SERUM VITAMIN A AND TOTAL PLASMA LIPID CONCENTRATIONS AS INFLUENCED BY THE ORAL ADMINISTRATION OF VITAMIN A TO CHILDREN WITH THE NEPHROTIC SYNDROME [1]

By B. M. KAGAN, ELAINE M. THOMAS, DAVID A. JORDAN, AND ARTHUR F. ABT

(From the Kunstadter Laboratories for Pediatric Research, the Sarah Morris Hospital for Children of Michael Reese Hospital and the Department of Pediatrics, University of Illinois College of Medicine, Chicago)

(Received for publication June 21, 1949)

The fasting serum vitamin A (1, 2) and total plasma lipid concentrations are abnormally high in children with the nephrotic syndrome. The following studies were planned to determine whether there is a relationship between the serum vitamin A and total plasma lipid concentrations in this condition; whether there is an abnormality in vitamin A absorption, excretion or utilization; and whether in this condition there is a difference in the metabolism of vitamin A administered orally as the ester in oil or as the alcohol form in aqueous dispersion.

MATERIAL AND METHODS

Six children with the nephrotic syndrome were studied. Their ages ranged from 18 months to 10 years. Eighteen normal children studied as controls varied in age from 10 months to 12 years.

The preparation of vitamin A ester in oil was Oleum Percomorphum [2] containing 60,000 U.S.P. units/gram. The vitamin A alcohol in aqueous dispersion contained 50,000 U.S.P. units/ml. with 36% Tween 20 as dispersing agent.[3] The amount of vitamin A per unit volume in these two preparations was purposefully planned to make them as similar as possible in concentration.

Each child was given a test dose of 6,000 U.S.P. units of vitamin A alcohol in aqueous dispersion per pound of body weight. Weights of the children were recorded daily. For purpose of the experiment, body weight was accepted to be the lowest weight determined prior to the test during the hospital stay. There was a rest period of at least three days between the times the test doses were given.

All food was withheld for at least 12 hours prior to collection of the fasting specimen and for 12 hours before the 24 and 48 hour specimens. In the interim the children were permitted small amounts of food low in fat and vitamin A (dry toast, jello, orange juice, grapefruit juice and carbonated beverages).

Blood was collected before administration of the test dose and again three, six and 24 hours after it had been given. In a few instances specimens were collected one and 48 hours after the test dose was given.

Serum vitamin A and carotene concentrations were determined by a minor modification of the Carr and Price method as described by Kramer *et al.* (2). We modified the method only as follows: 1 ml. of serum was extracted with successive 1 ml. and 2 ml. portions of petroleum ether; carotene and vitamin A were each determined in a final volume of 1.5 ml. in a Coleman spectrophotometer. Total lipid concentration was determined on alcohol-ether extract of 1 ml. of heparinized plasma by the Bloor oxidation technique (3).

RESULTS

Table I and Fig. 1 give the concentrations of serum vitamin A obtained in six children with the nephrotic syndrome before and after test doses of vitamin A alcohol in aqueous dispersion. One of these children (C. L.) was examined 10 months after an episode of anasarca during a time when he had no complaints, was free of edema and had a normal cholesterol and total lipid level but a low serum albumin concentration and persistent albuminuria. The results obtained on 18 normal children under the same conditions are also given.

The fasting serum vitamin A concentration of all but the convalescent case (C. L.) was higher than the highest normal. This has previously been reported by Josephs (1) and by Gottfried *et al.* (4). In addition, following a test dose of vitamin A alcohol in aqueous dispersion, the serum vitamin A concentrations reached much higher levels in children with the nephrotic syndrome than in normal children. The degree of elevation was

[1] This study was aided by a grant from Mead Johnson & Company, Evansville, Indiana.

[2] Supplied by Mead Johnson & Company, Evansville, Indiana.

[3] Prepared and supplied by Dr. Samuel M. Gordon of Endo Products Inc., Richmond Hill 18, New York.

TABLE I

Serum vitamin A concentrations in the fasting state and after administration of 6,000 U.S.P. units/pound of vitamin A alcohol in aqueous dispersion to children with the nephrotic syndrome and to controls

Case	Severity	Vitamin A U.S.P. units/100 ml.				
		Fasting	3 hours	6 hours	24 hours	48 hours
J. S.	Very severe	619	9,913	14,429	5,245	2,388
J. S.	Very severe	1,665	13,320	17,383	13,586	—
A. M.	Very severe	270	9,807	12,600	3,051	1,137
S. R.	Severe	1,343	8,059	7,542	5,068	—
S. R.	Severe	543	4,063	5,155	4,496	—
S. R.	Severe	1,659	6,204	7,443	11,269	5,688
M. K.	Severe	240	2,667	11,089	992	350
V. H.	Moderate	196	2,398	3,230	1,798	—
C. L.	*	153	5,495	4,669	673	153
18 Normal controls	Mean	125	3,280	854	198	—
	Std. deviation	32	1,337	410	63	
	Std. error	7.5	315	97	15	

* Ten months after acute episode. Patient was edema free at this time. Blood cholesterol and total lipid were normal. Total serum protein was 4.9, albumin 1.2, globulin 3.7. Urine showed 4+ albumin, occasional casts and 4–5 rbc/LPF not centrifuged.

generally greater in those more seriously affected (J. S., M. K., and S. R.) than in those less ill (C. L. and V. H.). Six hours after the test dose, the patients showed serum vitamin A concentrations which were two to 10 times as high as the highest normal at the same time interval. All the concentrations were elevated above the highest normal at the 24 hour interval. Even at 48 hours, three of the patients showed higher vitamin A concentrations than the highest normal at 24 hours. (The highest serum vitamin A concentration at the 24 hour interval in the normal children was 470 U.S.P. units/100 ml.)

Table II and Fig. 2 show the concentrations of serum vitamin A obtained in children with the nephrotic syndrome before and after a test dose of vitamin A ester in oil. The results are essentially the same as with the alcohol form except that in each patient the serum levels were considerably lower following the ester form than following the alcohol form.

There were large variations in the fasting total plasma lipid concentrations (815 to 5,114 mg./100 ml.) and in the fasting serum vitamin A concentrations (153 to 1,665 U.S.P. units/100 ml.). While the higher fasting vitamin A concentrations were seen in those with higher fasting total lipid levels, no quantitative relationship was demonstrated.

In the children with the nephrotic syndrome and in the normals, there was a distinct rise in the total plasma lipid concentration following administration of vitamin A alcohol in aqueous dispersion. In four normal children receiving vitamin A alcohol the highest rise in the lipid was seen at six hours (a rise of 105 mg./100 ml.). In the children with the nephrotic syndrome who received vitamin A alcohol rises greater than this were seen at three hours, but the maximum increases were seen at six hours (135 to 1,410 mg./100 ml.-av. 550 mg./100 ml.). At 24 hours in the normals the total plasma lipid concentrations were 73 mg./100 ml. or more lower than the level in the fasting state. On the other hand in the nephrotic syndrome they ranged from 49 to 1,190 mg./100 ml. (av. 400 mg./100 ml.) higher than in the fasting state.

In this connection, it may be recalled that when repeated large daily doses of vitamin A were given to normal rats, other animals and man, temporary rises in lipid levels occurred. Josephs (5) found that this was more marked and prolonged in vitamin deficient infants. Apparently there is mobilization of some lipid upon administration of vitamin A in large doses. Data in this study show that this occurs in the nephrotic syndrome after

TABLE II

Serum vitamin A concentrations in the fasting state and after administration of 6,000 U.S.P. units/pound of vitamin A ester in oil to children with the nephrotic syndrome

Case	Severity	Vitamin A U.S.P. units/100 ml.				
		Fasting	3 hours	6 hours	24 hours	48 hours
J. S.	Very severe	1,359	3,197	8,365	8,591	6,094
S. R.	Severe	416	839	2,051	1,732	1,212
V. H.	Moderate	176	480	2,271	942	330
C. L.	*	153	500	509	736	176
Normal		180	699	500	180	—

* Ten months after acute episode. See footnote to Table I.

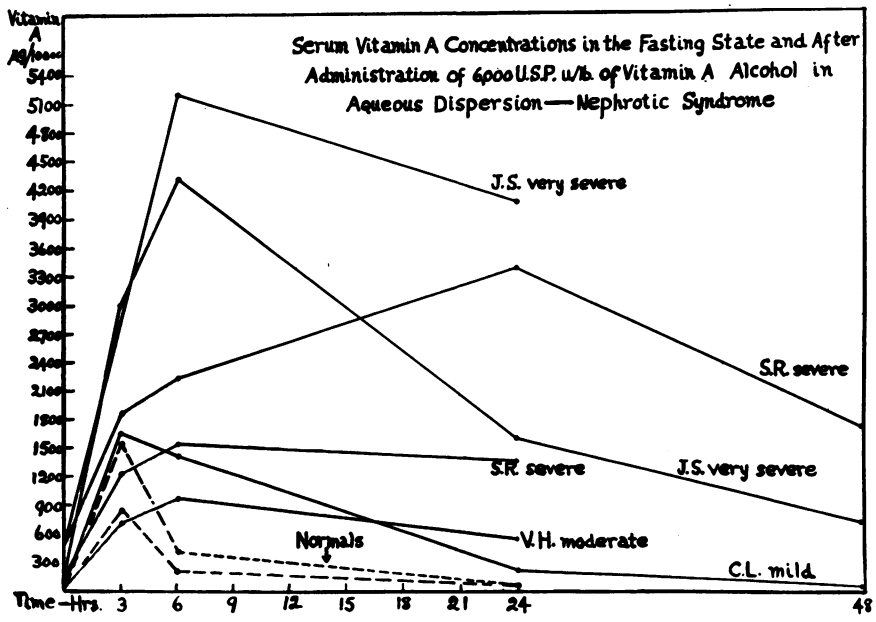

FIG. 1.

a single large dose of vitamin A. It may be that some of this mobilized lipid is related to the retention of vitamin A in the blood in the nephrotic syndrome.

In all but two of 13 determinations in the six children with the nephrotic syndrome the serum carotene concentrations in the fasting state were above normal (138 to 884 μg/100 ml.—av. 370 μg/100 ml. compared to normals, 72 to 259 μg/100 ml. —av. 125 μg/100 ml.). The fasting concentrations tended to be higher in those patients who were more seriously ill and who had higher total plasma lipid concentrations. This corroborates previous findings (1, 2). Following administra-

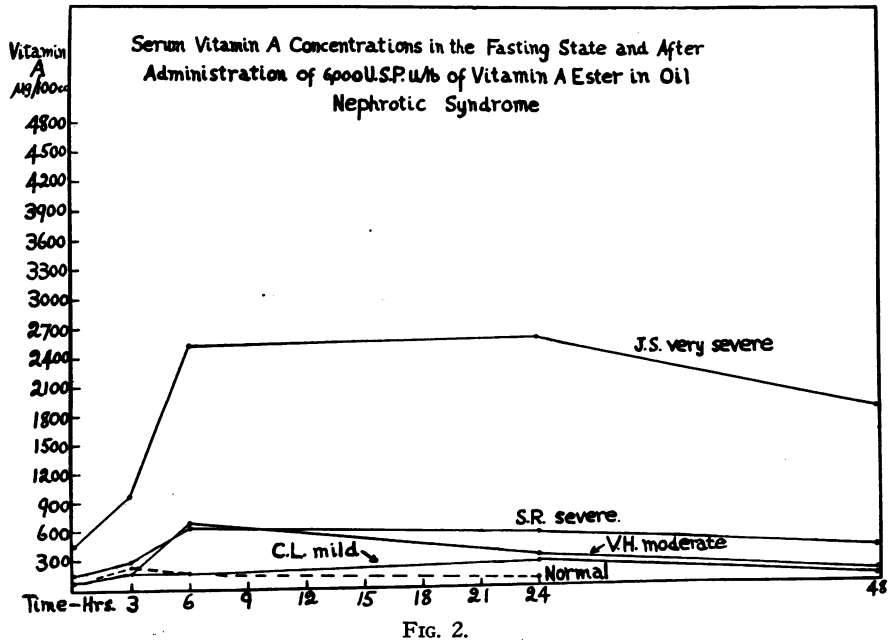

FIG. 2.

tion of the test dose of either vitamin A ester in oil or vitamin A alcohol in aqueous dispersion, the changes in the serum carotene concentrations were slight and inconstant in both the normal children and in those with the nephrotic syndrome.

DISCUSSION

The plotted curve of serum vitamin A concentrations in children with the nephrotic syndrome following a test dose of vitamin A resembles a diabetic glucose tolerance curve in that an abnormally high concentration is reached and there is a delayed fall. These changes in the nephrotic syndrome could be due to one or more of the following processes: increased absorption, decreased utilization or storage, or decreased excretion of vitamin A.

Serum vitamin A concentrations were determined one hour after the test dose on four normal children and three children with the nephrotic syndrome. The children with the nephrotic syndrome and the normals showed a similar change. This suggests that increased rate of absorption is probably not responsible for the high serum vitamin A concentrations found at longer intervals after the test dose.

The possibility of diminished urinary excretion of vitamin A seems remote in view of evidence that urinary excretion of vitamin A does not occur in the normal individual (6). Furthermore, we could detect no vitamin A in the urine of a normal or in the urine of a child with the nephrotic syndrome either before or during a 48 hour period after a test dose of vitamin A alcohol in aqueous dispersion.

The abnormally slow fall of the serum vitamin A concentration in children with the nephrotic syndrome following the administration of a test dose of vitamin A is, therefore, probably due to failure by the body to store or utilize the vitamin.

Failure of storage in the liver may be responsible for the slow fall in serum vitamin A concentration. Most of the body's vitamin A is stored in the liver (7). Clausen (7) and Sobel *et al.* (8) showed that there is an increase of vitamin A in the liver following its ingestion. Josephs' (9) work suggests that the liver normally has limited ability to take up a large quantity of vitamin A. It is possible, therefore, that in children with the nephrotic syndrome the prolonged elevation of serum vitamin A concentration is due to failure by the liver to utilize or store vitamin A even at the normal rate.

The possible role of the blood lipid has been discussed above. A change in body protein in this syndrome must also be considered as a possible factor. Luck (10) and also Addis *et al.* (11) showed that in rats fed a diet poor in protein, the protein content of the liver was reduced. Baumann *et al.* (12) showed that rats on such a diet were less able to store the vitamin in their livers. They suggested that the vitamin is held in the liver in the form of a protein complex. It may be, therefore, that in the nephrotic syndrome the liver protein component to which vitamin A is attached is depleted and that this is responsible for the observations in this study.

SUMMARY

This study demonstrated that there is an abnormality in the metabolism of vitamin A in children with the nephrotic syndrome. Although higher serum vitamin A concentrations were observed in patients with higher total plasma lipid concentrations, no quantitative relationship was demonstrated.

Higher serum vitamin A levels were found in nephrotic patients following a test dose of vitamin A alcohol in aqueous dispersion than following a test dose of vitamin A ester in oil. The highest concentrations of serum vitamin A were observed six hours or more after administration. At six hours, the levels were two to 10 times as high as the highest level noted in normal children at the same time interval. Statistical analysis of the data shows this finding to be significant. The concentrations remained elevated for 24 and even for 48 hours. Various possible causes for these differences were studied and are discussed. The most likely is that the liver in the nephrotic syndrome fails to utilize or store vitamin A as rapidly as in the normal.

BIBLIOGRAPHY

1. Josephs, H. W., Studies in vitamin A. Bull. Johns Hopkins Hosp., 1939, **65**, 112.
2. Kramer, B., Sobel, A. E., and Gottfried, S. P., Serum levels of vitamin A in children. Am. J. Dis. Child., 1947, **73**, 543.
3. Bloor, W. R., The determination of small amounts of lipid in blood plasma. J. Biol. Chem., 1928, **77**, 53.

4. Gottfried, S. P., Steinman, J. F., and Kramer, B., Chemical studies in children with the nephrotic syndrome. Am. J. Dis. Child., 1947, **74**, 283.
5. Josephs, H. W., Lipid response to vitamin A administration. Bull. Johns Hopkins Hosp., 1945, **77**, 402.
6. Lawrie, N. R., Moore, T., and Rajagopal, K. R., The excretion of vitamin A in urine. Biochem. J., 1941, **35**, 825.
7. Clausen, S. W., The absorption of vitamin A and its storage in the tissues. The Harvey Lectures, 1942–43, 199.
8. Sobel, A. E., Sherman, M., Lichtblau, J., Snow, S., and Kramer, B., Comparison of vitamin A liver storage following administration of vitamin A in oily and aqueous media. J. Nutrition, 1948, **35**, 225.
9. Josephs, H. W., Studies in vitamin A. Bull. Johns Hopkins Hosp., 1942, **71**, 265.
10. Luck, J. M., Liver proteins. I. The question of protein storage. J. Biol. Chem., 1936, **115**, 491.
11. Addis, T., Poo, L. J., and Lew, W., The quantities of protein lost by the various organs and tissues of the body during a fast. J. Biol. Chem., 1936, **115**, 111.
12. Baumann, C. A., Foster, E. G., and Moore, P. R., The effect of dibenzanthracene, of alcohol, and of other agents on vitamin A in the rat. J. Biol. Chem., 1942, **142**, 597.