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Vitamin A-Choline Interrelationship.* (23295)

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Abels, Gorham, Pack, and Rhoads(1) found that levels of vit. A in plasma of patients with malignant disease, particularly of the gastrointestinal tract, were below the normal range in 86% of cases examined. No response was obtained after vit. A administration, but feeding of yeast or pancreatic extracts, themselves free of carotenoids, raised the reduced plasma levels of vit. A. That this property in yeast or pancreas may be due to choline content has become apparent from later experiments. There was an average rise of 73% in plasma levels of vit. A in patients who were fed 1.5 g of choline chloride each day for 3 days. Popper and Chinn(2) showed that rats on a choline-poor diet containing liberal supplements of carotene developed fatty livers poor in vit. A. Therefore, it seemed worth while to determine choline levels of rat livers in normal, vit. A-supplemented, and vit. A-deficient animals.

Methods. In general, the procedure was to determine the amount of vit. A and choline in separate aliquots of rat livers from different dietary groups. Male, albino, Wistar-strain rats were used. The rats were divided into 3 dietary groups, normal (N) supplemented (S), and deficient (D). All animals were placed on a Red Range Dog Food† ration for at least 2 weeks. Animals of the supplemented group received an additional 5,000 International Units of vit. A‡ orally each day for one week before sacrifice. The deficient group was maintained on a vit. A test diet§ up to 7 weeks at which time vit. A analyses indicated that only trace amounts of vit. A remained in the liver. Each group was fasted for 24 hours before sacrifice by decapitation, accompanied by gravity exsanguination for approximately one minute. Usually 3 rats from the same dietary group were sacrificed at one time and their livers pooled. The livers were removed and placed in a freezer, at 10 to 15°C. The livers were then placed in a pre-cooled flask and weighed. The vit. A levels were run on freshly-chilled portions of liver, while total and phospholipid choline determinations were run on frozen livers. The livers, for vit. A analysis, were minced and one-half to one g portions were placed in a Potter-Elvehjem homogenizing tube containing 5 to 7 ml of ice-cold (0-5°C) distilled water. The homogenate was transferred to a 100 ml volumetric flask, and more liver was homogenized until 3 to 6 g of liver had been homogenized. The total homogenate was diluted to 100 ml with more cold distilled water. Triplicate aliquots of the homogenate were used immediately for determination of vit. A,

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† Red Range Dog Food sold by Southern States Feed Cooperative. Average Analysis (%), Protein 24.60, Fat 5.04, CHO 58.12; Vit. content/100 g, Vit. A activity-1100 I.U., Carotene 0.14 mg, Choline chloride 7.13 mg.

‡ Aqueous solution of vit. A prepared by Endo Products Inc. containing 25,000 I.U. of vit. A/ml of solution.

§ U. S. Pharmacopeia vit. A test diet distributed by Nutritional Biochemicals Corp. Average choline content 20 mg/100 g.

TABLE I. Total Choline, Phospholipid Choline, and Calculated Free Choline in Rat Livers on Normal, Deficient, and Supplemented Vit. A Diets.

Dietary group	Avg rat wt (g)	Liver vit. A (μg/g wet liver)	Total choline	Phospholipid choline	Calculated free choline
			(mg/g wet liver)		
N-1	239	184.2	3.12	1.96	1.16
2	105	170.0	3.31	2.30	1.01
3	116	182.0	3.21	2.03	1.18
D-1	129	32.3	2.57	2.04	.53
2	179	6.3	2.21	1.93	.28
S-1	114	483.5	2.50	1.86	.64
2	119	437.8	2.26	1.53	.73
3	143	480.0	2.71	2.10	.61

by the procedure of Oser *et al.*(3). Determination of total choline, by the procedure of Engel(4), was run in duplicate on samples of frozen livers, which ranged from 1.5 g to 2.5 g wet liver. For determination of phospholipid choline, the procedure of Entenman *et al.*(5) was used. The color produced by choline reineckate was measured on a Coleman Junior Spectrophotometer at a 520 mμ wave length setting. The standard used was prepared according to Beattie(6). Concentration of the standard corresponded to 0.26 mg choline chloride/ml.

Results. The results are presented in Table I. The μg of vit. A/g wet liver are given for the 3 dietary groups. It will be noted that the vit. A levels of the 2 deficient groups (6.3-32.3 μg) show only trace amount of vit. A, while the concentration of vit. A (437.8-483.5 μg) in the supplemented rats is well above that of the normal groups (170-184.2 μg).

Total choline, in mg of choline/g wet liver, was highest in normal groups, ranging from 3.12 to 3.31 mg. The lowest concentrations of total choline were reported in a deficient group (2.21 mg) and a supplemented group (2.26 mg).

Phospholipid choline did not vary as much as did total choline. The highest level was in a normal group with 2.3 mg and the lowest in a supplemented group with 1.53 mg.

The free choline was calculated by subtracting the phospholipid choline from the total choline.

From the data in Table I, it appears that the optimum level of choline in the liver is associated with a normal liver level of vit. A. Low or abnormally high liver vit. A levels are associated with a low level of choline in rat livers.

Discussion. The results indicate that there is some vit. A-choline interrelationship. The total choline concentration was highest in the 3 normal groups, but this concentration was reduced when an excessive amount or a deficient amount of vit. A was stored in the liver. Since the phospholipid choline content showed little variation under these experimental conditions, it appears that total choline variation is on the basis of a change in the free choline content. It is interesting to note that the maximum level of free choline is related to the normal level of vit. A.

This relationship between vit. A and choline has been further substantiated by the work of Thorbjarnarson and Drummond(7), who found that choline feeding stimulated removal of vit. A as well as fat from the liver. Popper and Chinn(2) also reported that a choline-poor diet revealed a similar dependence upon vit. A storage in the liver.

The low vit. A blood levels found in patients with malignant disease of the gastrointestinal tract may in some way be tied up with a disturbance of this vit. A-choline relationship in the liver.

Summary. The highest level of free choline in rat livers was obtained from rats with a normal level of vit. A storage in their livers. Rat livers which contained an excessive amount or a deficient amount of vit. A had low concentrations of free choline.

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Lymphocytosis Response in Mice and Its Relation to Thymus and Adrenal.* (23296)

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During the course of observations on the effect of thymectomy on circulating lymphocyte levels in the mouse, it was observed that sham-operated mice did not maintain stable white cell levels as expected, but invariably developed a lymphocytosis. Subsequently, it was found that normal mice developed a similar lymphocytosis when bled daily for the performance of white cell counts. This paper describes this lymphocytosis response in mice, its variation in mice of different strains and experiments throwing light on the mechanism by which the response is produced.

Materials and methods. Mice. Mice used throughout these experiments were obtained from the Jackson Memorial Laboratories and were of the following strains: AkR, Rf, $C_{57}Bl$, LAF_1, and C_{58}. All mice were fed on purina chow and maintained on oral terramycin to prevent intercurrent infections. Male mice were used throughout these experiments to eliminate possible white cell variations with the estrus cycle. However, some groups of female mice were tested to make sure no notable difference was present in the lymphocytosis response of the two sexes. No difference was observed. The age of the mice used was 4-5 weeks. All mice were autopsied at the conclusion of each experiment to check on the adequacy of the operative procedures. Results from any mice showing infections were discarded. *White cell counts.* Standard human white cell diluting pipettes and chambers were used. Differential white cell counts were made, counting 200 cells. Blood was obtained by pricking the tail veins with a sharp No. 13 scalpel blade. "Milking" of the tail to produce a flow of blood was avoided as was making too large a wound with the production of too free a flow of blood. Careful standardization of the bleeding procedure is necessary because of the difference between the absolute numbers of white cells in the peripheral and heart blood in the mouse(1). White cell counts were performed at fixed times during the day to eliminate any possible diurnal variations. Due to individual variations in white cell levels even within inbred strains, groups of 4 to 6 mice were used in each experiment, and the mean value for the group used as a single estimation. *Operations.* When required, thymectomy and/or bilateral adrenalectomy were performed, using I.P. nembutal anesthesia. Adrenalectomized mice were maintained on a single I.M. injection of 0.5 mg of percorten‡ and 0.5% NaCl in the drinking water.

Results. The lymphocytosis response. When daily white cell counts were performed on the tail blood of normal mice of $C_{57}Bl$ strain, a lymphocytosis appeared on the second day. This persisted for as long as daily observations were made. The lymphocytosis levels fell towards the baseline levels of randomly bled mice several days after cessation

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