Survey of Liver Vitamin A Stores of Canadians

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MANY studies have been undertaken in different countries to determine the vitamin A reserves in the normal human being and the influence of disease on these reserves.

Sherman and Boynton[14] and Moore[8] found that the liver is the main storage organ of vitamin A and that the distribution of the vitamin throughout the body is similar for many mammals. About 90% of the body store of vitamin A is in the liver.[11] Determination of the concentration of the vitamin in the liver, therefore, provides a convenient index of vitamin A status.

Numerous nutritional surveys have used blood vitamin A levels as an index of body stores. It is recognized, however, that serum and liver levels are only correlated at or near deficiency states.[5, 11] Meyer *et al.*[7] found in man no relationship between levels of vitamin A in liver biopsy specimens and those of plasma.

Over 30 years ago, Wolff,[17] Green,[4] Moore[9] and others[2, 13] conducted major surveys to assess nutritional status from liver stores found at autopsy and to correlate these with disease conditions. More recently, similar studies have been reported by Dagadu[1] and Smith and Malthus.[15] The evidence obtained from these and numerous animal studies[16] indicates that the vitamin A status is affected and the requirements are increased in conditions of malabsorption, certain liver disorders, infectious diseases, parasitic diseases, sustained fever, renal disease and interference with the conversion of carotene to vitamin A.

To our knowledge no such survey has ever been conducted in Canada.[3] During the last decade many changes in nutritional habits and dietary regimens have been initiated and new therapeutic agents have been introduced. The influence of these on the metabolism of fat-soluble vitamins is not known.

The present study was undertaken to establish standard levels of vitamin A in Canadians in relation to age, sex and disease conditions, and to establish a baseline by which to judge in the future the effect of changes in nutritional habits, dietary regimens and therapeutic agents.

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MATERIALS AND METHODS

Approximately 10 g. of liver tissue from the central portion of the right lobe of the liver removed at autopsy was immediately frozen at -20° C. and analyzed within two weeks for vitamin A content.

The amount of vitamin A was determined by procedures described by Mervyn and Morton[6] and by Phillips.[12] Duplicate analyses were performed on 5-g. liver samples by saponification in ethanolic KOH containing pyrogallol. The isolated non-saponifiable material was separated on deactivated alumina and the vitamin A concentration was determined spectrophotometrically at 325 mμ. (E 1%/1 cm. 1750). Most of the adult liver tissue also contained lycopene in varying concentrations. This material appeared as a pronounced pink zone on the alumina column. Separation from vitamin A posed no difficulties, since most of the material was eluted with the 2% diethyl ether in the petroleum ether fraction.

RESULTS

One hundred human liver samples were analyzed for vitamin A and the results compared with respect to age, sex and disease conditions. Ranges, means and medians have been used as criteria in summarizing the results for the different groups.

The results in relation to age and sex regardless of the cause of death are shown in Table I. Children from 1 to 10 years of age had a greater mean liver vitamin A store than any other group, and considerably higher than what was found in stillborn infants and in children under 1 year of age. High mean vitamin A values were observed in the age groups 11 to 20 and 51 to 60 years. Although the mean levels appeared adequate, some subjects in the latter group contained no detectable vitamin A while high levels were found in all of the subjects in the younger age groups. The lowest mean value for adults was found in the group 31 to 40 years of age. Vitamin A liver stores of male subjects did not differ from those of female subjects.

For the purpose of studying the interrelationship of vitamin A stores and disease conditions, the subjects were classified into five groups according to primary cause of death. Stillborn infants and infants under 1 year were not in-

TABLE I.—VITAMIN A LEVELS IN HUMAN LIVER IN RELATION TO AGE AND SEX

Age (years)	No. of subjects	Vitamin A (μg./g. liver) Range	Mean	Median	Standard deviation
Stillborn	4	6.2 - 104.2	38.0	20.9	± 45.0
0 - 1	7	*ND - 129.6	44.6	40.5	± 41.0
1 - 10	8	15.2 - 533.6	223.0	192.30	±173.8
11 - 20	3	105.0 - 233.8	152.3	118.0	± 70.9
21 - 30	8	ND - 230.0	76.9	62.1	± 79.9
31 - 40	7	21.4 - 95.8	52.5	39.1	± 28.7
41 - 50	10	12.0 - 411.2	98.8	74.3	±113.6
51 - 60	16	ND - 535.5	150.8	108.0	±149.0
61 - 70	11	ND - 191.7	69.5	66.7	± 47.0
71 - 80	15	ND - 173.7	65.2	72.0	± 52.7
81 and over	11	ND - 438.0	92.0	36.4	±128.3
Male	55	ND - 535.5	105.4	68.4	±120.5
Female	45	ND - 438.0	89.8	78.0	± 95.9

*Not detected.

cluded. As shown in Table II, the mean liver vitamin A stores were lower in subjects dying from specific disease conditions than in subjects dying from accidental causes. With respect to the disease groups, subjects with disease of the heart and/or coronary arteries had the highest mean liver vitamin A stores and subjects in the cancer group had the lowest.

age groups of New Zealanders were considerably above those found for Canadians. Liver vitamin A stores declined after the age of 20 to a low level between 31 and 40 years of age; they increased considerably between 51 and 60 years of age and again were lower in the old age groups. Whether this reflects nutritional neglect during the 20- to 40-year age period and

TABLE II.—VITAMIN A LEVELS IN HUMAN LIVER IN RELATION TO DISEASE

Cause of death	No. of subjects	Vitamin A (μg./g. liver) Range	Mean	Median	Standard deviation
Accidental	13	24.6 - 533.6	172.9	118.0	±145.3
Heart and coronary disease	30	*ND - 535.5	111.4	76.9	±132.0
Cancer	13	ND - 108.5	54.6	41.1	± 39.7
Respiratory disease	11	ND - 219.1	87.7	88.8	± 68.2
Others (over 1 year of age)	24	ND - 427.0	95.1	68.6	± 97.8

*Not detected.

No vitamin A was detected in the autopsy liver samples of eight subjects, using the described method of analysis.

DISCUSSION

The concentration of vitamin A varies with the site in the liver from which the sample is taken. In a preliminary study of five subjects it was found that this variation was least in the central core taken from the right lobe. The vitamin A concentration of tissue from this site was more representative of total liver homogenates.

The finding that vitamin A stores in the livers of infants under 1 year of age were considerably below those of older children and adults is in agreement with previous reports.[2, 15] Smith and Malthus[15] likewise reported that the highest hepatic vitamin A stores occurred in children from 1 to 10 years of age, but the levels for all

during old age, and the high plane of nutrition associated with middle age, remains an interesting speculation, since no nutritional histories were available and the age comparison included subjects who died from various causes.

Subjects in the accidental death group were assumed to be in normal health and used as such in relating the liver vitamin A stores with the disease groups. The mean of 173 μg. vitamin A per g. in the accidental death group was greater than the means in the disease groups. The age distribution of the former group was biased by the high proportion of accident cases in the 1- to 20-year age bracket.

The levels of liver vitamin A stores with respect to disease conditions are similar to the findings of Moore[9, 10] and others.[2, 13, 17] However, the low liver stores which were found in the cancer group in this study may indicate a greater influence on the depletion of body stores

of vitamin A by this disease. Included in the group of "others (over 1 year of age)" were cirrhosis of the liver, cerebral hemorrhage, kidney diseases, gastric and duodenal ulcerations, etc. Of the eight cases with no detectable vitamin A, two had cirrhosis of the liver and one had kidney disease. The remainder were distributed among the other disease groups. No case of complete absence of vitamin A occurred in the accidental death group. Although the influence of disease conditions is evident, the effect on susceptibility to disease of low vitamin A status cannot be excluded. It has been shown that an optimal vitamin A supply retarded the clinical outbreak of tuberculosis and restricted the size of tuberculosis lesions in chicks and also promoted natural resistance to parasitic diseases in sheep and poultry.[16]

TABLE III.—DISTRIBUTION OF LIVER VITAMIN A STORES IN 81 CANADIAN SUBJECTS OVER 10 YEARS OF AGE

Vitamin A content (μg./g.)	*Number of subjects*	*% of total*
0 - 40	26	32.1
41 - 80	18	22.2
81 - 120	20	24.7
121 - 160	6	7.4
161 - 200	5	6.2
201 - 240	2	2.5
over 240	4	4.9

Excluding infants and children under 10 years of age, the range of distribution of liver vitamin A stores in the remainder of the 100 subjects is shown in Table III. In 32% of these the liver stores ranged from 0 to 40 μg. per g. Obviously the vitamin A status of these subjects was poor when compared to that of accident victims (173 μg. per g.) and the "normal" human liver reserves of 100 to 300 μg. per g. quoted by Pearson.[11] From food consumption figures the apparent vitamin A intake for Canadians in 1964 was estimated at about 6800 I.U. per person per day. In view of this estimate, it must be assumed that disease conditions, poor nutritional habits and other unknown environmental factors were the main contributing cause of the low vitamin A status in the high percentage of subjects. These data strongly suggest that greater attention be directed towards improved nutrition with remedial and prophylactic treatment in acute and chronic disease states.

Since this was a local survey only, it is not known whether the results are representative of the state of affairs that prevails across the country.

Summary Vitamin A analyses were performed on 100 human livers obtained at necropsy.

Age influenced liver vitamin A stores. Children from 1 to 10 years of age had higher liver stores (223 μg. per g.) than any other age group, much higher than those for stillborn infants and children up to 1 year of age (38 and 45 μg. per g., respectively). Among adult subjects, the lowest liver stores occurred in the 31- to 40-year age group and the highest between the ages of 50 and 60.

Comparisons were made of the vitamin A status in health and disease by classification into five major categories as determined from the primary cause of death: normal individuals dying accidentally, heart and coronary diseases, cancer, respiratory diseases and a miscellaneous group of diseases. The mean of 173 μg. vitamin A per g. of liver tissue in the accidental death group was greater than for any of the disease groups, but this was biased by the high proportion of cases of accidental death in the 1- to 20-year age bracket. Within the disease groups, subjects with heart and coronary vessel disease had the highest (111 μg. per g.) and the cancer group the lowest (55 μg. per g.) levels.

Vitamin A was not detected in the liver of eight subjects. Thirty-two per cent of the liver vitamin A values in subjects over 10 years of age were in the range 0 to 40 μg. per g. These data strongly suggest that greater attention should be directed towards improved nutrition with both remedial and prophylactic treatment in acute and chronic disease conditions.

Résumé Des analyses de vitamine A ont été pratiqués dans 100 foies humains obtenus à la nécropsie.

L'âge a influencé les réserves hépatiques de vitamine A. Les enfants de 1 à 10 ans possédaient des réserves hépatiques (223 μg par g) supérieures à celle de n'importe quel autre groupe d'âge, beaucoup plus élevées que chez des bébés mort-nés et des enfants jusqu'à 1 an (38 et 45 μg par g respectivement). Parmi les adultes, les réserves hépatiques les plus basses ont été constatées dans le groupe de sujets d'âge variant entre 31 et 40 ans et les réserves les plus élevées dans les âges allant de 50 à 60 ans.

On a procédé à la comparaison des réserves de vitamine A dans l'état de bonne santé et dans la maladie. A cet effet, on a classé les sujets en cinq catégories principales, selon la cause primaire de la mort: personnes en bonne santé morts accidentellement et sujets décédés de maladies cardiaques, coronariennes, de cancer, d'affections respiratoires et un groupe de sujets morts de pathologies diverses. La moyenne de 173 μg de vitamine A par gramme de tissue hépatique trouvée dans le groupe de mort accidentelle était supérieure à celle qu'on notait dans tous les autres groupes de pathologies, mais cette différence est faussée par la proportion élevée de

cas de mort accidentelle dans le groupe d'âge de 1 à 20 ans. Dans la catégorie des malades, c'étaient les personnes mortes de maladies cardiaques et coronariennes qui avaient les taux les plus élevés (111 μg par g) et les cancéreux le taux le plus bas (55 μg par g).

On n'a pu déceler de vitamine A dans le foie de huit sujets. La teneur de vitamine A hépatique chez 32% des sujets de plus de 10 ans était dans la gamme de 0 à 40 μg par g. Ces constatations permettent de croire qu'il faudrait accorder plus d'attention à l'amélioration de l'alimentation, en même temps qu'aux traitements à la fois curatif et prophylactique dans les pathologies aiguës et chroniques.

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Studies on the Inhibition of Intestinal Absorption of Radioactive Strontium:

VIII. The Effect of Alginate-Containing Diets on Water Metabolism

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ALGINATE is a high-molecular-weight polysaccharide which is not absorbed in the gastrointestinal tract. In the course of studies on the suppression of intestinal absorption of radioactive strontium by administration of sodium and calcium alginate,[1a-e] we observed that the fecal material in rats fed with wet or dry sodium alginate contained large amounts of water, while the volume of urine was also increased. Detailed studies of water metabolism are necessary to understand and predict long-term changes occurring during alginate administration. Orally ingested sodium alginate has been shown not only to be a valuable antidote in human subjects with acute strontium poisoning[2-5] but also to be of value in reducing the body burden of deposited radiostrontium in chronically labelled cats.[6]

The current wide use of non-absorbable filling agents as a means of controlling weight makes studies of the water content of the gut following alginate administration particularly interesting. Alginate, like agar, cellulose and other bulk materials, exercises its effect by binding large amounts of water. The concomitant feeling of fullness depresses appetite. A problem, however, arises when sodium and calcium alginates are ingested, because the Na^+ and Ca^{++} can exchange with other cations,[7] thereby contributing to the tonicity of the diet and influencing thirst and water intake. Bruce and Kennedy[8] have shown that water intake ultimately depends on the demands imposed by the renal secretion of the products of metabolism, and that hypertonic diets are well tolerated, provided there is free access to drinking water. It is apparent, therefore, that several factors will play a role in the water balance when alginate is administered, namely, the hypertonicity of the ingested diet, the hydrophilic nature of, and bulk provided by, the alginate itself, and the amount of water which the animal will consume either by drinking or by eating premoistened food.

This paper reports the effect on the urinary and fecal excretion of water by the rat of feeding various types of alginate, in both wet and

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