Effects of Dietary Vitamin A Deficiency, Retinoic Acid and Protein Quantity and Quality on Serially Obtained Plasma and Liver Levels of Vitamin A in Rats

BARBARA A. UNDERWOOD, JOHN D. LOERCH AND KEVIN C. LEWIS
Nutrition Program, Division of Biological Health, The Pennsylvania State University, University Park, Pennsylvania 16802

ABSTRACT Rats were fed vitamin A-deficient diets either alone, supplemented with retinoic acid (RA), or of limited protein quality or quantity (7% rice or 7% casein protein); one group was fed 7% rice protein supplemented with vitamin A. Plasma and liver levels of vitamin A were determined serially. Plasma levels in rats fed otherwise adequate vitamin A-deficient diets remained above 30 μg/dl until liver reserves were below 10 μg/g tissue, at which point plasma levels decreased in some but not all rats while liver levels continued to decline (at a slower rate) to levels as low as 3 μg/g. Supplementation with RA caused an immediate and sustained reduction of 15 to 20 μg/dl in circulating vitamin A. At 7% dietary protein, plasma levels of vitamin A remained above 30 μg/dl when casein protein was fed or when the rice protein diet was supplemented with dietary vitamin A, but not when the rice protein diet was fed without an exogenous source of the vitamin. A scheme is proposed suggesting possible regulatory mechanisms that might control homeostatic levels of plasma vitamin A.

INDEXING KEY WORDS plasma vitamin A • liver vitamin A • retinoic acid • protein quality • protein quantity

It is difficult to assess quantitatively by acceptable biochemical procedures whether an individual is well-nourished with respect to vitamin A, except under extreme conditions of tissue depletion or saturation. Under such extreme conditions, clinical signs and symptoms are usually present and blood levels are remarkably low or high. The level of vitamin A in the liver is the best reflector of relative vitamin A status, but the liver is not a tissue that can be directly assayed in living human populations. Between the extremes of vitamin A depletion or saturation, blood levels of vitamin A remain relatively constant with widely varying levels of hepatic reserves and do not reflect either the concentration of vitamin A in the liver or that of the immediate dietary supply (1-7). Current knowledge about plasma levels of vitamin A might be summarized as follows: Individuals (animals and man) have a homeostatic mechanism which, in the presence of optimum supplies of nutrients and in the

Received for publication January 4, 1978.

1 Supported in part by NIH Grant AM-16578.
3 Current address: Department of Nutrition and Food Science, Massachusetts Institute of Technology, Room 20A-207, Cambridge, Massachusetts 02139.
absence of disease and unusual stress, establishes a level of circulating vitamin A that can be regarded as normal for that individual. Acute or chronic deviations in physiological or external conditions may cause transitory or long-term readjustment in the circulating level of vitamin A, influenced only in part by the availability of the vitamin from endogenous or exogenous supplies. Distribution curves describing given adult populations for rats and man approach a Gaussian distribution with values falling between 20 and 60 μg/dl (8-10). The curve is shifted somewhat to the left in younger age groups (10-13), by some acute and chronic disease states (14-16) and by emotional (17) and dietary, environmental, and physiological stresses (9, 18-23). In such instances, circulating levels of vitamin A may fall to 15 to 30 μg/dl and be relatively refractory until the constraining dietary, environmental or physiological factor is alleviated.

The regulatory factors that cause adjustments of the homeostatic set point and the precise reactions by which control is exerted are incompletely understood. The availability of retinol from endogenous and/or exogenous sources (24, 25), of retinol-binding protein (RBP) (26, 27) and of adequate protein nutriture in general (28-33) are known to be important factors. Other nutrients, variations in hormonal levels, and the rate of tissue utilization of retinol have also been implicated (19, 20, 22, 23, 34-37). However, the literature contains few reports of serially determined blood and liver values of vitamin A in rats during progressive vitamin A depletion or repletion, or during imposition of other alterations proposed to affect the homeostatic set point (3, 23, 38).

In this paper we report results of three experiments in which plasma and liver levels of vitamin A were studied during progressive vitamin A depletion or repletion, or during imposition of other alterations proposed to affect the homeostatic set point (3, 23, 38).

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### MATERIALS AND METHODS

#### Experiment 1
Twenty-six male weanling Sherman rats were fed a vitamin A-deficient diet for up to 33 days. The composition of the diet is given in table 1; the diet met the requirements for all nutrients other than those intentionally deleted (39). All rats were fed and given double-distilled water ad libitum and were housed in individual stainless steel cages in an isolated room with automatic temperature (22 to 26°), humidity (60%), and 12-hour (0700 to 1900) light cycle controls. Body weight was measured periodically. None of the rats showed signs of vitamin A deficiency during the study. At approximately weekly intervals, blood samples were taken from a randomly selected group of 5 to 11 rats; the selected rats were then killed for determination of hepatic levels of vitamin A.

#### Experiment 2
Weanling male Sprague-Dawley rats were fed the vitamin A-deficient diet shown in table 1. One group of 82 rats received the deficient diet for 21 days. Forty-four rats were killed at random intervals during this period. The selected rats were then killed for determination of hepatic levels of vitamin A.

### TABLE 1

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (g/kg diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein, vitamin-free</td>
<td>180.00</td>
</tr>
<tr>
<td>Oil, cottonseed</td>
<td>50.00</td>
</tr>
<tr>
<td>Starch, corn</td>
<td>649.99</td>
</tr>
<tr>
<td>Yeast, brewer's powder</td>
<td>80.00</td>
</tr>
<tr>
<td>Mineral mix, USP XIV</td>
<td>40.00</td>
</tr>
</tbody>
</table>
| Ergocalciferol, in corn oil | (400,000 units/g)  | 0.0110

1 The vitamin A-deficient diet was purchased commercially from Teklad Mills, Madison, Wisconsin. The diet contains 12.1 ppm zinc (based on tables of composition from Teklad Mills); of these, casein contributed 7.2 ppm, cornstarch 0.3 ppm, and brewer's yeast 4.6 ppm. The mineral mix contains (g/kg diet): \( \text{AlNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O} \) (0.0036); \( \text{CaCO}_3 \) (2.7440); \( \text{Ca}_2(\text{CH}_3\text{CO}_2)_2 \cdot 4\text{H}_2\text{O} \) (12.332); \( \text{Ca}_{(\text{H}_2\text{PO}_4)}_2 \cdot \text{H}_2\text{O} \) (4.5120); \( \text{CuSO}_4 \cdot 5\text{H}_2\text{O} \) (0.0032); ferric ammonium citrate (16.5 to 17.5% Fe) (0.6112); \( \text{MgCO}_3 \) (1.4080); \( \text{MgSO}_4 \) (1.5320); \( \text{MnSO}_4 \cdot \text{H}_2\text{O} \) (0.0080); \( \text{KI} \) (0.0016); \( \text{K}_2\text{HPO}_4 \) (8.7520); \( \text{NaCl} \) (3.0640); and \( \text{NaF} \) (0.0200).
TABLE 2

Composition of the vitamin A-deficient diet for experiment 3

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice flour</td>
<td>880 (g/kg)</td>
<td>Oil, cottonseed</td>
<td>60 (g/kg)</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>10 (g/kg)</td>
<td>Starch, corn</td>
<td>808 (g/kg)</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>50 (g/kg)</td>
<td>Vitamin mix</td>
<td>10 (g/kg)</td>
</tr>
<tr>
<td>Casein</td>
<td>80 (g/kg)</td>
<td>Minerals</td>
<td>50 (g/kg)</td>
</tr>
</tbody>
</table>

All ingredients were obtained from Teklad Mills, Madison, Wisconsin, except the Vitamin Mix. The rice protein diet containing vitamin A (3 mg retinol equivalents/kg diet) was prepared from the deficient diet by adding to each kg diet 0.020 g dry stabilized retinyl palmitate containing 150 mg retinol equivalents/g. Rice flour contained 81% carbohydrate, 8% protein and 0.8% lipid. Casein was 91% protein. The Vitamin mix was the vitamin diet fortification mixture without vitamin A, purchased from ICN Pharmaceuticals, Inc., 26201 Miles Road, Cleveland, Ohio 44128. It contained in g/100 kg: cholecalciferol (400,000 units/g), 0.55; a-tocopherol, 11.0; ascorbic acid, 99.1; inositol, 11.0; choline chloride, 185.2; menadione, 5.0; p-aminobenzoic acid, 11.0; niacin, 9.9; riboflavin, 2.2; pyridoxine hydrochloride, 2.2; thiamine hydrochloride, 2.2; calcium pantothenate, 6.6; biotin, 0.04; folic acid, 0.2; vitamin B12, 0.03. The Mineral mix contained the following (in percentages): CaCO3, 29.29; CaHPO4·2H2O, 0.43; KH2PO4, 34.31; NaCl, 25.06; MgSO4·7H2O, 9.98; Fe(C6H5O6)·6H2O, 0.123; ZnCl2, 0.020; Kl, 0.0005; (NH4)6MoO24·4H2O, 0.0025; Na2SeO3·5H2O, 0.0015. The Mineral mix was purchased from Teklad Mills (Cat. No. 170760).

remaining 38 rats were divided into two subgroups: group 1, consisting of 27 rats, was fed retinoic acid (RA) (12 mg/kg diet) for 17 days and was designated [-A + RA]. Group 2, consisting of 11 rats, continued to eat the unsubplemented vitamin A-deficient diet for the same length of time and was designated [-A - RA]. A third group, consisting initially of 40 rats, ate the vitamin A-deficient diet supplemented with RA (12 mg/kg diet) for the entire period of the study and was designated [-A + RA]. Housing conditions and the schedule for sampling and killing rats were approximately as described above for experiment 1.

Experiment 3. Weanling Sherman rats were divided into three groups of seven rats each: group 1 was fed a diet deficient in vitamin A, composed of 7% protein from rice flour, and designated [RP - A]. Group 2 was fed the same basic vitamin A-deficient diet with 7% casein replacing the rice protein [CP - A] and pair-fed to group 1. Group 2 was fed ad libitum the 7% rice protein diet supplemented with 3 mg retinol equivalents/kg diet as dry stabilized retinyl palmitate and designated [RP + A]. The composition of the diets is given in table 2. Housing conditions were as described above for experiment 1. At approximately weekly intervals, plasma was obtained from all rats for determination of vitamin A levels. Liver levels of vitamin A were determined on day 0 for a randomly selected group of three rats and on days 34 and 49 of the study for a subgroup of three to four rats from each dietary group.

In all experiments approximately 75 µl tail blood were drawn into each of four heparinized capillary tubes. The tubes were sealed, centrifuged for 6 minutes, measured for hematocrit, and 50 µl of plasma from pairs of tubes were combined, sealed under nitrogen, frozen, and stored in the dark for determination of vitamin A the next day. Preliminary studies showed no losses of vitamin A when such samples were stored for several days. For analysis of vitamin A levels, 50 µl plasma were transferred to a small tube for extraction. This and all subsequent procedures were conducted in dim light. To the samples were added 50 µl of fluorometric-grade ab-
PLASMA AND LIVER LEVELS OF VITAMIN A

solute or 95% ethanol and 0.5 ml of fluorometric-grade hexane. The samples were vortexed for 2 minutes, centrifuged for 10 minutes, and 0.3 to 0.4 ml of the hexane layer was transferred directly to microfluorometric cells. Determination of vitamin A was by the method of Thompson et al. (40, 41).

Each rat selected for analysis of liver vitamin A levels was killed by decapitation after the tail blood sample was obtained. Livers were removed without perfusion, chilled, blotted dry, weighed, and homogenized in a motor-driven Potter-Elvehjem glass homogenizer with sufficient water added to give a final volume in ml equal to five times the liver weight in g. Duplicate aliquots of homogenates were saponified and analyzed for vitamin A. A 0.5-ml volume of homogenate, 0.5 ml aqueous 60% potassium hydroxide, and about 10 mg pyrogallol were mixed in a 12-ml stoppered glass tube and put into a boiling water bath for 15 minutes with occasional vortexing. After the saponified mixture was chilled, 1 ml water, 1 ml ethanol, and 5 ml hexane were added and the mixture was vortexed for 2 minutes to extract vitamin A. The sample was centrifuged for 10 minutes, the hexane layer was removed completely and saved, 5.0 ml fresh hexane were added to the sample, and the extraction procedure was repeated. Equal volumes of the two hexane extracts were combined for the fluorometric measurement. Determination was as described for plasma vitamin A except standard 5-ml cells were used in the spectrofluorometer.

Statistically significant differences in means between groups were determined by the Student's t-test (42).

RESULTS

Growth rates. No difference was seen in growth rates of rats in either experiments 1 or 2; all rats gained weight continuously throughout the study and showed no signs of vitamin A deficiency until the 3rd to 4 days, when the weights of some rats fed the unsupplemented vitamin A-deficient diet began to plateau, an early sign of vitamin A deficiency. Initial weights of rats in experiments 1 and 2 averaged 50 g and final weights averaged 210 g, a gain of about 160 g during the 29 to 33 days, a daily weight gain of 4.8 to 5.5 g. This rate of gain is in accord with that considered normal for rats fed an adequate diet (39).

In contrast, rats fed the protein-restricted diets in experiment 3 gained weight very slowly. Initial body weights of all rats ranged from 48 to 52 g. Rats fed the 7% rice protein diet, whether or not supplemented with vitamin A, weighed 95 ± 5 g (mean ± SEM) after 49 days, an average daily weight gain of 1 g. Rats pair-fed to group 1 a diet of 7% casein protein weighed 130 ± 7 g after 49 days, an average daily weight gain of 1.6 g.

Plasma and liver levels of vitamin A. Plasma and liver levels of vitamin A for rats in experiment 1 are shown in figure 1. For rats fed the vitamin A-deficient diet,
Fig. 2 Effect of vitamin A-deficient diets with or without supplementation with retinoic acid (RA) on plasma and liver levels of vitamin A in rats. Weanling rats were fed a vitamin A-deficient diet for up to 38 days. One group ate the deficient diet for 21 days, then was divided into two subgroups at the point indicated by arrows: Group 1, fed RA (12 mg/kg diet) for 17 days [−A + RA], and group 2, fed the vitamin A-deficient diet without RA for the same period [−A − RA]. Group 3 ate the RA-supplemented (12 mg/kg diet) vitamin A-deficient diet for the entire period [−A + RA]. Group 1: △ = plasma, △ = liver vitamin A. Group 2: ○ = plasma, ● = liver vitamin A. Group 3: □ = plasma, ■ = liver vitamin A. Data are presented as means ± SEM. Number of rats in each group is indicated above the corresponding data point.

Mean plasma values increased from 20 to 30 μg/dl at the first blood sampling on day 7 to 40 to 50 μg/dl at day 14, and then remained relatively stable in the range considered normal for rats (30 to 50 μg/dl) for at least 7 additional days, even though liver reserves had declined to less than 10 μg/g wet weight. Plasma values declined to a mean of 33 μg/dl after 33 days of feeding, at which time liver values were 5 μg/g liver or less. Fluctuations in plasma values did occur in samples from individual rats from each group killed at a specified interval, particularly at the beginning and near the termination of the study, when liver stores were approaching depletion. For example, plasma values for day 7 of the deficient diet were 7.4 μg/dl for one rat, 19.8 μg/dl for a second, and 33 to 41 μg/dl for the remaining three. Corresponding liver values were 10, 13, and 14 to 20 μg/g wet weight. Less variability was noted in plasma levels of the 11 rats killed after 13 days of feeding the deficient diet, with values for all but one rat between 40 to 57 μg/dl and liver values 5 to 15 μg/g; the exceptional rat had the lowest plasma level (21 μg/dl) but the highest liver level (16 μg/g) of the group. At the termination of the study, the mean plasma value had fallen below 30 μg/dl, with individual determinations ranging from 21 to 35 μg/dl, whereas liver values varied only from 3 to 5 μg/g.

The effects of the vitamin A-deficient diet supplemented with RA for short or longer periods on plasma and liver levels of vitamin A are shown in figure 2. Both plasma and liver levels for the Sprague-Dawley strain of rats of experiment 2 were higher on the first sampling date (day 7) than those of the Sherman strain of rats sampled at a comparable time in experiment 1. The pattern of plasma response, however, was similar for rats fed diets unsupplemented with RA for the first 3 weeks of the study, with mean plasma levels rising and generally remaining above 30 μg/dl even though liver stores had declined to less than 10 μg/g. When RA was fed on day 21 to the [−A − RA] subgroup, with hepatic levels of vitamin A equivalent to those of the [−A − RA] subgroup, plasma levels declined by at least 10 to 15 μg/dl within 2 days of feeding RA. A drop in plasma levels also occurred in the group fed the RA-supplemented diet from the start of the study ([−A + RA]), also at a time when there were minimal differences between groups in liver stores of vitamin A (Fig. 2). Between days 24 and 38, plasma levels of the [−A − RA] and the [−A + RA] groups, with similar liver reserves of less than 5 μg/g, declined rapidly to mean levels of 18 μg/dl and 9 μg/dl, respectively, whereas mean plasma levels of the [−A +
PLASMA AND LIVER LEVELS OF VITAMIN A

Rats fed no source of vitamin A-active nutrient lost liver stores at an average rate of 4.3 µg/day from day 7 to 24 of the study; this depletion rate was twice that of rats whose diet was supplemented with RA, sampled over a comparable interval. A marked decline in the depletion rate to an average of 0.8 µg/day occurred when the liver concentrations of the [-A - RA] group had fallen below 5 µg/g; this was accompanied by a continuous decline in mean plasma values (fig. 2). During the same period the depletion rate of the [-A + RA] group with liver stores above 5 µg/g increased to 3 µg/day; this was not associated with any change in mean plasma levels.

The Sherman rats in experiment 3 after 7 days of feeding had plasma levels similar to rats of the same strain in experiment 1 and lower than those of the Sprague-Dawley rats of a similar age of experiment 2. Plasma levels (fig. 3) rose to the normal range of 30 to 50 µg/dl within 2 weeks (as in experiments 1 and 2) for rats fed the [RP + A] diet (group 2) and for rats fed the [CP - A] diet (group 3), but not for rats fed the [RP - A] diet (group 1). Rats of the [RP - A] group consistently had the lowest plasma values (19 to 27 µg/dl) while their pair-fed controls [CP - A] had the highest values (24 to 44 µg/dl). Plasma values of rats of the [RP + A] group were between 24 and 40 µg/dl.

| TABLE 3 | Depletion rates of endogenous liver vitamin A stores for weanling rats fed vitamin A-deficient diets with or without dietary retinoic acid supplementation |
|----|----|----|---|
| Dietary Group | Rates of hepatic depletion (µg/day) | Days 7 to 24 | Days 24 to 30 |
| -A - RA | 4.3 | 0.8 |
| -A + RA | 2.2 | 3.2 |

1 Based on 99 µg/liver on day 7.

Liver vitamin A levels initially and on days 34 and 49 of the study are also shown in figure 3. On day 34 mean liver reserves of the [RP - A] and [CP - A] groups were not significantly different (19 and 16 µg/g, respectively) while their plasma levels were significantly different (27.2 ± 1.0 and 44.1 ± 1.6 µg/dl, respectively). In contrast, mean liver levels of vitamin A for rats of the [RP + A] group were at least 7-fold higher (137 µg/g) than those of the [RP - A] and [CP - A] groups, but their plasma levels were not significantly different from those of [CP - A] rats. Two weeks later, on day 49, differences in liver concentrations between these groups were 15-fold, whereas plasma levels differed by less than 10 µg/dl. Rats fed the [RP + A] diet all had plasma levels above 30 µg/dl.
whereas plasma levels had fallen to 20 to 30 "g/dl for both the [RP - A] and the [CP - A] groups.

**DISCUSSION**

Weanling rats fed an otherwise adequate vitamin A-deficient diet established and maintained from endogenous reserves a relatively stable concentration of the vitamin in the blood (30 to 60 "g/dl) which was not correlated to the level of vitamin A in the liver. The plasma level did not drop below 30 "g/dl until liver reserves were at least under 10 "g/g tissue. Individual variations occurred in plasma values of rats fed identical diets, living under identical conditions and with similar liver reserves, particularly when liver stores were less than 5 "g/g. Some rats maintained plasma levels above 30 "g/dl when liver reserves were as low as 2 "g/g, while others with a similar level of hepatic reserve had plasma values below 30 "g/dl.

When RA, a substance with partial vitamin A activity (43-48) not normally present in significant amounts in foods but produced in vivo (49, 50) was added to the vitamin A-deficient diets, plasma levels were immediately reduced and sustained at 10 to 15 "g/dl below those of rats fed the vitamin A-deficient diet without RA supplementation, irrespective of the level of liver reserves.

When protein quantity and quality were restricted (7% rice protein), plasma levels of vitamin A were maintained in a range above 30 "g/dl only when vitamin A was added. The diet deficient in vitamin A and limited in protein quantity, but of high protein quality (7% casein) allowed plasma levels to be established and maintained in the normal range (30 to 50 "g/dl) even when liver reserves were depleted to under 10 "g/g.

The above data extend the limited literature reporting progressive changes in plasma and liver levels of vitamin A when diets are varied in the quantity and form of vitamin A-active nutrient provided and in protein quantity and quality. Our results confirm the generally held view that, under a given set of dietary conditions, an individual's plasma level of vitamin A is set by a homeostatic mechanism peculiar to that individual. The level established can be altered by conditions other than the status of vitamin A nutrition (51).

Although their plasma levels of vitamin A differed significantly, the growth rates of rats fed vitamin A-deficient diets with or without RA supplementation were not significantly different, indicating that tissue needs for growth were fully met by both diets. Apparently tissue needs for retinol were also fully met at the lowered plasma levels for the period of this study. The decreased vitamin A depletion rate of the RA-supplemented rats compared to those receiving no RA for days 7 through 24 of the study suggests that a decreased rate of mobilization rather than an increased rate of catabolism caused the lowered plasma levels. Our observation that RA has a sparing effect on liver reserves of vitamin A concurs with findings by other investigators in several species (45-47), including the rat (44, 48) but contrasts with the findings of others (43, 52).

We are not aware of other reports on the influence of RA-supplemented vitamin A-deficient diets on serially obtained blood levels of vitamin A. Muto et al. (24) noted lowered levels of retinol-binding protein (RBP) in RA-supplemented rats already depleted of vitamin A. When RA was fed at high levels to pregnant rabbits, plasma retinol levels were lower than in unsupplemented animals; this was not true, however, of RA-fed pregnant rats (53). Pigs and hamsters fed RA had lower terminal blood vitamin A levels than those fed retinyl palmitate (45, 46) but it is not clear whether the lowered levels occurred in response to hepatic depletion only or whether they might have been observed had the blood levels been examined prior to depletion.

We interpret our observations from experiments 1 and 2 to indicate that an inadequate dietary supply of vitamin A was compensated for by an internal regulatory mechanism which maintained plasma levels from endogenous reserves at a concentration presumably needed to supply tissues at an optimal level, as long as some vitamin remained in the liver. When the tissue needs were partially met by RA, the homeostatic set point was adjusted down-
ward, reducing the rate of hepatic mobilization (table 3) to that necessary to supply tissue needs for retinol still unmet by the RA.

Rats fed the 7% rice protein diet, whether or not supplemented with vitamin A, grew poorly. Rats fed the [CP — A] diet pair-fed to the [RP — A] diet grew at a faster rate after 2 weeks of feeding the diet, indicating a more efficient utilization of nutrients with the higher quality protein, even though quantity was still limited. This more efficient physiological functioning was reflected in the ability of the casein-fed rats to establish and maintain plasma levels of vitamin A above 40 μg/dl until hepatic stores had declined to less than 10 μg/g, at which time the plasma levels fell. At the termination of the study, mean plasma levels for the [RP — A] and the [CP — A] groups were between 20 and 30 μg/dl and declining; liver stores were less than 4 μg/g (fig. 3). It is particularly significant that the more rapidly growing rats (casein-fed) with only endogenous stores to supply their vitamin A needs nonetheless maintained higher plasma levels of the vitamin than less rapidly growing rats (rice protein-fed) with or without dietary vitamin A. The results are consistent with earlier suggestions that plasma levels reflect, at least in part, tissue utilization rates of the vitamin (20, 33, 36).

Although no difference was seen in the growth of rats fed the rice protein diets with or without vitamin A supplementation, differences did occur in plasma and liver levels of vitamin A. After the first week of the diet, the [RP + A] group established and maintained plasma levels between 30 and 40 μg/dl, somewhat lower than those of the food-restricted but more efficiently growing [CP — A] group but higher than those of the group growing at an identical rate and fed the same rice protein diet without vitamin A supplementation [RP — A]. Hence, in rats, limiting protein in quantity and quality to a point where growth is slowed but not stopped allowed the homeostatic control mechanism to maintain plasma levels of vitamin A in the normal range as long as adequate vitamin A was supplied with the protein-inadequate diet. However, a diet without vitamin A supplementation and with poor quality protein caused the homeostatic set point to be lowered and maintained at less than 30 μg/dl.

In studies on rats, Muhilal and Glover (31, 32) fed vitamin A-deficient diets containing 5% soy bean or rice protein for 5 to 8 weeks and found plasma holo-RBP (RBP with retinol attached) levels under 5 μg/ml in rats fed the soy bean diets even though endogenous liver reserves were 35 to 40 μg/g tissue. They reported zero holo-RBP levels for plasma and liver reserves of rats fed the rice-based diets (32). Hepatic apo-RBP (RBP unattached to retinol) was available in these rats since providing a dose of vitamin A caused an immediate rise in plasma holo-RBP. The peak plasma value reached following dosing was higher as protein quality improved. In the present study, plasma levels of vitamin A (we did not measure holo-RBP) remained between 22 and 27 μg/dl for rats fed the vitamin A-deficient 7% rice-protein diet and were considerably higher from the second to the seventh week of the study for rats pair-fed the vitamin A-deficient 7% casein protein diet (36 to 44 μg/dl). Liver reserves had declined over the 7-week period from an initial average of 53 to 9 μg/g and 3 μg/g for rats fed the rice and casein protein diets, respectively. Hence, under the conditions of our study, RBP synthesis appeared to be depressed slightly by the poorer quality rice protein diet but was sufficient to maintain adequate circulating levels of the vitamin generated from endogenous reserves. These findings concur with results reported by Friend et al. in pigs (30).

Muto et al. (24) and Smith et al. (25) have demonstrated that the availability of retinol, exogenous or endogenous, is a factor regulating holo-RBP secretion. Data which we have reported elsewhere (20), like that of others (24, 25, 31, 32), show a surge in plasma levels of retinol in response to an oral dose or an intrajugular injection of vitamin A to rats whose livers are replete or depleted of the vitamin. These observations suggest that the feedback control regulating the hepatic synthesis of RBP is at least partially independent of that which regulates secretion of holo-RBP. The regu-
SUMMATION OF VITAMIN A METABOLISM

A. PREHEPATIC

- **DIET**
  - Vitamin A
- **INTESTINAL LUMEN**
  - Micelles
- **MUCOSAL CELL**
  - Carotene
- **LYMPH-PLASMA**
  - Carotene

B. HEPATIC

- **UPTAKE**
  - Chymuscrine
- **STORAGE**
  - Acceptor Site
- **MOBILIZATION**
  - R
  - R-RE

C. POSTHEPATIC

- **PLASMA**
  - R-RBP
  - PA-RBP
  - Retinyl acid
- **TISSUES**
  - CRABP
  - CRBP
  - R
  - FA

- **KIDNEY**
  - RBP
  - R

- **URINE**
  - PA

Fig. 4 A schematic summation of the metabolism of vitamin A: prehepatic, hepatic and posthepatic. Points where feedback signals might be generated are numbered 6, 7, and 8. Reactions in the liver where control might be exerted depending on adequate vitamin A are numbered 1 and 3 for exogenous sources and 2 and 4 for endogenous sources. Control of secretion of holo-RBP (number 5) could be regulated partially independently of other control points and by the same or by different signals. R = retinol, RE = retinyl ester, FA = fatty acid, PA = prealbumin, RBP = retinol-binding protein, CRBP = cellular retinol-binding protein, CRABP = cellular retinoic acid-binding protein, RBP = partially denatured retinol binding protein.

The feedback mechanisms governing secretion appear to be generated extrahaepatically, perhaps in response to the rate of tissue uptake of retinol and/or its rate of tissue utilization.

Figure 4 presents a working model summarizing the metabolism of vitamin A and suggests points in the metabolic scheme at which feedback signals might be generated, exerting a regulatory effect over the homeostatic level of circulating retinol. The effectiveness of RA in depressing plasma levels of vitamin A suggests that the signals for secretion of holo-RBP, if generated as a metabolite during the intracellular metabolism of retinol, occur prior to oxidation to RA (fig. 4, numbers 7 and 8). On the other hand, the signal might be generated by the level of partially degraded circulating unbound RBP that has released retinol to intracellular binding sites (fig. 4, number 6). The site of action of the signal might be in controlling the rate of release of retinol from its esterified form [originating from the reserve storage pool (fig. 4, numbers 2 and 4) or from the diet (fig. 4, numbers 1 and 3)], or in the processing involved in the formation of holo-RBP and its release, presumably from the microtubules \(^{14}\) (fig. 4, number 5). These possibilities are consistent with observations that the homeostatic level of plasma retinol under a variety of dietary and physiological conditions is determined by its rate of utilization in the functioning organism.

LITERATURE CITED


