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Vitamin A–Nutrient Interrelationships

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I. INTRODUCTION

Vitamin A deficiency affects primarily the most underprivileged populations around the world. These are the same population groups that because of their limited socioeconomic condition are not only vulnerable to being vitamin A deficient, but also suffer from the lack of a variety of other essential nutrients.

The majority of epidemiological studies show that usually vitamin A deficiency does not occur as a single entity. Instead, it is almost always accompanied by other nutritional deficiencies. For example, severe vitamin A deficiency in children is often accompanied by protein-energy malnutrition and in fact this latter condition has been considered a coadjuvant factor in the development of xerophthalmia and keratomalacia. Furthermore, depending on the prevailing nutrient deficiencies in a given geographical area, hypovitaminosis A can also be accompanied by specific local deficiencies of other vitamins and minerals.

It is now well established that the nutrients do not act alone and that in order to be properly utilized and to carry out their biological function, in a harmonious and integrated fashion, they have to interact among each other (Hanck, 1983). This phenomenon is of great biological and nutritional significance since in this context the lack or excess of one nutrient, such as vitamin A, may conversely influence the nutritional need and metabolism of others.

The practical importance of this nutrient interaction becomes very relevant, and should be a primary consideration, when making efforts to combat either vitamin A deficiency or any other specific nutrient deficiency in populations. Otherwise we may not obtain our desired biological

impact or, on the other hand, we could even exacerbate the nutritional need for other nutrients.

The purpose of this chapter is to discuss those vitamin A interrelationships that, based on their implications, could be considered the most relevant in the public health context.

II. RELATIONSHIP BETWEEN PROTEIN NUTRITURE AND VITAMIN A METABOLISM

Protein deficiency can markedly affect vitamin A nutriture and in turn vitamin A deficiency can also influence protein metabolism [International Vitamin A Consultative Group (IVACG), 1977].

Protein deficiency affects primarily the intestinal absorption, the release from the liver, and the blood transport of vitamin A. Thus, despite a relative adequate vitamin A intake, protein deficiency may lead to a secondary deficiency of this vitamin. Vitamin A deficiency, on the other hand, can affect the metabolism of proteins by lowering the plasma levels of retinol-binding protein (RBP), impairing nitrogen balance, and decreasing the synthesis of some specific proteins.

Most of the information on the effect of protein deficiency on vitamin A metabolism has been obtained in studies of severe forms of protein-energy malnutrition (PEM) in children. PEM is most frequent in preschool age children and so is vitamin A deficiency. Therefore, in practical terms the information obtained from children in relation to this interaction is of foremost importance.

Children suffering from PEM almost always have low serum levels of vitamin A (Mejía *et al.*, 1984). These low levels of retinol are in part related to the dietary deficit of vitamin A prevailing in the region, but their protein undernutrition also plays an important role. It was shown many years ago that in PEM the intestinal absorption of vitamin A is drastically impaired (Arroyave *et al.*, 1959). Figure 1 shows the response in serum levels of retinol after a single oral dose of 75,000 μg of fat-soluble vitamin A given to children suffering from severe PEM on admission and again after 5 days of PEM treatment. There was practically no absorption of the vitamin on admission to the hospital, but adequate absorption was restored shortly after initiation of successful dietary treatment of the patients. This failure to absorb vitamin A is related in part to the fact that in severely malnourished children there is a low output of conjugated bile acids, which impairs micellar formation and as a consequence the absorption of fat and fat-soluble substances (Viteri and Schneider, 1974). Furthermore, in protein deficiency there is a decreased intestinal hydrolytic

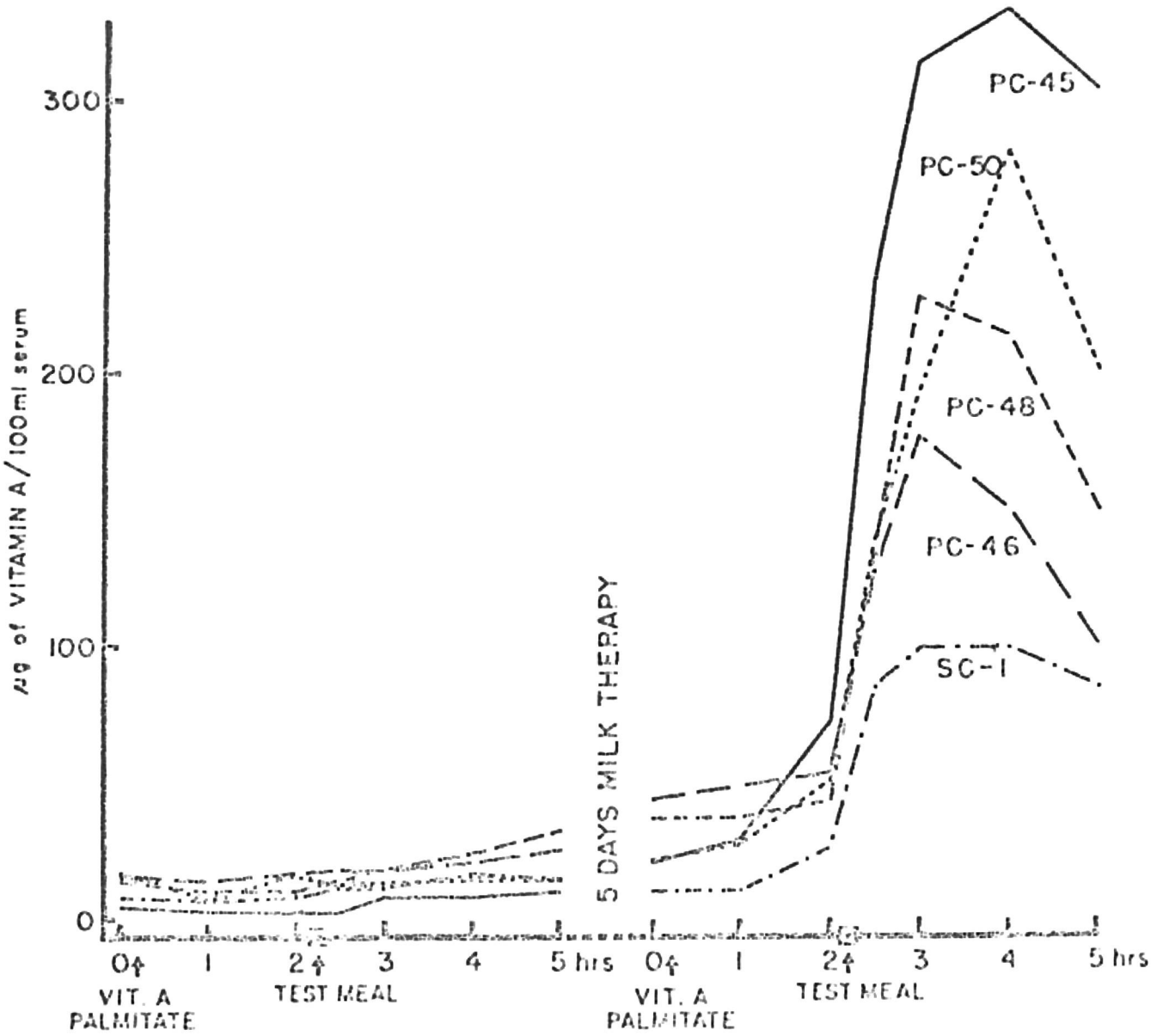

Fig. 1. Response in serum vitamin A levels after the oral administration of retinyl palmitate (75,000 μg) to children suffering from kwashiorkor, before and after 5 days of nutritional therapy. In each case, a test meal consisting of a glass of skimmed milk or corn starch gruel was given 2 hr after the vitamin A dose to stimulate its absorption. (Reprinted with permission from Arroyave *et al.*, 1959. © *Am. J. Clin. Nutr.*, American Society for Clinical Nutrition.)

activity of retinyl esters which leads to a very slow hydrolysis of vitamin A esters in the diet (Nir *et al.*, 1967). In addition, the secretion of glycoproteins from the goblet cells is lowered and both the diffusion layer which traps the micelles around the mucosal cell membrane as well as the viscosity of the lumen fluid are reduced. As a result, the micelles containing vitamin A are not so readily brought into contact with the intestinal mucosa in the optimal region for absorption (IVACG, 1977).

Despite this condition, however, children with PEM can have a modest amount of vitamin A in the liver (Arroyave *et al.*, 1961). This hepatic vitamin A is probably obtained before protein deficiency becomes severe, or it may also mean that severe PEM does not impose a complete blockage to the intestinal vitamin A absorption. Regardless of the mechanism,

TABLE I

Effect of Dietary Level of Protein in the Activity of Carotene Dioxygenase in Rat Intestine[a]

Dietary protein level (%)	Retinal/mg ADN ($\times 10^{-4}$)
2.5	1.0 ± 0.2
5	1.4 ± 0.1
10	2.9 ± 0.2
20	1.8 ± 0.2

[a] Adapted with permission from Gronowska-Senger and Wolf (1970). (© *J. Nutr.*, American Institute of Nutrition.)

it is important to be aware that this limited amount of stored vitamin A is not enough to support growth and normal development once these children are provided with nutritional treatment.

Carotenoid utilization at the intestinal level is particularly affected in severe PEM. Not only is carotenoid absorption reduced by the mechanism already described, but its cleavage to retinaldehyde by carotenoid dioxygenase in the intestinal mucosa is depressed (Gronowska-Senger and Wolf, 1970). Table I shows the effect of the level of dietary protein on the ability of carotenoid dioxygenase to convert carotenoids into retinal in the rat. It can be seen that there is a clear and significant increase on retinal production when the protein in the diet changes from 2.5 to 10%. This may mean that children suffering from protein deficiency also have an impaired capability of utilizing dietary carotenoids and this in turn could affect their vitamin A status.

Another marked effect of protein deficiency on vitamin A metabolism is on the release from the liver and blood transport of retinol (F. R. Smith *et al.*, 1973). It has already been mentioned that in severe PEM there is a modest accumulation of vitamin A in the liver. In this regard, Arroyave *et al.* (1961) have shown that when children suffering from severe PEM are given nutritional treatment with a protein-rich diet and without supplementary vitamin A, there is not only the expected increase in serum proteins but also a rapid initial significant rise in serum retinol. This phenomenon is shown in Figure 2. It can be concluded from this observation that in protein deficiency there is a failure in the release of retinol from the liver and that its transport mechanism is impaired. This impairment is due to the fact that protein deficiency decreases the biosynthesis of RBP and prealbumin, both of which are in charge of vitamin A transport and release from the liver (F. R. Smith *et al.*, 1973). Additional

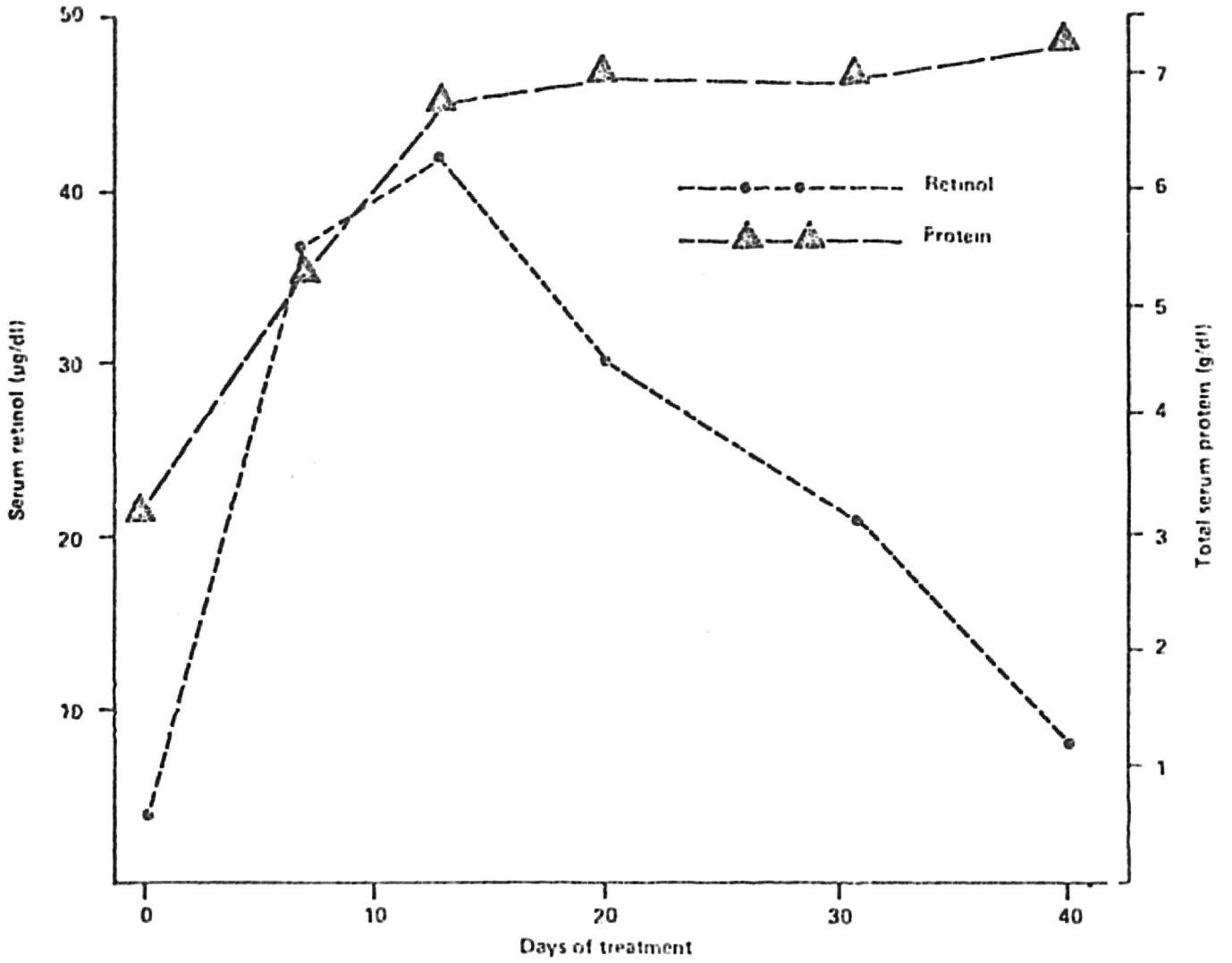

Fig. 2. Serum levels of retinol and total proteins during treatment of severe protein-energy malnutrition in children. (Adapted with permission from Arroyave *et al.*, 1961. © *Am. J. Clin. Nutr.*, American Society for Clinical Nutrition.)

results by F. R. Smith and co-workers (1975) showing a positive effect of protein treatment on vitamin A transport confirms this relationship. Furthermore, RBP and prealbumin are rich in essential amino acids and therefore their synthesis could be highly sensitive to the amount and quality of dietary protein. In this regard, Glover and Muhilal (1976) have shown the effect of the quantity and quality of dietary protein on plasma RBP and albumin levels. In this experiment, weanling rats were fed for a period of 5 weeks on diets containing different levels of soybean and rice proteins. The effect of supplementing the rice protein with its limiting amino acid lysine was also tested. The results are presented in Table II. It can be seen that the groups of animals fed with the low soybean protein level (50 gm/kg diet) had significantly lower levels of RBP than those animals fed the higher level of the same protein (200 gm/kg diet). Even more striking is the effect on plasma RBP levels when the animals were fed the rice protein. Their RBP levels were 16.3 ± 0.6 μg/ml as compared to the level of 22 ± 2.0 μg/ml encountered in the group fed the same level of the higher-quality soybean protein. When lysine was added to the rice

TABLE II

Effect of Amount and Quality of Dietary Protein on Plasma RBP Levels in the Rat[a]

Group	Protein (gm/kg diet)[b]	Plasma RBP (μg/ml)
L	Soybean 200	26.0 ± 0.4
N	Soybean 50	22.0 ± 2.0
P	Rice 50	16.3 ± 0.6
R	Rice 50 + lysine	20.0 ± 2.0

[a] Adapted from Glover and Muhilal (1976).
[b] Contained vitamin A at a level of 3 mg/kg.

protein, however, the RBP levels improved significantly, approaching those levels found in the rats receiving the 50 gm/kg diet of soybean protein. It is clear that both the quantity and quality of protein, which affect the protein nutritional status, can at the same time affect vitamin A transport.

Figure 2 also illustrates another important phenomenon which can have significant practical implication when treating PEM or perhaps even milder forms of protein deficiency. Nutritional treatment of PEM reactivates anabolic activity, as shown by the rise in total serum proteins, with a resulting increase in lean body mass and growth. Under these circumstances, the demand for vitamin A is suddenly increased and the usually meager vitamin A stores are rapidly used up. It can be observed in the graph that although there was an initial rise in serum retinol, this was followed by a gradual drop, reaching again a deficient level. This important demonstration implies that if the vitamin is not provided, clinical deficiency of the vitamin may develop.

It is clear that when improving protein nutriture either in the hospital or in undernourished populations, caution should be taken to provide adequate amounts of vitamin A, particularly in areas where the dietary deficiency of vitamin A is endemic. Conversely, an adequate protein nutriture is also required for proper vitamin A utilization.

III. ROLE OF DIETARY FAT ON VITAMIN A ABSORPTION

It has long been established through a series of investigations that dietary fat has a marked stimulatory effect on vitamin A absorption, particularly of carotenoids [Food and Agriculture Organization/World Health Organization (FAO/WHO), 1967]. In a classical experiment, Roels *et al.*

TABLE III

Effect of Fat on Carotene Balance and Absorption after Carotene Supplementation in Boys[a]

Group	Dietary addition	Average carotene supplement/day (mg)	Average carotene excretion/day (mg)	Estimated net absorption of supplement
Control (E)	Placebo[b]	Insignificant	0.9	—
Control (D)	20 ml of olive oil	None	1.0	—
A	200 gm of carrots	18.8	18.8	<5%
B	200 gm of carrots plus 20 ml of olive oil	18.8	15.1	25%
C	28 mg of carotene plus 20 ml of olive oil	28	16.5	45%

[a] Adapted with permission from Roels *et al.* (1958). (© *J. Nutr.*, American Institute of Nutrition.)
[b] Cassava flour in a gelatin capsule.

(1958) showed that in humans the addition of fat to the diet significantly increased the absorption of carotenoids. In this particular study (Table III), Ruandan boys between 9 and 16 years of age were divided into five groups and their customary diet, which is low in vitamin A and fat (7% of the calories), was supplemented with carotene as follows: 200 gm of carrots (group A), 200 gm of carrots plus 20 ml of olive oil (group B), and 28 mg of carotene plus 20 ml of olive oil (group C). There were two control groups (E and D) given, respectively, a placebo consisting of cassava flour inside a gelatin capsule and 20 ml of olive oil. This dietary regime was maintained for a period of 1 month and carotene balance was determined by measuring daily carotene intake and excretion in the feces. The results showed that the boys in group A ingested an average of 18.8 mg/day of carotene contained in the 200 gm of carrots and that their carotene excretion was also 18 mg/day. Considering that the control groups excreted approximately 1.0 mg/day, it was estimated that the net absorption of carotene in this group was less than 5%. When adding fat (group B) the carotene excretion decreased to 15.1 mg and the net absorption of carotene had a marked rise to 25%. The impact of fat on carotene absorption was even greater when given pure carotene (group C). This observation illustrates that dietary fat increases carotene absorption not only when given in a pure form but also as a natural constituent of foods.

Because vitamin A is a lipid-soluble substance, the effect of fat on its absorption would seem very logical and expected. In the first place, fat is the dietary vehicle for the transport of vitamin A, including carotenoids from the stomach into the intestinal lumen. Second, the presence of fat in

the duodenum enhances the secretion of the intestinal hormone cholecystokinin, which in turn stimulates gallbladder contraction and as a consequence secretion of bile salts (Guyton, 1971). An ample supply of bile salts is necessary not only for the formation of micelles and the solubilization of the apolar β-carotene but more specifically for stimulating pancreatic lipase and assisting β-carotene to penetrate the plasma membrane (IVACG, 1977). Bile salts are also needed for proper intestinal solubilization of vitamin A, thus favoring enzyme action for the hydrolysis of both retinyl esters and carotenoids (Wolf, 1980). In summary, bile salts are absolutely required for intestinal carotenoid utilization and are important for both retinyl ester cleavage and the absorption of retinol. On this basis, the role of fat in vitamin A absorption is fairly well defined.

It has also been observed that the type of dietary fat may also have some effect on absorption and cleavage of carotenoids (Wolf, 1980). Increased unsaturation of fat causes a decrease in efficiency of carotenoid uptake and cleavage. This, however, is only an indirect effect related to the fact that unsaturated fats are more susceptible to oxidation, which in turn may also affect the vitamin A content of the diet.

In areas of the world where the dietary intake of vitamin A is limited, the amount of dietary fat is also low. Unfortunately, the significance of the amount of fat in the diet on vitamin A utilization at the population level has not been evaluated. Therefore, probably due to the lack of this type of information, expert groups have not given importance to the effect of the level of dietary fat on vitamin A nutriture (FAO/WHO, 1967). However, the existing data indicate that in populations with low fat intake, the intestinal utilization of dietary vitamin A can be improved by increasing the amount of dietary fat (Roels *et al.*, 1958; Geovani and Devi, 1981). This is particularly true when the main source of vitamin A is in the carotenoid form as has been shown in more recent experiments (Richardson and Cook, 1983).

On the basis of the mechanism and physiological requirements for proper vitamin A absorption already discussed, it is also important to consider the absorption of pharmacological doses of vitamin A when given on an empty stomach, as is often done for prophylactic and therapeutic measures. For example, vitamin pills are often ingested during fasting or apart from meals; under these circumstances, there is a lack of the stimulatory effect of dietary fat. Preliminary unpublished results from our laboratory (L. A. Mejía and N. W. Solomons, unpublished) have shown that the absorption of vitamin A from commercial multivitamin–mineral preparations is impaired when ingested by human subjects under fasting conditions. The simultaneous ingestion of corn oil increased significantly the speed of vitamin A absorption from the pill. This type of obser-

vation can also have tremendous importance in vitamin A therapy. All this information indicates that dietary fat is essential for proper intestinal absorption of vitamin A, especially of carotenoids.

IV. THE EFFECT OF VITAMIN E ON VITAMIN A UTILIZATION

The interaction between vitamins A and E has been known since the early 1940s (Moore, 1940; Davies and Moore, 1941). This interaction has been established primarily on the basis of a series of animal studies showing that the liver storage of vitamin A is greatly increased when adding vitamin E to the diet and that when vitamin E is not provided the liver vitamin A is more rapidly depleted (Guggenheim, 1944; Roels *et al.*, 1964; Harril *et al.*, 1965; Sondergaard, 1972). There is only little information about this relationship in humans. The vitamin A and E interrelation has been reviewed by Bauernfeind *et al.* (1974), Anrich (1978), and more recently in 1985 (Anonymous). Possible forms in which vitamin E may exert this effect on vitamin A have been discussed by Green and Bunyan (1969).

In humans, a positive direct effect of vitamin E supplements on vitamin A absorption has not been consistently observed (Kusin *et al.*, 1974). Although this type of response may depend on the vitamin E status of the individual, on the basis of the existing data it can be established that most, if not all, of the vitamin E action on vitamin A utilization is related to its antioxidant properties.

Vitamin E is an effective lipid-soluble antioxidant (Tappel, 1962). Through this mechanism, vitamin E may protect vitamin A and particularly carotenoids from oxidation in the intestinal lumen. This can be particularly true when there is a high amount of fat in the diet containing elevated levels of polyunsaturated fatty acids, which are known to increase vitamin E requirements (Witting, 1972). Thus, vitamin E could increase the luminal availability of vitamin A for mucosal uptake and only indirectly favors its absorption.

At the intracellular level, this interaction is less well defined since the addition of vitamin E to oral preparation of vitamin A has failed to significantly improve the vitamin A retention in malnourished children (IVACG, 1977). In this regard, the antioxidant action of vitamin E in protecting against disruption of membrane lipoprotein structure caused by high levels of vitamin A is perhaps more important (Lucy and Dingle, 1964). This means that vitamin A could protect against the cellular and subcellular effects of vitamin A toxicity. Toxicity of vitamin A has been

alleviated by adding vitamin E to diets of growing chicks and rats (McCuaig and Motzok, 1970; Young *et al.*, 1972; Young and Mitchell, 1975). Hypervitaminosis A brought about in chickens by feeding of excessive vitamin A (3,250,000 IU/kg diet) resulted after 32 days in 78% mortality, whereas another group receiving the same diet plus vitamin E (10,000 IU) had no mortality at all. Furthermore, the high vitamin A intake markedly depressed growth which was largely restored by the inclusion of vitamin E (McCuaig and Motzok, 1970). It is also known that excessive amounts of vitamin A can be teratogenic (Giroud, 1970). In this regard, Soliman (1972) gave to rats, 8 and 10 days after mating, high doses of vitamin A with and without vitamin E administration. When the animals were killed on the twentieth day after mating and the offspring were examined, a lower percentage of anomalies was observed in the group treated with vitamin E.

These observations (increased utilization of vitamin A and protection against hypervitaminosis A) are the theoretical basis for recommending the simultaneous administration of vitamin E (50–200 IU) when given massive doses of vitamin A as treatment or prophylactic measures (Bauernfeind *et al.*, 1974). Because some studies have failed to show any beneficial effect of such an approach, this issue has been somewhat controversial (Kusin *et al.*, 1974; IVACG, 1977). However, it is indirectly supported by the experience obtained in vitamin A massive dose programs conducted in developing countries in which the oral administration (twice a year) of 200,000 IU of vitamin A accompanied with 40 IU of vitamin E has produced a significant improvement in the vitamin A status of children and no toxicity has been observed (Bauernfeind *et al.*, 1974).

More recently the work of Jagadeesan and Reddy (1978) has shown that supplementation with vitamin E may have a beneficial effect on vitamin A-deficient children. Forty-five Indian children aged between 3 and 10 years were studied. Twenty of them had clinical signs of vitamin A deficiency. In the whole sample, the plasma vitamin E and A levels ranged from 60 to 1285 and from 15 to 33 μg/dl, respectively. Twenty-five percent of the children were below the "normal" level of 500 μg/dl of vitamin E and 31% had plasma vitamin A values below 20 μg/dl. Seven "normal" children (plasma vitamin A $\geq$ 20 μg/dl) and seven vitamin A-deficient children (plasma vitamin A $<$ 20 μg/dl) were supplemented daily for 2 weeks with 100 mg of DL-α-tocopherol acetate. Seven other children received a placebo and served as controls. The results of this intervention in plasma vitamin E and vitamin A levels are presented in Table IV. It can be observed that independent of their vitamin A status, the children who received vitamin E supplements experienced a significant increase not only in vitamin E levels but also in the levels of vitamin A. No significant

TABLE IV

Effect of Vitamin E Supplements to Children on Their Plasma Vitamin E and Vitamin A Levels[a]

Group	*n*	Plasma levels (μg/dl) Vitamin E	Vitamin A
(a) Normal			
Initial	7	578 ± 124	22.9 ± 1.1
Final	7	1045 ± 240*	38.3 ± 4.6*
(b) Vitamin A deficient			
Initial	7	505 ± 119	13.3 ± 2.0
Final	7	810 ± 195*	20.1 ± 3.7*
(c) Control			
Initial	7	746 ± 56	23.1 ± 2.4
Final	7	692 ± 34	21.1 ± 2.3

[a] From Jagadeesan and Reddy (1978).
* $p < 0.05$ or better.

change in these parameters was observed in the placebo group. It is unfortunate that the diet of these children was not described, but the results strongly suggest that vitamin E can increase the plasma concentration of vitamin A which in turn could become more available for tissue utilization. Whether these effects are due to the antioxidant properties of vitamin E or to an increased mobilization of vitamin A from the liver cannot be established.

Vitamin E deficiency is not a common condition in the general population except in the premature infant (Oski and Barness, 1967). Yet it may be a common finding in severely malnourished children as reported from different parts of the developing world (Rahman *et al.*, 1964; Scrimshaw *et al.*, 1956; Throwell *et al.*, 1954; Tulloch and Sood, 1967; Woodruff, 1965). Furthermore, McLaren *et al.* (1965) have reported that malnourished children with xerophthalmia have significantly lower serum vitamin E levels than malnourished children without ocular lesions. On the basis of these observations, the malnourished child may benefit most from vitamin E action. In this regard, considering the high prevalence of malnutrition of various degrees and its coexistence with vitamin A deficiency in developing countries, the addition of vitamin E to vitamin A supplements seems justified.

Conversely, early reports in the literature also indicate that large doses of vitamin E can lower the utilization of carotenoids and the liver stores of vitamin A in the rat (Johnson and Bumann, 1948; Swick and Baumann, 1952). On this basis, Bieri (1973) has warned about ingesting excessive

amounts of vitamin E. In this context it is interesting to see that in humans daily supplementation with 200 IU of vitamin E for 3 weeks without supplementary vitamin A can significantly decrease the serum vitamin A levels (Oaks *et al.*, 1978). Additional studies in adults have shown that pharmacological doses of vitamin E (147 mg daily for 3 weeks) lower not only serum vitamin A levels but also the levels of RBP (Garret-Laster *et al.*, 1981). Whether this phenomenon is related to dietary carotenoid utilization or to an enhancement of tissue uptake of vitamin A is not clear.

The possible mechanism for the effect of excess vitamin E on the biological utilization of carotenoids has been investigated in the rat (Arthur *et al.*, 1979). It was found that raising the tocopherol intake in young rats to 50 times the requirement almost abolished hepatic deposits of vitamin A from β-carotene while utilization of retinyl esters was not affected. Since fecal losses of unconverted β-carotene was elevated by high tocopherol, it seemed possible that the enzymatic cleavage of carotenoids was reduced. This was investigated by giving [^{14}C]-β-carotene by duodenal injections and measuring 1 hr later the retinol, its metabolites, and unconverted β-carotene in the intestine. The activity of the β-carotene cleavage enzymes was also assayed. The results obtained failed to give any indication that excess tocopherol had any inhibiting effect on the conversion of carotenoids into vitamin A. Although the mechanisms of this interaction have not been established, this undesirable effect of vitamin E should be kept in mind especially in populations with a high prevalence of hypovitaminosis A and whose dietary source of vitamin A is mainly carotenoids.

V. ROLE OF ZINC IN VITAMIN A TRANSPORT AND FUNCTION

As early as 1956 Stevenson and Earle observed that zinc deficiency in swine led to a decrease in plasma vitamin A concentration. Years later, Sarawswat and Arora (1972) reported that in lambs deficient in both zinc and vitamin A, zinc supplementation was necessary for maximum efficacy of vitamin A therapy. These observations suggested a regulatory role of zinc in vitamin A metabolism.

In a classical study, J. C. Smith *et al.* (1973) confirmed the above observations using the rat as experimental model. They showed that zinc deficiency was accompanied by low levels of plasma vitamin A. Furthermore, when rats deficient in both of these nutrients were supplemented with only vitamin A, there was an accumulation of vitamin A in the liver and the plasma levels of the vitamin remained depressed. Supplementation of zinc and vitamin A to the doubly deficient animals, however,

produced not only a significant rise in plasma vitamin A concentration but there was also no abnormal elevation of vitamin A stores. In a subsequent experiment they demonstrated that the levels of RBP in both plasma and liver of zinc-deficient rats were lower than those in zinc-sufficient animals, suggesting that in zinc deficiency there is an impairment of the vitamin A transport system (J. C. Smith *et al.*, 1973). Based on these observations and additional studies conducted by the same investigator it has been postulated that the metabolic defect produced by zinc deficiency is a failure to mobilize vitamin A from the liver (Brown *et al.*, 1976; Smith *et al.*, 1976; Smith, 1980).

This concept has been seriously questioned by the work of Carney *et al.* (1976). In a well-controlled rat study using pair-feeding they showed that zinc deficiency does not produce accumulation of hepatic vitamin A. More important, using tritium-labeled retinyl acetate it was demonstrated that zinc deficiency had no effect on vitamin A mobilization. Urinary excretion of vitamin A was also unaltered. They concluded that the low level of plasma vitamin A observed in zinc deficiency is not a direct effect of the lack of zinc but rather the result of growth retardation.

It is known that in zinc deficiency there is a significant reduction in food intake and growth. In fact, it can be observed in the work of Smith *et al.* (1976) that severe food restriction in rats to the point of growth arrest can lead, in some of the animals, to plasma vitamin A depressions of the same magnitude as that observed in zinc depletion.

Additional data in animals has come from the work of Duncan and Hurley (1978), who studied the interaction between zinc and vitamin A in pregnant and fetal rats. It was thought that since both zinc deficiency and vitamin A deficiency are teratogenic in rats, further evidence of an interrelationship between these two nutrients could be obtained. In this experiment, vitamin A-depleted rats were bred overnight with males fed a stock-normal diet. The pregnant females were then divided into three groups and were fed a vitamin A-free diet containing three different levels of zinc (100, 9, and 0.5 μg/gm of diet). Each group was further divided into three subgroups which were given daily by stomach tube three different levels of vitamin A as retinyl palmitate (400, 8, and 0 μg/kg BW/day). A control group was provided with sufficient levels of both vitamin A and zinc throughout the study and followed the same protocol in terms of mating. On day 21 of gestation the animals were sacrificed and the fetuses were removed by cesarean section. To determine the effect of the different treatments, various reproductive parameters were evaluated and the plasma and liver concentrations of zinc and vitamin A were measured in both maternal and fetal tissue. Table V shows the effect of the treatments on the percentage of fetal implantation sites with either a reabsorbed or

TABLE V

Effect of Various Dietary Levels of Zinc and Vitamin A on Reproduction and on Vitamin A Concentration in Blood and Livers of 21-Day-Old Rat Fetuses[a]

Dietary treatment					
Zn content of diet (μg/g)	Vitamin A fed (μg/kg BW/day)	Implantation sites affected (%)[b]	Malformed fetuses (%)	Plasma vitamin A (μg/dl)	Liver vitamin A (μg/mg protein)[c]
100[d]	400	0	0	48.0	0.224
100	400	0.3	0	46.4	0.203
100	8	35.4	30.1	26.0	0.014
100	0	72.0	65.5	6.0	0
9	400	14.0	14.0	34.9	0.173
9	8	52.1	48.3	25.8	0.007
9	0	79.5	70.1	4.1	0.001
0.5	400	100.0	95.0	15.8	0.189
0.5	8	100.0	100.0	3.9	0.01
0.5	0	100.0	100.0	1.0	0.0002
		Analysis of Variance (p values)			
Effect of zinc (Zn)		0.01	0.01	0.01	NS
Effect of vitamin A (A)		0.01	0.01	0.01	0.01
Interaction (Zn × A)		0.05	0.05	0.01	NS

[a] Adapted with permission from Duncan and Hurley (1978). (© *J. Nutr.*, American Institute of Nutrition.)

[b] With either a reabsorbed or malformed fetus.

[c] On wet weight basis.

[d] Nondepleted control group.

malformed fetus. The percentage of malformed fetuses is also presented. It can be observed that the levels of each of these nutrients had a significant effect on these parameters and, more important, two-way analysis of variance revealed a significant interaction between vitamin A and zinc ($p < 0.05$). Similar results were obtained on the plasma levels of vitamin A of the fetuses, but there was no significant interaction between zinc and vitamin A in relation to the amount of vitamin A stored in the liver (Table V). These observations were consistent with the results obtained in maternal tissues. The data suggested that although zinc deficiency did not produce a significant accumulation of vitamin A in the liver, based on the other parameters there was a significant interrelationship between zinc and vitamin A which was probably not related to growth retardation but perhaps instead to an impairment of vitamin A mobilization.

Taken together, the existing controversial data do not allow one to define whether zinc deficiency has a direct or only an indirect effect on vitamin A transport. These conflicting results are probably related to the

fact that both zinc deficiency and vitamin A deficiency are similarly affected by other factors imposed by a decreased intake of food. For example, it has been shown that the level and quality of dietary protein can significantly affect the synthesis of RBP (Glover and Muhilal, 1976). Although zinc is not a constituent part of RBP, it is known that zinc is an essential element for RNA synthesis and as a consequence zinc deficiency could impair the synthesis of proteins in general (Terhune and Sanstead, 1972). Because zinc deficiency leads to low levels of RBP, this might mean that RBP synthesis could be particularly sensitive to the lack of this essential element. In this regard several studies have shown that in severely malnourished children protein supplementation results in a significant increase in the levels of both vitamin A and RBP (Arroyave, 1969; Ingenbleek *et al.*, 1975; Smith *et al.*, 1975). Children with PEM are often zinc deficient as well (Kutumbale *et al.*, 1976). Although in this case the protein deficit is the main cause of low RBP levels, zinc deficiency could also be a contributing factor. The data from Shingwekar *et al.* (1979) support this speculation. In this study, vitamin A-deficient children with relative adequate protein-energy nutriture (mean albumin = 3.1 gm/dl) and children suffering from severe edematous-type PEM (mean albumin = 2.2 gm/dl) were supplemented with zinc for a short period of time. The results revealed that this single treatment produced a significant elevation in plasma vitamin A and RBP levels only in the children with PEM and not in children with higher albumin levels.

On the basis of all these considerations, a logical mechanism by which zinc deficiency affects vitamin A transport is through an indirect action on protein synthesis.

In practical terms, the existing information suggests that when treating vitamin A deficiency particularly in malnourished children, not only their protein status but also their zinc nutriture should be of concern.

Another aspect of the relationship between zinc and vitamin A is related to the oxidation–reduction process of vitamin A alcohol (retinol) at the tissue level. During vitamin A metabolism, retinol is interconverted to retinal and this process requires a specific alcohol dehydrogenase enzyme known as retinol dehydrogenase. In mammalian tissues, various alcohol dehydrogenases are known to be zinc-metalloenzymes, that is, they require zinc for proper enzyme activity (Vallee and Hoch, 1957).

This function of zinc becomes very important in certain tissues, such as the retina, where, as part of the visual cycle, retinol has to be converted to retinal (Wald, 1950). Huber and Gershoff (1975) have studied the effect of zinc deficiency at this level in the rat. In this experiment, the content of zinc and other trace metals such as copper, manganese, and chromium were determined in the eye of zinc-deficient animals. Furthermore, the

TABLE VI

Zinc Content of the Eye and Alcohol Dehydrogenase Activity in the Retina of Zinc-Deficient Rats[a,b]

	Deficient	Control	*p*
Zn content of eye (ppm Zn/dry weight)	68.4 ± 2.8	1113.6 ± 4.9	<0.001
ADH activity (mμmole NADH/min/retina)			
Substrate: Retinol	11 ± 0.6	27 ± 6.5	<0.02
Ethanol	15 ± 0.5	26 ± 4.0	<0.02

[a] Adapted with permission from Huber and Gershoff (1975). (© *J. Nutr.*, American Institute of Nutrition.)

[b] Each value is the mean ± SEM of 10 rats.

enzyme activity of the alcohol dehydrogenase (retinol dehydrogenase) was also assayed in the retina using as substrate both retinol and ethanol. Some of the results obtained in this study are presented in Table VI. The zinc content of the eye was significantly lower in the zinc-depleted animals in comparison with the zinc-sufficient controls. The fact that the content of the other trace minerals was not altered indicated that this phenomenon was not produced by a reduction in food intake. The activity of the alcohol dehydrogenase enzyme was also significantly depressed in the retina of the zinc-deficient animals. No difference in enzyme activity was seen in the liver. These observations suggest that the eye is particularly sensitive to zinc deficiency and that the dietary lack of this mineral could easily impair the visual process. In humans, Morrison *et al.* (1978) reported six patients with alcoholic cirrhosis and abnormal dark adaptation who responded to zinc supplementation by lowering their final dark-adapted threshold to normal in comparison with six age-matched control subjects. The results of this interesting study are shown in Table VII. Two of the patients (1 and 2) had been first treated with vitamin A (1,000,000 IU/day) for 2 to 4 weeks without satisfactory response. Only after oral supplementation of zinc (220 mg of zinc sulfate/day) for 1 to 2 weeks did their final dark-adapted threshold return to normal. Three other patients (2–5) were given just zinc sulfate for 1 to 2 weeks and their impaired dark adaptation also became normal. Patient 6 responded similarly upon supplementation of both vitamin A and zinc. In this study, zinc supplementation did not raise serum levels of RBP. Therefore, considering that retinal must be constantly supplied to the rods for normal vision, the author concluded that the effect of zinc supplementation was to enhance the alcohol dehydrogenase activity in the retina. Similar observations have been made after zinc supplementation by McClain *et al.* (1979).

TABLE VII

Effect of Zinc Supplementation on Dark Adaptation in Cirrhotic Patients[a,b]

Patient	Before treatment	After treatment: Vitamin A, 10,000 IU (2–4 weeks)	After treatment: Zinc sulfate, 220 mg/day (1–2 weeks)	After treatment: Vitamin A and zinc (2 weeks)
1	3.2	2.6	2.1	
2	3.0	3.2	2.2	
3	3.1		2.2	
4	2.5		2.1	
5	4.3		3.1	
6	2.8			2.3

[a] Reprinted with permission from Morrison *et al.* (1978). (© *Am. J. Clin. Nutr.*, American Society for Clinical Nutrition.)

[b] Dark adaptation units are log lux. Normal dark-adapted threshold for six age-matched controls was 2.1 ± 0.2.

The influence of zinc deficiency on alcohol dehydrogenase has also been investigated in other tissues. A reduced activity of retinal reductase (retinol dehydrogenase) has been observed in the testes but not in the liver of zinc-deficient rats (Sundaresan *et al.*, 1977). Since this change in enzyme activity observed in the testes was not related to food restriction, it was concluded that the phenomenon was a direct effect of the lack of zinc. However, the significance of this observation in relation to gonadal metabolism and reproductive function is unclear.

Together, the existing data from animals indicate that zinc deficiency can alter vitamin A metabolism not only by directly or indirectly affecting vitamin A mobilization but also by impairing the oxido-reduction of vitamin A in the retina, testes, and probably other tissues as well.

On the other hand, all this basic knowledge of vitamin A and zinc interaction has had a significant influence and stimulatory effect in clinical research, particularly in those pathological conditions such as liver and pancreatic disease in which the levels of zinc or the levels of vitamin A are known to be altered. For a complete description and discussion of the zinc–vitamin A interaction under pathological conditions, the reader is referred to the review by Solomons and Russell (1980).

VI. EFFECT OF HYPOVITAMINOSIS A ON IRON NUTRITION AND METABOLISM

Early information on the effect of hypovitaminosis A on hematopoiesis, which may reflect alterations in iron metabolism, has appeared since 1922

and revealed two different lines of contrasting evidence: on one hand the occurrence of anemia and on the other that of polycythemia. Although these early findings have been reviewed by Hodges *et al.* (1978), it is worth describing the work performed by Amine *et al.* (1970) in which, even though there was not a demonstration of anemia per se caused by vitamin A deficiency, there was good evidence for an interaction between vitamin A and iron. The purpose of their study was to evaluate the combined effect of deficiencies of iron and vitamin A in the weanling rat. Four groups of animals were fed as follows: (1) low iron and low vitamin A, (2) low iron, (3) low vitamin A, and (4) a control group. Groups 2 and 3 gained approximately the same amount of weight, which was less than that of the control group. Doubly deficient animals, however, gained significantly less weight than those deficient in each nutrient alone. Animals fed the low vitamin A diet had a higher hemoglobin concentration than the control group. The hemoglobin and hematocrit values of iron deficiency or double deficiency decreased comparably throughout the experiment. An interesting finding was the fact that those animals fed diets low in either iron or vitamin A developed microcytosis, whereas animals fed the doubly deficient diet tended to have a normocytic anemia. In addition, each of the deficient groups had a mean corpuscular hemoglobin concentration significantly less than that of the control.

More recent data on the subject come from the human vitamin A deficiency study conducted by Hodges and associates (1978). In this particular experiment vitamin A deficiency was induced by feeding diets deficient or low in vitamin A to eight middle-aged male volunteers. As expected, the concentration of total carotenoids in their serum fell rapidly and the concentration of retinol fell slowly during the vitamin A depletion period, which varied in the different subjects from 359 to 771 days. Despite a daily intake of 18–19 mg of iron in their diet, the men gradually began to manifest a mild degree of anemia accompanied by low serum iron levels. This phenomenon is shown for one of the subjects in Figure 3. It can be observed that during the depletion period there was a simultaneous drop in the levels of both serum vitamin A and blood hemoglobin. Furthermore, as the anemia became apparent, the men were given oral medicinal iron (310 mg/day). It turned out that the iron treatment had little or no value so long as the men remained deficient in vitamin A. In the case of one particular subject, he responded transiently to medicinal iron but relapsed despite continued therapy. Soon after vitamin A repletion was started with β-carotene, he made a prompt and complete hematological recovery while continuing to eat the same diet. This interesting observation strongly suggested the need of vitamin A for normal hematopoiesis.

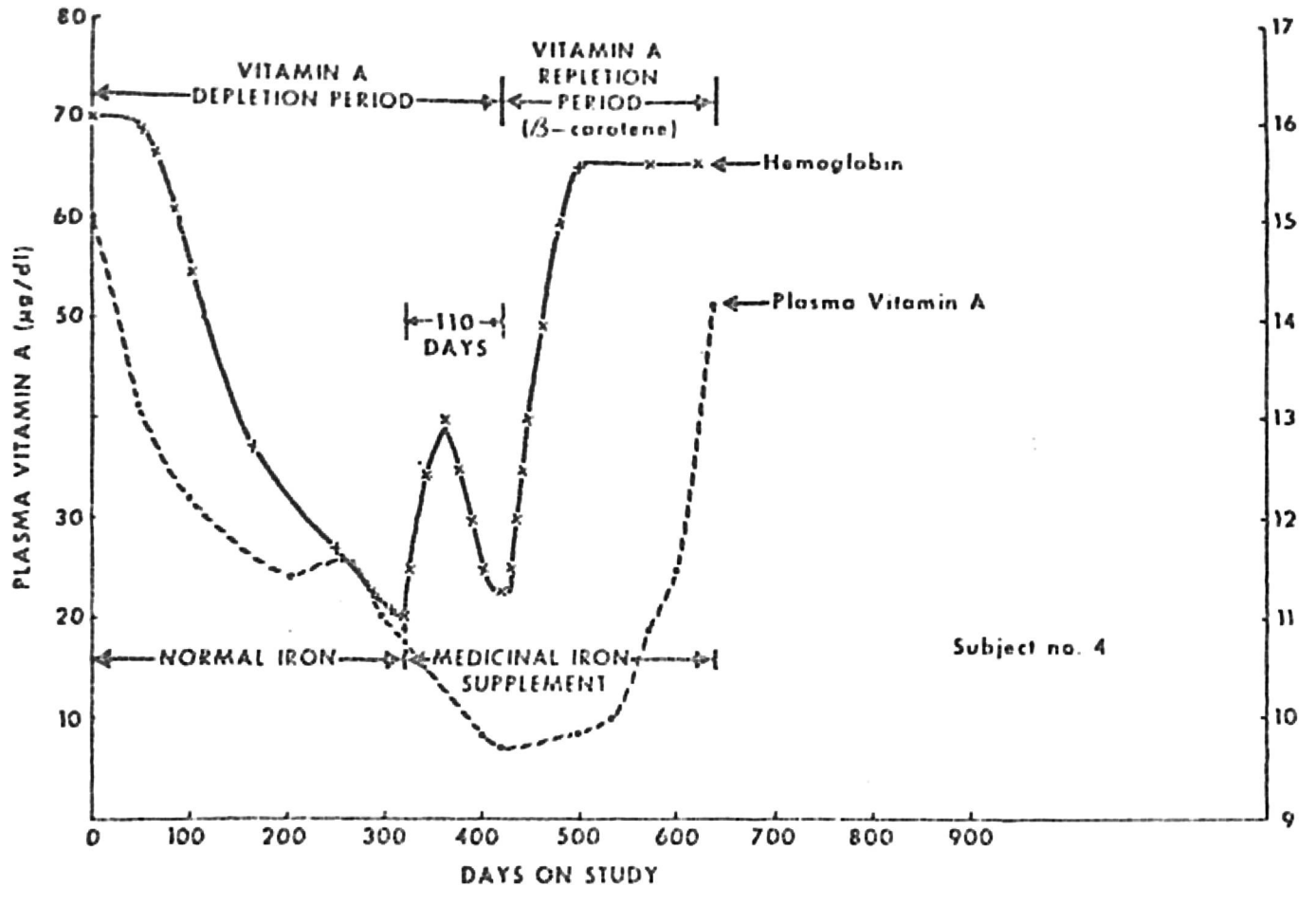

Fig. 3. Effect of vitamin A and iron supplements on plasma vitamin A and hemoglobin in a vitamin A-depleted human volunteer. (Reprinted with permission from Hodges *et al.*, 1978. © *Am. J. Clin. Nutr.*, American Society for Clinical Nutrition).

From the epidemiological point of view it can be demonstrated not only that both vitamin A deficiency and anemia coexist but also that there are significant positive associations between serum vitamin A and hemoglobin and other biochemical indicators of iron nutriture (Hodges *et al.*, 1978; Mejía *et al.*, 1977). These associations have been confirmed by similar observations made by Mohanram *et al.* (1977) and Jagadeesan and Reddy (1978) in Indian children and by Wegner *et al.* (1979) in a group of elderly persons in Vienna.

Animal studies have also shown that anemia may result from the lack of vitamin A (Mejía *et al.*, 1979a). As illustrated in Figure 4, after approximately 40 days of feeding a vitamin A-free diet to young adult rats, they exhibited lower hematocrit and hemoglobin levels than those observed in the control groups. At around 90 days, however, hematocrit and hemoglobin levels become elevated even reaching higher levels than those observed in the control. Isotopic dilution studies have shown that this latter phenomenon is due to a reduction in blood and plasma volume which occurs when the vitamin deficiency becomes severe (Mejía *et al.*, 1979b). Alterations in water metabolism in vitamin A deficiency which may cause hypovolemia have also been reported by others (López *et al.*, 1973; Mahant and Eaton, 1976). Thus, at this stage the anemia is masked by hemo-

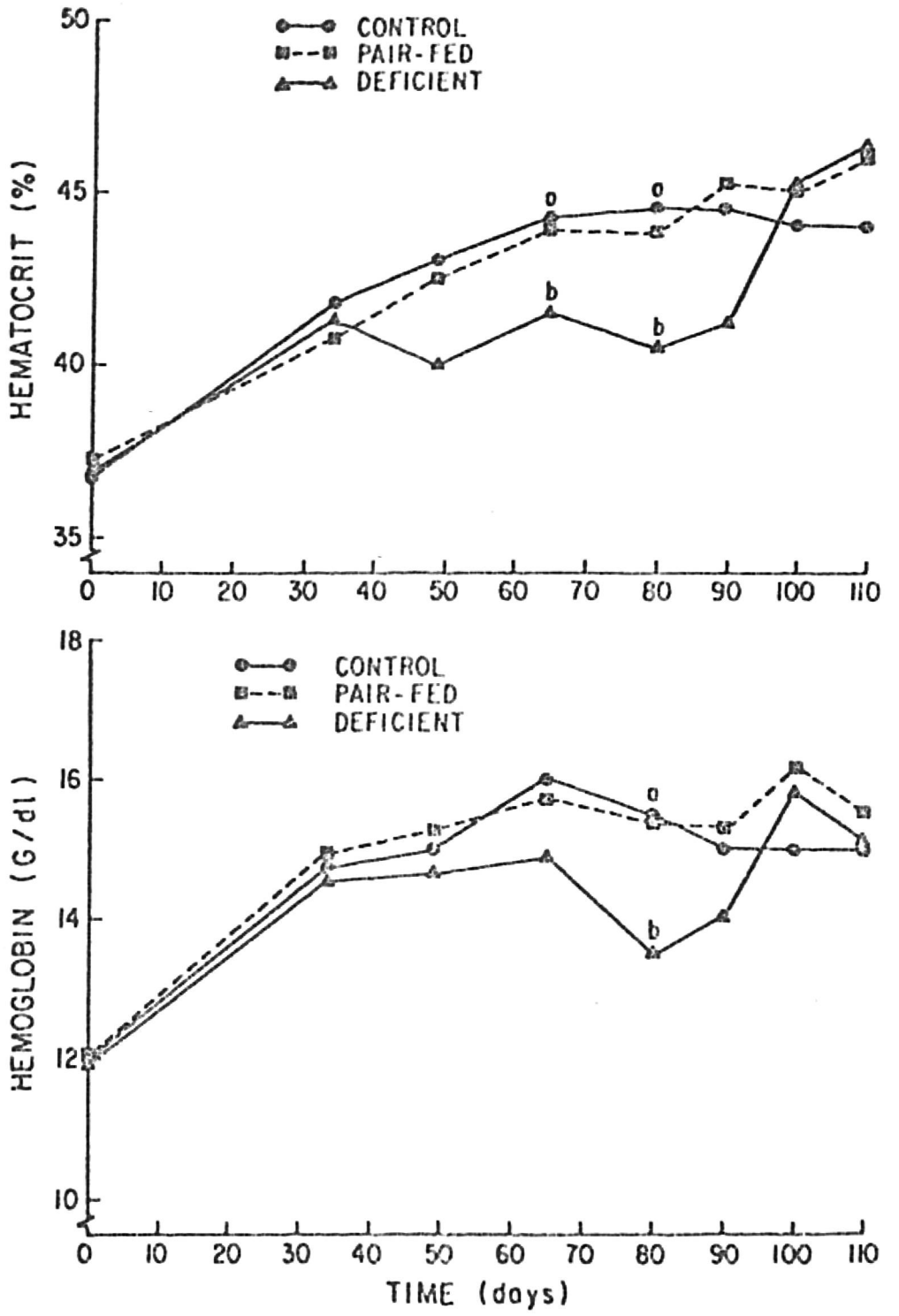

Fig. 4. Changes in hematocrit (upper panel) or hemoglobin (lower panel) in rats fed diets sufficient or deficient in vitamin A. Each point represents the average from six animals. Values bearing a different superscript are significantly different at $p < 0.05$. (Reprinted with permission from Mejía *et al.*, 1979a. © *Am. J. Clin. Nutr.*, American Society for Clinical Nutrition.

concentration and this may be the reason why several investigators have failed to observe anemia in vitamin A-deficient animals. When the anemia develops, the animals also showed a reduction in serum iron and a concomitant elevation of the amount of iron in the liver. Spleen iron also increases when the deficiency becomes severe. These observations are supported by the isotope data presented in Table VIII, which show that when an oral dose of radioactive iron is given to vitamin A-deficient rats

TABLE VIII

Absorption of ^{59}Fe and Specific Activities of Liver and Spleen in Experimental Rats[a]

Group	Percentage absorption	Specific activity (CPM/gm tissue)	
		Liver	Spleen
Control	43.9 ± 14.6[a,*]	242 ± 22.4[a]	533 ± 97.2[a]
Pair-fed	12.0 ± 12.8[b]	180 ± 18.1[a]	280 ± 77.3[b]
Deficient	38.3 ± 6.7[a]	552 ± 85.4[b]	1217 ± 185.0[c]

[a] Adapted with permission from Mejía *et al.* (1979b). (© *J. Nutr.*, American Institute of Nutrition.)

* $\bar{X}$ ± SEM. Different superscript letters indicate $p < 0.05$.

there is no significant alteration in iron absorption, but there is a greater incorporation of the isotope in the liver and spleen of the deficient animals as compared to controls (Mejía *et al.*, 1979b). A 50% increase in ^{59}Fe in the spleen from vitamin A-deficient rats has also been observed by Gardner and associates (1979) after administration of prelabeled red blood cells. These observations indicate that in vitamin A deficiency iron accumulates in storage depots. In fact one of the early pathological findings in vitamin A deficiency was hemosiderosis of the spleen and liver (Wolbach and Howe, 1925; Blackfan and Wolbach, 1933). Considering that iron absorption is not altered in vitamin A deficiency, it can be concluded that neither the low serum iron levels nor the increased stored iron observed in hypovitaminosis A can be related to changes in this parameter. Iron absorption was first suggested to be increased in vitamin A deficiency (Amine *et al.*, 1970). Further studies, however, have shown that the absorption of this mineral is not significantly affected in both the vitamin-A deficient rat and in vitamin A-deficient children (Mejía *et al.*, 1979b; Mohanram, 1977).

Although there is need of additional data to refine a mechanism for the effect of vitamin A on iron metabolism and hematopoiesis, the existing metabolic information indicates that in hypovitaminosis A there is a shifting of body iron resulting in an elevation of iron stores and a decrease in plasma iron. Thus, iron may become less available to erythropoietic tissues for red cell formation. Furthermore, it has been demonstrated that in the vitamin A-deficient rat there is a lower incorporation of ^{59}Fe into erythrocytes (Mejía *et al.*, 1979b). This observation suggests that in vitamin A deficiency there is also an impaired utilization of iron for the synthesis of red blood cells. This latter phenomenon has been confirmed in vitamin A-deficient children (Mohanran and Reddy, 1982).

Effect of Vitamin A Interventions

In Central America, nutrition surveys have revealed a high prevalence of hypovitaminosis A. In an attempt to overcome this nutritional problem, the Institute of Nutrition of Central America and Panama in conjuction with the government of Guatemala began in late 1975 a vitamin A nutrification program at the national level using table sugar as the dietary vehicle. The experimental design and methodology used in this program have been reported and the results of its evaluation have been published (Arroyave *et al.*, 1979). In summary, preschool children and lactating mothers from 12 small rural communities were studied for 2 years in five consecutive surveys, one prior to vitamin A nutrification (Survey I) and four additional ones (Survey II–V) at 6-month intervals after the intervention began. The dietary data revealed that in comparison with the prenutrification survey, the implementation of sugar nutrification resulted in a significant threefold increase in the average daily intake of retinol equivalents. As a result, there has been a highly significant reduction in the prevalence of low and deficient levels of serum retinol in children. Similar results were obtained in the levels of retinol in breast milk of lactating women. The average intake of iron, however, did not change throughout the 2-year period of evaluation. This vitamin A program provided a unique opportunity to evaluate the effect of this single intervention on iron nutrition and metabolism at the population level. The results showed that vitamin A nutrification had a positive impact on iron nutrition and metabolism (Mejía and Arroyave, 1982). Figure 5 shows the changes observed in serum retinol in relation to changes in iron biochemical indicators in a group of preschool children sampled in the initial survey and at 6 months after nutrification began. In these children there were significant positive correlations between the experienced change in serum retinol and changes in serum iron, total iron-binding capacity (TIBC), and saturation of transferrin. In contrast, stored iron, as defined by serum ferritin levels, correlated negatively. These results suggest that vitamin A mobilized the stored iron into the circulation, supporting previous observations in experimental animals. It is interesting to note that in these children, despite the increase in serum iron levels, there was also an unexpected elevation in the levels of TIBC, suggesting that vitamin A could have affected the iron carrier glycoprotein, transferrin. This possibility has been tested in children and no significant association has been found between the vitamin A status and the plasma levels of this protein (Mejía and Arroyave, 1983).

After a more prolonged intervention the effect of vitamin A on iron nutriture was different, particularly in relation to iron stores. As shown in

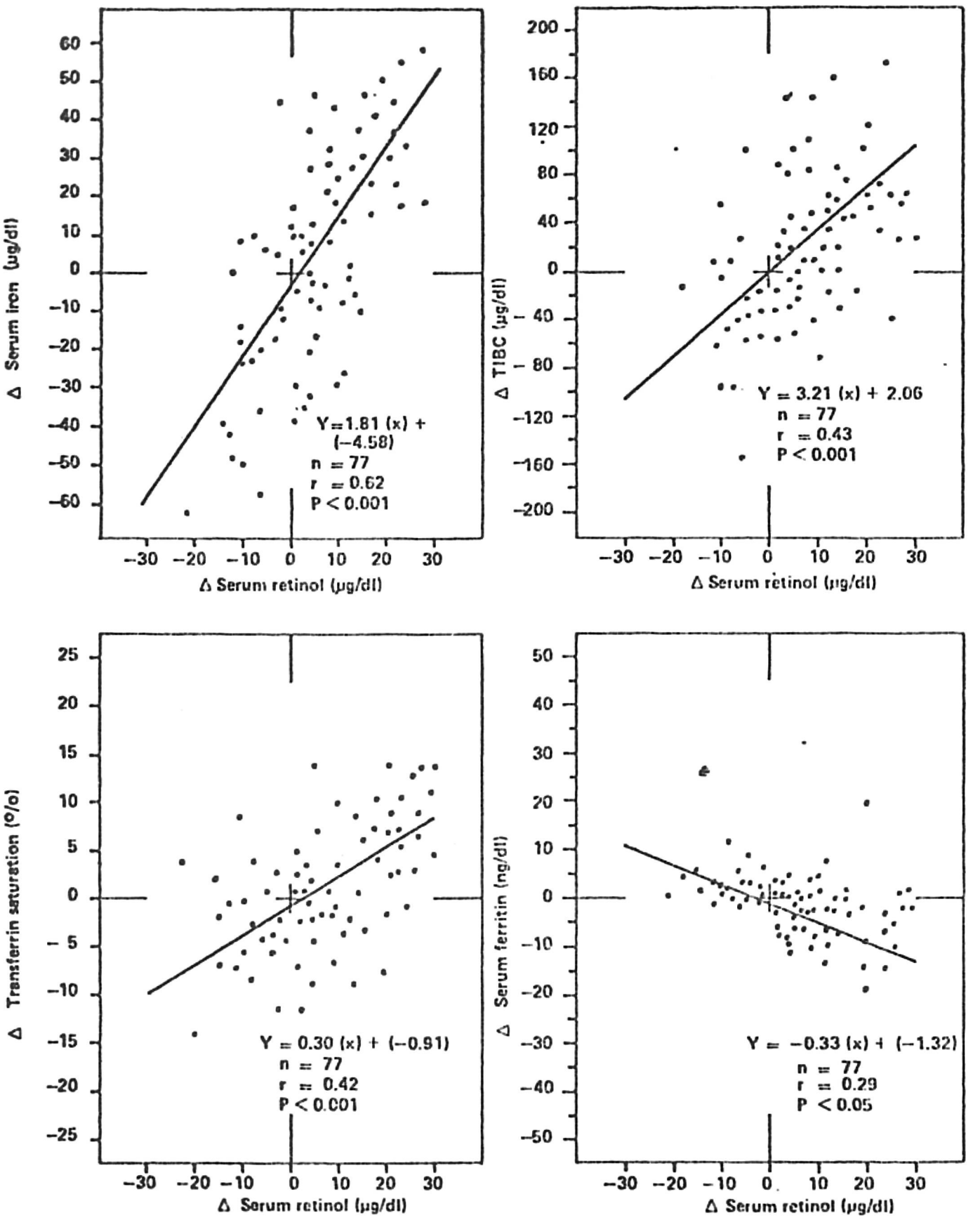

Fig. 5. Correlations between changes in serum vitamin A and changes in serum levels of iron parameters after 6 months of vitamin A nutrification of Guatemalan children (Δ, change). (Reprinted with permission from Mejía and Arroyave, 1982, *Am. J. Clin. Nutr.*, American Society for Clinical Nutrition.)

TABLE IX

Percentage Distribution of Cases in Surveys I and V by Categories of Iron Parameters[a]

	Survey I[b] (%)	Survey V[c] (%)
Serum iron (μg/dl)		
<50	43.1	25.5*
50–75	27.5	39.2
>75	29.4	35.3
Serum TIBC (μg/dl)		
<250	5.9	2.0
250–350	37.3	49.0
>350	56.9	49.9
Percentage ST		
<15	39.2	27.5
15–20	33.3	33.3
>20	27.5	39.2
Serum ferritin (ng/ml)		
<10	64.7	21.6*
10–20	23.5	58.8
>20	11.8	19.6

[a] Reprinted with permission from Mejía and Arroyave (1982). (© *Am. J. Clin. Nutr.*, American Society for Clinical Nutrition.

[b] Initial survey.

[c] Two years after initiation of vitamin A nutrification.

* The distributions are significantly different ($p < 0.05$ or better).

Table IX, when comparing in a group of children the distribution of cases by categories of levels of adequacy of iron indicators between the initial and last survey, an overall improvement in the levels of iron indicators can be observed. This favorable effect was more marked in relation to the amount of stored iron. There was a lower prevalence of children with low serum ferritin levels in Survey V than in Survey I prior to vitamin A nutrification. Siimes *et al.* (1974) have shown that in healthy children the levels of serum ferritin remain constant from 6 months to 16 years of age. Therefore, the observed improvement cannot be attributed to the fact that the children were 2 years older at the end of the study. Most probably this elevation in iron stores observed in the long run was due to an enhancement of dietary iron absorption triggered as a response to the initial depletion of iron reserves experienced after 6 months of vitamin A nutrification and, in addition, to a possible increment in hematopoietic utilization of this mineral. Because this was a retrospective study performed by using

stored serum samples, it was not possible to assess the hematological impact of the intervention. One study, however, has demonstrated that if children suffering from vitamin A deficiency are supplemented orally with 8000 μg of retinyl palmitate they improve not only their serum levels of iron but also their hematological condition (Mohanram *et al.*, 1977).

Although there is need of additional information on the mechanism of the effect of vitamin A on iron metabolism and hematopoiesis, the existing data indicate that there is a biological interaction between vitamin A and iron. Through this relationship vitamin A deficiency may be a contributing factor in the occurrence of anemia, especially in areas where hypovitaminosis A is highly prevalent and endemic. When planning nutritional interventions proper consideration should be given to this interaction.

VII. RELATIONSHIP BETWEEN VITAMIN A AND OTHER NUTRIENTS

For many years vitamin A has been linked with iodine metabolism, thyroid function, and goiter (Drill, 1943; Jaya Rao and Khan, 1974; Ingenbleek and DeVisscher, 1979; Moore, 1957; Morley *et al.*, 1978; Stowe *et al.*, 1980). The role of vitamin A nutriture on thyroid function and the development of goiter is somewhat controversial. Although the most accepted concept is that vitamin A deficiency favors the hyperactivity of the thyroid gland (Jaya Rao and Khan, 1974; Morley *et al.*, 1978). others have suggested that the deficiency of the vitamin may lead to the occurrence of goiter in populations (Ingenbleek and DeVisscher, 1979). The concept of increased thyroid function in vitamin A deficiency is well supported by the fact that in both humans and experimental animals the levels of thyroid hormones as well as those of the thyroid-stimulating hormone (TSH) and the thyroid-releasing hormone (TRH) can be elevated (Jaya Rao and Khan, 1974; Morley *et al.*, 1978). Despite this phenomenon, however, no signs of hyperthyroidism have been observed in humans (Jaya Rao and Khan, 1974). On the other hand, the possibility that hypovitaminosis A may lead to goiter has not been proven on an experimental basis. The existing epidemiological data are insufficient to conclude that vitamin A deficiency can contribute to the occurrence of endemic goiter in populations (Anonymous, 1980). In general, what seems to be more evident about this interaction is that vitamin A may play an important regulatory role in iodine metabolism and thyroid function as suggested by Morley *et al.* (1978, 1979).

Another interaction that has been reported is that between vitamins A and C (Mohanram *et al.*, 1976; Gruber *et al.*, 1976). In the vitamin A-depleted rat, the levels of ascorbic acid in blood liver and urine are signifi-

cantly lower than those in vitamin A-sufficient animals (Mohanram *et al.*, 1976). Similar observations have been made by Gruber *et al.* (1976). In this animal, a logical explanation for this phenomenon could be a decrease in ascorbic acid synthesis. However, it had been previously found that vitamin A deficiency does not affect the enzyme gulanolactone oxidase in rat liver (Rogers, 1969). This means that the low levels of ascorbic acid found in the vitamin A-deficient rat are probably not related to a depressed ascorbic acid synthesis. Furthermore, despite this effect of vitamin A on ascorbic acid, large doses of vitamin C do not protect the rat from the consequences of vitamin A deficiency (Zile and DeLuca, 1968). Because the rat, in contrast to man, can synthesize its own vitamin C, the significance of these observations to human nutrition has not become an important issue.

A relationship between vitamins A and K in experimental animals has also been known for many years (Walker *et al.*, 1947; Maddock *et al.*, 1948). In these studies it has been observed that hypervitaminosis A can cause hemorrhages related to a decline in prothrombin which in turn results in an increased clotting time. Furthermore, this abnormality can be either prevented or corrected by the administration of vitamin K. It is not clear whether this is a direct or indirect interaction between vitamins A and K. In this regard, it has been postulated that the excess of vitamin A can produce a secondary vitamin K deficiency by antagonizing the intestinal absorption of vitamin K (Matschiner *et al.*, 1967). These observations are worth keeping in mind in human clinical nutrition.

Other relationships between vitamin A and several other nutrients, such as calcium (Navia and Harris, 1980), copper (Moore, 1969), and vitamin D (Veltmann *et al.*, 1982), have also been described. Due to space limitations, however, the reader is referred to the references provided.

VIII. CONCLUDING COMMENTS

The data presented indicate that in biological systems both vitamin A deficiency and vitamin A excess can be conversely related to other nutrients. The effect of these relationships can alter not only their utilization but also their biological function.

The mechanisms of these interactions are often not well established. In some cases, it is not clear whether these are direct relationships or just secondary effects mediated through other biological factors which may even involve other nutrients. In this regard, growth depression and reduced food intake, often unavoidable in animal experimental nutrition, have been important confounding factors.

Despite existing uncertainties, however, the interactions, either direct or indirect, between vitamin A and other nutrients can have important implications in human health and nutrition. These interactions should be kept in mind particularly when treating or preventing vitamin A deficiency both at the clinical and at the population levels.

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