LIVER VITAMIN A RESERVES OF NEONATES, PRESCHOOL CHILDREN AND ADULTS DYING OF VARIOUS CAUSES IN SALVADOR, BRAZIL

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SUMMARY

In and around the metropolitan district of Salvador, Bahia, Brazil, 141 liver samples of individuals ranging in age from premature infants to 82 years, were analyzed for vitamin A and carotenoids. Premature, stillborn or short-lived ($<$ 6 days) infants had a median liver reserve of 24 μg/g retinol, with only 3°/o at high risk ($\leqslant$ 5 μg/d). After 6 weeks of age, the median vitamin A concentration fell, reaching a value of about 60 μg/g in the 3-12 month age group, and then rose again to $\sim$ 25 μg/g from 1-4 years of age. The percentage of children in the high risk category ($\leqslant$ 5 μg/g) increased to about 30°/o between 3 weeks and 2 years of age, and then declined. Major cited causes of death in the 1 week-4-year age group were gastroenteritis (33°/o), bronchoneumonia (29°/o) and dehydration (19°/o), either singly or in combination. Children suffering from dehydration, with or without

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gastroenteritis, had the highest percentage (45%) of very low ($\leqslant 5$ µg/g) liver reserves of vitamin A.

Older children (4-15 years of age) had median reserves of 80 µg/g. Of the three children in this group with liver reserves < 10 µg/g, two died of acute liver schistosomiasis and one of gastroenteritis. The liver reserves of all adults ($\geqslant$ 15 years) examined were satisfactory (> 10 µg/g), except for 4 persons with severe liver or kidney disease. The median value for adults was 57 µg/g, in keeping with values reported from other countries.

Median carotenoid concentrations in the liver of neonates were low ($\sim$3 µg/g) and remained around 4 µg/g until 4 years of age. The concentration then increased to an overall median of about 15 µg/g in adults. Although the concentrations of vitamin A and carotenoids in the liver were positively correlated, the variance was large, the correlation coefficient low and the P value high.

Our sample of children who died between 0 and 4 years of age seems to be representative of all children of like age dying of various causes in the Salvador region. The relation of the present sample to the living population of like age, however, cannot be precisely defined. If one assumes that the nutritional status of *twice* the number of surviving children 0-4 years of age is the same as those of like age who die of various causes, then about 3% of children 0-4 years old in the general Salvador population have inadequate vitamin A reserves and about 1% are at high risk.

INTRODUCTION

In the past 15 years many studies have been conducted in Brazil relative to the vitamin A status of preschool children (1-23). These studies, which include dietary surveys (1, 2, 5, 6, 9, 10, 11, 15, 16, 20-22), clinical examinations (1, 2, 5, 6, 8, 15-17, 19), blindness surveys (12, 13) and biochemical analysis (1-8, 14-16, 18, 19, 23) have indicated that preschool children from certain socioeconomic groups in specified areas of Brazil suffer from at least a marginal state of vitamin A nutriture. Of the biochemical indicators selected, plasma vitamin A values have been used in all but two investigations, where liver reserves were assessed (14, 18). Inasmuch as plasma values of vitamin A are reliable indicators of vitamin A status only when the liver is largely depleted of its stores (24-26), the direct analysis of autopsy specimens of liver, which normally contain 90% or more of the total body reserves, rather uniquely allows evaluation of the adequacy of vitamin A reserves in the population studied. In

order to assess the vitamin A nutritional status as a function of age, therefore, the vitamin A concentrations in the livers of 141 persons, varying in age from premature infants to 82 years, who died of various causes in the Salvador region of Bahia from January-July, 1975 have been examined in the present study. A preliminary report of this work has been published (14).

MATERIALS AND METHODS

Liver Samples

Samples of liver (5-10 g) were obtained mainly from the mid-central portion of the right lobe at autopsy, placed in labeled snap–top plastic containers, frozen at $-20^{o}C$, and stored until analysis. The time between death and autopsy was less than 24 hours in all but a few cases. The age, sex, color, economic status, home area and cause of death were obtained in nearly all of the 141 cases. Cooperating hospitals were the Institute of Legal Medicine (Nina Rodriguez) and the Hospital Edgard Santos, both in the city of Salvador. Only samples which showed no signs of autolysis were analyzed. Although the vitamin A in frozen liver samples is remarkably stable (27), analyses were generally conducted within one week of freezing.

Geographic Distribution of Cases

Of the neonates and children under 15 years of age for whom home areas were specified (n = 62), 38 came from 26 different sections of the city of Salvador, 12 from various Salvador suburbs, and 12 from other cities or towns in the Salvador region. Of the adults for whom home information was available (n = 13), 9 came from various localities in the city of Salvador, 1 from Governador Mangabeira, a town in the Salvador region, and 3 from neighboring regions, 2 from Feira de Santana, and one from Serrinha. Thus, the sample was derived rather broadly from the Salvador region and its environs rather than solely from specific urban or rural localities where health conditions were particularly poor.

Vitamin A Analyses

Vitamin A and carotenoids were determined by a simple

dual assay technique involving spectrophotometric and Carr-Price analysis (28). In brief, a weighed ~1 g liver sample is placed in a 9 ml screw-top vial, gently mashed against the side of the vial together with 2.5 g of anhydrous sodium sulfate by use of a spatula, and covered with 5.0 ml of chloroform. After gentle mixing, the vial is hermetically sealed and placed at 0° overnight, i.e, 8-24 hours. An aliquot (0.30 ml) of the chloroform extract is then diluted to a total of 3.0 ml with ethanol, mixed, and the absorbancies are read in 1 cm cuvettes (4 ml capacity) in a Zeiss PMQ-III spectrophotometer at 280, 330, 380, and 450 nm.

Contaminant absorption in the ultraviolet region, traces of turbidity in the sample and β-carotene end-absorption are corrected by the formula:

$$\text{Corr. A330} = 0.5\ (2.27 \times \text{A330} + 0.17 \times \text{A450} - \text{A280} - \text{A380})$$

Then, ug retinol/g liver =

$$\frac{\text{Corrected A330} \times \text{dilution factor (e.g. 50 in the cited case)}}{0.1835 \times \text{sample weight (g)}}$$

And, µg carotenoids/g liver =

$$\frac{\text{A450} \times \text{dilution factor (e.g. 50 in the cited case)}}{0.25 \times \text{sample weight (g)}}$$

Another aliquot (0.20 ml) of the same chloroform extract is placed in a 1 cm cuvette, 1.8 ml of freshly prepared trichloroacetic acid in anhydrous chloroform is quickly and forcefully pipetted into the cuvette, and the absorbancy at 620 nm is measured at its maximum, usually 10 seconds thereafter. If the absorbancy is very low (< 0.06) and does not decrease over 20-30 seconds, the vitamin A content is taken as zero. The amount of vitamin A in the test is determined from a standard curve run at the same time with reference retinyl acetate. When appreciable amounts of carotenoids are present, vitamin A is corrected in the following way:

$$\text{Corr. retinol (µg/g)} = \text{observed retinol (µg/g)} - \frac{\text{carotenoids (µg/g)}}{20}$$

Retinol concentrations were expressed as a mean of the two analyses, the median per cent difference of which was 7.7% over a wide range of retinol concentrations (28).

Statistical Analysis

Data were coded, key-punched on IBM data cards and analyzed by an IBM 360/65 computer using the Statistical Analysis System (SAS), Version 76.6 (29). Computed values included the median, the mean $\pm$ the standard deviation, frequency distribution and the range for the full sample and for various sub-samples relative to age, sex, socioeconomic status and skin color. Regression analysis and correlation coefficients were computed for vitamin A and carotenoid values. When the number of items in a group is even, the reported median is the average of the two middle numbers.

RESULTS

Overall Distribution of Vitamin A and Carotenoid Concentrations in the Liver

The distribution of various concentrations of vitamin A and carotenoids in the liver for the whole group of 141 cases is presented in Figure 1. The median, mean $\pm$ SD and range of values for vitamin A concentrations are 32, 62 $\pm$ 104 and 0-858 µg/g, respectively, and for carotenoid concentrations, are 4, 9 $\pm$ 11 and 1-63 µg/g. In both cases the distribution is highly skewed towards the right, a point de-emphasized in Figure 1 by the changing scale on the abscissa. Because of this skewness, standard deviations often exceeded mean values. Median values are consequently of greater value in characterizing the vitamin A status of a group.

The distribution of vitamin A and carotenoid values in these different age groups (premature and stillborn infants, children 7 days to 15 years of age, and adults over 15 years of age), are also shown in Figure 1. Newborns had marginal vitamin A reserves and little carotenoid in the liver, children (7 days-15 years) had a higher percentage of very low vitamin A values and a broader range of both vitamin A and carotenoid concentrations, while the adult group had higher vitamin A and carotenoid values.

The linear regression line formula for a plot of the vitamin A

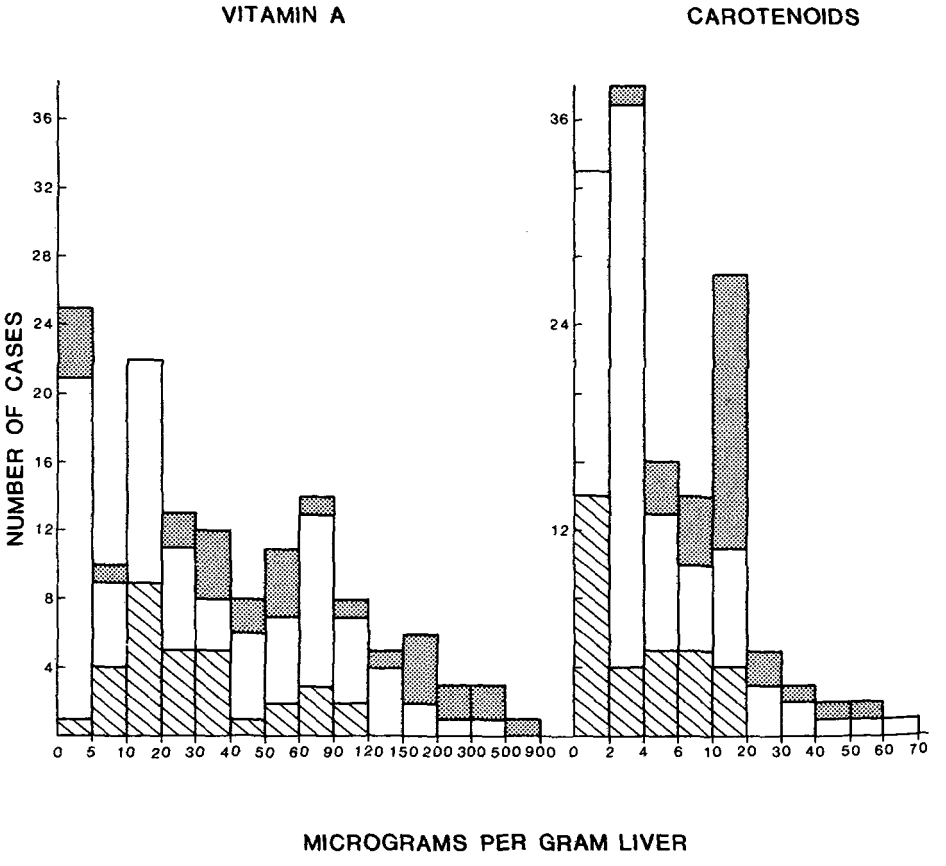

FIGURE 1

Distribution of concentrations of vitamin A and carotenoids in the liver of 141 human subjects. Values of premature and stillborn infants (n = 32) are denoted by ▨, of young persons (7 days to 15 years; n = 80) by □ and of adults (>15 years n = 29) by ▩.

values (y) against the carotenoid values (x) of individuals was y = 52 + 1.09 x. Although the correlation between these two values was positive, the scattering of points in the plot was extensive, as indicated by a coefficient of variation of 168, a correlation coefficient of only 0.12 and a P value of 0.17. Thus the relationship has little predictive value.

Distribution of Vitamin A and Carotenoid Values as a Function of Age

The median, mean and range of values for different ages are given in Table 1, together with the percentage of a given group with vitamin A concentrations equal to, or less than 5 and 20 µg/g. With respect to vitamin A concentration, median and mean values at birth were 24 and 33 µg/g, respectively. The median remained about the same until three months, when it fell, and ultimately reached a minimum of 10 µg/g for the 6-12 month period. Thereafter it rose to about 25 µg/g for 1 to 4-year-olds and then rose further after 4 years into the adult range. Perhaps of greater interest is the percentage of children at extreme risk, e.g., with $\leqslant$ 5 µg retinol/g. This percentage was only 3% at birth, but then increased markedly to a maximum of about 31% from 3 months to 2 years, after which it declined. The percentage of children with *less* than an adequate vitamin A reserve, defined as 20 µg retinol/g, was expectedly about 50% at birth. Thereafter this percentage rose to a maximum of 74% between 3 and 12 months of age, and then declined. The median value for adults, defined here as persons 15 years of age or older, was 57 µg/g. All had adequate stores of vitamin A with the exception of 4 persons with poor liver or kidney function. If those cases are excluded, the median is raised to 64 µg/g. Individual vitamin A and carotenoid values with the age group medians are presented in Figures 2 and 3.

Effect of Disease and the Immediate Cause of Death on Liver Vitamin A Reserves

Of the 41 persons 4 years of age or older, four of the seven with liver values velow 10 µg/g had severe liver ailments, i.e., 8 and 14-year-old males with acute liver schistosomiasis (3 and 7.3 µg retinol/g, a 37-year-old female with extensive liver sarcoma ($\sim$ 1 µg/g/ and a 64-year-old male with severe liver cirrhosis (2 µg/g).

TABLE 1

VITAMIN A AND CAROTENOID VALUES AS A FUNCTION OF AGE (n = 141)

		Vitamin A*					Carotenoids		
Age	n	Median (μg/g)	Mean ± SD (μg/g)	Range (μg/g)	%≤5μg/g	%≤20μg/g	Median (μg/g)	Mean ± SD (μg/g)	Range (μg/g)
Premature and stillborn to 6 days	32	24	33 ± 27	2.2±112	3	44	3	4.7 ± 4.4	1±19
1 wk<3 wk	8	23	25 ± 18	0±53	13	25	5	10 ± 13	1±34
3 wk<6 wk	7	50	82 ± 98	2.5±281	29	29	4	6.0 ± 7.2	1±22
6 wk<3 mo	9	18	43 ± 45	0.8±130	22	56	4	9.4 ± 17	2±55
3 mo<6 mo	11	11	35 ± 51	0.8±153	36	73	4	8.8 ± 11	2±38
6 mo<1 yr	12	10	29 ± 41	0±117	25	75	4	11 ± 20	1±63
1<2 yr	16	28	33 ± 29	0±93	31	38	4	6.1 ± 6.7	1±26
2<4 yr	5	18	25 ± 21	1.2±55	20	60	3	3.6 ± 0.9	3±5
4<15 yr	12	80	104 ± 108	2.6±392	17	25	6	7.1 ± 4.5	2±17
15 – 30 yr	2	39	39 ± 1.4	38±40	0	0	15	14 ± 4.9	11±18
31 – 40 yr	6	21	180 ± 339	1±858	33	50	12	12 ± 5.3	5±19
41 – 50 yr	4	123	185 ± 208	20±473	0	0	16	23 ± 17	13±48
51 – 60 yr	6	179	206 ± 160	47±473	0	0	23	28 ± 15	16±57
61 – 70 yr	7	57	71 ± 63	2±168	14	14	11	12 ± 7.5	5±25
>70 yr	3	55	38 ± 32	0.8±57	33	33	11	11 ± 3.5	8±15
Adult (unknown)	1		92		0	0		4	

* Expressed as μg retinol/g wet weight of liver.

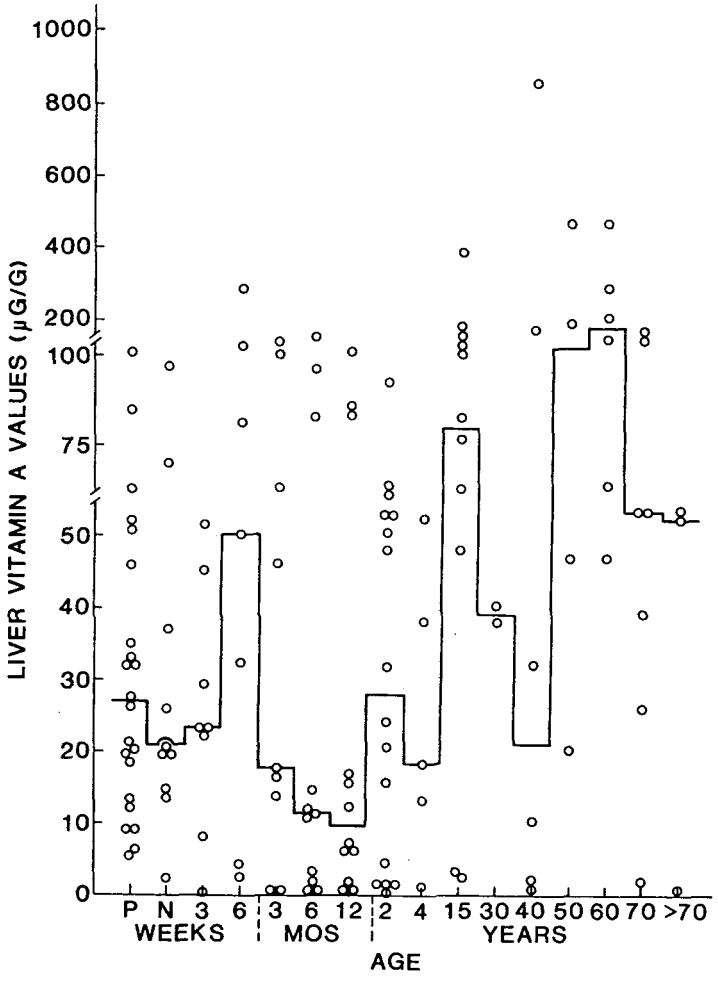

FIGURE 2

Liver vitamin A values of individuals as a function of age (n = 140). Median values are denoted by bars (—) which are connected by vertical lines. The ordinate has *three* scales, with changes at 50 and 100 μg/g. The age group symbols on the abscissa are: P, premature infants; N, full-term neonates up to 1 week; 3 weeks, 1 week to 20 days; 6 weeks, 3 up to 6 weeks; 3 months, 6 weeks up to 3 months; 6 months, 3 up to 6 months; 12 months, 6 up to 12 months; 2 years, 1 up to 2 years; 4 years, 2 up to 4 years; 15, 4 up to 15 years; 30 years, 15 through 30 years; 40 years, 31 through 40 years, etc.

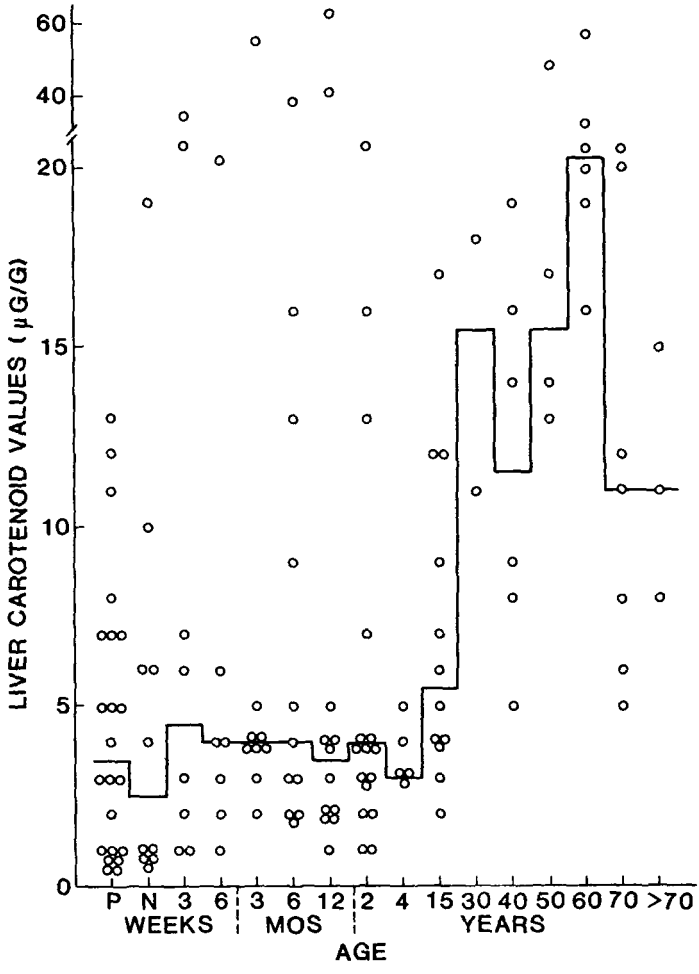

FIGURE 3

Liver carotenoid values of individuals as a function of age (n = 140). Median values are denoted by bars which are connected by vertical lines. The ordinate has *two* scales which change at 20 µg/g. The age group symbols on the abscissa are the same as those defined in the legend of Figure 2.

The other three were a 7-year-old girl with gastroenteritis (2.6 µg/g), a 36-year-old male with chronic renal insufficiency (2.5 µg/g) which also lowers liver vitamin A reserves (30), and an 82-year-old woman with general arteriosclerosis and hepatic congestion (1 µg/g). Others of this group died from a wide variety of causes, including cardiac and respiratory ailments, neoplasms primarily affecting tissues other than the liver, acute bacterial infections, other kidney disease, and accidents. No clear pattern between vitamin A reserves and cause of death was evident in this vitamin A sufficient group.

Among the 32 premature infants, stillborns and neonates who died during the first week of life, the major listed causes were anoxia, probably due to hyaline membrane disease, and just "prematurity". Of the 68 children 1 week to 4 years of age, the major causes of death were gastroenteritis (33%), bronchopneumonia (29%) and dehydration (19%), as shown in Table 2. Dehydration, either alone or in combination with gastroenteritis,

TABLE 2

RELATION OF CAUSE OF DEATH TO LIVER VITAMIN A RESERVES IN CHILDREN 1 WEEK TO 4 YEARS OF AGE (n = 68)

		Vitamin A in liver		
Cause of death	n	Median (µg/g)	Mean ± SD (µg/g)	%<5µg/g
Total listed causes	116			
Gastroenteritis	38	19	36 ± 38	24%
Bronchopneumonia	34	31	49 ± 57	15
Dehydration	22	12	22 ± 31	45
Bronchitis capillary	4	14	21 ± 25	50
Respiratory insufficiency	4	53	59 ± 58	25
Tuberculosis	3	6	7 ± 5	33
Malnutrition	3	81	66 ± 59	33
Accidents	2	43	43 ± 15	0
Other	6	18	20 ± 15	17

gave low median vitamin A values (12 and 7 μg/g) and the highest percentage (45 and 50%) of children at high risk. Other childhood diseases associated with low liver reserves and high risk were tuberculosis and bronchitis capillary.

It is instructive to compare the major causes of death in our sample to those reported for the administrative region of Salvador in 1973 (31). Of a population of 2,010,984, a total of 19,046 deaths were recorded, or 0.95%. Of these deaths, 7,716 or 40.5% occurred in children 0-4 years of age. Of this latter group, the primary cause of death was recorded in 4,778 cases, which is compared to the present sample of the same age group in Table 3. With few exceptions, the frequencies of major causes of death are similar in the two samples.

TABLE 3

A COMPARISON BETWEEN MAJOR CAUSES OF DEATH IN THE SALVADOR REGION (1972) AND IN THE PRESENT SAMPLE (1974-5) OF CHILDREN UP TO 4 YEARS OF AGE

Cause of death	Salvador region		Present sample	
	n	%	n*	%
Total sample	4,778	100	154	100
Gastroenteritis and/or diarrhea	1,441	30.1	60	39.0
Pneumonia	982	20.5	37	24.0
Anoxia, obstetrical problems, etc.	1,007	21.1	33	21.4
Nutritional deficiencies	248	5.2	5	3.3
Tuberculosis	89	1.9	3	1.9
Measles	66	1.4	0	0
Tetanus	73	1.5	1	0.6
Meningitis	151	3.2	2	1.3
Accidents	56	1.2	2	1.3
Bronchitis	53	1.1	5	3.3
Other infections	122	2.6	1	0.6
All other causes	490	10.2	5	3.3

* Total causes listed for 104 cases.

Effect of Socioeconomic Status, Sex and Skin Color

Of the 94 cases drawn from the Nina Rodríguez Institute, family support or indigency was declared in 92 cases, and skin color was noted in 91. Sex was indicated in all but one case of the total sample. As shown in Table 4, family-supported children had higher median and mean vitamin A values than indigent children. On the other hand, the percentages at high risk (<5 μg/g), and with inadequate reserves were essentially the same in both groups. Male adults had 3-5-fold higher median and mean values than female adults. Among premature and stillborn infants and children 1 week to 15 years old, however, median and mean values and percentages at risk were essentially the same in both sexes. In regard to skin color, no major differences were noted among the four major groups cited. If anything, children with brown skin had somewhat higher median and mean values of vitamin A and were somewhat less at risk than the other three groups. Because of the large variance and relatively small groups, however, these differences are not helpful as predictive indicators.

DISCUSSION

Inasmuch as 90% or more of the total body reserve of vitamin A is normally stored in the liver, measurement of the vitamin A concentration in a liver sample taken at autopsy gives a direct indication of the vitamin A status of that individual (32). If one assumes that the half-life ($t_{1/2}$) of vitamin A in the liver is 50 days in infants and young children and that a minimal acceptable vitamin A reserve for preschool children should last for a 100 days protection period, a minimal acceptable liver concentration of vitamin A for this age group can be calculated to be 20 μg/g liver (24). A similar calculation for adults yields a value of 10 μg/g liver. Since children suffering from xerophthalmia usually have $\leqslant 5$ μg retinol/g liver (33), any child with such low reserves has been considered to be at high risk.

The general application of these criteria, i.e., 20 μg/g as a minimally acceptable level for children and $\leqslant 5$ μg/g as a high risk concentration, is complicated by the fact that children at birth have relatively low liver reserves, regardless of the nutritional state of the mother (30). Thus, the change in liver reserves as a function of age in children becomes a useful indicator of the vitamin A status during the early crucial years of life.

TABLE 4

EFFECT OF SOCIOECONOMIC STATUS, SEX AND SKIN COLOR ON LIVER VITAMIN A VALUES

Category	Age group	n	Median (μg/g)	Mean ± SD (μg/g)	% ⩽ 5 μg/g	% ⩽ 20 μg/g
Socioeconomic						
Family supported	0–8 years	36	29	62 ± 83	19%	44%
Indigent	0–8 years	56	21	33 ± 35	21	48
Sex						
Male	All ages	78	32	76 ± 130	18	37
	Stillborn – 6 days	17	27	34 ± 20	0	35
	1 week – 15 years	42	22	45 ± 67	28	48
	15 – 82 years	19	149	180 ± 217	11	21
Female	All ages	62	26	44 ± 52	18	45
	Stillborn – 6 days	15	20	32 ± 34	7	53
	1 week – 15 years	38	26	50 ± 61	20	47
	15 – 82 years	9	40	36 ± 22	20	33
Skin color						
White (Branco)	0 – 8 years	12	15	24 ± 23	33	58
Light brown (Moreno)	"	4	20	42 ± 60	50	50
Brown (Pardo)	"	65	23	52 ± 68	20	43
Black (Prete)	"	10	15	27 ± 31	10	70

In the Salvador region the median and mean values of liver stores in premature, stillborn and short-lived infants were 24 and 33 μg/g, respectively. Although premature infants tend to have higher liver vitamin A concentrations than full-term neonates (25), these differences were small in the present case, i.e., median and mean values for premature infants (n = 22) of 26.5 and 33.2 μg/g, and for full-term infants (n = 10) of 20.9 and 32.2 μg/g respectively. Only one of 32 infants in this category was at high risk. Incidentally these vitamin A reserves are considerably higher than those recently reported for neonates in India (34), Thailand (25) or the United States (35), or in the 1930's in Holland, Norway or England (30, 36). Quite possibly, the extensive ingestion of red palm oil in the Salvador region might enhance fetal stores of vitamin A, or a genetic component might influence the placental transfer and/or fetal retention of vitamin A. Mean values and ranges for liver carotenoids in neonates less than 1 day of age were higher in Bahia (3.3 μg/g and 1-13 μg/g) than in the United States (2.4 μg/g and 0-7 μg/g), and brown-skinned Brazilian neonates had higher mean vitamin A values (40 μg/g) than white Brazilian newborns (25 μg/g).

The distinct fall in the median vitamin A values of infants at 3 months of age indicates a worsening of the vitamin A status. This poor status, also indicated by the high percentages with inadequate reserves and at high risk, persisted through 2 years of age before improvement occurred. This pattern might be contrasted with that found in England (36) and the United States (35), where the median vitamin A reserves increase dramatically in children between 2 and 4 months of age. On the other hand, the vitamin A status of all adults studied in Salvador, with the exception of those with severe liver disease or chronic renal insufficiency, was satisfactory, with median values similar to those reported for other countries (24, 30). Thus our primary concern about vitamin A nutriture in the Salvador region focuses on children 0-4 years old, or more specifically on those 3 months to 2 years of age.

Of the major listed causes of death in the 1 week to 4-year-old age group, dehydration, either alone or in combination with gastroenteritis, was most commonly associated with very low vitamin A values. Bronchitis capillary and tuberculosis were similarly characterized, whereas the vitamin A status of children dying of bronchopneumonia was better than average. By contrast, the median liver reserves in England (36) of 4-year-old children dying

of tuberculosis were higher and of respiratory diseases were lower than the norm. In all likelihood, therefore, the cited causes of death may be useful in identifying a potential vitamin A problem *in a specific ambient,* but are not of universal utility.

Only five individuals, ranging in age from 20 days to 62 years, died of accidents in our sample. The median and mean values were 48 and 47 ± 12 µg/g, respectively, somewhat below the overall median and mean values of 57 and 62 µg/g. Although Ellison and Moore (36) reported that accident victims 4 months to 14 years of age in England had median and mean values very close to their overall sample, the present sample is clearly different in structure from that which they analyzed.

In Recife, Brazil, Flores and Araújo (18) have conducted similar analyses of 142 liver autopsy samples from children up to 72 months of age. Of this group 36°/o had liver values < 20 µg/g, with a larger incidence of low values in infants < 1 year of age. Low vitamin A values were also common in children with ⩽ 70°/o of the reference weight for age. Our values agree well with those reported from Recife, namely, that 49°/o of the Salvador autopsy samples from children 1 week to 6 years of age (n = 74) had liver reserves of ⩽ 20 µg/g, with the lowest median reserves in the 3 months to 1-year-age group.

Only limited data are available from other countries. In Bangladesh, the median liver retinol concentration in 13 autopsy samples from children 3-10 years old was 15 µg/g, with 70°/o <20 µg/g but none < 5 µg/g (37). In Canada the reported median values for infants <1 year old (n = 16) and for children 1-10 years of age (n = 10) were 25.5 µg/g and 248 µg/g, respectively (38). In New Zealand, where liver stores are extremely high, median liver vitamin A levels increased from 31 µg/g at birth to 49 µg/g at < 1 year of age and then reached 595 µg/g in the 1-10 year old age group (39). In two studies in the United States (40-41) *mean* values (medians were not cited) for small groups (n = 8, 12) of children < 10 years of age were 171 µg/g and 304 µg/g. In another study carried out in the United States (42), the median values for a few children (n = 5, 17) 2-12 months and 1-10 years of age were 387 µg/g and 177 µg/g, respectively. The percentages < 5 µg/g clearly were low but not zero in these latter surveys. In none of these limited studies, however, has careful attention been given to changes of vitamin A concentration as a function of age.

The present sample seems to be representative of all children of like age dying of various causes in the Salvador region. First of

all, the major cited causes of death for the 0-4-year-old age group in our sample and in the whole population closely correspond (Table 3). In making this comparison, one must bear in mind that autopsy reports tend to be concise, and consequently the interaction of contributing factors such as malnutrition, to the severity of infectious disease tend to be overlooked. Secondly, the sample used for our study was clearly drawn from the whole Salvador region, and not solely from specific economically deprived areas of the city. And finally the samples were obtained more or less at the same rate over a 6-month period, which would minimize the effects of seasonal influences, acute economic deprivation, or epidemics.

The relation of our sample to the living population is much more difficult to define, and can at best only be approximated. First of all, chronic liver and kidney diseases are rare among young children; indeed such causes were never cited in our sample of 0-4-year-old children. Thus a low dietary intake of carotenoids and vitamin A must account for most cases of very low vitamin A reserves. Secondly, the percentages of children at high risk and with inadequate reserves in the family-supported and indigent groups were essentially the same, even though the frequency of high values, and consequently the mean, was higher in the family-supported group. Thirdly, early weaning (3-6 months) is a common practice in the Salvador region, and postweaning foods often consist of cassava gruel, molasses and perhaps some diluted milk or mashed banana (43). Thus, conventional weaning and postweaning feeding practices of a significant segment of the Salvador population would tend to produce a marginal state of vitamin A nutriture in infants. Finally, the income distribution in the Salvador region is strongly skewed towards the lower income groups. For example, in 1971 the percentage of families earning less than one or two so-called "minimal" salaries (170 cruzeiros per month in 1971) were 16 and 42%, respectively, whereas the family size for these groups averaged about four persons (44). Thus, a significant percentage of the population is in an economic category characterized as well by a below average intake of protein, fat and vitamin A rich foods, such as meats, fresh milk, eggs, butter and vegetable oils (44). In all likelihood, therefore, children who die of various causes early in life are only slightly worse off nutritionally than a several-fold larger segment of the population of like age who survive.

The possible relationship of the present sample to the living

population can also be approached in a more quantitative way. Of the two million people living in the Salvador region, the steady-state size of the 0-4-year-old age group would be about 12%, or 240,000 (44). The annual mortality rate in children up to 1 year is roughly 10%, or 6,000 of 60,000 infants; whereas that for children 1-4 years of age is about 1%, or 1,800 of 180,000 children (31, 44).

Now a major assumption is made in this analysis, namely that *the number of surviving children 0-4 years old who are in essentially the same nutritional state, is twice the number of those who died.* Among children 0-4 years old who died (Table 1), 50% had inadequate reserves ($\leqslant$ 20 µg/g) and 18% were at high risk ($<$ 5 ug/g). Thus, among surviving children 0-4 years old in the Salvador region, about 7,800 might have inadequate reserves and 2,800 might be at high risk. By extrapolation to the whole population, over 3% of all children 0-4 years of age might have inadequate reserves and over 1% might be at high risk.

Inasmuch as liver reserves are low at birth and only reach satisfactory levels with proper nutrition at about 3 months of age (35, 36), the above estimate should be modified. The percentages of children from 0.25-4 years of age with inadequate and high risk reserves are 59% and 27%, respectively. If 3,000 infants die within the first 3 months of life and the above major assumption is retained, the number of children 0.25-4 years old with inadequate and high risk reserves would be about 5,700 and 2,600, respectively. By extrapolation, about 2.4% of all children 0.25-4 years old in the whole population would have inadequate reserves and over 1% would be at high risk.

These estimates, it must be stressed, are based on the above-states assumptions. The actual vitamin A status of the living population in these age groups might be decidedly better or significantly poorer than that suggested. Data from liver autopsy studies clearly have intrinsic limitations as an epidemiological tool and cannot be used in a more predictive way than that described. In the absence of more definitive information, however, the above analysis might serve for the present as a rough guide to the extent of the vitamin A problem in the Salvador region.

Investigations of plasma levels of vitamin A in northeastern Brazil tend to support the viewpoint that a sizable number of infants and young children are at significant risk relative to vitamin A deficiency (1-4, 6, 15). Dietary surveys in the state of Pernambuco have also indicated that the average daily intake of

vitamin A is roughly 30°/o of the recommended daily allowance for very young children (1, 9, 15, 20). On the other hand, the Salvador region differs from most other areas of the northeast in that red palm oil, which contains 0.5 mg of α– and β–carotene per ml, is a common constituent of the diet. Thus the *per capita* intake for all ages of retinol equivalents in Salvador is around 900 µg/day, or roughly 150°/o of the RDA (11, 15).

In Salvador, red palm oil accounts for 54°/o of all sources of vitamin A and its precursors, with only 5°/o coming from eggs, 9°/o from milk products, 7°/o from legumes and vegetables and 1°/o from fruits. Upon correcting these percentages for differences in total vitamin A intake, a smaller percentage of carotenoids and vitamin A is derived from these other conventional sources in Salvador than in Recife, Rio de Janeiro, and other major urban centers of Brazil (11, 15). As already noted, the poor in the Salvador region tend to ingest less vegetable oils and dairy products than those more economically advantaged (44). Inasmuch as red palm oil costs roughly twice as much as corn oil and considerably more than lard, it is probably used sparingly among a significant segment of the poorer economic group despite its general cultural acceptance.

Thus, the major conclusions of the present study, namely that an appreciable number of children between 3 months and 4 years of age are inadequately nourished in vitamin A, whereas the adult population, other than those suffering from chronic liver and kidney disease, are in a satisfactory status, are concordant with most other studies conducted in the Northeast.

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RESUMEN

LAS RESERVAS DE VITAMINA A EN EL HIGADO DE RECIEN NACIDOS, PREESCOLARES, Y ADULTOS QUE MUEREN POR DIVERSAS CAUSAS EN SALVADOR, BRASIL

Se analizaron 141 muestras de hígado de individuos con edades que fluctuaban desde niños prematuros hasta 82 años, del distrito metropolitano de Salvador, Bahía, Brasil, para determinar su contenido de vitamina A y carotenoides. Los infantes prematuros, los nacidos muertos o los de corta vida (<6 días) acusaron una reserva hepática media de 24 μg/g de retinol, con sólo 3% de alto riesgo ($\leqslant 5$ μg/g). La concentración media de vitamina A descendió después de las 6 semanas de edad hasta un valor aproximado de 10 μg/g en el grupo de 3 a 12 meses de edad, y luego ascendió de nuevo a $\sim$25 μg/g en los niños de 1 a 4 años. El porcentaje de niños en la categoría de alto riesgo ($\leqslant 5$ μg/g) aumentó a cerca de 30% en aquéllos de 3 semanas a 2 años de edad, y luego declinó. Las principales causas de muerte notificadas para el grupo de edad de 1 semana a 4 años fueron, gastroenteritis (33%), bronconeumonía (29%), y deshidratación (19%), ya fuese solas o combinadas. Los niños que sufrieron deshidratación, con o sin gastroenteritis, acusaron el porcentaje más alto (45%) de muy bajas reservas hepáticas de vitamina A ($\leqslant 5$ μg/g).

Los niños mayores (de 4 a 15 años de edad) tenían reservas medias de 80 μg/g. De los tres niños en este grupo, con reservas hepáticas <10 μg/g, dos murieron de esquistosomiasis aguda del hígado, y uno de gastroenteritis. Las reservas hepáticas de todos los adultos ($\geqslant 15$ años) fueron satisfactorias (>10 μg/g) salvo 4 personas que padecían de enfermedades severas del hígado o del riñón. El valor de la mediana en el caso de los adultos fue de 57 μg/g, lo que concuerda con valores notificados en otros países.

Las concentraciones medias de carotenoides en el hígado de los recién nacidos fueron bajas (~ 3 μg/g), y permanecieron en alrededor de 4 μg/g hasta los 4 años de edad. Luego, la concentración aumentó hasta alcanzar una media global de alrededor de 15 μg/g en los adultos. Aunque las concentraciones de vitamina A y carotenoides en el hígado guardaron una correlación positiva, la varianza fue grande, el coeficiente de correlación fue bajo, y el valor de P fue alto.

La muestra de los niños estudiados por nosotros, que murieron entre las edades de 0 a 4 años, parece ser representativa de todos los niños de la misma edad que en la región de Salvador mueren por diversas causas. Sin embargo, la relación de la presente muestra a la población viviente de edades semejantes, no puede definirse con precisión. Si se asume que el estado nutricional del doble del número de niños sobrevivientes de 0 a 4 años de edad es el mismo que el de aquéllos de edades similares que mueren por diversas causas, entonces cerca del 3% de niños de 0 a 4 años de edad de la población general de Salvador, tienen reservas inadecuadas de vitamina A y están cercanos a un alto riesgo de 1%.

RESUMO

AS RESERVAS DE VITAMINA A NO FIGADO DE RECEM NASCIDOS, CRIANÇAS PRE-ESCOLARES E ADULTOS MORTOS POR VARIAS CAUSAS EM SALVADOR, BRASIL

No distrito metropolitano e nos arredores de Salvador, Bahía, Brasil, foram analisados, quanto a vitamina A e carotenoides, espécimes de fígado de 141 indivíduos cujas idades iam de infantes prematuros a oitenta e dois anos. Os infantes prematuros, os nascidos mortos, ou os que viveram por pouco tempo ($<$ 6 dias) tinham uma reserva hepática média de 24 μg/g de retinol, com apenas 3% em grave périgo ($\leqslant$ 5 μg/g). Depois de 6 semanas de idade, a concentração média de vitamina A baixou, chegando a um valor de cerca de 10 μg/g no grupo de 3 - 12 meses de idade, e depois, subiu de novo para $\sim$25 μg/g de 1 - 4 anos de idade. A porcentagem de crianças na categoria de grave périgo ($\leqslant$ 5 μg/g) aumentou para 30% entre 3 semanas e 2 anos de idade, e depois diminuiu. As causas de morte mais frequentemente citadas no grupo de 1 semana a 4 anos de idade formam: gastrenterite, broncopneumonia e desidratação, occorrendo sozinhas ou conjuntamente. Crianças sofrendo de desidratação, com ou sem gastrenterite, tinham a maior porcentagem (45%) de reservas hepáticas muito baixas ($\leqslant$ 5 μg/g) de vitamina A.

As crianças mais velhas (4 - 15 anos) tinham reservas médias de 80 μg/g. Das tres crianças neste grupo com reservas hepáticas de 10 μg/g, duas morreram de esquistossomíasis aguda do fígado, e uma, de gastrenterite. As reservas hepáticas de todos os adultos ($\geqslant$15 anos) examinados foram satisfatórias ($>$ 10 μg/g), com exeção de 4 pessoas com sérias enfermidades do fígado e dos rins. O valor mediano para adultos foi de 57 μg/g, comparável a valores constatados em outros paises.

Concentrações médias de carotenoide no fígado de recém-nascidos foram baixas ($\sim$3 μg/g) e continuaram for volta de 4 μg/g até a idade de 4 anos.

Dai em diante, a concentração aumentou até uma média total de 15 μg/g em adultos.

Embora as concentrações de vitamina A e carotenoides no fígado fossem positivamente correlacionadas, a variação foi grande, o coeficiente de correlação, baixo e o valor de P, alto.

Nossa amostra, de crianças que morreram entre 0 e 4 anos de idade, parece ser representativa de tôdas as crianças de mesma idade que morreram de várias causas na região de Salvador. A relação entre a presente amostra e população viva da mesma idade, não pode ser, no entanto, precisamente definida. Supondo-se que a condição nutricional do *dobro* do número de crianças sobreviventes, de 0–4 anos de idade, seja a mesma que a das crianças da mesma idade que morreram de diversas causas, então, cerca de 3% das crianças de 0—4 anos de idade na população total de Salvador têm reservas inadequadas de vitamina A e cerca de 1% estão em categorias de grave périgo.

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