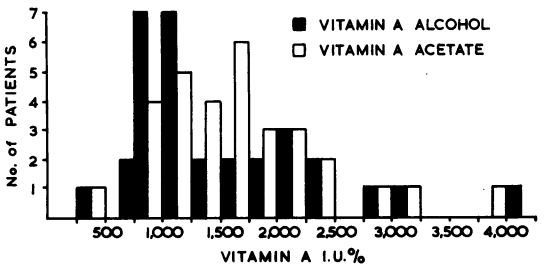

FIG. 1. *Frequency distribution of post-absorption peaks of vitamin A acetate and vitamin A alcohol (dose 250,000 i.u. vitamin): 31 tests in normal controls.*

EFFECT OF FOOD ON PEAK In Table II the curves in subjects with and without a fatty meal during the test are compared.

As can be seen, six of these controls showed post-absorption peaks, without a fatty meal, which would be judged as quite abnormal and indicating mal-absorption; however, the administration of a fatty meal during the test corrected this error in all except one subject who was nauseated during the test.

EFFECT OF EXERCISE ON PEAK We noted many low curves in our ambulant subjects, so these tests were compared with those of resting subjects.

As can be seen from Table III, Cases 3 and 4 in the ambulant group showed unusually low curves. The mean rise in the ambulant group was lower than that for the resting group. While it has been suggested that exercise might have an effect on these curves no figures have been previously reported. There are not sufficient results for a reliable statistical analysis ($P = 0{\cdot}05$).

TABLE I

RESULTS OF VITAMIN A ABSORPTION TESTS IN NORMAL ADULT SUBJECTS USING 250,000 i.u. OF VITAMIN A ACETATE OR ALCOHOL PREPARATION

Case No.	*Sex*	*Fasting Carotene (mg.%)*	*Vitamin A Acetate (i.u.%)*			*Vitamin A Alcohol (i.u.%)*	
			Fasting	*3 Hours*	*5 Hours*	*3 Hours*	*5 Hours*
1	M	144	290	1,300	1,000	1,000	475
						2,592[1]	1,150
2	M	185	110	1,210	750	875	575
3	F	230	83	465	215	2,085	661
4	M	—	150	1,070	450	1,050	450
5	M	190	190	Not done	Not done	1,420	750
6	M	185	190	1,020	460	1,120	350
7	F	237	178	1,803	1,828	1,690	2,327
8	F	156	250	1,600	1,460	1,600	950
9	M	—	220	1,100	—	1,150	1,350
10	M	100	333	1,516	1,499	1,131	1,089
Mean		178	199	1,231	958	1,428	830
Range		100-237	83-333	465-1,803	215-1,828	875-2,592	350-2,327
Median		185	190	1,205	750	1,140	618

[1]This was the third test carried out over a 10-day period on this subject.

TABLE II

PEAK SERUM LEVEL OF VITAMIN A AFTER ADMINISTRATION OF 250,000 i.u.% WITHOUT AND WITH FATTY MEAL

No.	*Sex*	*Without Fatty Meal*		*With Fatty Meal*	
		Vitamin Preparation	*Peak Figure*[1]	*Vitamin Preparation*	*Peak Figure*
1	M	Alcohol	135	Alcohol	988
		Acetate	296	Alcohol	1,136
				Alcohol	2,592
				Acetate	1,340
2	M	Alcohol	575	Alcohol	870
		Acetate	700	Acetate	1,249
3	F	Alcohol	480	Alcohol	2,055
		Acetate	295	Acetate	460
4	M	Alcohol	1,050	Alcohol	1,080
5	F	Alcohol	750	Alcohol	1,420
6	M	Alcohol	450		
		Alcohol	480		
7	M	Alcohol	320		
		Alcohol	731		
Mean			521		1,320
Median			480		1,136
Range			125-1,050		460-2,592

[1]Peak expressed as actual maximal rise from fasting level in i.u.%.

TABLE III

PEAKS OF VITAMIN A ABSORPTION TESTS IN RESTING AND AMBULANT SUBJECTS

No.	*Sex*	*Resting Vitamin A (i.u.%)*	*Ambulant Vitamin A (i.u.%)*
1	M	1,250	—
2	F	1,200	350
3	M	1,014	89
4	F	2,175	330
5	M	2,522	1,350
6	M	960	1,249
7	F	1,377	465
8	F	1,859	2,175
9	M	963	1,080
10	M	1,420	1,420
Mean		1,475	912
Range		963-2,522	30-2,175
Median		1,314	1,080

DISCUSSION

There is still some difference of opinion as to the relative value of the tests used in assessing intestinal absorption.

The fat balance test is mainly affected by absorptive capacity. The vitamin A test is affected by many factors other than absorption. Emulsification has been shown to increase the serum absorption curve and reduce the faecal excretion of the vitamin in abnormals as well as in normal subjects (Fox, 1949; Barnes *et al.*, 1950). The difference in absorption between emulsified and non-emulsified preparations is more evident in patients with malabsorption. When the particles are of small size in a water-soluble phase further emulsification may not be needed (May and Lowe, 1948). In preliminary tests of absorptive function emulsifiers such as Tween 80 should be excluded from preparations used in absorptive tests, unless one is specifically looking for an emulsification defect (lack of bile) as distinct from a digestive (pancreatic or succus entericus) or absorptive (intestinal wall) defect.

The differential absorption of vitamin A alcohol relative to that of vitamin A esters, such as vitamin A acetate, has been previously noted by Lewis *et al.* (1947), Kagan, Thomas, Jordan, and Abt (1950), and by Barnes *et al.* (1950). They concluded that vitamin A alcohol was better absorbed than the esterified form in normal and in abnormal human subjects. Our present findings suggest that there is little differential absorption of the two forms of the vitamins in normal subjects.

The dose used by various authors has shown such a variation that it is difficult to compare results from different papers. The dosage ranged from 90,000 to 600,000 i.u., some doses being related to body weight. Mendelhoff (1954) found a rise of over 100 i.u.% following 90,000 to 180,000 i.u. taken orally. Kramer *et al.* (1947) had noted a rise of over 4,000 i.u, following 6,000 i.u./lb. body weight. In our experience 250,000 i.u. is a satisfactory dosage in terms of body weight when dealing with concentrated preparations as small errors in volume could mean up to 100,000 i.u. difference in dosage. This dosage is equivalent to 3,571 i.u./kg. or 1,630 i.u./lb. in a 70 kg. subject. Dosage up to 150,000 i.u. may be taken up too rapidly by the tissues so that good absorption curves do not result; however, with dosage over 200,000 i.u. a good curve usually results in normal subjects.

Mendelhoff (1954) considered that a rise to four times the fasting vitamin A level indicated normal absorption but, for a number of reasons, he held that one should not evaluate the normality of a curve from the relation of post-absorptive level to fasting level. As pointed out by Popper, Steigman, Mayer, and Zevin (1943) the fasting level may be an index of vitamin A storage rather than absorption and they concluded that the curve was not necessarily related to the fasting level. It should be remembered that the chemical estimation of vitamin A is very much affected by the carotene level since the factor of 0·4 times the carotene figure in milligrams per cent, as suggested by Moore (1957), must be subtracted from the final vitamin A figure in order to correct for the blue colour produced by carotene with the antimony trichloride. At levels of carotene over 300 mg.% the correction of 0·4 is probably excessive and a smaller

TABLE IV

VARIATIONS IN DOSE OF VITAMIN A USED FOR INVESTIGATIONS BY DIFFERENT AUTHORS

Author	*Dose (i.u.)*	*Expected Peak in Normal Subjects (i.u.%)*
Adlersberg and Sobotka (1943)	(a) 90,000 (b) 80,000 (c) 600,000 (occasionally)	53% rise for (a) and (b)
Kramer, Sobel and Gottfried (1947)	6,000/lb. body weight	4,000
Mendelhoff (1954)	100,000	4 times fasting level or 100 μg.%
Week and Sevigne (1950)	440,000	Over 2,000
Kagan, Thomas, Jordan and Abt (1950)	5,000 /lb. body weight	2,480-2,120
Barnes, Wollaeger, and Mason (1950)	7,500	500
This paper	250,000	800-4,000

factor (0·3 or even 0·25) might be more accurate. Paterson and Wiggins (1954) considered that if the serum level rose to over 500 i.u. after administration of 250,000 i.u. of vitamin A steatorrhoea was almost certainly excluded. As seen in Table I, the lowest fasting vitamin A level was found in a subject with nearly the highest carotene figure (fasting vitamin A, 83 i.u.%, fasting carotene, 230 μg%).

A meal definitely raised the absorption curves, as shown in Table II, and already pointed out by Hillman and Becker (1957). These authors noted that fatty meals gave the best response. We have relied on meals containing 50 g. fat given one to two hours after vitamin A dosage. In Table II it is shown that there is a rise in absorption from a mean level of 531 i.u. without a fatty meal to 1,320 i.u. when a fatty meal is added during the test. Many tests which were carried out with the patient fasting right through the test showed abnormally low curves but on adding the fatty meal during the test the curves became quite normal. A possible explanation for this difference is that the small volume in which the vitamin A is given is not sufficient to stimulate secretion of digestive enzymes, or an added force is needed to drive the absorbed vitamin A into the lacteal and systemic circulations (Mendelhoff, 1954).

The effect of exercise on the curve is not generally appreciated, though Mendelhoff (1954) did discuss the possible effect. We have noted that vitamin A tests carried out on ambulant controls have frequently shown lower absorption curves than those in resting controls. This difference is shown in Table III. It is possible that many of the unsatisfactory results obtained in controls by Hillman and Becker (1957) were related to the fact that their controls were mainly medical personnel in the course of their duties.

Vitamin A absorption curves from the literature show much variance as a result of the factors mentioned. A coincidental meal must be given and exercise must be avoided in all tests, as it has been shown that excessive variations occur if the patients are kept fasting during the test or if exercise is allowed.

SUMMARY

The absorption of vitamin A alcohol and acetate in normal subjects is discussed.

There is no significant difference between the serum level after the administration of vitamin A alcohol and vitamin A acetate.

The use of a fatty meal during the test is recommended.

Exercise appears to affect the curve; it is important to keep subjects resting during the test.

The use of two samples (at three and five hours) is more accurate in detecting peak serum absorption figures than one sample at four hours.

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