

AN ESTIMATION OF PLASMA VITAMIN A AND THE VITAMIN A ABSORPTION TEST

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Following the adaptation of the Carr and Price (1926) colour reaction to the determination of the vitamin A content of the blood by McCoord and Luce-Clausen (1934), Chesney and McCoord (1934) reported impaired absorption of vitamin A from the intestine in coeliac disease, and devised the vitamin A absorption test as an index of the absorption of fat from the intestine. The chemical method was improved by Kimble (1939) and by May, Blackfan, McCreary, and Allen (1940), in each case a photoelectric colorimeter of the Evelyn (1936) type being employed in place of the earlier visual matching of the colours. The vitamin A absorption test has since been used by a number of workers in the United States, who confirmed the findings of low fasting plasma vitamin A levels and flat absorption curves in coeliac disease (Breese and McCoord, 1939; Clausen and McCoord, 1938; May *et al.*, 1940; May, McCreary and Blackfan, 1942; Adlersberg and Sobotka, 1943a), in cystic fibrosis of the pancreas (Blackfan and May, 1938), in sprue and idiopathic steatorrhoea (Adlersberg and Sobotka, 1943b; Ingelfinger, 1943; Cayer, Ruffin and Perlzweig, 1945; Darby, Kaser, and Jones, 1947), in congenital atresia of the bile duct (May *et al.*, 1940), and in colitis (Page and Bercovitz, 1943).

It was soon found, however, that low plasma vitamin A levels and flat absorption curves may occur in conditions other than those characterized by steatorrhoea, particularly in chronic infectious diseases, e.g., in tuberculosis (Breese, Watkins, and McCoord, 1942), in giardiasis (Katsampes, McCoord, and Phillips, 1944), in catarrhal jaundice (Breese and McCoord, 1940), in cirrhosis of the liver (Ralli, Bauman, and Roberts, 1941), in malnutrition, inflammatory lesions, and cretinism (May and McCreary, 1941), and in several diseases of the skin (Ruch, Brunsting, and Osterberg, 1946; Peck, Glick, Sobotka, and Chargin, 1943).

It is thus evident that the low plasma vitamin A level and flat absorption curve is not in itself

diagnostic of steatorrhoea although both findings are invariably present when fat absorption is impaired. Ingelfinger (1943) considers that in assessing the low vitamin A level and flat absorption curve much is gained by a knowledge of the plasma carotene level, which is also low in steatorrhoea (Cayer *et al.*, 1945).

Our purpose in this communication is to present a method of plasma vitamin A estimation and to record data which we have found relevant to the performance and interpretation of the absorption test.

Method

Although the modifications of the Carr and Price blue reaction for the estimation of the vitamin A content of the plasma present little practical difficulty, these have in common several inherent drawbacks, viz., (1) the very rapid development and disappearance of the blue colour requires that the antimony trichloride reagent be added to the test solution in the photoelectric colorimeter, necessitating some technical dexterity; (2) the blue colour is not specific for vitamin A, being also given by the carotene compounds. Although this difficulty is to some extent overcome by the use of suitable filters or of a spectrophotometer, the methods in use generally require the application of a theoretical correction factor; (3) at low vitamin A levels the intensity of the blue colour is inadequate to permit sufficiently accurate determination; (4) a practical difficulty arises in the cleaning of cuvettes on which, if they are allowed to become wet, a white deposit forms. For these reasons we found the Carr and Price blue reaction to be inconvenient for routine clinical work and finally employed a modification of the spectrophotometric methods involving the destruction of vitamin A by ultra-violet irradiation (Bessey, Lowry, Brock, and Lopez, 1946; Karmarkar and Rajagopal, 1952).

Heparinized plasma (4 ml.), 8 ml. absolute alcohol, and 8 ml. *n*-heptane (Hopkin and Williams, "free from aromatic hydrocarbons"), are shaken in a mechanical shaker for 15 minutes. No troublesome emulsions are formed and the layers are allowed to separate. The heptane layer is pipetted off and divided into two approximately equal portions. One portion in a soda-glass

tube (12 mm. bore, 1 mm. wall and fitted with a glass stopper) is irradiated for three hours at a distance of 5 cm. from the Woods glass of a "hanovia" fluorescence type portable ultra-violet ray lamp. The optical density of the non-irradiated portion is determined at a wave-length of 327 m μ , using the irradiated portion for the blank setting, in a Beckman DU quartz spectrophotometer. The observed optical density is converted to international units vitamin A per 100 ml. plasma by reference to a standard curve constructed by plotting the difference between the optical densities of irradiated and non-irradiated dilutions of a sample of international standard vitamin A in heptane. The curve is a straight line and the following equation may be derived:

$$E_{1\text{cm.}} \text{ for 1 i.u. vitamin A per ml. heptane} = 0.048 \text{ at } 327 \text{ m}\mu.$$

Thus optical density non-irradiated sample - optical density irradiated sample $\times 4,200 =$ i.u. vitamin A per 100 ml. plasma.

The length of time during which irradiation is carried out will vary with the type of lamp employed. In Fig. 1 it is shown that, with the ultra-violet lamp available to us, destruction of vitamin A is complete at three hours.

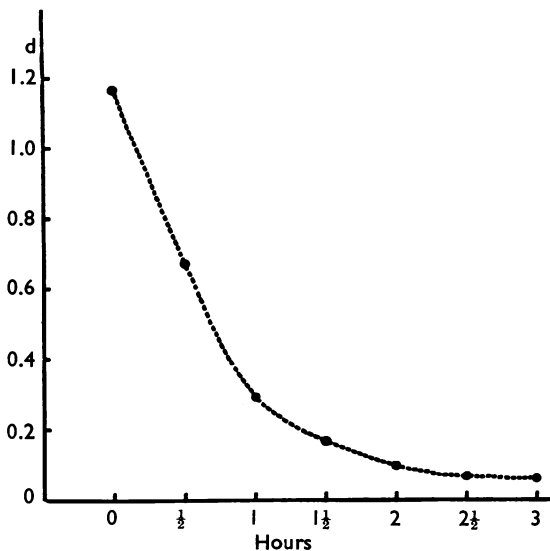


FIG. 1.—Destruction of a vitamin A solution in heptane by irradiation at a distance of 5 cm. from the Woods glass of a "hanovia" fluorescence type portable ultra-violet ray lamp. Ordinate: Optical density vitamin A solution at 327 m μ . Abscissa: Time in hours.

Vitamin A can be satisfactorily estimated by this method, which has also proved convenient for the following reasons: (1) Plasma and heptane extract (either before or after irradiation) may be kept at 4° C. for periods up to 48 hours without appreciable loss of vitamin A. (2) With experience, the amount of fluorescence of the extracts at the start of irradiation gives to the eye a rough indication of the vitamin A content of the samples, particularly when carrying

out a vitamin A absorption test. (3) If the optical density of the heptane extract be determined using a heptane blank, and again after irradiation, 0.75 ml. plasma will suffice without resort to microestimation equipment.

The "Wetting" Factor in Vitamin A Absorption

One of the principal factors affecting the absorption of vitamin A from the intestine is the degree of "wetting" or dispersion of the vitamin A administered. In Fig. 2 are shown the variable

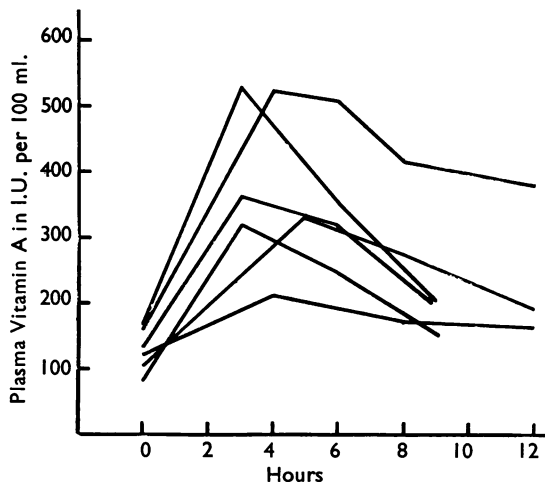


FIG. 2.—Plasma vitamin A levels in six normal individuals following 200,000 i.u. crystalline vitamin A orally, no attention being paid to whether the individual was fasting or not.

plasma vitamin A curves found in six normal individuals to each of whom a dose of 200,000 i.u. vitamin A ester in crystalline form was given in the morning without regard to whether breakfast was eaten, or, if eaten, the type of breakfast. In Figs. 3 and 4 the effect of prior solution of the vitamin in oil is evidenced by the much higher plasma levels reached in two normal individuals.

In recent years considerable experience has been gained both in experimental animals and in man (normal individuals and cases of steatorrhoea) of the comparative absorption of vitamin A administered in oily medium and in media in which the vitamin is in greater dispersion, viz. propylene glycol, dextri-maltose and the "tween" compounds (Lewis, Bodansky, Birmingham, and Cohan, 1947; Kramer, Sobel, and Gottfried, 1947; May and Lowe, 1948, 1949; Jones, Culver, Drummey, and Ryan, 1948; Fox, 1949; Danielson, Binkley, and Palmer, 1949; Barnes, Wollaeger, and Mason, 1950). In general, absorption of vitamin A is greater and more rapid, faecal loss is diminished, and the plasma vitamin A levels are higher when vitamin A in these high-dispersion media is admin-

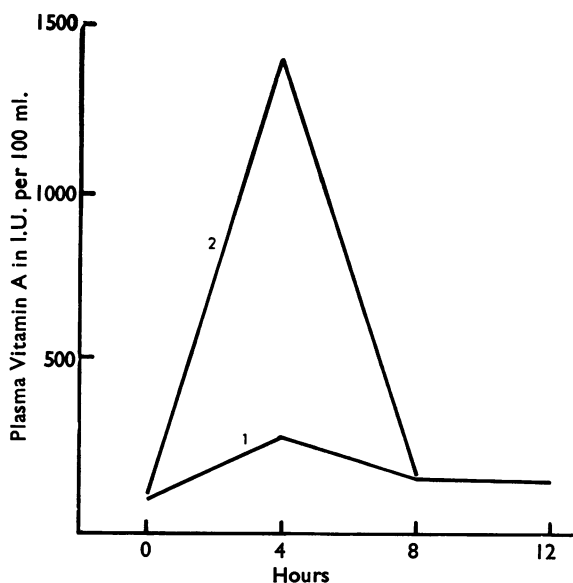


FIG. 3.—Plasma vitamin A levels in a normal individual (not fasting) (1) following 350,000 i.u. vitamin A in crystalline form orally, and (2) following 350,000 i.u. vitamin A in oil orally.

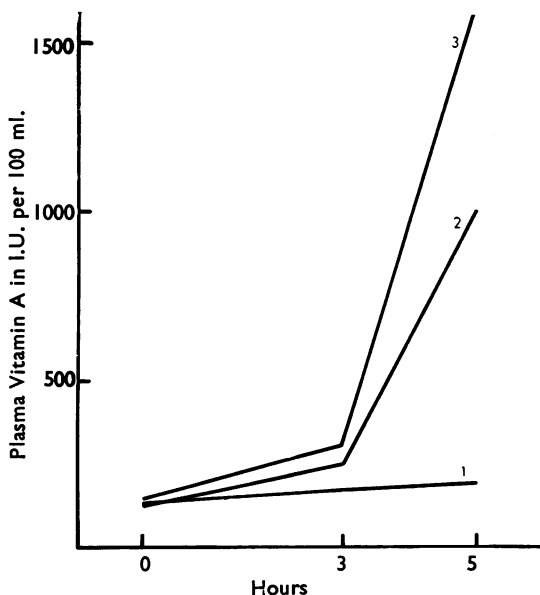


FIG. 4.—Plasma vitamin A levels in a normal individual (1) following 350,000 i.u. crystalline vitamin A orally fasting; (2) following 350,000 i.u. vitamin A in 1.5 ml. oil orally fasting; (3) following 350,000 i.u. vitamin A in 10 ml. oil orally not fasting.

istered to animals and to normal man. In pancreatic fibrosis there is an improvement in the absorption curve to near normal levels, but such improvement has been reported in only a few cases of coeliac disease, sprue, and idiopathic steatorrhoea (May and Lowe, 1949; Fox, 1949; Daniel-

son *et al.*, 1949; Barnes *et al.*, 1950). In Table I are recorded the plasma levels of vitamin A in two cases of idiopathic steatorrhoea. In each case an absorption curve was determined after an oral dose of 350,000 i.u. vitamin A ester in oil. Polyoxethylene sorbitan mono-oleate ("tween 80") was given to the first patient for 10 days and to the second for four days in dosage of 6 g. per day. The absorption curves were then repeated, using the same dose of vitamin A in oil emulsified with sorbitan mono-oleate. It is seen that the effect of the emulsifying agent is to raise the fasting level and also that there is a slight but definite improvement in the absorption curve.

TABLE I
EFFECT OF POLYOXETHYLENE SORBITAN MONO-OLEATE ON THE VITAMIN A ABSORPTION CURVES IN TWO CASES OF IDIOPATHIC STEATORRHOEA

Patient	Method of Administration	Fasting	4 Hrs.	8 Hrs.	12 Hrs.
K. S.	Vitamin A in oil	43*	43	54	54
	Vitamin A in oil and polyoxethylene sorbitan mono-oleate	67	65	92	104
A. B.	Vitamin A in oil	46	102	74	45
	Vitamin A in oil and polyoxethylene sorbitan mono-oleate	69	125	95	86

* Figures are i.u. vitamin A per 100 ml. plasma.

Intermediate Absorption of Vitamin A

Emmet and Bird (1937) suggested that the naturally occurring esters of vitamin A were better absorbed than the free alcohol by the rat. Gray, Morgareidge, and Cawley (1940), however, demonstrated in the same species that hydrolysis of the esters occurred and that the alcohol form was present in the mucosal cells of the intestine. Drummond, Bell, and Palmer (1935) observed that after feeding the free alcohol to a patient with a fistula of the thoracic duct the vitamin was recovered from the chyle mainly as an ester.

In *in vitro* experiments with equal volumes of 90% methanol and heptane, vitamin A alcohol is equally distributed between the solvents, but vitamin A ester (palmitate) remains in the heptane layer. This method of separation (Popper, Steigmann, Dubin, Dyniewicz, and Hesser, 1948) was applied to the plasma obtained four hours after the oral administration of a dose of vitamin A alcohol. The heptane extract was shaken with 90% methanol but none of the vitamin A present in the heptane layer was found to go into the methanol layer. It seems probable, therefore, that vitamin A alcohol undergoes esterification during

the process of absorption. This may take place after the vitamin has been taken up by the mucosal cell.

In normal individuals and in patients with steatorrhoea neither Lewis *et al.* (1947) nor the present authors have found any significant difference between the absorption curves obtained after the administration of vitamin A alcohol or palmitate.

We have not, however, had the opportunity of making an adequate comparison in cases of pancreatic fibrosis.

Nature of the Vitamin A Absorption Curve

Barnes *et al.* (1950) have suggested that the height to which the plasma vitamin A level rises after oral dosage of vitamin A is dependent upon the degree of saturation of the vitamin A storage depots, or, in other words, that the test is a saturation or tolerance test. They therefore advocate the preliminary feeding of vitamin A for at least four days before carrying out the test in order to saturate the tissue stores. Popper, Steigmann, and Zevin (1943) have shown that massive doses of vitamin A orally did not alter the vitamin A absorption curve. This, too, has been our own experience both in normal individuals and in those with steatorrhoea. The absorption curve, however, must be the result of the rates of absorption from the intestine and of storage. It is thus possible that in vitamin A deficiency not due to an absorptive defect a very low vitamin A absorption curve would result.

Aron (1949) reviews suggestive evidence that there exists a mechanism which controls the level of vitamin A in the plasma, and shows that this mechanism may be upset in various disease processes. Thus it has been our experience that despite repeated experiments involving heavy dosage with vitamin A, our normal subjects maintained from day to day almost constant fasting plasma vitamin A levels. Aron considers that the liver plays an important part in the regulatory mechanism, and attributes the fall in the plasma vitamin A level seen in liver disease and in inflammatory processes to disturbance of the mechanism. It is not surprising, therefore, that it has been the experience of several investigators that the vitamin A absorption curve fails to reflect fat absorption in the presence of liver disease (Popper and Steigmann, 1943; Popper, Steigmann, and Zevin, 1943; Legerton, Texter, and Ruffin, 1953). As a rule the plasma vitamin A levels are low and the absorption curves flat. This is only in part due to the exclusion of bile from the intestine. Low levels

and flat absorption curves are also seen in the presence of inflammatory disease.

In two cases of obstructive jaundice a yellow pigment has been extracted by the heptane from the plasma. This pigment has an absorption peak at $410\text{ m}\mu$ and is broken down to colourless compounds by irradiation. These have a broad peak at about $300\text{ m}\mu$ and interfere with the vitamin A extinction at $327\text{ m}\mu$. This is not carotene, which is stable in ultra-violet light, and we have been unable to find any reference to a heptane-soluble fraction of bile pigment. So far we have not been able to extract a similar pigment from bile obtained in the post-mortem room.

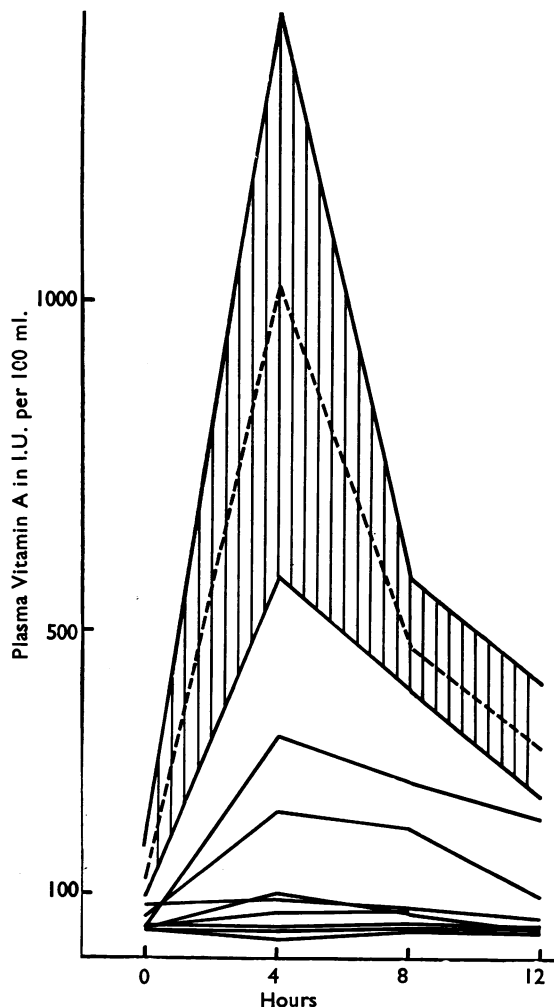


FIG. 5.—Plasma vitamin A levels in 11 normal individuals and eight patients with steatorrhoea following 350,000 i.u. vitamin A in oil (60,000 i.u. per g.) not fasting. Hatched area enclosed by the highest and lowest curves of 11 normal individuals, the dotted line being the arithmetic mean.

The Vitamin A Absorption Test in Steatorrhoea

The need for a standard procedure in carrying out the test is clear. Most investigators have administered the vitamin in the form of oleum percomorph (60,000 i.u. per g.) in dosage of 7,500 i.u. per kilogram body weight, with an upper limit of 350,000 i.u. We have done likewise, but have prepared solutions of crystalline vitamin A alcohol or palmitate in arachis oil in order to overcome the nausea which many individuals suffer after a dose of fish oil. To dissolve vitamin A in oil is difficult, and prior solution in a small quantity of alcohol is required. Since we felt that the object of the test should be restricted simply to determine whether vitamin A is or is not adequately absorbed from the intestine, we considered that no purpose is served in withholding meals. In Fig. 5 the highest and lowest plasma curves, together with the mean curve of 11 normal subjects, are drawn. Vitamin A absorption curves of eight cases of steatorrhoea, confirmed by fat balance studies, are also shown. These results are in close accord with those of Legerton *et al.* (1953) and earlier authors cited above, and permit the conclusion that although a flat vitamin A absorption curve is not necessarily diagnostic of steatorrhoea, the finding of a plasma vitamin A level of 500 i.u. or more between four and five hours following the oral dose of vitamin A in oil would almost certainly exclude a diagnosis of steatorrhoea. The vitamin A absorption test is thus a valuable screening procedure.

Summary

A spectrophotometric method utilizing the destruction of vitamin A by ultra-violet irradiation has been employed for the determination of the plasma vitamin A level.

The vitamin A absorption test and factors which relate to its performance and interpretation are discussed.

A low vitamin A absorption curve is not diagnostic of steatorrhoea, but the test as described is of value in that adequate absorption of vitamin A excludes a diagnosis of steatorrhoea.

We wish to express our thanks to colleagues who acted as control subjects, and to Professors E. J. King and J. MacMichael in whose departments the work was carried out.

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