Alterations in serum concentration of vitamin A associated with the hypoproteinemia of severe protein malnutrition

Children recovering from kwashiorkor may show an increase in serum vitamin A concentration during the first 1 to 2 weeks of treatment even when receiving a diet based on skim milk free of vitamin A. This occurs only when the liver of the patient initially contains vitamin A reserves. Under these conditions a high correlation is found between serum vitamin A and proteins. Studies in rats with a wide range of serum proteins and albumin, produced by experimental protein deficiency, and/or adrenalectomy, desoxycorticosterone, or cortisone treatments, show a close direct relationship between vitamin A and albumin levels in the serum. The results are evidence of impairment of vitamin A transport in severe protein deficiency.

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THE possibility that severe protein deficiency or other pathologic conditions conducive to a marked decrease in plasma proteins might impair blood transport of vitamin A was mentioned by McLaren[9] in 1958. His suggestions were based on epidemiologic observations made in regions where signs of both protein and vitamin A deficiency are found concomitantly. Supporting evidence has since been obtained from experimental work in animals carried out at the National Institute for Medical Research, Mill Hill, London (Friend and his associates[5]), and from observations of severely protein-malnourished children at

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INCAP's laboratories (Arroyave and his colleagues[1]).

The present paper gives the results of recent investigations on 5 children with kwashiorkor, as well as data obtained from rats, in which several levels of albumin concentrations in plasma were experimentally produced.

MATERIAL AND METHODS

1. **Observations in malnourished children.** The data were obtained from children with the clinical picture of kwashiorkor as described elsewhere (Scrimshaw and his coworkers[10]) admitted to INCAP's hospital ward for metabolic studies. After the first 16 to 20 hours, during which they received only water and electrolyte therapy, a blood sample was taken. They were then put on skim milk–based therapeutic diets, adequate in all respects except that they lacked vitamin A or carotene.

During the initial phase of their recovery, a number of the children showed a marked increase in serum vitamin A over their very low initial levels despite the absence of vitamin A or carotene in the therapeutic diet. This rise indicated, on the basis of evidence published elsewhere (Arroyave and his coworkers), that these children had a significant hepatic vitamin A reserve at the time they were admitted to the hospital. From among this group, 5 boys, aged 1 year and 5 months to 4 years and 8 months, were selected for the study of the relationship between plasma vitamin A and proteins. At about weekly intervals, additional blood samples were taken during the remainder of their recovery period. The serum was separated by centrifugation and analyzed for total serum proteins and vitamin A by the methods of Lowry and Hunter[8] and Bessey and co-workers,[2] respectively. Twenty-one values for each of those two serum components, obtained between admission and the time at which the serum vitamin A reached its highest value, were correlated statistically.

2. **Observations in experimental rats.** During previous investigations in INCAP's laboratories of the effect of cortisone and desoxycorticosterone on weaning protein-malnourished rats of the Sprague-Dawley strain, with (INCAP, 1962[7]) and without (Castellanos and Arroyave[4]) adrenalectomy, animals under different treatments showed very diverse serum albumin levels. A corn protein–deficient diet whose composition has been published (Castellanos and Arroyave[4]), was used to produce protein deficiency. During the course of the studies, data on serum albumin and vitamin A were obtained from the following groups of animals.

Group 1. Six rats were maintained for 21 days on the corn protein–deficient diet, followed by 8 days on the same diet plus 3.12 mg. of cortisone acetate in 0.125 ml. of aqueous suspension given subcutaneously.

Group 2. Six rats received the same diet as Group 1, but 1 mg. of desoxycorticosterone acetate in 0.2 ml. of oil solution was substituted for the cortisone.

Group 3. Six rats also received the same diet as Group 1 but 0.125 ml. of 0.8 per cent sodium chloride solution replaced the steroid preparations.

Group 4. Four rats, after weaning, received laboratory "chow" for 8 days, then were adrenalectomized on the eighth day, and thereafter offered the corn protein–deficient diet for a week.

Group 5. Five rats were fed and adrenalectomized as were those in Group 4, but they were on the corn protein–deficient diet for 2 weeks and received 3.12 mg. of cortisone acetate for the last week.

Group 6. Six rats under the same treatment given to Group 5 received 1 mg. of desoxycorticosterone acetate as replacement for the cortisone.

The animals were sacrificed by decapitation after light ether anesthesia; the blood was collected, allowed to clot, and the serum was separated by centrifugation. The livers were excised, made into a brei, and representative aliquots were homogenized with ice cold 0.8 per cent sodium chloride solution in a glass homogenizer. Vitamin A was determined in both the liver homogenates

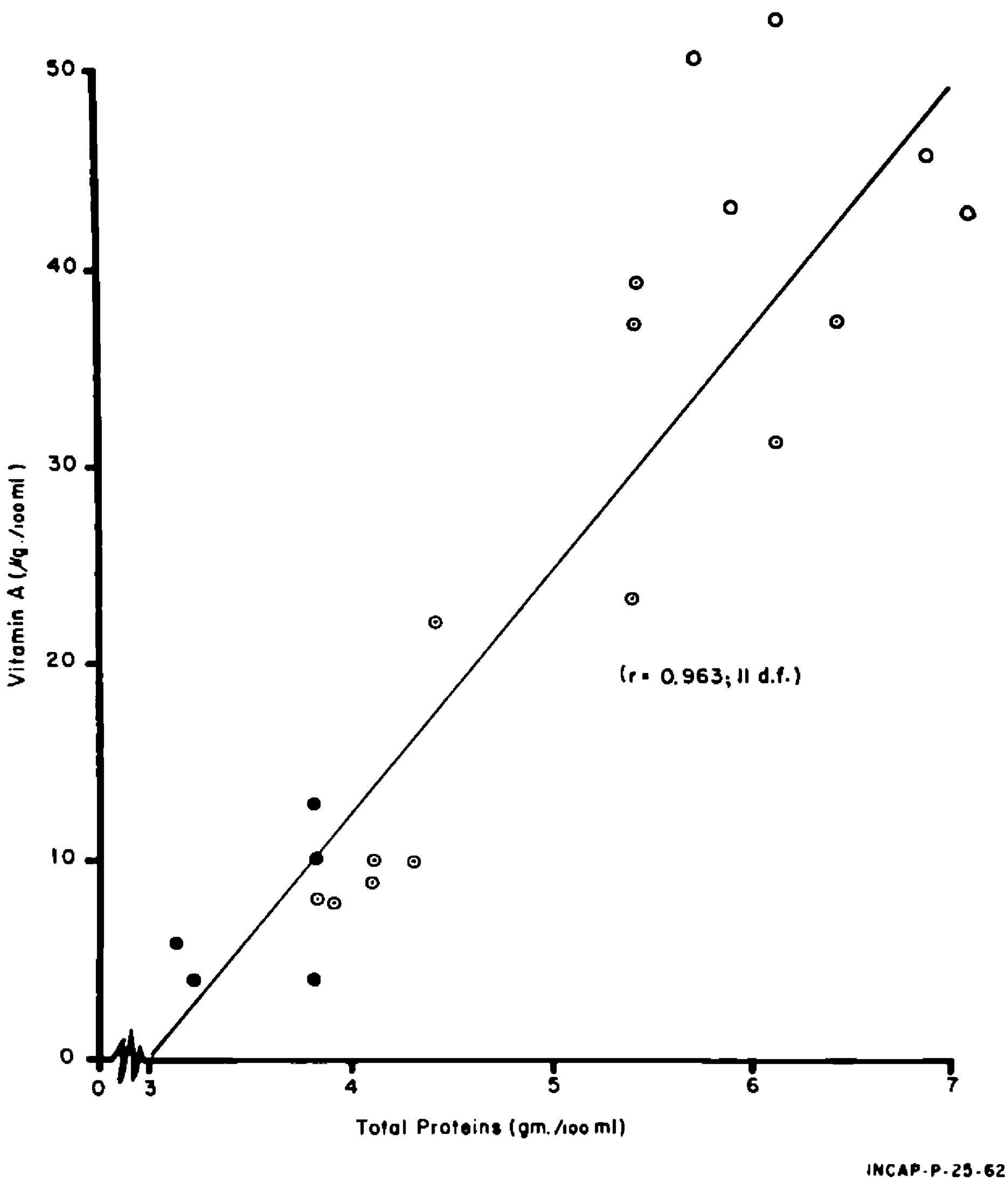

Fig. 1. Relationship of serum vitamin A to serum proteins in 5 children with kwashiorkor on admission to the hospital and during initial recovery on a vitamin A–free diet. Admission values (●); intermediate values (⊙); values at the time the peak of serum vitamin A was observed (○).

and the serum by the method of Bessey and his associates.[2] Serum albumin was estimated by paper electrophoresis with sodium veronal buffer pH 8.6 for 16 hours, at 100 volts, amido-black staining, elution, and colorimetry (Block, Durrum, and Sweig[3]).

3. **Collection of food consumption data.** Food consumption was measured for 3 days during the hormone or placebo treatments in Groups 1, 2, and 3, and for 8 days in Groups 5 and 6. No food consumption data was obtained for Group 4.

RESULTS

Observations in children. Fig. 1 is a plot of the 21 values for serum vitamin A and serum total proteins of the five kwashiorkor cases, found at different intervals during the period between admission to the hospital and the attainment of the peaks in vitamin A serum level. A high "pooled-within-child" correlation (0.963; 11 d.f.) was observed between these two serum components.

Observations in experimental rats. Food consumption data for the different groups of rats are included in Table I. Groups 1, 2, and 3, which were run simultaneously as part of one trial, consumed practically the same amounts of the diet. Groups 5 and 6 were studied together as part of another trial; Group 5 consumed 17 per cent more food than Group 6.

Additional unpublished data, collected in a subsequent 12 day rat experiment with

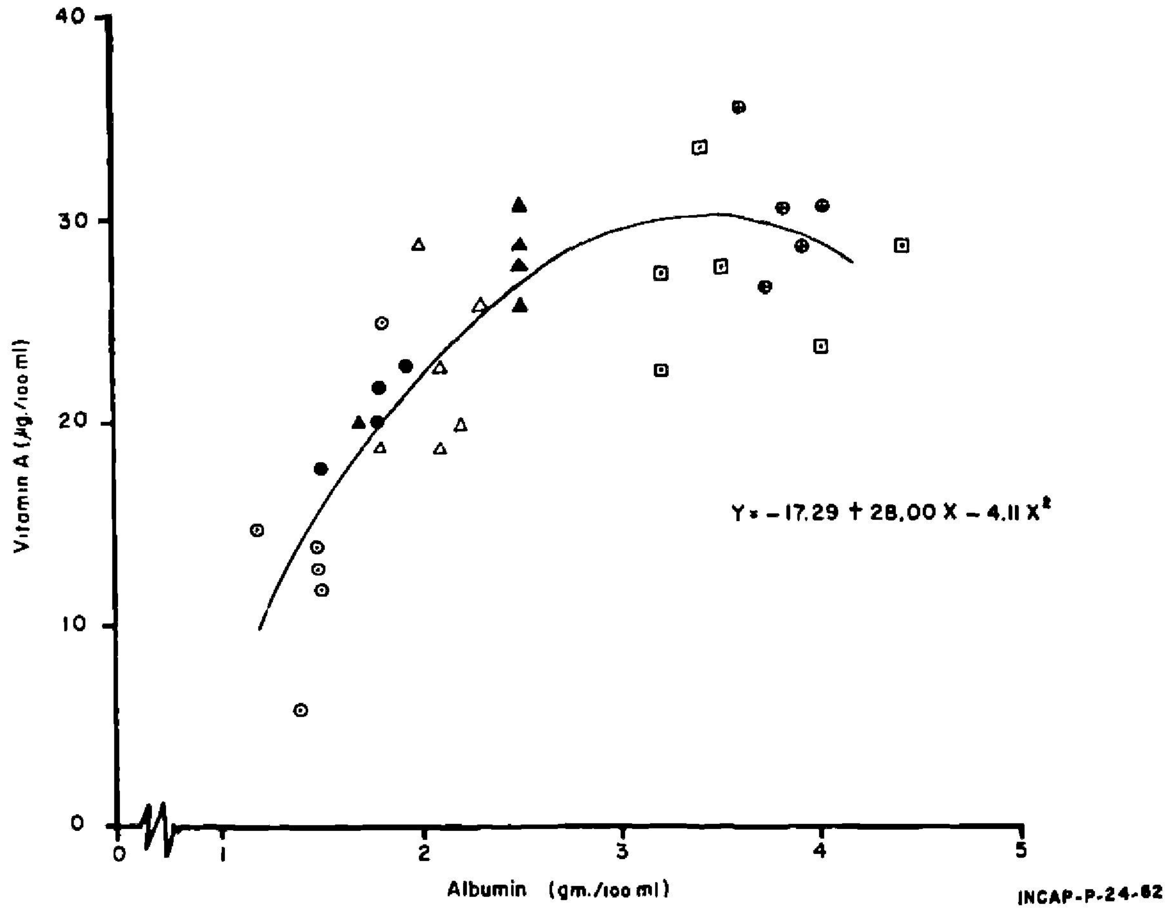

Fig. 2. Relationship of serum vitamin A to serum albumin of rats. Group 1, Intact-cortisone ⊡; Group 2, Intact-DOCA △; Group 3, Intact-NaCl ▲; Group 4, Adrenalectomized ●; Group 5, Adrenalectomized-cortisone ⊕; Group 6, Adrenalectomized-DOCA ⊙.

13 animals per group, in which the same treatments as had been given to Groups 1 through 3 were used, revealed no differences in food consumption among the groups. Since all the groups were eating the same diet, the relative figures for food consumption also represent relative figures for vitamin A intake.

The different treatments to which the protein-deficient experimental rats were subjected resulted in varied serum albumin levels, ranging from very low in the adrenalectomized animals receiving desoxycorticosterone acetate to high albumin levels in those treated with cortisone. The relationship between the wide-ranging albumin values, on the one hand, and the corresponding serum vitamin A concentrations, on the other, is shown in Fig. 2. The fitted quadratic form is shown by the equation.

Table I gives the average values for serum albumin, for serum vitamin A, and for liver

Table I. Serum albumin, and serum and liver vitamin A in protein-deficient rats under different treatments

Groups	*No.*	*Treatment*	*Food consumption (Gm./rat/day)*	*Serum* Albumin (Gm./100 ml.)	*Serum* Vitamin A (µg/100 ml.)	*Liver vitamin A (fresh tissue) (µg/100 Gm.)*
1	6	Intact—cortisone	8.0	3.6	27.7	3550
2	6	Intact—DOCA	7.8	2.1	22.6	5410
3	6	Intact—NaCl	8.0	2.2	25.9	6230
4	4	Adrenalectomized—none	--	2.2	20.0	2537
5	5	Adrenalectomized—cortisone	11.0	4.2	30.8	2878
6	5	Adrenalectomized—DOCA	13.3	1.7	14.1	3050

vitamin A, for the different groups of rats. It is evident, from these data, that there is no positive relationship between the concentrations of vitamin A in serum on the one hand, and the liver reserves on the other. In fact, there is a tendency for this relationship to be inverse, as illustrated by the fact that, both in the intact animals and in the adrenalectomized ones, the groups with low serum vitamin A and low serum albumin had also the highest liver vitamin A contents. The close relationship between the individual values of the two serum components, albumin and vitamin A, also explains the good agreement between their group averages, as shown in Table I.

DISCUSSION

It must be emphasized that throughout the initial phase of recovery, the period when they were studied, the children received a high protein diet *devoid* of vitamin A or carotene. The marked increases in serum vitamin A, accompanying increases in protein, were caused, therefore, by vitamin A liberated from the tissue reserves. Discussion of this point has been covered in detail in a previous publication.[1]

Albumin values were not available from these five kwashiorkor patients. It is known, however, that the rapid rise in total serum proteins observed in kwashiorkor children during initial recovery is accounted for largely by a rise in the albumin fraction. In view of the association between vitamin A plasma transport and albumin or a plasma protein fraction electrophoretically close to it (Ganguly[6]), a good correlation between their increases is to be expected. Friend and his collaborators[5] showed such correlation in pigs in which severe protein malnutrition, similar to the human kwashiorkor type, had been produced experimentally. The data from the rat experiments reported here agree with the observations of Friend[5] but the type of relationship obtained (Fig. 2) may indicate that as the albumin values increased the amount of vitamin A consumed by the rats became the factor which limited further increments in serum vitamin A. The electrophoretic method used separated only 4 distinct rat plasma protein fractions, albumin, and alpha, beta, and gamma globulins. Whether protein of the alpha-1 type moved with the albumin or with the alpha-2 globulin was not determined. The alpha fraction, however, did not correlate with the vitamin A values.

The significantly higher hepatic vitamin A values in the group of rats with the lowest serum albumin may reflect the lack of mobilization of the vitamin reserves accumulated in the liver tissue at the time impairment in blood transport ensued. On the other hand, the consistently lower liver vitamin A content of the rats with the highest serum albumin and vitamin A values is compatible only with the enhanced transport and utilization of the vitamin in these animals.

The data presented contribute additional evidence that, under conditions of severe protein deficiency characterized by marked hypoproteinemia, liver stores of vitamin A are not reflected by its blood plasma levels, but that the latter are related rather to the concentration of plasma albumin.

SUMMARY

Children recovering from kwashiorkor may show a marked increase in vitamin A serum levels during the first 1 to 2 weeks of treatment even when receiving a diet based on skim milk free of vitamin A. This occurs only when the hepatic tissue of the patients initially contains vitamin A reserves. The vitamin increase parallels the increase in serum protein and albumin concentration. This study shows that under these conditions a high correlation is found between serum vitamin A and proteins. It also shows that in rats with a wide range of serum proteins and albumin, produced by experimental protein deficiency, and/or adrenalectomy, desoxycorticosterone, or cortisone treatments, there is a close direct relationship between vitamin A and albumin levels in the serum. The results are further evi-

dence of impairment of vitamin A transport in severe protein deficiency, possibly as a consequence of the marked lowering of the plasma protein carriers of the vitamin.

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REFERENCES

1. Arroyave, G., Wilson, D., Méndez, J., Béhar, M., and Scrimshaw, N. S.: Serum and liver vitamin A and lipids in children with severe protein malnutrition, Am. J. Clin. Nutrition 9: 180, 1961.
2. Bessey, O. A., Lowry, O. H., Brock, M. J., and López, J. A.: The determination of vitamin A and carotene in small quantities of blood serum, J. Biol. Chem. **166:** 177, 1946.
3. Block, R. J., Durrum, E. L., and Sweig, G.: A manual of paper chromatography and paper electrophoresis, New York, 1955, Academic Press, Inc.
4. Castellanos, H., and Arroyave, G.: Role of the adrenal cortical system in the response of children to severe protein malnutrition, Am. J. Clin. Nutrition **9:** 186, 1961.
5. Friend, C. J., Heard, C. R. C., Platt, B. S., Stewart, R. J. C., and Turner, M. R.: The effect of dietary protein deficiency on transport of vitamin A in the blood and its storage in the liver, Brit. J. Nutrition **15:** 231, 1961.
6. Ganguly, J.: Symposium on vitamin A and metabolism. V. Absorption, transport and storage of vitamin A, Vitamins & Hormones **18:** 387, 1960.
7. Institute of Nutrition of Central America and Panama, 1962 (unpublished data).
8. Lowry, O. H., and Hunter, T. H.: The determination of serum protein concentation with a gradient tube, J. Biol. Chem. **159:** 465, 1945.
9. McLaren, D. S.: Hypovitaminosis A. VI. The pathogenesis of hypovitaminosis A, Fed. Proc. 17 (suppl. 2): 136, 1958.
10. Scrimshaw, N. S., Béhar, M., Arroyave, G., of kwashiorkor (Síndrome pluricarencial de Viteri, F., and Tejada, C.: Characteristics la infancia), Fed. Proc. 15: 977, 1956.