Impairment of Intestinal Absorption of Vitamin A Palmitate in Severe Protein Malnutrition (Kwashiorkor)

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ONE of the consequences of the severe protein malnutrition underlying kwashiorkor is a decrease in the activity of various enzymes as measured in blood serum, tissues, and duodenal contents. Veghélyi,[1] Thompson and Trowell[2] and Gómez *et al.*[3] have reported that the activities of lipase, trypsin, and amylase are reduced in the duodenal secretions of kwashiorkor patients. Of the three enzymes lipase appears to be the first to be affected, followed by trypsin.[1] These biochemical findings are consistent with the histologic damage observed in the pancreas and intestine when the disease is severe.[4,5]

A decrease in pancreatic lipase and in bile salts as well as functional alterations of the small intestine, are known to impair the absorption of vitamin A. In the course of studies in children with kwashiorkor in Central America[6] very low serum levels of vitamin A have been found and ocular lesions suggestive of vitamin A deficiency are observed.

Even though a dietary deficiency of sources of vitamin A or carotene may be partially responsible for these biochemical and clinical findings, the contribution of metabolic and pathologic factors which alter the utilization of the vitamin should also be considered. The present work was designed to study the effect of oral administration of vitamin A palmitate on serum vitamin A levels in children with kwashiorkor before and after therapy.

METHODS AND TECHNIC

A vitamin A "absorption test" was performed according to the recommendations of Mendeloff.[7] A vitamin A palmitate concentrate¶ was diluted with corn oil shortly before use to a final concentration of 10,000 μg/ml; the preparation was kept refrigerated and protected from direct light. The test solution was then administered by stomach tube to the fasting child to provide a net dose of 75,000 μg of vitamin A. Two hours later the subject was given a test meal consisting of either 200 ml of skimmed milk or the same quantity of corn starch gruel; both gave the same type of response. Fingertip blood samples were taken just before the administration of the vitamin and then 1, 2, $2^1/_2$, 3, 4 and 5 hours following the vitamin dose. The two-hour sample was taken immediately prior to the test meal. In this way, seven samples were collected during a period of five hours. A surgical blade was employed to make only one fingertip incision from which the seven 0.5 ml

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¶ General Biochemicals, Inc., Chagrin Falls, Ohio. Vitamin A palmitate in corn oil, 1,000,000 i.u./g.

TABLE I

Total Serum Protein in Serial Fingertip Blood Samples of Children with Kwashiorkor

Case	Days of treatment	Total serum proteins (g/100 ml) at time intervals (hrs) after test dose						
		0	1	2	2½	3	4	5
PC-46	0	—	4.55	4.42	4.45	4.43	4.05	4.05
PC-48	0	—	4.12	4.20	4.14	4.12	4.14	4.02
PC-49	0	3.19	3.17	3.12	3.08	3.00	2.88	2.93
PC-50	0	4.40	4.40	4.40	4.34	4.30	4.00	4.16
PC-52	5	4.32	4.16	4.10	4.20	4.18	4.10	3.70
PC-46	5	5.98	5.60	6.00	5.53	5.42	5.50	5.42
PC-50	5	5.20	5.24	4.92	5.02	4.82	4.85	4.62
A-3	3	5.37	5.37	4.84	5.21	5.07	4.93	4.90
A-4	3	4.85	5.09	5.40	5.35	5.20	5.12	5.37
Completely recovered cases of kwashiorkor								
RPC-1	67	7.26	—	7.23	7.26	7.24	7.28	—
RPC-2	130	6.76	—	7.08	7.12	6.98	7.01	—
RPC-3	315	6.72	—	6.83	6.81	6.83	6.81	—

blood samples could ordinarily be collected by re-opening the wound with pressure, thus causing a minimum of discomfort to the child.

The serum from each sample was stored at −20° C until analyzed for vitamin A and carotene by the microtechnic of Bessey *et al.*[8] In order to evaluate the possible contamination of the blood samples with extracellular exudate (particularly in patients with edema), total proteins were also measured in each individual sample of serum by the method of Lowry and Hunter.[9]

EXPERIMENTAL PROCEDURE AND RESULTS

Children admitted with frank clinical kwashiorkor either to the General Hospital or to the hospital of the "Sociedad Protectora del Niño" in Guatemala City were tested. In the original description of the method a positive response was recorded when the test dose resulted in an increase of the serum vitamin A level to at least four times the fasting concentration or in an absolute peak value greater than 100 μg/100 ml during the test. This gives a large margin of safety in distinguishing a positive response from changes in vitamin A concentration due to variations in plasma volume or to contamination with extracellular exudate. Since in the present work vitamin A palmitate absorption was tested in children employing a technic previously used only in adults, it was

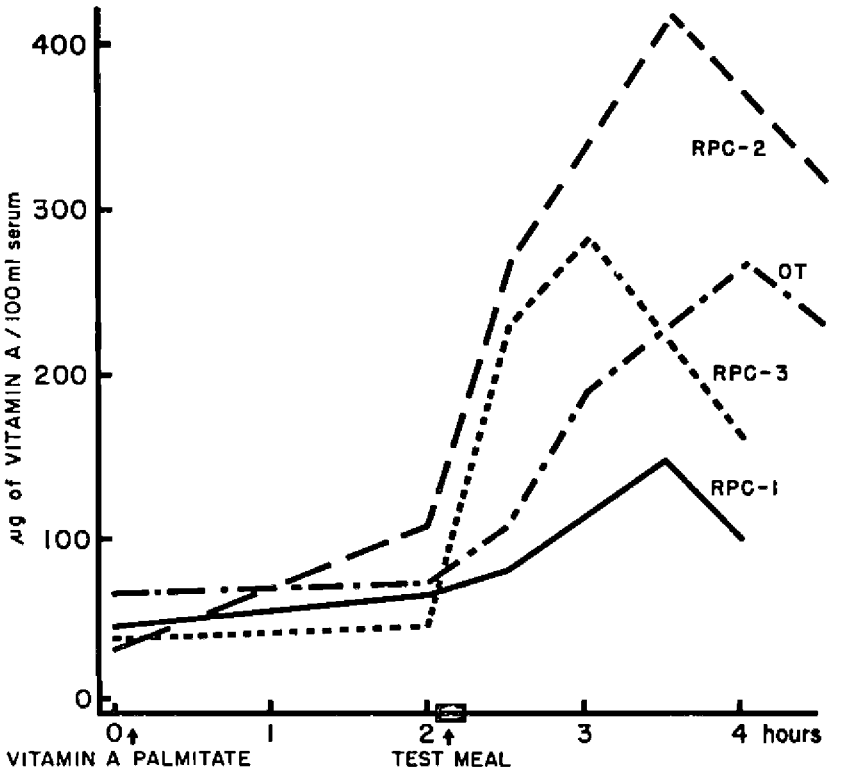

Fig. 1. Response to oral vitamin A palmitate in adequately nourished children. A test dose of 75,000 μg was given to each child and followed by a glass of skimmed milk two hours later. Blood samples for vitamin A analysis were taken at the intervals indicated. The sex and age of these children were as follows: RPC-1 (M), 2 yr, 10 mo; RPC-2 (M), 1 yr, 7 mo; RPC-3 (M), 2 yr, 2 mo and O.T. (F), 3 yr, 2 mo.

decided to re-evaluate the procedure in order to determine the time and magnitude of maximum response in children.

For this purpose, the test was run in three hospitalized children who had completely recovered from kwashiorkor (RPC-1,2,3) and on

TABLE II

Serum Carotene in Serial Fingertip Blood Samples of Children with Kwashiorkor

Case	Days of treatment	Serum carotene (μg/100 ml) at time intervals (hrs) after test dose						
		0	1	2	2½	3	4	5
PC-46	0	—	7	3	4	4	4	4
PC-48	0	13	11	10	10	10	10	—
PC-49	0	4	6	4	5	6	5	5
PC-50	0	4	4	3	3	3	3	4
PC-52	5	2	1	1	1	1	1	1
PC-54	5	3	4	2	4	2	3	3
PC-55	5	4	4	3	4	4	4	4
A-3	3	12	11	12	15	13	13	13
No-4	3	5	5	4	5	5	7	7
Completely recovered cases of kwashiorkor								
RPC-1	67	103	—	103	103	102	100	—
RPC-2	130	45	—	46	45	45	44	—
RPC-3	315	12	—	12	12	13	13	—

an outpatient child not suffering from severe malnutrition (OT). Figure 1 shows the rapid rises in serum vitamin A, reaching maximum concentrations within 45 minutes and 1 hour 45 minutes after taking of food, i.e., within three to four hours after the vitamin A administration. The timing of these responses is the same and the magnitude similar to that reported by Mendeloff *et al.*[7] for adults and accordingly their criteria for a positive response have been used in this report. It should be noted that the technic is quite different from that of McCoord *et al.*[10] which requires four to five hours to obtain maximum serum levels after the test dose.

Variations in serial values of total serum proteins for 12 cases randomly selected from the different experiments are illustrated in Table I and indicate changes of such a small magnitude as to have no significant effect on the result of the test. This is also true of the results of carotene determinations in a similarly selected group presented in Table II, which also illustrates the narrow range of variation among the values for each individual child and the extremely low levels of this provitamin in the serum of kwashiorkor patients.

Experiment 1

Eight children with kwashiorkor were admitted for study and given electrolyte therapy for a period of 24 hours according to routine procedures described elsewhere.[11] They were then tested as described above for the ability to absorb vitamin A palmitate. After the trial, a strictly controlled diet was given to the children, consisting of acidified half skimmed milk to provide therapeutic amounts of protein (3 to 5 g/kg body weight); on the fifth day of treatment the test was repeated on five of the patients.

The results obtained in five children who were tested both on hospital admission and the fifth day of therapy are presented in Figure 2. It shows the lack of response to the test on admission as well as the positive response on the fifth day of controlled therapy. Three additional children were tested on admission with negative results; their pre-test vitamin A serum levels were changed from 9, 8 and 14 μg per 100 ml to 13, 10 and 14 μg, respectively.

Experiment 2

In view of the results obtained in experiment 1, the possibility was investigated that the vitamin A given on admission could be determining or influencing the positive response to the test of the same individuals on the fifth day. This could occur either as the result of a therapeutic effect or of adding to the body reserves of vitamin A through slow absorption subsequent to the initial test. Omitting the

test on admission, four children with kwashiorkor were tested after five days and one after four days of the same therapy as in experiment 1. The data presented in Table III show that under these conditions the responses after therapy were still positive, but the peak serum vitamin A levels were not as high as in the children given an initial test dose in experiment 1. The differences between the peaks in these two experiments suggest that significant quantities of the vitamin ester given on admission in experiment 1 were eventually absorbed.

Experiment 3

Another group of children with kwashiorkor was tested as in experiment 2 in order to study the effect of the palmitate after only three days of the therapeutic diet. Comparison of the pre-test serum vitamin A levels with the highest levels obtained after the test dose (Table IV) reveals that only three of the five children showed more than a four-fold increase and that only one gave a response as great as the subjects of experiments 1 and 2 after five days of treatment. Three days appears to be close to the lower limit of time necessary for recovery of the normal ability to absorb the ester under the conditions of the experiment, and not all patients recovered this rapidly even with good therapy.

Experiment 4

Nine additional cases of kwashiorkor in the pediatric ward of the General Hospital were studied after three or five days of hospitalization without the initial test on admission. In contrast to the three previous experiments, these children received the regular ward diet *ad libitum* and were not under research control. Table V shows the lack of satisfactory

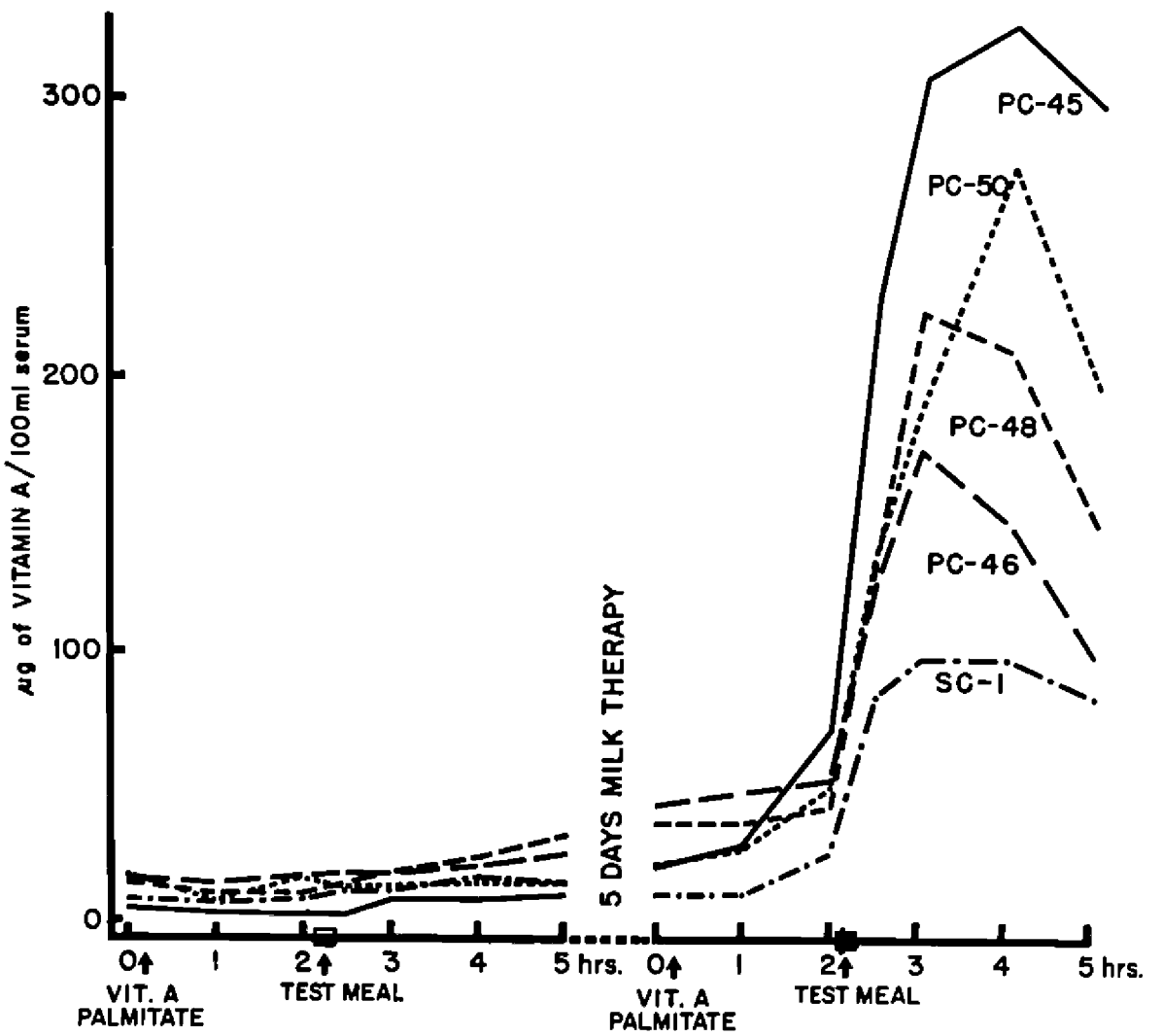

Fig. 2. Response to oral vitamin A palmitate in children with kwashiorkor before and after five days of therapy. After 24 hours of electrolyte therapy children hospitalized for kwashiorkor were given 75,000 μg of vitamin A as palmitate orally followed by a glass of either skimmed milk or corn-starch gruel two hours later. Blood samples for vitamin A analysis were taken at the intervals indicated and the entire test repeated after five days of intensive therapy based on the administration of adequate quantities of half-skimmed milk.

TABLE III

Vitamin A Palmitate Absorption Test in Kwashiorkor after Five Days of Research-Controlled Milk Therapy

Case	Sex and age in years	Serum vitamin A (μg/100 ml)		Peak as multiple of initial value
		Before dose	Peak after dose	
PC-52	M 1.7	2	26	13.0
PC-54	M 2.0	3	46	15.3
PC-55	M 1.4	10	76	7.6
SC-2	F 1.1	24	218	9.1
No-1*	M 4.0	10	87	8.7

* Four days of treatment.

TABLE IV

Vitamin A Palmitate Absorption Test in Kwashiorkor after Three Days of Research-Controlled Milk Therapy

Case	Sex and age in years	Serum vitamin A (μg/100 ml)		Peak as multiple of initial value
		Before dose	Peak after dose	
No-2	M 4.0	16	38	2.4
No-3	M 10.0	2	12	6.0
No-4	M 11.0	3	13	4.3
A-3	F 2.0	13	25	1.9
A-4	F 3.5	34	254	7.5

TABLE V

Vitamin A Palmitate Absorption Test in Kwashiorkor after Three to Five Days of Treatment not under Research Control

Case	Age†	Serum vitamin A (μg/100 ml)		Peak as multiple of initial value
		Before dose	Peak after dose	
Na-1	6.8	11	10	0.9
Na-2*	5.0	14	27	1.9
Na-3*	4.0	9	14	1.6
Na-5*	4.0	20	20	1.0
Na-7	4.0	2	3	1.5
Na-8	4.0	1	3	3.0
Na-9	5.0	20	30	1.5
Na-10	3.2	23	45	2.0
Na-11	5.1	19	28	1.5

* After five days of treatment, others after three days.

† All subjects were female.

response to the test under these therapeutic conditions. Since anorexia is a characteristic of the syndrome, it is reasonable to assume that the *ad libitum* dietary intake of the patients during the three to five-day experimental period was insufficient to produce a therapeutic response.

DISCUSSION

The results suggest that the vitamin A "absorption test," as recommended by Mendeloff,[7] is a sensitive indicator of functional impairment of the intestinal uptake of vitamin A palmitate. The direct cause, however, of the failure of vitamin A palmitate to appear in the blood stream in kwashiorkor, following an oral test dose, cannot be definitely determined from the results of the present investigation, since the disease results in a number of structural and biochemical changes which could be wholly or partly responsible. Histologic studies of the liver have revealed in all cases a high degree of fatty change. Although most of the classic liver function tests have failed to indicate a generalized hepatic dysfunction[12,13] direct examination of the bile obtained from the gallbladder in necropsy studies has shown it to be scanty, thin and mucus-like in consistency and with a golden yellow color instead of the normal dark green.[14]

Furthermore, histologic studies of the intestine itself leave no doubt as to the extensive atrophy with marked diminution in the number and size of the Kerckring's valves, atrophic degeneration of the Lieberkühn's crypts, as well as a reduction in epithelial and Paneth cells.[5] Finally there is a marked decrease in lipase activity, secondary to serious pancreatic disease.[4,5,15]

Whatever the causes may be, the response shown by patients fed therapeutic amounts of milk proteins is remarkably rapid. Studies of digestive enzyme changes during treatment have also clearly demonstrated a return to physiologically satisfactory levels in three to four days of therapy.[1] It is possible that the response to the vitamin A "absorption test" is dependent primarily on the availability of pancreatic lipase. Certainly tests of this type are commonly used as indicators of the capacity to absorb fat. Moreover, Gómez *et al.*[16] have shown a significantly reduced fat absorption in malnourished children admitted to the hospital whether macroscopic steatorrhea was present or not.

The low carotene and vitamin A content of

the Central American diets which result in kwashiorkor[17] may contribute to the lower serum levels of these factors. If, however, the deficiency of protein is so severe that the normal intestinal absorption of vitamin A is markedly decreased, satisfactory vitamin A serum levels will not be maintained regardless of the dietary content of this vitamin. Low levels of vitamin E also have been found in kwashiorkor by Trowell, Moore and Sharman[18] in Uganda and Scrimshaw *et al.*[6] in Central America. Trowell suggests that the reduced levels of serum vitamin E may be another consequence of a general impairment in the intestinal absorption of fat-soluble factors.

SUMMARY

The effect of an oral dose of 75,000 μg of vitamin A as palmitate on vitamin A serum levels has been tested in children suffering from kwashiorkor. It was found that on admission, this amount of the vitamin ester had no detectable effect on serum levels. After dietary treatment based exclusively on therapeutic amounts of acidified half skimmed milk, the test dose resulted in very marked serum vitamin A increases as early as the fifth day, and in some patients even by the third day of treatment. In another group of children an unsupervised *ad libitum* diet failed to produce this change in three to five days.

The possible relation of these observations to histopathologic and biochemical alterations in the pancreas, liver and intestine is discussed. It is suggested that the low levels of serum vitamin A found in kwashiorkor may be a consequence of a generalized failure to absorb fat-soluble factors.

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