Growth Effect and Tissue Distribution of Vitamin A Following Intravenous Injection of Vitamin A-Rich Chyle.* (29785)

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Parenterally administered vitamin A, in oil-in-water emulsion, is generally less effective for promoting growth in deficient rats than orally administered vitamin A(1). Study concerning the utilization of intravenously administered vit A is complicated by the problem of producing an oil-in-water emulsion of the vitamin that is stable and nontoxic(2).

In this report: 1) the growth response of assay rats subsequent to oral and intravenous administration of rat chyle rich in vit A is compared; 2) the tissues of assay rats are examined for their vit A content by fluorescence microscopy subsequent to intravenous injection of vit A-rich rat chyle or vit A-containing artificial emulsions; 3) distribution of the vit A of rat chyle between the chylomicron and the soluble fraction is estimated.

Materials and methods. Vitamin A-deficient rats (Sprague-Dawley weanlings) were prepared according to the procedure outlined in the U.S.P. XII. They were used at the beginning of their weight plateau at which time one-dose bioassays, similar to the method of Sherman and Todhunter(3) were carried out, and other procedures were performed. At this time, randomly selected rats were sacrificed as controls; their livers contained no histologically(4) or chemically(5) detectable vit A.

The intravenous injections were made slowly (0.5 ml per 60-120 seconds) into the femoral vein, which was exposed by a small incision while the rats were lightly anesthetized with ether. The oral doses were administered by stomach tube into unanesthetized rats. The rats used for the histological studies were sacrificed by decapitation 30 minutes after injection.

Three vit A-containing materials were injected or fed: 1) fatty chyle containing from 35 to 250 μg of vit A per ml that was collected from the main abdominal lymph duct of donor rats(6) subsequent to oral dosage with 20,000 μg of vit A acetate in corn oil; 2) a 10% oil-in-water emulsion, stabilized with 6% Knox gelatin, into which 550 μg of vit A acetate per ml was incorporated (A-Gel); 3) and Lipomul emulsion into which 550 μg of vit A palmitate per ml was incorporated (Lipomul-A).‡

The sizes of the lipid particles of the chyle and of the emulsions were measured directly by means of a calibrated ocular micrometer and a phase-contrast microscope. The mean diameters of the lipid particles of chyle ranged from 0.5 to 1.0 μ with no larger particles; the mean diameters of the particles of A-Gel ranged from 1.5 to 2.0 μ but there were particles as large as 3.0 μ; the mean diameters of the particles of Lipomul-A ranged from 0.5 to 1.0 μ with rare particles as large as 2.0 μ.

Several lots of rat chyle that contained vit A were fractionated into chylomicron and soluble fractions either by centrifugation at 10,000 *g* for 30 minutes in the cold(7), or by addition of 0.20 mg of protamine sulfate per ml of chyle followed by centrifugation at 500 *g* for 15 minutes in the cold(8).

The vit A content of the artificial emulsions and of the whole chyle and the respective subfractions was determined by Kimble's modification of the Carr-Price reaction(5). Ether extracts of the livers of the control rats which had been depleted of vit A were analyzed for vit A content in a similar manner after saponification of the livers in ethanolic

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‡ Lipomul-A was made available through the courtesy of Dr. Paul E. Shurr, Upjohn Co., Kalamazoo, Mich. Lipomul is a cottonseed oil emulsion suitable for intravenous injection.

TABLE I. Growth Response of Vitamin A Deficient Rats Dosed with Vitamin A-Rich Rat Chyle.

Dose vit A* (μg)	Route	No. of rats	Avg wt gain (g)	Avg days to peak wt
50	Orally	4	23.5†	19
50	I.V.	4	46.5†	21
25	Orally	5	12.8‡	15
25	I.V.	4	29.0‡	20

* One lot of chyle used in all doses; vit A content 57.5 μg/ml.
† $t = 2.45$, $Df = 6$, $P = 0.05$.
‡ $t = 2.8$, $Df = 7$, $P = 0.01$-0.05.

KOH and subsequent extraction with ethanol and petroleum ether.

Fluorescence microscopic examinations were made of sections of the livers, adrenals, spleens, and lungs of the injected rats; the presence or absence of the green fading autofluorescence, characteristic of vit A, was determined(4). The intracellular localization of the vit A was noted and quantitative estimates of the intensity and extent of its distribution were made.

Results. Intravenous injection of the vit A-rich rat chyle into vit A-deficient assay rats resulted in a weight gain twice that produced by the same dose given orally (Table I). The differences in weight gain between the groups were significant at the 1 to 5% level using Student's t test(9).

The rat chyle was easily separable into a chylomicron fraction, containing most of the visible turbidity, and a clear fraction. Roughly 50 to 90% of the vit A content of rat chyle was in the form of chylomicrons (Table II). The concentration of vit A contained in the chylomicron fraction was from 6 to 181 times that of the clear lipoprotein fraction.

Subsequent to the intravenous injection of comparable amounts of vit A as fatty chyle, A-Gel, or Lipomul-A, an apparently normal tissue distribution of the vit A autofluorescence(4) resulted only after chyle injection.

The distribution of vit A autofluorescence subsequent to the injection of A-Gel or Lipomul-A was significantly different from the normal; there was marked phagocytosis of the fluorescent material by the reticulo-endothelial cells of the lung, liver, and spleen. The frequency of occurrence of vit A autofluorescence in the spleens and lungs of the 3 groups of injected rats is summarized (Table III). The confidence levels of the differences in occurrence in each group were estimated using a Chi square analysis(9). The p values are presented in Table III. Note the infrequent

TABLE II. Separation of Vitamin A-Rich Rat Chyle into Chylomicron and Lipoprotein Fractions.

Sample No.	Whole chyle vit A (μg/ml)	Chylomicron fraction % of total vit A	Chylomicron fraction vit A (μg/ml)	Lipoprotein fraction vit A (μg/ml)	Ratio concentration chylomicron/ lipoprotein vit A
5*	4	53	8	1.4	6
17*	7	26‡	36	1.7	21
11*	15	61	114	4.6	25
21*	17	72	99	4.5	22
10†	21	78	562	3.1	181
7*	165	90	2040	29.5	69

* Gofman separation method(7).
† Brown " " (8).
‡ Technically poor separation.

TABLE III. Frequency of Occurrence of Vitamin A Fluorescence in Spleen and Lung Subsequent to Intravenous Injection of Chyle or Artificial Emulsions.

Material injected	No. of rats	Spleen vit A present	% present	No. of rats	Lung vit A present	% present
Chyle	14	2* ($p = .01$)	14	14	4* ($p = .01$)	28
A-Gel	10	10	100	10	8	80
Lipomul-A	9	9	100	9	1* ($p < .01$)	11

* Trace amount.

occurrence of vit A in the spleen following chyle injection, and the infrequent occurrence of vit A in the lung subsequent to both chyle and Lipomul-A injection.

The vit A fluorescence in the spleen following the A-Gel and Lipomul-A injections was localized routinely in reticulo-endothelial cells in the marginal zone between the red and white pulp. Subsequent to the largest doses, the reticulo-endothelial cells throughout the entire red pulp were filled but the heaviest concentration was still present in the marginal zone.

The vit A fluorescence in the lung subsequent to Lipomul-A injection and especially following A-Gel injection was localized in reticulo-endothelial cells in the septa and as small emboli in the capillaries. The vit A present after chyle injection occurred in only trace amounts.

The vit A fluorescence in the liver subsequent to A-Gel and Lipomul-A injections was seen primarily in fine droplets in the Kupffer cells and less notably in the parenchymal cells adjacent to them. In contrast, the vit A fluorescence subsequent to chyle injections was present as a diffuse cytoplasmic inclusion primarily in parenchymal cells, as in the normally fed rat, and was seen occasionally in the Kupffer cells.

The vit A fluorescence in the adrenal gland subsequent to the injection of chyle, A-Gel, and Lipomul-A was in the form of a diffuse cytoplasmic inclusion within the cells of the zona fasciculata and the zona reticularis but was never seen in the cells of the zona glomerulosa. Subsequent to the smallest doses of vit A (30 to 80 μg), a narrow fluorescent band was observed in the mid-fasciculata; with higher doses (440 to 550 μg) the entire zona fasciculata and zona reticularis were filled with vit A.

Discussion. Intravenously administered vit A contained in rat chyle had roughly twice the growth-promoting ability compared with the same dose given orally. This finding was not expected, according to the review of Moore(1), but was suggested by the earlier results of Lease *et al*(10). Our markedly improved utilization of vit A following intravenous injection was due probably to its previous incorporation into chyle which is its usual physiologic state. Possibly the usual decreased utilization of parenterally administered vit A in the form of emulsions was due to the increased reticulo-endothelial cell uptake, as we observed, as compared with chyle. Presumably vit A-containing particles, trapped by the reticulo-endothelial cells as "foreign bodies," may be acted on by various esterases and lipases known to be present in the Kupffer cell(11) which destroy their growth promoting activity before release into circulation occurs. Also micro-embolization in the lungs, as observed, may act as a non-specific stress tending to decrease growth response. However, it is of interest to note that we observed little pulmonary embolization of vit A following intravenous injection of Lipomul-A; whether or not the vit A contained in this emulsion could produce a growth response more nearly equal to that produced by intravenous injection of the same amount of vit A in chyle remains to be determined.

As reported here, the bulk of vit A contained in rat chyle was transported in the chylomicrons rather than the soluble phase in proportions similar to that reported for the cholesterol content of chyle(12); however, post-absorptive rat plasma contains the bulk of vit A not in the chylomicrons but in a fraction precipitated at 50% saturation with ammonium sulfate(13). Also post-absorptive human plasma contains the bulk of vit A not in the chylomicrons but in the Sf 3 to 9 and the Sf 10 to 100 lipoprotein fractions(14). Perhaps vit A is transferred from the chylomicrons of the chyle to the high and low density lipoproteins of the plasma in a manner analogous to the known transfer of chylomicron lipid to plasma lipoprotein by the heparin clearing reaction(15).

The markedly diminished phagocytic uptake of vit A after intravenous injection of chyle and the markedly increased phagocytic uptake of vit A after intravenous injection of artificial lipid emulsions were comparable to the data of Murray and Freeman(16), who used a somewhat different approach involving lipid localization by Sudan staining techniques. The difference which we found where-

in vit A was localized in parenchymal cells after the chyle injections as compared with the reticulo-endothelial cell localization following injection of the artificial emulsions, may have been a function of the smaller particle size of chyle, or perhaps it was due to the relative ease with which the chyle chylomicrons enter into the heparin clearing reaction(17).

Summary. Intravenous injection of vitamin A in the form of rat chyle into vit A-deficient assay rats produced a growth response twice that produced by the same dose given orally. The characteristic vit A autofluorescence was absent from the parenchymal cells of liver and adrenal cortex of assay rats depleted of vit A; the autofluorescence was restored to the distribution in these tissues characteristic of the normally fed rat only after intravenous injection of rat chyle which contained vit A. In contrast, intravenous injection of artificial emulsions which contained vit A resulted in a marked localization of vit A fluorescence in the reticulo-endothelial cells of spleen, lungs and liver and to a smaller extent in the liver and adrenal parenchymal cells. Most of the vit A content of rat plasma has been reported to be in the soluble fraction; however, we found most of the vit A content of post-absorptive rat chyle in the chylomicron fraction.

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Effect of Exclusion of Pancreatic Juice on Digestion and Mucosal Absorption of Fat in Dogs.* (29786)

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The well-established fact that pancreatic insufficiency results in defective lipid absorption has been based largely on the recovery and analysis of fecal lipids of animals lacking the external secretion of the pancreas(1-3). However, since the small intestine is probably the primary region in which such defects are manifested, it was felt that it would be fruitful to investigate in detail the intraluminar and mucosal lipid changes which occur in

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