Serum and Liver Vitamin A and Lipids in Children with Severe Protein Malnutrition

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SERUM cholesterol levels are very low in children suffering from the kwashiorkor type of severe protein malnutrition, but increase rapidly after a few days of therapy.[1–5] The significance of this rise became more apparent when it was found that it paralleled changes in other serum lipid constituents.[5] These increases were observed even when the therapeutic diet contained negligible amounts of fat. It has been proposed that the lipids appearing in the circulating plasma under these conditions are of tissue origin, probably from the liver,[5] although it is conceivable that some fat could have been synthesized from carbohydrates and some cholesterol from acetate.

The very reduced serum levels of vitamin A seen in children with kwashiorkor upon admission to the hospital,[6] were at first assumed to be a result of a dietary deficiency. In view of the changes in serum lipids just noted, however, the pattern of change of vitamin A serum levels during initial recovery was studied in children with kwashiorkor who received a diet practically devoid of vitamin A activity. An increase in vitamin A serum levels under these conditions could occur only at the expense of stored vitamin A since this vitamin is not synthesized *de novo* in the body. Such a finding would strongly imply that the mechanism of blood transport of lipids is impaired in acute kwashiorkor and that this impairment is rapidly corrected with treatment.

Since fat accumulates in large quantities in the liver of children with kwashiorkor, and because liver tissue is the main reservoir of vitamin A, the relative changes in the lipids and vitamin A content of the serum and liver tissue were studied during the initial phase of recovery from the disease.

MATERIAL

The subjects were boys admitted to the hospital with acute kwashiorkor, conforming to the typical clinical picture of the disease as it occurs in Central America, including edema, skin lesions, hair changes, anorexia, apathy and severe hypoproteinemia.[6]

Preliminary observations on the changes in serum levels of lipids, vitamin A and carotene were made in the three following cases:

DEC. This child, aged two years and five months, was treated from the time of admission with skim milk and calcium caseinate at a level of intake increasing from 6.6 gm. of protein and 75 cal. per kg. of body weight per day initially, to 11 gm. of protein and 94 cal. per kg. per day by the end of the one-month observation period.

VDC. This child, aged five years and six months, was given a diet of corn and beans at a level of intake of 2 gm. of protein and 70 cal. per kg. of body weight per day, for nine days following hospital admission. Forty-five per cent of the protein came from corn and 55 per cent from beans. Since the clinical condition of the child was not improving satisfactorily, the diet was changed at this time to skim milk which supplied 4.5 gm. of protein and 80 cal. per kg. per day.

BLO. This infant, aged one year and five months, was given a diet of corn and beans during the first four weeks of hospitalization at a level of intake of 2 gm. of protein and 90 cal. per kg. of body weight per day.

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The proportion of corn and beans was the same as for VDC. At the end of the second week, 60 gm. of a puree of a green leaf (Amaranthus hybridus) were added to the daily diet as a source of natural carotene. This supplied 5,000 I.U. of vitamin A activity. At the end of the fourth week, the corn-bean diet was replaced by skim milk, at which time the protein intake was increased gradually from 2 to 5 gm. per kg. of body weight per day and the caloric intake from 90 to 120 cal. per kg. per day. The observation period lasted two months

In order to study changes in liver composition that accompany the changes in serum, both liver tissue samples and serum samples were studied in the following four cases:

JRA. This child, aged four years and eight months, received skim milk with calcium caseinate at a level that increased from 2 gm. of protein and 80 cal. to 5 gm. of protein and 120 cal. per kg. of body weight per day.

JRE. This child, aged three years and nine months, was treated with skim milk from the time of admission. The intake increased from 2 gm. of protein and 60 cal. to 4 gm. of protein and 90 cal. per kg. of body weight per day by the eighteenth day of study.

PAP. This three year old child was treated from the day of admission with skim milk at a level of intake which increased from 2 to 8 gm. of protein and 60 to 90 cal. per kg. of body weight per day during the observation period of three weeks.

LCA. This child, aged one year and eleven months, received skim milk from the day of admission at a level of intake of 2 gm. of protein and 60 cal. per kg. of body weight per day, increasing to 4 gm. of protein and 90 cal. per kg. per day by the end of the observation period of three weeks.

METHODS

Blood samples were obtained at approximately weekly intervals by either venipuncture or fingertip prick. The serum was separated within two hours and serum proteins were immediately estimated by the method of Lowry and Hunter.[7] Aliquots of the rest of the serum were frozen at −20°C. until analyzed. The micromethod of Bessey et al.[8] was used for the estimation of vitamin A and carotene. Cholesterol was measured by a microadaptation of the method of Abell et al.[9] using 0.050 ml. of serum. An aliquot of 0.040 ml. of serum was extracted with Bloor's solvent mixture for the determination of lipid phosphorus by the method of Maclay[10] adapted to a microscale, and total lipids by the dichromate oxidation method of Bragdon.[11] The factor of 25 was used to convert lipid phosphorus to phospholipid values.

In addition, in four cases (JRA, JRE, PAP and LCA) a liver biopsy was performed, not only before any dietary therapy was instituted, but also after satisfactory initial response to therapy, as shown by laboratory and clinical evidence. After previous verification of a normal prothrombin time and local anesthesia, the liver biopsy specimens were taken with a Franseen needle by aspiration through the pleural space. From 15 to 25 mg. of tissue were thus obtained and a 1:25 homogenate was prepared in ice cold 0.85 per cent sodium chloride solution. Not more than one hour elapsed between the taking of the sample and the homogenization, and the sample was kept in a test tube immersed in ice during that time.

Total lipids, phospholipids and vitamin A and carotene were determined in this sample by the same technics as for serum, and proteins with the reagent of Folin-Ciocalteu as described by Lowry et al.[12]

RESULTS

Table 1 gives the changes observed in the serum of the first three children studied. The clinical improvement of DEC with the milk diet was very satisfactory. His total serum proteins increased 2.2 gm. per 100 ml. in seven days and 3.6 gm. in thirteen days. The increases in phospholipids, cholesterol and vitamin A were very marked; their levels reached peak values at essentially the same time. The variations in the carotene levels in serum were insignificant.

The clinical condition of VDC did not improve satisfactorily on the corn-bean diet, and the total serum proteins did not change significantly; neither the concentration of vitamin A nor that of carotene increased during this period. Upon changing the diet to skim milk, a marked clinical improvement was seen with a parallel increase in serum proteins in ten days from 4.1 to 5.8 gm. per 100 ml. Vitamin A, phospholipids and cholesterol serum levels also increased rapidly from this time but carotene levels remained low.

On the corn-bean diet, BLO did not show appreciable recovery; some edema, irritability,

TABLE
Serum Proteins, Lipids, Vitamin A and Carotene

	DEC (Diet 1)						VDC (Diet 2)			
	Days of Treatment						Days of Treat-			
	0	7	13	20	31	40	0	6	16	22
Serum components per 100 ml.										
Proteins (gm.)...	3.2	5.4	6.8	7.0	7.0	7.4	3.8	4.1	5.8	6.3
Vitamin A (μg.).	4	37	43	30	21	8	12	9	48	47
Carotene (μg.)...	7	11	7	4	5	5	5	1	11	5
Cholesterol (mg.)..	74	219	236	...	134	94	65	...	171	144
Phospholipids (mg.)	165	320	315	...	200	135	150	...	282	238

NOTE: Diet 1—Skim milk plus calcium caseinate. Diet 2—Corn-beans for thirty days, then changed to skim milk for next thirty-five days.

TABLE
Serum and Liver Composition During

	JRA					JRE	
	Days of Treatment					Days of	
	0	7	12	15	19	3	6
Serum components per 100 ml.							
Proteins (gm.).....	3.1	4.4	5.4	5.9	6.5	3.8	4.1
Vitamin A (μg.).....	6	22	39	41	30	10	10
Carotene (μg.).....	3	5	6	6	1	5	4
Total lipids (mg.).....	520	887	940	933	840	448	502
Cholesterol (mg.).....	69	166	227	210	153	89	62
Phospholipids (mg.).....	155	252	280	255	212	120	99
Liver components per 100 gm.							
Proteins (gm.).....	13.1	...	...	...	17.3	9.3	...
Vitamin A (μg.).....	2500	...	...	...	944	1950	...
Carotene (μg.).....	47	...	...	...	51	121	...
Total lipids (gm.).....	18	...	...	...	10	...	...
Phospholipids (gm.).....	5.1	...	...	...	5.3	...	...

* All children received skim milk as the only dietary protein source.

anorexia and skin lesions were still present at the end of one month, and the serum proteins had increased only 0.5 gm. per 100 ml. Frank improvement in all the clinical signs was noted shortly after the diet of skim milk was started. The total serum proteins increased 1.1 gm. per 100 ml. in only seven days. Parallel to this improvement, serum levels of vitamin A, cholesterol and phospholipids rapidly increased; serum carotene also rose in this child.

The biochemical results of the four children in whom both changes in serum and liver composition were studied, are shown in Table II. Each of these four children was treated from the time of admission with a therapeutic diet in which the protein was supplied by milk. Except for some dehydration during the first few days, all showed satisfactory clinical recovery and their total serum proteins increased steadily with treatment.

During Initial Recovery from Kwashiorkor

			BLO (Diet 3)							
ment			Days of Treatment							
29	36	43	0	8	22	29	36	38	45	54
6.2	6.9	7.0	3.8	3.9	3.8	4.3	5.4	6.1	6.4	7.0
38	38	28	4	8	8	10	23	31	37	40
5	6	9	8	6	7	6	27	32	66	94
144	149	144	135	144	121	104	168	188	176	149
185	192	208	225	...	220	190	...	270	250	190

milk, green leaf carotene source added beginning on the fifteenth day. Diet 3—Corn-beans for nine days, skim

II

Initial Recovery from Kwashiorkor*

		PAP				LCA			
Treatment		Days of Treatment				Days of Treatment			
13	17	0	7	14	21	0	7	15	20
6.2	6.6	3.3	4.9	6.2	6.5	3.3	4.4	5.5	5.8
49	24	2	0	2	0	0	0	0	3
4	3	2	2	4	4	8	4	4	1
721	...	...	...	...	...	334	367	488	440
193	...	61	94	120	122	80	88	115	132
235	...	108	135	185	180	66	50	79	86
...	17.1	9.5	...	...	12.4	9.9	...	...	...
...	1090	150	...	...	0	104	...	...	...
...	116	150	...	...	97	103	...	...	...
...	7	21	...	...	8	19	...	...	...
...	4.6	3.1	...	...	3.6	3.5	...	...	...

Two of these children (JRA and JRE) had appreciable reserves of hepatic vitamin A (2,500 and 1,950 μg. per 100 gm. of fresh liver, respectively). Both vitamin A and the serum lipid fractions studied rose markedly although carotene did not. The increase in protein content of the liver was paralleled by a drop in hepatic vitamin A content in both children, while the carotene in the liver did not change appreciably.

In JRA, total liver lipids dropped from 18 per cent initially to 10 per cent on the nineteenth day with practically no variation in phospholipid content. In JRE, no initial total lipid and phospholipid analyses were possible, but the figures obtained on the seventeenth day agree with the reduced figures for JRA after initial recovery.

There were two main differences between the results in these two children and those for

PAP and LCA. In the latter, no elevation of the very low serum vitamin A levels was obtained with treatment, and furthermore, the initial vitamin A content of their liver tissue was negligible (150 and 104 μg. per 100 gm. of fresh tissue, respectively). The liver tissue analyses performed for PAP on admission and after twenty-one days of treatment, show that hepatic protein increased, carotene and fat decreased and phospholipid practically remained unchanged. No second biopsy analysis could be made on LCA, but the initial sample had a composition similar to that of PAP.

COMMENTS

With treatment, serum levels of vitamin A increased even though the diets, with one exception, did not contain vitamin A or carotene. These observations suggest that the sudden appearance of the vitamin in the circulation was due to mobilization of tissue stores brought about by protein therapy. This is confirmed by the corresponding rapid decrease in vitamin A in the liver tissue, and by the lack of serum vitamin A increases in the two children whose initial hepatic reserves of the vitamin were negligible (PAP and LCA).

By analogy, the parallel changes in total serum and liver lipids could also be explained by the improvement of an initially impaired capacity of plasma to transport them from the liver to other tissues of the body. The fact that the phospholipid concentration of liver tissue did not decrease parallel to the increase in serum, suggests that the hepatic biosynthesis of these compounds during initial recovery is sufficient to compensate for the amounts which enter the circulation.

The observed changes are closely associated with recovery, and particularly with regeneration of serum proteins. In kwashiorkor, failure to mobilize lipids and vitamin A may be a direct consequence of the severe hypoproteinemia since several fractions of the plasma proteins are important in the transport of lipid compounds. It is known, for example, that endogenous vitamin A is mobilized in the alcohol form bound to the albumin fraction of the plasma proteins.[13] Phospholipids and cholesterol are present in highest concentrations in the β-globulin fraction of proteins.[14]

Very low serum albumin levels are a universal finding in kwashiorkor and a decreased β-globulin fraction occurs frequently in Central American patients.[15] These changes may well be the primary cause of the failure in mobilization of vitamin A and lipid fractions in kwashiorkor. The rapid synthesis of the carrier-plasma proteins during recovery may be the factor determining the prompt re-establishment of normal fat transport when adequate treatment is given. This hypothesis is strengthened by the findings of Cravioto et al.[16] that serum levels of α and β-lipoproteins are initially low in kwashiorkor and increase rapidly with recovery.

When given large doses of vitamin A palmitate in oil, children with acute kwashiorkor do not show the marked increase in plasma vitamin A which occurs in well nourished subjects under these circumstances.[17] This apparent lack of absorption may well be explained by the transport disturbance indicated by the present results. In Indonesia, Yap Kie Tiong[18] found that ten children with frank keratomalacia, the most advanced eye lesion of vitamin A deficiency, presented very low levels of the vitamin as well as severe hypoalbuminemia (average 1.7 gm. per 100 ml.; range 1.3 to 2.1 gm. per 100 ml., while ten other children with only xerophthalmia also had very low serum levels of vitamin A but their serum albumin was not nearly as low (average 3 gm. per 100 ml.; range 2.5 to 3.7 gm. per 100 ml.).

The results suggest that in severe protein deficiency, the vitamin A of the diet does not reach the liver; furthermore, whatever reserves may remain in the hepatic tissue are not available to the other tissues of the body for utilization. It is thus postulated that, independently of both the dietary intake and liver stores of vitamin A, a functional deficiency of this vitamin may occur in kwashiorkor and other forms of severe protein deficiency, as a consequence of the reduced plasma proteins.

SUMMARY

The serum levels of total lipids, phospholip-

ids, cholesterol, vitamin A and carotene were measured in children with kwashiorkor on admission to the hospital, and at regular intervals during the first weeks of recovery. Marked increases in all these serum constituents were observed, except for vitamin A in some of the children. Liver biopsy specimens were also studied for the same lipid constituents, except cholesterol. The data indicate that total lipids and vitamin A decrease in liver tissue simultaneously with an increase in these serum components. The findings are suggestive of an initial impairment in lipid and vitamin A blood transport possibly associated with decreases in the plasma protein fractions to which these lipid compounds are normally bound. Phospholipids differ in that they are apparently synthesized in the liver at a rate sufficient to prevent their decrease in hepatic tissue despite increased removal in the circulation.

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