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Vitamin A: Concentration in the Rat Liver Golgi Apparatus

Abstract. *Vitamin A compounds (principally as retinyl esters) are concentrated in Golgi apparatus fractions from rat liver. The amounts vary with the vitamin A status of the liver and show an inverse relation to the concentration of ubiquinone. The results suggest a specific role of the Golgi apparatus in the mobilization or action, or both, of vitamin A compounds.*

Vitamin A compounds are essential dietary constituents for man and vertebrates. In many parts of the world, their deficiency is a major cause of juvenile disease and blindness (*1*, *2*). Problems of vitamin A deficiency are partially averted through mechanisms of storage and slow release of the vitamin. During periods of sufficiency, excess vitamin A is stored in the liver where levels reach several hundred times the minimum daily requirement (*1*, *2*). The stored vitamin is then secreted from the liver into the circulation from which it is withdrawn at the many sites where the vitamin plays a functional role. The liver also provides the main store of vitamin A alcohol (retinol), which is important to vision and other body processes. Thus an understanding of the mechanism of hepatic sequestration and secretion of vitamin A compounds is important to an understanding of vitamin A nutrition and molecular function.

High concentrations of ubiquinone in fractions of Golgi apparatus from rat liver (*3*) are accompanied by large amounts of a compound with an absorption maximum at 328 nm. Similar extracts from endoplasmic reticulum or mitochondrial fractions reveal none of this compound on a protein equivalent basis. In this report, we show that the compounds absorbing at 328 nm are derived principally from vitamin A esters concentrated in the Golgi apparatus. Previous studies of the distribution of vitamin A in rat liver (*1*, *4*) have not considered the Golgi apparatus. Yet the hepatocyte Golgi apparatus is a major component in the mechanism for secretion of lipoproteins into the blood (*5*). We suggest that this functional role may be more general and include fat-soluble vitamins.

Livers of male Holtzman rats were used for preparing subcellular fractions. Animals deficient in vitamin A were obtained by feeding weanling rats, without restriction, a test diet (General Biochemicals) deficient in vitamin A until their weight plateaued (5 to 6 weeks). Purified Golgi apparatus, endoplasmic reticulum, and mitochondrial fractions were obtained as described previously (*6*).

Two procedures were used to determine vitamin A compounds. Large quantities (from more than 50 mg of Golgi apparatus protein) were determined spectrophotometrically after an extraction procedure similar to that employed for determination of ubiquinone (*3*) except that the vitamin A was eluted in the fraction containing 1 to 2 percent diethyl ether. Smaller quantities (from less than 10 mg of Golgi apparatus protein) were determined after alkaline hydrolysis, either the Carr-Price reaction (*7*) or a fluorometric method being used (*8*). Protein was determined according to the method of Lowry *et al.* (*9*).

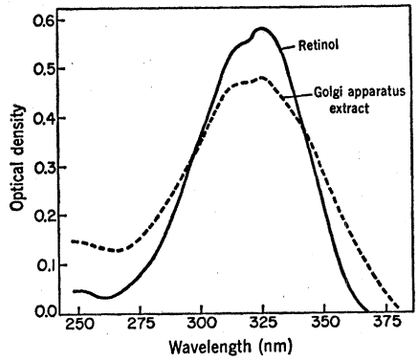

Fig. 1. Ultraviolet absorption spectrum of a Golgi apparatus extract [1 to 2 percent diethyl ether in petroleum ether fraction eluted from an alumina column (*2*)] compared with standard retinol, both in ethanol as the solvent.

When Golgi apparatus extracts were fractionated on alumina columns, the 1 to 2 percent diethyl ether eluate showed an ultraviolet absorption spectrum characteristic of vitamin A compounds (Fig. 1). With the Carr-Price reagent, the spectrum characteristic of vitamin A reaction products (absorption maximum at 620 nm) was obtained.

Retinol and retinyl palmitate standards were cochromatographed with partially purified vitamin A from Golgi apparatus extracts. Silica gel G thin-layer plates were developed in a mixture of cyclohexane and diethyl ether (80 : 20, by volume) or benzene. Spots were visualized with antimony trichloride reagent or observed in ultraviolet light (*10*). Most of the vitamin A of the Golgi apparatus extracts had an R_F similar to that of retinyl palmitate. Other thin-layer chromatography solvent systems used were those listed by Stahl (*10*) and the R_F's are provided in that reference. No attempt was made to identify specific retinyl esters. Neither the characteristic ultraviolet spectrum nor the Carr-Price reaction were obtained with extracts similarly prepared from equivalent amounts (on a protein basis) of purified mitochondria or endoplasmic reticulum fractions.

Table 1. Recovery of vitamin A in the preparation of a Golgi apparatus fraction from a fed, male rat (250 g) (*7*).

Fraction	Vitamin A content as retinol	
	Micrograms	Micrograms per milligram of protein
Total homogenate	1150	0.43
Unbroken cells, cell fragments, and nuclei	597	.62
Supernatant (2000*g*)	488	.44
Large particle fraction (sedimenting at 2000*g* with the Golgi apparatus and containing mitochondria, endoplasmic reticulum fragments, lysosomes, and plasma membrane)	96	.37
Purified Golgi apparatus	13	1.48

Table 2. Vitamin A content (as retinol) of whole livers and Golgi apparatus fractions of rats, under varying conditions of age, sex, and dietary condition, determined by means of the Carr-Price reaction (*6*).

Age (days)	Weight (g)	Sex	Dietary condition	Vitamin A content (μg)		Vitamin A concentration (μg/mg of protein)		Percent of total vitamin A in Golgi apparatus fraction
				Whole liver	Golgi apparatus fraction	Total homogenate	Golgi apparatus fraction	
60	272 ± 22	Male	Fed	1915 ± 455	17.4 ± 0.3	1.4 ± 0.3	3.5 ± 0.7	1.0 ± 0.4
	244 ± 8	Male	Starved	1650 ± 160	55.6 ± 7.0	1.7 ± .2	6.7 ± 1.4	3.4 ± .9
	208 ± 2	Female	Fed	2070 ± 400	18.5 ± 0.5	2.1 ± .5	6.1 ± 0.3	0.9 ± .02
100	419 ± 4	Male	Fed	3920 ± 340	17.2 ± .1	2.3 ± .2	2.5 ± 1.0	.4 ± .03
	328 ± 33	Female	Fed	7025 ± 125	25.7 ± 1.1	5.5 ± .3	8.0 ± 1.0	.4 ± .02

In recovery experiments, in which the fluorometric method was used (*8*), the bulk of the vitamin A was recovered in the supernatant and the fraction containing unbroken cells (Table 1). This agrees with previous studies (*4*). However, among the membranous fractions, the greatest concentration was in the Golgi apparatus. If one assumes a recovery of 30 to 40 percent for Golgi apparatus (*6*), as much as one-fourth to one-third of the total particulate vitamin A of the liver cell appeared within the Golgi apparatus fraction. A significant portion of the remainder was found in the heterogeneous membrane fraction containing mitochondria, lysosomes, endoplasmic reticulum, and plasma membrane which sedimented at 2000*g* with the Golgi apparatus. However, no vitamin A compounds were detectable spectrophotometrically in extracts of purified mitochondrial or endoplasmic reticulum fractions.

To relate the vitamin A content of the Golgi apparatus to the vitamin A status of the animal, Golgi apparatus fractions were prepared from a number of rats of both sexes and of various ages and dietary backgrounds (Table 2). As reported previously (*1*, *2*), rats accumulate more vitamin A in their livers with increasing age, females accumulating more than males. With the older females, the concentration of vitamin A in the Golgi apparatus reached about 8 μg per milligram of protein, and the fractions were noticeably yellow before extraction. With old males, the concentration of vitamin A in the Golgi apparatus was about 2.5 μg per milligram of protein, but the percentage of vitamin A in the Golgi apparatus fraction was approximately the same for both males and females. The latter was due to compensating differences in the total amounts of vitamin A in the livers. Similar relations were observed in comparing younger males and females, but the proportion of the total vitamin A recovered in the Golgi apparatus fraction was increased nearly threefold to about 1 percent of the total (Table 2). When male rats weighing 250 g were fasted for 48 hours, the vitamin A content of the Golgi apparatus exceeded that of females of comparable age. Under these conditions, approximately 3.5 percent of the vitamin A of the liver was recovered in the Golgi apparatus fraction.

In parallel experiments, vitamin A was not detected in Golgi apparatus fractions or total homogenates prepared from livers of rats deficient in the vitamin (indicated by a weight plateau), although the ubiquinone content of the Golgi apparatus fraction (9.6 nmole per milligram of protein) was increased nearly fourfold over that of control animals that had been fed similar diets supplemented with vitamin A. A similar reciprocal relation between ubiquinone and vitamin A concentrations has been observed in whole liver, the increase of ubiquinone in vitamin A deficiency being most pronounced in the crude microsomal fraction rather than in the mitochondrial fraction (*11*).

The results show that the concentrations of vitamin A in the Golgi apparatus reflect both the rate of supply to the circulation and the total vitamin A content of the liver. For example, starvation is known to increase the mobilization of reserve lipids and the secretion of very low density lipoproteins into the blood; a similar effect on vitamin A compounds might be expected (*12*). Thus a role of the Golgi apparatus in the mobilization and transport of vitamin A compounds is suggested. We do not discount the possibility that vitamin A participates in some regulatory function at the level of the Golgi apparatus. Retinol is a membrane-active substance that changes the properties of cytoplasmic membranes (*2*, *13*, *14*). Additionally, interest has focused on effects of vitamin A on sulfated mucopolysaccharides (*1*, *2*, *15*) and lysosomal membranes (*1*, *2*, *14*), both of which are derived from the Golgi apparatus (*16*). Yet a role of the Golgi apparatus in vitamin A mobilization seems clearest. Perhaps the vitamin A is carried to the blood stream via the same Golgi apparatus–secretory vesicle–plasma membrane export route postulated for secretion of plasma lipoproteins (*5*). A similar functional role for Golgi apparatus–derived vesicles as transporters of vitamin A–containing photopigments was suggested previously for photosensory cells in the eyes of a pulmonate snail, *Helix aspera* (*17*).

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Suppression of Food Intake with Intragastric Loading: Relation to Natural Feeding Cycle

Abstract. *Rats were infused through chronically implanted intragastric tubes with 100 percent of their normal total daily food intake. The infusion was given either continuously over 24 hours or divided into discrete meals programed to simulate the rats' natural eating pattern. The same diet was also available for consumption by mouth. In neither case did the animals completely stop eating. During slow infusions excessive consumption ranged from 30 to 50 percent. During simulated meal infusion of the same total quantity of diet, they compensated far better, overeating by only 2 to 18 percent. Periodic filling of the stomach between scheduled meals was no more effective than a continuous slow infusion. Therefore, factors related to the natural feeding cycle make a significant contribution to the effectiveness of food in maintaining satiety and controlling food intake.*

It is generally accepted that mammals control food intake under a variety of conditions to meet caloric requirements. Thus rats eat more in the cold, less in the heat (*1*), and compensate for fasting by overeating when refed and for dilution of the diet by increasing intake (*2, 3*). However, it has been suggested that need for calories may not be the sole factor controlling food intake, because when 100 percent of food normally supplied by mouth is supplied by stomach load, animals do not compensate by precisely reducing food intake (*4*). This finding has led workers to postulate that oral factors may play an important role in controlling food intake (see *4, 5*).

It is possible, however, that the observed failure to regulate during intragastric infusions may have resulted from the temporal distribution of the infusions. For example, Thomas and Mayer (*6*) used either a slow continuous infusion or an infusion which began when the rat began to eat a meal. In each case total caloric intake significantly exceeded the preinfusion intake.

We report here a study which shows that rats will more precisely compensate for intragastric loads if they are given in discrete meals at times which closely approximate their normal eating habits.

Four separate experiments, using a total of 23 rats, were carried out. All rats had previously been implanted with chronic nasopharyngeal tubes (*7*) permitting direct injection of a nutritionally adequate liquid diet into the stomach (*8*). The same diet was also available for the rats to eat. Infusion of the diet into the stomach was accomplished by using a Holter peristaltic pump which delivered diet at a preset rate and which was controlled automatically by behavioral programing equipment. Water was always available.

Our first experiment was designed to determine the degree to which rats

Table 1. Mean daily food intake, meal sizes, and number of meals before and during slow continuous infusion and infusion in discrete meals simulating each animal's normal meal pattern. The two rows for each animal are the two trials under each load condition. A period of at least 5 minutes without eating was used to separate meals.

Rat No.	Body weight (g)	Preinfusion			24-hour slow infusion			Discrete meal infusion		
		Mean oral intake (ml)	Mean number of meals	Mean meal size (ml)	Oral intake (ml)	Number of meals	Mean meal size (ml)	Oral intake (ml)	Number of meals	Mean meal size (ml)
62	278	30	10	3.0	9	6	1.5	3	3	1.0
					9	2	4.5	2	2	1.0
53	279	35	17	2.1	17	7	2.4	5	7	0.7
					12	6	2.0	4	6	0.66
45	289	33	12	2.75	12	4	3.0	4	4	1.0
					23	6	3.8	6	4	1.5
40	320	36	22	1.63	20	13	1.54	3	2	1.5
					21	4	5.25	4	2	2.0
39	324	33	12	2.75	12	5	2.4	3	4	0.75
					10	4	2.5	4	2	2.0
38	370	50	29	1.7	16	9	1.77	1	1	1.0
					29	14	2.07	5	2	2.5
30	280	27	10	2.7	11	3	3.6	4	3	1.3
					10	4	2.5	5	4	1.2