Rat Liver Storage of Vitamin A from Fortified Nonfat Dried Milk

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Vitamin A palmitate in oil and vitamin A desoxycholate at 1.2 and 40 I. U. levels per day were fed to weanling rats for 51 days in low fat dried milk diets. Under the experimental conditions, both vitamin preparations were equally effective in increasing liver stores of vitamin A. Addition of 5% fat to the low fat diet did not significantly increase vitamin A liver stores. Vitamin A absorption from the low fat dried milk diet was superior to absorption from a stock diet containing 5% fat.

SEVERAL recent reports (*1*, *8*, *17*) have indicated that nonfat dried milk (NFDM) should be fortified with vitamins A and D to aid in the nutrition of both infants and adults, particularly those of low income families. While the literature reveals that vitamin D utilization from milk is not related to dietary fat, the evidence is less conclusive with regard to utilization of vitamin A from milk products of very low fat content.

Studies with hens (*16*) and rats (*7*) indicate that both absorb greater amounts of carotene in the presence of 4 and 5% dietary fat than from diets very low in fat. However, this depression of absorption is not evident when vitamin A replaced carotene in the ration (*2*). The pathway of transport of both carotene (*13*) and vitamin A (*6*, *11*) can be influenced by the dispensing agent. Carotene or vitamin A in oil is transported via the thoracic duct, whereas emulsified (water-dispersible) carotene or vitamin A is transported via the portal vein.

Bile (*4*) and bile acids (*12*) also play an important role in carotene and vitamin A absorption. The absorption of vitamin D from certain diets is facilitated by the presence of fat, but excess levels of fat give less favorable results (*3*,*10*). That the dietary effectiveness of vitamin D in milk has no relationship to fat has been well elucidated (*2*, *3*, *9*).

The present study was undertaken to elaborate on the role of fat in utilization of vitamin A from NFDM and to determine the relative availability of vitamin A desoxycholate in supplying vitamin A needs. Vitamin A desoxycholate is a complex of vitamin A acetate with desoxycholic acid to produce a product that is very stable in NFDM, and imparts no undesirable flavor to this product (U. S. Patent No. 2,758,924). At fortification levels for NFDM, vitamin A desoxycholate is water-soluble and, hence, should be transported via the portal vein.

Experimental

Fifty male weanling rats of the Sprague-Dawley strain were purchased from a commercial supplier. These rats were reared to have low liver storage of vitamin A. The rats were housed in individual, raised-wire floor cages in a battery. The rats were divided into 10 equal lots of five each. Lot weights varied between 256 and 261 grams. All rats were individually weighed once weekly, and fed the vitamin A supplements daily in addition to the day's offering of feed.

One lot of rats was sacrificed at the start of the experiment, and the vitamin A content of the pooled excised livers was determined to establish the storage level of liver vitamin A at the start of the experimental period. A second lot was fed the higher level of vitamin A supplementation and carried for pilot liver vitamin A determinations as the study progressed. The other eight lots of rats were fed for 7 weeks on their various regimens, and were then sacrificed and the livers excised, weighed, and frozen for individual vitamin A determinations.

Two rations were employed in this study—the U. S. Pharmacopeia XIV vitamin A test diet, and NFDM fortified with vitamin D3 desoxycholate (3 I.U. per gram), dry *dl*-alpha tocopheryl acetate (0.2 I.U. per gram), and 0.035% of a trace mineral mixture containing 14.2% manganese, 10.4% iron, 1.5% copper, 0.26% cobalt, 0.54% iodine, and 30% zinc. Dried skim milk contains an excess of the rats' growth requirements (*14*) for B-vitamins. The NFDM was an equal mixture of three instantized milks purchased on the open market and was assayed to contain 1.2% fat (Rose-Gottleib) and less than 5 I.U. vitamin A per 100 grams.

The vitamin A oil was prepared by dissolving crystalline vitamin A palmitate in cottonseed oil so that 0.1 ml. contained either 1.2 or 40 I.U. of vitamin A. Vitamin A content of the oils was checked weekly by assay (*15*). The vitamin A desoxycholate was mixed with NFDM so that 1.0 gram contained 1.2 or 40 I.U. of vitamin A—checked by assay (*15*).

The livers from killed animals were excised, weighed, placed in small plastic bags, and immediately frozen at −20° C. The frozen livers were minced for determination of vitamin A content. Vitamin A determination was made by the Carr-Price colorimetric method (*15*).

Results and Discussion

The experimental design and results secured are offered in Table I. The rates of gain were greater on the USP XIV test ration, which contains 5% fat, than on any of the NFDM supplemental diets. The USP XIV ration is calculated to contain 359 calories of metabolizable energy (ME) (*18*) per 100 grams, while NFDM offers only 272 calories. ME per 100 grams, and NFDM with 5% added cottonseed oil presents an increased value to 301 calories ME per 100 grams. Thus, caloric density alone could account for much of the difference in rate of gain. This is substantiated by the increased rate of gain in lots 7 and 8, wherein caloric density of the NFDM diet was increased by adding 5% of cottonseed oil. The powdery nature of the NFDM diets and their tendency to become sticky when wet decreases feed intake.

The differences of growth rate are also reflected in liver A depletion for rats fed 1.2 I.U. of vitamin A per day (lot 1 *vs.* lots 3 and 5). The percentage storage on the USP XIV ration of vitamin A dissolved in oil at a 40-I.U. daily dose (13.2%) is in good agreement with the findings of Bird *et al.* (*5*), who secured 11.1% storage at an intake of 63 I.U. daily. Liver storage of vitamin A improved with the NFDM ration.

The percentage liver storage of vitamin A dissolved in cottonseed oil with rats fed 40 I.U. vitamin A per day was greater on the NFDM ration (lot 4, 23.2%) than on the USP XIV ration (lot 2, 13.2%), and was about equal to the storage secured with vitamin A desoxycholate (lot 6, 25%). Thus, fat had no apparent

Table I. Results Secured with Male Weanling Rats in a 51-Day Liver Vitamin A Storage Study with Two Diets and Two Dispersion Materials

Lot No.	Test Ration	I.U. Vit. A Daily	Carrier of Vit. A	Av. Daily Gain, Grams	Av. Wt. Livers, Grams	Av. I.U. Vit. A. per Gram Liver	I.U. Vit. A per Liver	I.U. Vit. A Increase per Liver	I.U. Vit. A Stored per Day	% of Daily Dose Stored
				(Five rats started)						
Control	None	...	...	...	2.3	115.2	265	...	...	...
1	USP XIV	1.2	CS Oil[a]	3.7	9.6	0	0	(−265)	...	...
2	USP XIV	40	CS Oil	3.8	10.2	52.7	538	273	5.4	13.2
3[b]	NFDM	1.2	CS Oil	1.2	3.9	17.3	68	(−197)	...	...
4	NFDM	40	CS Oil	1.5	4.7	157.4	740	475	9.3	23.2
5[c]	NFDM	1.2	DCA[d]	1.2	3.5	30.1	105	(−160)	...	...
6[b]	NFDM	40	DCA	1.6	5.0	154.7	774	509	10.0	25.0
7	NFDM[e] + 5% Fat	40	Oil	2.3	5.7	135.1	770	505	9.9	24.8
8	NFDM[e] + 5% Fat	40	DCA	2.3	4.5	176.1	792	527	10.3	25.8

[a] Cottonseed oil. [b] 1 Rat died. [c] 2 Rats died. [d] Desoxycholic acid. [e] 5% Cottonseed oil added.

influence on the utilization of vitamin A from NFDM. Further evidence that fat does not influence utilization of vitamin A from NFDM is apparent from lots 7 and 8, wherein inclusion of 5% cottonseed oil did not increase liver vitamin A storage from vitamin A in oil or vitamin A desoxycholate over that secured without additional fat (lot 6). Therefore, vitamin A utilization from NFDM is unrelated to added fat content and appears, like vitamin D (*4–6*), to be absorbed in association with milk proteins. If fat is needed for effective utilization of vitamin A in NFDM by the rat, the 1.2% fat in NFDM is adequate. Absorption of vitamin A from the NFDM ration is superior to absorption from the stock diet containing 5% fat.

Literature Cited

(1) Anderson, H. S., Lease, E. J., Lease, J. G., Malphrus, R. K., *J. Am. Dietet. Assoc.* **43,** 34 (1963).

(2) Ansbacher, S., Bender, R. C., Flanigan, G. E., Supplee, G. C., *J. Biol. Chem.* **114,** 95 (1936).

(3) Bender, R. C., Flanigan, G. E., Supplee, G. C., *J. Dairy Sci.* **17,** 483 (1934).

(4) Bernhard, K., Ritzel, G., Steiner, K. U., *Helv. Chim. Acta* **37,** 306 (1954).

(5) Bird, O. D., Brown, R. A., Emmett, A. D., Lemley, J. M., *J. Nutr.* **33,** 53 (1947).

(6) Birmingham, J., Bodansky, O., Cohlan, S. Q., Lewis, J. M., *J. Pediat.* **31,** 496 (1947).

(7) Burns, M. J., Hauge, S. M., Quackenbush, F. W., *Arch. Biochem.* **30,** 341 (1951).

(8) Dodds, M. L., Pontzer, M. E., *J. Am. Dietet. Assoc.* **42,** 128 (1963).

(9) Flanigan, G. E., Hess, A. F., Kahlenberg, O. J., Supplee, G. C., *J. Biol. Chem.* **91,** 773 (1931).

(10) Floody, Robert J., Knudson, A., *Nutrition* **20,** 317 (1940).

(11) Gottfried, S. P., Kramer, B., Sobel, A. E., *Am. J. Diseases Children* **73,** 543 (1947).

(12) Greaves, N. D., Schmidt, C. L. A., *J. Biol. Chem.* **105,** XXXi (1934).

(13) Henrotein, E., Kowalewski, K., Van Feertruyden, J., *Acta Gastro-enterol. Belg.* **14,** 7 (1951).

(14) *Natl. Acad. Sci., Natl. Res. Council, Publ.* **990** (1962).

(15) "Official Methods of Analysis," Assoc. Offic. Agr. Chemists, 9th ed., p. 652, Washington, D. C.

(16) Polskin, L. J., Russell, W. C., Taylor, M. W., Walker, H. A., *J. Biol. Chem.* **140,** CIX (1941).

(17) Scrimshaw, N. S., *J. Am Dietet. Assoc.* **42,** 203 (1963).

(18) Titus, H. W., "The Scientific Feeding of Chickens," 2nd ed., p. 258, Table 18, Interstate Printers and Publishers, Danville, Ill., 1955.

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CATTLE AS FALLOUT MONITORS

Iodine-131 in Bovine Thyroid Glands from 1957 through 1961

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IODINE-131 accumulation by cattle thyroids has been studied under a variety of circumstances. Van Middlesworth (*16, 17, 19, 20*), Wolff (*22*), and Blincoe and Bohman (*2*) reported on I^{131} concentrations subsequent to nuclear weapons tests. Iodine-131 accumulation by the thyroid glands of domestic ruminants was also reported after a nuclear reactor accident by Van Middlesworth (*18*) and Robertson and Falconer (*13*). Because of the short half-life of I^{131} (8.08 days), its presence indicates recent releases of fission products and is uncomplicated by older fallout.

This paper reports on thyroid I^{131} concentrations of cattle maintained in desert environments under a variety of fallout conditions.

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Methods

Two general categories of cattle were used for these studies: cattle maintained on desert range areas in Nevada, and cattle slaughtered by a packing plant in Reno, Nev.

Figure 1 indicates the locations of the three test herds of range cattle used in this study. All animals were grade or purebred Herefords. Cattle at the two southern locations (DV and NTS) sub-