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Distribution of vitamin A in various organs of rats in relation to the quality and the quantity of dietary proteins*

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Summary: The influence of the quality and the quantity of dietary proteins on the distribution of a single massive dose of vitamin A in various organs of growing Wistar strain rats has been studied by using casein and bengal gram diets at 20 % and 10 % protein levels. The distribution of [^{3}H]-retinyl acetate in various tissues was also investigated in these dietary conditions. The results show that the hepatic storage of dietary as well as a single massive dose (20,000 I.U.) of vitamin A was profoundly decreased in the rats fed on bengal gram diets as compared to those fed on casein diets. Regardless of hepatic stores, the plasma vitamin A levels were comparable in all the groups. Feeding of low quality of protein reduced the tissue distribution of [^{3}H]-retinyl acetate in control as well as rats given a massive dose of vitamin A. This study suggests that both the poor quality and the inadequate quantity of dietary protein are detrimental influences on the vitamin A status of the growing rats.

Zusammenfassung: Es wurde der Einfluß von Qualität und Quantität von Nahrungsproteinen auf die Verteilung einer einzigen massiven Dosis von Vitamin A in verschiedenen Organen wachsender Wistar-Ratten untersucht. Die Untersuchungen wurden mit Casein- und Bengal-Gram-Diäten mit 20 % und 10 % Proteingehalt und auch mit radioaktiv markiertem Retinylacetat durchgeführt. Die Ergebnisse zeigen, daß die Leberspeicherung sowohl von Vitamin A aus der Nahrung als auch die einer einzigen starken Vitamin-A-Gabe (20 000 I.U.) stark herabgesetzt war bei Ratten, die nach der Bengal-Gram-Diät gefüttert wurden, verglichen mit Ratten, die auf Casein-Diät gesetzt waren. Im Gegensatz zur Leberspeicherung ist das Vitamin-A-Niveau im Plasma in allen Gruppen vergleichbar. Füttern niedriger Protein-Qualität reduzierte die Gewebeverteilung von [^{3}H]-Retinylacetat sowohl bei Kontrollratten als auch bei solchen, denen eine massive Dosis Vitamin A gegeben wurde. Diese Untersuchung läßt vermuten, daß sowohl schlechte Qualität als auch unzureichende Mengen von Nahrungsproteinen nachteilige Einflüsse auf den Vitamin-A-Zustand wachsender Ratten haben.

Key words: Dietary protein – Vitamin A – Rat

Introduction

Vitamin A deficiency is one of the major and wide spread public health nutritional problems. It mostly takes a heavy toll of infants and preschool children of the socio-economically depressed population in many develop-

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ing countries. This deficiency, when superimposed on protein energy malnutrition gets aggravated and thus leads to higher incidences of mortality and occular signs (1). Several sudies have shown the existence of a close interrelationship between the dietary proteins and the vitamin A absorption and its metabolism in a number of species (2–7). Inadequacy of the dietary protein has been reported to affect the hydrolysis of vitamin A ester in the intestine, its absorption, hepatic stores, and transport from the liver to the target tissues (3, 8–10).

A possible approach for the control and prevention of vitamin A deficiency would be the administration of a single massive dose of vitamin A in lieu of daily dietary intake. Single massive dose of vitamin A has been advocated as an effective prophylactic public health measure (11). Bengal gram (cicer arietinum), which belongs to the order leguminosae, is one of the most commonly consumed pulses in India. It is a significant source of dietary protein, but is relatively deficient in a number of essential amino acids. Effect of dietary proteins on the vitamin A status of rats has largely been evaluated in parameters of hepatic storage and plasma vitamin A levels (12, 13). It is not yet clear that how the quality and the quantity of dietary proteins affect the distribution of vitamin A in various tissues of rat. In the present study, therefore, we have attempted to evaluate the influence of the quality and the quantity of dietary proteins on the distribution of vitamin A in various organs of rats.

Materials and Methods

Experimental animals:

Male rats of the Wistar strain weighing 40–60 g with moderate hepatic vitamin A stores (15–20 μg/g liver) from the Institute maintained colony were used in the present study. Rats were housed in individual cages with raised wire mesh floor. Rats were randomly allocated into four groups on an equal average body weight basis. Animals were fasted overnight prior to feeding the diets shown below:
Group I: 20 % casein diet (referred to as C-20),
Group II: 20 % bengal gram diet (referred to as G-20),
Group III: 10 % casein diet (referred to as C-10),
Group VI: 10 % bengal gram diet (referred to as G-10).

The composition of the experimental diets was the same as described earlier (5). The protein levels in the experimental diets were varied at the expence of potato starch. Each diet was iso-caloric. Vitamins and salt mixture at 0.25 % and 5.0 % level and choline chloride at 0.15 % level were added to the diet. The rats were pair-fed these diets for a period of two weeks and water was available all the time. The body weights of the animals were recorded on alternate days. These groups of rats were used for the fllowing experiments:

I Effect of dietary proteins on the distribution of single massive dose of vitamin A in different tissues

On the 14th day of feeding, rats from each of the above groups were further subdivided into two groups. To one subgroup (referred to as experimental group) 20,000 I.U. of vitamin A in groundnut oil/100 g body weight was given orally in a single doses, while the other subgroup (referred to as control group) received vehicle alone. 24 hrs after administration of vitamin A, blood was collected from the juglar vein into heparinized tubes and all rats were killed by decapitation. Liver,

lungs heart, kidneys, spleen, adrenals, and small intestine were immediately removed, rinsed in ice-cold physiological saline, (0.9 % w/v), blotted and processed for the isolation of vitamin A. The vitamin A, from the plasma and all tissues was extracted with petroleum ether (40–60 °C) (14). Suitable aliquots were taken for the estimation of vitamin A according to the method of Neeld and Pearson (15) using retinyl acetate as standard.

II Distribution of [^{3}H]-retinyl acetate in various organs of rats fed different dietary proteins

24 hrs before sacrifice, half of the rats from all the dietary groups were orally given 20,000 I.U. of vitamin A/100 g body weight. On the 15th day, 3 hrs before sacrifice each rat in each group was intraperitonealy injected with [^{3}H]-retinyl acetate (10 μCi/100 g body weight) in physiological saline containing tween-80 (10 mg/ml). Prior to use the ^{3}H-retinyl acetate (specific activity 380 mCi/mg retinyl acetate) was purified by column chromatography on 10 % (v/w) water deactivated alumina column (14). At the time of sacrifice blood was collected from the juglar vein into heparinized tubes. Liver, lungs, heart, kidneys, spleen, adrenals, and small intestine were removed and processed for the vitamin A extraction. Suitable aliquots were taken into scintillation vials for the determination of [^{3}H]-radioactivity in Kontron MR-300 liquid scintillation counter using toluene based scintillation cocktail.

The results were analyzed statistically and the significance of differences was calculated using student's „t" test.

Results

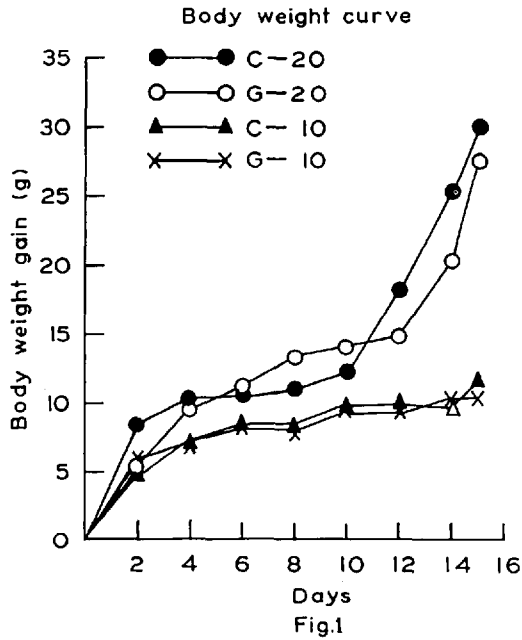
Body weights

The growth pattern shows that even at 20 % protein level, bengal gram is not as good as casein at 20 % protein level in supporting growth (Fig. 1). The gain in body weight was significantly lower in rats fed on either casein or on bengal gram diet at 10 % protein level as compared to those fed these diets at 20 % protein level (Fig. 1).

Distribution of a single massive dose of vitamin A:

The effect of feeding casein and bengal gram diets at different protein levels on the vitamin A contents of plasma and various organs is shown in table 1. In the liver, contents of vitamin A were profoundly reduced in rats fed on casein and bengal gram diets at 10 % protein level as compared to the respective diets at 20 % protein level and the reduction was greater in bengal gram fed rats as compared to casein fed rats (Table 1). This was evident for endogenous vitamin A levels both in control rats and in rats given 20,000 I.U. of vitamin A (Table 1). Administration of a single massive dose of vitamin A significantly raised the plasma vitamin A levels in bengal gram fed rats than in casein fed rats at both the dietary protein levels (Table 1). However, the plasma vitamin A levels in rats fed on either diet at the 10 % protein level were significantly lower than in rats fed on these diets at the 20 % protein level (Table 1).

Feeding of the bengal gram diets resulted in reduced vitamin A levels in the small intestine of control as well as of rats given 20,000 I.U. of vitamin A (Table 1). Regardless of the quality of the dietary protein, the vitamin A



Effect of the quality and the quantity of dietary proteins on the gain in body weight of rats

Male growing rats (40–60 g) were pair fed casein and bengal gram diets at 20 % and 10 % protein levels for a period of two weeks. Values are mean of gain in body weight of four rats in each group. C-20: 20 % casein diet; G-20: 20 % bengal gram diet; C-10: 10 % casein diet and G-10: 10 % bengal gram diet.

contents of adrenals were significantly lower at 10 % protein level as compared to 20 % protein level in the control and in the experimental rats. The vitamin A contents of the lungs of control rats fed on bengal gram diets at 20 % and 10 % protein levels were significantly higher than of the rats fed on casein diet at respective protein levels. In rats given 20,000 I.U. of vitamin A, the lung vitamin A levels were significantly higher in G-20 and C-10 groups as compared to C-20 and G-10 groups respectively (Table 1). No effect of either the quality or the quantity of dietary proteins was observed in the endogenous vitamin A contents of the heart in control rats. When the rats were given 20,000 I.U. of vitamin A, the vitamin A contents in the heart were significantly increased in rats fed on a low quantity of the dietary protein as compared to those fed on a high quantity of the dietary protein (Table 1). In case of the kidney, the vitamin A levels were comparable in control rats, but in rats that were given 20,000 I.U. of vitamin A, these levels were significantly higher in the group G-20 as compared to the C-20 or the G-10 groups (Table 1). The vitamin A contents of the spleen were not affected by either the quality or the quantity of dietary proteins in the control as well as in the experimental rats (Table 1).

Table 1. Vitamin A contents of different organs of rats fed casein and bengal gram protein diets.

Tissue	Vitamin A given (I.U.)	Dietary groups			
		C-20	G-20	C-10	G-10
			μg/organ		
Liver	None	93.9±5.4	54.5±4.3[a]	61.5±2.3[c]	36.1±3.8[b,d]
	20 000	227.7±10.4	188.5±8.5[a]	155.5±7.7[c]	91.6±6.9[b,d]
Plasma*	None	17.6±0.7	17.1±0.9	8.5±1.0[c]	9.4±0.8[d]
	20 000	85.6±2.3	122.5±7.0[a]	46.8±4.1[c]	72.4±6.2[b,d]
Small	None	27.4±0.8	16.5±0.7[a]	21.2±0.7[c]	16.9±1.4[b]
intestine**	20 000	140.1±9.6	107.9±8.5[a]	95.4±5.5[c]	97.3±6.4
Adrenals	None	7.8±0.7	6.8±0.5	3.1±0.3[c]	5.0±0.7
	20 000	20.8±1.6	20.2±1.2	11.9±2.4[c]	13.3±0.6[d]
Lungs	None	15.3±1.1	22.2±1.2[a]	8.3±0.2[c]	11.5±1.1[b,d]
	20 000	38.9±1.6	51.8±2.5[a]	49.9±2.6[c]	29.1±1.6[b,d]
Heart	None	4.8±0.8	3.4±0.4	5.5±0.5	4.6±0.3
	20 000	7.1±0.9	10.4±1.2	14.7±1.0[c]	18.9±1.5[d]
Kidneys	None	13.7±2.4	13.2±1.5	13.6±1.9	14.0±1.8
	20 000	20.5±1.8	28.3±1.2[a]	22.9±2.2	19.3±1.2[d]
Spleen	None	2.1±0.3	2.3±0.2	2.7±0.2	2.5±0.4
	20 000	7.8±1.3	9.4±0.8	11.0±1.1	7.9±1.0

Values are mean ± SEM from four rats in each group.
* Values are expressed as μg/dl.
** Values are expressed as μg/g tissue.
a, b, c and d show statistically significant values (where $P \leq 0.05$).
a = G-20 vs C-20; b = G-10 vs C-10; c = C-10 vs C-20; d = G-10 vs G-20.

Distribution of [^{3}H]-retinyl acetate:

In control rats, the vitamin A radioactivity (DPM/organ or dl plasma) in the liver and plasma was significantly higher in the bengal gram fed rats as compared to casein fed rats (Table 2). When 20,000 I.U. of vitamin A was given, the radioactivity in the liver was significantly lower in rats fed on bengal gram diets as compared to the rats fed on casein diets at both dietary protein levels. No effect of either the quality or the quantity of dietary proteins could be observed on the distribution of vitamin A radioactivity in plasma of rats given 20,000 I.U. of vitamin A (Table 2).

The distribution of vitamin A radioactivity in small intestine of control rats was lower in the group G-20 than in the group C-20 but higher in groups C-10 and G-10 as compared to groups C-20 and G-20 respectively (Table 2). In the small intestine of experimental rats, the radioactivity was significantly reduced by a low quantity of dietary protein but it was not influenced by the quality of dietary protein (Table 2). The quality and the quantity of dietary protein did not significantly affect the distribution of vitamin A radioactivity in adrenals and lungs of control rats. However, in rats that were given 20,000 I.U. of vitamin A the radioactivity was decreased in adrenals and increased in lungs by the low quantity of dietary protein (Table 2). In the control experiment, the distribution of the

Table 2. Distribution of [^{3}H]-retinyl acetate in various organs of rats fed casein and bengal gram protein diets.

Organ	Vitamin A given (I.U.)	Dietary groups			
		C-20	G-20	C-10	G-10
		DPM × 10^{-3}/organ			
Liver	None	46.4±3.1	60.1±3.9[a]	25.3±4.4[c]	31.6±3.5[d]
	20 000	879.2±80.5	483.4±45.5[a]	202.5±17.5[c]	102.7±15.9[b,d]
Plasma*	None	10.4±0.8	32.1±3.8[a]	6.5±0.5[c]	10.6±0.3[b,d]
	20 000	35.1±2.7	40.9±2.5	44.0±4.5	36.6±3.2
Small	None	5.4±0.3	2.8±0.2[a]	11.3±0.8[c]	9.5±0.5[d]
intestine**	20 000	23.0±3.2	28.8±3.0	10.1±1.1[c]	8.3±0.5[d]
Adrenals	None	0.16±0.01	0.15±0.01	0.15±0.02	0.12±0.01
	20 000	3.7±0.3	3.5±0.3	1.2±0.1[c]	1.1±0.1[d]
Lungs	None	1.9±0.2	2.1±0.2	2.1±0.2	1.9±0.4
	20 000	18.2±1.1	16.4±2.4	27.3±2.9[c]	24.2±2.8[d]
Kidneys	None	4.3±0.3	1.2±0.1[a]	2.8±0.1[c]	1.9±0.1[b,d]
	20 000	8.4±0.6	6.9±0.4	7.1±0.3	3.5±0.2[b,d]
Heart	None	0.95±0.1	0.90±0.04	0.84±0.08	0.96±0.06
	20 000	2.7±0.3	1.9±0.2[a]	1.6±0.2[c]	1.8±0.1
Spleen	None	4.56±0.42	8.15±0.46[a]	3.60±0.28	1.57±0.07[b,d]
	20 000	1.87±0.14	2.61±0.15[a]	1.45±0.07	0.85±0.04[b,d]

Values are expressed as mean ± SEM from four rats in each group.
*Values are expressed as DPM/dl.
** Values are expressed as DPM/g tissue.
a, b, c and d show the significant values (where $P \leq 0.05$) as mentioned in table I.

radioactivity in the kidneys of groups G-20 and the G-10 was lower than in that of groups C-20 and C-10 respectively. Upon administration of 20,000 I.U. of vitamin A, the distribution of the radioactivity in the kidneys was reduced only in the group G-10 as compared to groups G-20 and C-10 (Table 2). In the heart of control rats, the vitamin A radioactivity distribution was not influenced by the dietary proteins, whereas, in the experimental rats it was increased in the group C-20 as compared to groups G-20 and C-10 (Table 2). In the spleen of control as well as in rats that were given 20,000 I.U. of vitamin A, the radioactivity was higher in the group G-20 than in the group C-20 and lower in the group G-10 than the groups C-10 and G-20 (Table 2).

Discussion

The objective of the present study was to elucidate the effect of the quality and the quantity of dietary proteins on the absorption of dietary vitamin A from the gut, its storage in liver and distribution in the extrahepatic tissues. The results obtained indicate that both the quality and the quantity of dietary proteins have multiple effects on these steps of vitamin A metabolism in the rat. The observed higher growth rate in the rats fed on casein diets is probably due to its higher contents of essential aminoacids

and the biological availability of these essential nutrients. Other workers have also reported the reduced growht rates in the rats fed on the inferior protein diet (16). The decreased hepatic storage of vitamin A in rats fed on bengal gram diets regardless of dietary levels could be attributed to the inferior quality of the dietary proteins. The higher plasma vitamin A after oral dosing for rats fed bengal gram suggests that uptake might be slower in these rats. On the other hand the major effect of the dietary levels of protein, regardless of its source, would appear to be at the level of vitamin A absorption, since low quantity protein diets were associated with decreased vitamin A contents of the intestine, liver, and plasma after an oral vitamin A load (Table 1). Moreover, the decreased plasma levels of endogenous vitamin A in the low protein fed rats compared to highprotein fed rats is unlikely to be due to a decreased release of vitamin A from liver per se despite the reduced endogenous hepatic vitamin A stored in low protein fed rats. The reduced absorption of vitamin A and its decreased levels in the liver and blood have been observed by several workers in experimental animals maintained on inadequate dietary proteins (3, 10, 12, 13). The impaired absorption has been ascribed to the reduced intestinal vitamin A hydrolase activity in low protein fed rats leading to reduced hepatic levels of vitamin A (3, 17).

The reduced accumulation of vitamin A in the extra-hepatic tissues of rats fed on bengal gram diets as compared to those fed on casein diets reflects the negative effect of the poor quality protein on the uptake capacity of the target cell. The details of the mechanism(s) of the vitamin A transport from holoserum-RBP across the cell membrane of target tissue are not precisely understood, however, the participation of specific cell surface receptors has been suggested (18, 19). The role of cellular retinol binding protein in the intra-cellular translocation of vitamin A has also been reported (20). The results of the present study on vitamin A contents of various tissues suggest that both the quality and the quantity of dietary proteins may affect this process differently in different tissues and this may be related to effects either at the cell surface receptor level or at the cellular-RBP level or both.

Studies regarding the metabolism of vitamin A in the cell have revealed that vitamin A might be undergoing many group substitutions and/or addition reactions with the production of water soluble metabolites. There are several reports which demonstrate that the low quality and the inadequate quantity of dietary proteins reduce the mixed function oxidases involved in these types of biotransformations resulting in the accumulation of the parent compound (21, 22). The increased levels of vitamin A observed in certain tissues in the present study (viz. adrenals, lungs, and heart) may alternatively be ascribed to the reduced biotransformation of vitamin A in these tissues of rats fed on a poor quality protein.

It is clear from the present investigation that regardless of the precise mechanisms involved, the vitamin A status of the animal is profoundly influenced and in different ways, by either the quality or the quantity of dietary proteins consumed. This has important implications as regards the proposed prophylactic measure against vitamin A deficiency. Since not only such measures take account of the adequacy of protein content in the diet but account must also be taken of the quality and type of the dietary

protein, which can influence the dynamics of the vitamin A status of an individual even at seemingly adequate quantitative levels.

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