THE VANDERBILT COOPERATIVE STUDY OF MATERNAL AND INFANT NUTRITION [1]

X. ASCORBIC ACID

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INTRODUCTION

The basic findings of the Vanderbilt cooperative study of maternal and infant nutrition have been reported (Darby et al., '53a, b; McGanity et al., '54), but little of the detail of the interrelationships of various factors was included. Analyses of the interrelationships of biochemical and dietary data in large groups of persons are rare. Such analyses are necessary, however, to the soundest interpretation of nutritional data. Accordingly, we have studied each of several nutrient groups separately and explored numerous possible relations. This paper deals with the dietary intake and the serum levels of ascorbic acid, the variation in each of these with time of gestation, season of the year, and with age, parity, height, weight, and physical findings of the mother. The variation in serum vitamin C with intake levels and the

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relation of both intake and serum levels to the course and outcome of pregnancy and to lactation are also explored. The data are for 2,129 consecutively encountered pregnant women, the outcome of the pregnancy being observed during the course of the investigation. The methods employed have been described in the earlier reports.

OBSERVATIONS AND DISCUSSION

Intake levels. The distribution of recorded intakes of vitamin C is given in table 1. This is based on a one-week diet record obtained from each patient once during each trimester of pregnancy. A wide range in reported intake is apparent, and there occurs a slight overall decrease in intake level from the first to the third trimester.

Median consumption in the study group was lower than the allowances recommended by the Food and Nutrition Board ('53) of 70 mg per day for adult women and 100 mg per day during the third trimester of pregnancy. Of those who had diet records for both the second and third trimesters, 14% reported daily intakes of less than 40 mg of vitamin C on both occasions, and 2% reported intakes of less than 20 mg on both records. Only 15% had intakes of 80 mg or more on both records.

Serum levels. As previously reported (Darby et al., '53b), serum vitamin C levels declined during pregnancy and were lowest at the time of the postpartum examination. Distributions are shown in table 1. Postpartum values were clearly lower for lactating than for non-lactating women, but a corresponding difference between these groups was not observed during pregnancy. In other words, the low values of serum ascorbic acid which are associated with lactation appear to be a reflection of lactation rather than the result of a pre-existing difference between those who lactated and those who did not.

Serum vitamin C levels during pregnancy and lactation have been studied by many investigators (Ingalls et al., '38;

TABLE 1

Distribution of dietary intakes and serum levels of vitamin C by period of the reproductive cycle

INTAKE			
Intake of vitamin C	First trimester	Second trimester	Third trimester
mg/day	*Number of women*		
0– 19	12	76	174
20– 39	49	205	374
40– 59	59	308	392
60– 79	61	244	308
80– 99	34	174	180
100–119	33	91	104
120–139	13	69	68
140–159	9	27	24
160–179	5	14	15
180–199	2	4	10
200 and over	1	9	16
Total subjects	278	1221	1665
Median intake (mg/day)	66	62	54

SERUM LEVELS					
Serum vitamin C	First trimester	Second trimester	Third trimester	Postpartum [1] Lactating	Postpartum [1] Non-lactating
mg/100 ml			*Number of women*		
Under 0.20	72	315	638	510	235
0.20–0.39	80	289	458	158	179
0.40–0.59	53	236	316	67	84
0.60–0.79	56	217	235	51	58
0.80–0.99	32	146	163	24	49
1.00–1.19	23	95	89	13	22
1.20–1.39	17	48	41	2	7
1.40–1.59	1	17	12	1	2
1.60–1.79	3	3	5	1	1
1.80–1.99	1	1	2		
2.00 and over		2	1		
Total subjects	338	1369	1960	827	637
Median (mg/100 ml)	0.46	0.47	0.35	0.16	0.29

[1] Postpartum data do not include women who had abortions or twins.

Teel et al., '38; Snelling and Jackson, '39; Lund and Kimble, '43; Anderson et al., '46; Young et al., '46; Munks et al., '47; Hoch and Marrack, '48; and Moyer et al., '54). The present analysis considers the influence of both intake levels and period of gestation on serum levels and hence offers an opportunity to extend our interpretation of such data.

Seasonal pattern. Seasonal variation occurred in both the intake and the serum vitamin C content (figs. 1, 2). The months of lowest recorded intake during the second trimester were January, February, June, July and September, and in the third trimester June, July, August, September and October. Serum vitamin C levels were lowest in the period of February through June. In other words, the season (July-September) of highest blood levels was a period when recorded intakes were relatively low. This inconsistency will be considered again in a later section. We have no explanation as to why the variation in intake with season was not identical for the second and third trimesters.

Correlation of values obtained for the same patient in different trimesters. In nutrition surveys or in the clinical assessment of nutriture the use of a one-week diet record or of a single laboratory determination is based on the assumption that each of these gives a reasonably reliable measure of the intake or serum level for the period in question. If the values vary greatly from one time to another, then a single record is obviously inadequate. It is of interest, therefore, to compare the records of the same patient in different trimesters in order to observe the constancy of findings over a time interval.

There is a moderate degree of correlation between the recorded vitamin C intake of the same individuals in different trimesters, as well as between serum vitamin C levels taken at different times. Tables 2 and 3 typify the degree of these correlations. Although there were some individuals having high values at one time and low values at another, the majority of cases showed reasonably good agreement. However, because of the large differences observed in some cases, it seemed

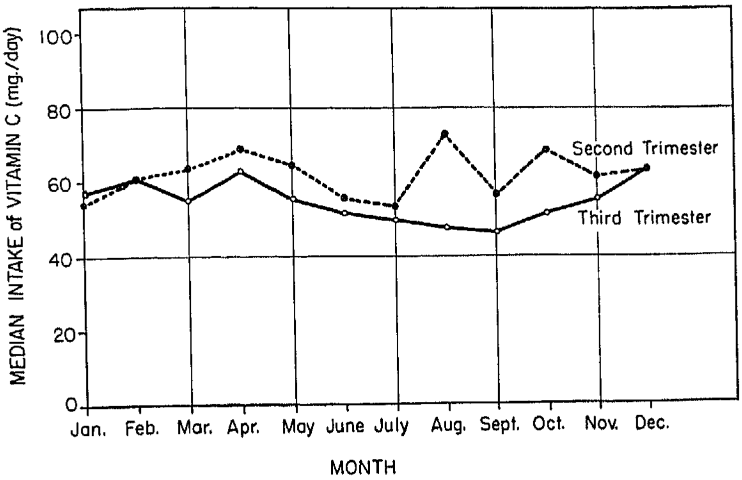

Fig. 1 Median dietary intake of vitamin C by month.

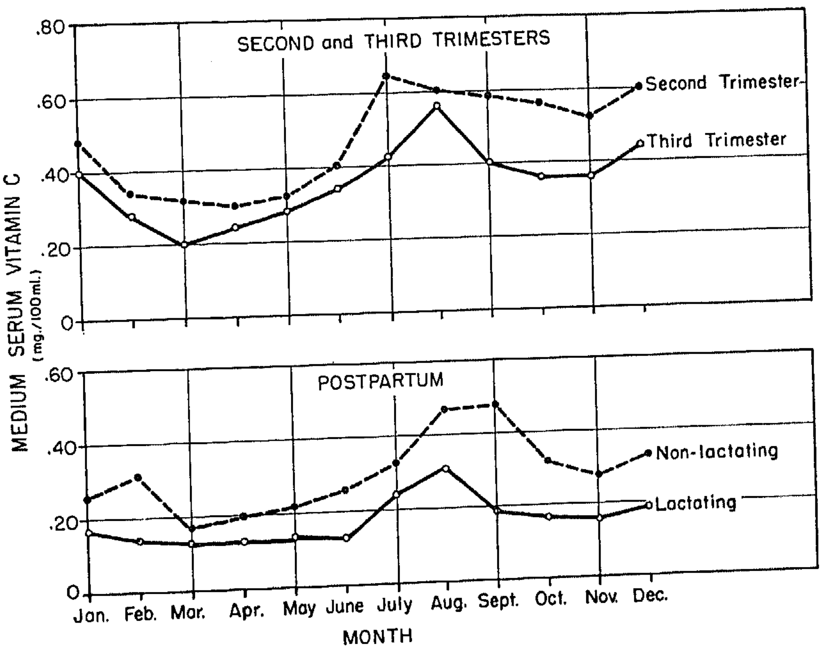

Fig. 2 Median serum levels of vitamin C by month.

likely that a comparison between those individuals who had either low or high values on each of two occasions might yield sharper contrasts than comparison made on the basis of a single determination. Consequently, in the latter part of

TABLE 2

Relation of dietary intakes of vitamin C in second and third trimesters

(Winter and spring seasons)

THIRD TRIMESTER INTAKE (MG/DAY) (SPRING SEASON)	SECOND TRIMESTER INTAKE (MG/DAY) (WINTER SEASON)							
	Under 20	20–39	40–59	60–79	80–99	100 and over	Total number patients	Median
	Number of cases							
Under 20	3	6	8	3	2		22	45
20–39	6	16	16	11	4	3	56	48
40–59	2	8	12	11	12	6	51	66
60–79	2	3	10	6	10	9	40	77
80–99	2	2	6	3	6	6	25	77
100 and over		1	6	7	6	16	36	93
Total no. patients	15	36	58	41	40	40	230	63
Median	35	35	48	52	64	87	55	

TABLE 3

Relation of serum levels of vitamin C in second and third trimesters

(Winter and spring seasons)

THIRD TRIMESTER LEVELS OF SERUM VITAMIN C (MG/100 ML) (SPRING SEASON)	SECOND TRIMESTER LEVELS OF SERUM VITAMIN C (MG/100 ML) (WINTER SEASON)						
	Under 0.20	0.20–0.39	0.40–0.59	0.60–0.79	0.80 and over	Total number patients	Median
	Number of cases						
Under 0.20	51	27	12	8	8	106	0.21
0.20–0.39	10	22	17	6	9	64	0.40
0.40–0.59	10	9	12	8	8	47	0.48
0.60–0.79	3	3	4	7	4	21	0.61
0.80 and over	3	3	4	5	26	41	0.96
Total no. patients	77	64	49	34	55	279	0.40
Median	0.15	0.25	0.35	0.48	0.72	0.30	

this paper groups having "consistenly" low, medium or high intakes or serum levels of vitamin C are reported.

Correlation of serum vitamin C with vitamin C intake. Serum levels of vitamin C vary with recorded intake, but the degree of correlation is not high (table 4). However, there were distinct differences in average values of serum vitamin C for groups at different intake levels. In each trimester daily intakes were grouped as follows: less than 40 mg, 40 to 79 mg, 80 to 119 mg, and 120 mg and over. Median serum vitamin C values were determined for each intake group, postpartum values being studied in relation to third trimester intakes. Cases were limited to those for whom the interval between the diet record and the serum determination was not more than three weeks (except for the postpartum group), and subdivision of the data by season (on the basis of laboratory values) was made as follows: winter (January through March), spring (April through June), summer (July through September), and fall (October through December). Table 5 gives median serum vitamin C values for the third trimester. Data for second trimester and postpartum show similar differences. Median serum levels increased with increasing intake. In addition, clear seasonal differences in the levels in the blood for similar recorded intakes were present, except possibly in the first trimester. For a given nutrient level, serum vitamin C values were highest in summer followed by fall, winter and spring, in that order. These seasonal differences were statistically significant except those between winter and spring. This same phenomenon was reflected in the difference in seasonal patterns between nutrient and serum vitamin C levels noted above. Among the influences which may contribute to these seasonal inconsistencies are seasonal variation in the vitamin C content of some foods (for which no allowance was attempted in calculations), and seasonal shifts in the composition of the intake groups. It is also possible that there is seasonal variation in vitamin C requirements, or in certain metabolic processes which affect serum levels of vitamin C.

Correlation of serum vitamin C with other laboratory determinations. Serum vitamin C levels were positively correlated with serum carotene levels and to a slight extent with serum vitamin A levels.[2] Intakes of vitamin C and carotene were also correlated. This is not unexpected, inasmuch as dietary

TABLE 4

Relation between nutrient intake and serum levels of vitamin C, winter season, third trimester

SERUM VITAMIN C	INTAKE OF VITAMIN C (MG/DAY)					
	Under 40	40–79	80–119	120 and over	Total	Median intake
mg/100 ml			*Number of cases*			
Under 0.39	107	91	45	14	257	47
0.40–0.79	26	51	22	14	113	66
0.80–1.19	8	19	18	10	55	81
1.20 and over	4	5	5	7	21	95
Total patients	145	166	90	45	446	57
Median serum level	0.21	0.36	0.40	0.62	0.33	

TABLE 5

Median levels of serum vitamin C in relation to nutrient intake, by season, third trimester

INTAKE	MEDIAN SERUM VITAMIN C				NUMBER OF CASES			
	Winter	Spring	Summer	Fall	Winter	Spring	Summer	Fall
mg/day		*mg/100 ml*						
Under 40	0.21	0.20	0.36	0.30	145	133	125	109
40–79	0.36	0.34	0.52	0.44	166	191	141	145
80–119	0.40	0.31	0.61	0.54	90	78	41	58
120 and over	0.62	0.57	0.80	0.52	45	31	14	35
All intake levels	0.33	0.29	0.46	0.40	446	433	321	347

sources of carotene and of ascorbic acid are often the same — especially greens of various sorts.

Serum vitamin C levels were also positively correlated with urinary excretion values, after a test dose, of niacin and, to perhaps a slight degree, of riboflavin and thiamine.

[2] Martin, Bridgforth, McGanity and Darby, unpublished data.

These correlations seem to reflect a corresponding correlation in intakes.

There is no apparent relationship between ascorbic acid level and serum proteins.

A positive relationship between certain hematological values and the intakes and serum levels of vitamin C will be discussed in the sections on "consistent" groups.

Parity and other factors. With increasing parity there occurred a decrease in both vitamin C intakes and serum levels. Data for the third trimester are shown in table 6. The trend in vitamin C level in the serum associated with

TABLE 6

Median intakes and serum vitamin C levels by parity, third trimester

PARITY	DAILY INTAKE	NUMBER OF CASES	MEDIAN SERUM VITAMIN C	NUMBER OF CASES
	mg		*mg/100 ml*	
0	56	546	0.42	621
1–2	56	645	0.36	752
3–4	52	230	0.28	274
5 and over	48	213	0.24	272

parity seems to be over and above that associated with differences in recorded intakes. It may reflect the depleting effect of successive pregnancies (and lactation). The present data, however, do not permit a decisive analysis of this possibility.

No association was found between serum ascorbic acid content and age, height, or weight of mother.

Vitamin C in relation to physical findings and disease conditions in the mother. The study and interpretation of data on physical findings were complicated by the differences among examiners in the frequency with which a given condition was diagnosed (McGanity et al., '54). Nevertheless, two physical findings, gingival changes and edema, seemed possibly to exhibit some relationship to vitamin C nutriture.

The percentage of cases with gingival lesions (red or swollen gums or pyorrhea) was 23, 24, 28, and 27 for successive periods from the first trimester through the postpartum. The incidence of gingival changes was higher among women with low concentrations of vitamin C in their serum but the differences were not large. Gum findings ran high in the women of higher parities, averaging about 40% for those of parity three and over, and there was little relation to dietary intakes or serum levels of vitamin C in this group. At the lower parities there was some evidence of a relation. For example, when groups with "consistent" levels of serum vitamin C (see below) were compared, the percentages with gingival findings in the second trimester were found to be 21, 17 and 8 for low, medium and high concentrations in the serum, respectively, for parity two and under. These differences are significant ($P = 0.002$). There were not significant differences in the other trimesters or postpartum.

A significant relationship between gingival findings and intake of vitamin C appeared only when groups with "consistently" low, medium, and high vitamin C intakes (see below) were compared on the basis of the number having gum findings on two or more physical examinations. Among women of parity two or less with "consistently" low vitamin C intakes, 19% had gum findings on at least two examinations. Among the "consistently" medium and high intake groups the corresponding percentages were 14 and 9, respectively. Thus it seems likely that vitamin C may play a role in gingival lesions, but that it is not of major importance.

Linghorne et al. ('46) in an experiment in which dietary intake was controlled, showed a relationship between the occurrence of gingivitis and the intake of vitamin C, but they found no effect of the administration of vitamin C on pre-existing gingivitis. Our data are in agreement with these findings. While ascorbic acid nutriture may be a factor in the development of gingivitis in young mothers of low parity, other factors are primarily responsible for the continuation of gingivitis observed among the older multiparae.

The number of women having edema increased from 2.4% in the first trimester to 10.8% in the third and returned postpartum to 2.1%. Only in the second trimester was there a significant relationship between serum level of ascorbic acid and edema. We were unable to detect a relationship between intake of vitamin C and the occurrence of edema in any trimester, even among the "consistent" groups. We, therefore, conclude that vitamin C is not an important factor in the occurrence of edema in our study group.

It was found (McGanity et al., '54) that women with anemia during pregnancy had lower intakes and serum levels

TABLE 7

Frequency of occurrence of single, live-born premature infants according to intake and serum levels of vitamin C in the third trimester of pregnancy

VITAMIN C INTAKE	TOTAL CASES	PREMATURES		SERUM VITAMIN C	TOTAL CASES	PREMATURES	
		No.	*%*	*mg/100 ml*		*No.*	*%*
mg/day							
Under 20	170	12	7.1	Under 0.20	622	34	5.5
20–39	371	10	2.7	0.20–0.39	449	19	4.2
40–59	385	8	2.1	0.40–0.79	537	17	3.2
60–99	475	14	2.9	0.80 and over	311	11	3.5
100 and over	233	6	2.6				
Total	1634	50	3.1	Total	1919	81	4.2

of vitamin C than the rest of the study group. Also, women with disease conditions complicating pregnancy [3] had somewhat lower intakes and serum levels during pregnancy. Relation of vitamin C to hematologic values will be considered again later in the present paper.

Vitamin C in relation to the course and outcome of pregnancy. As reported in a previous paper (McGanity et al., '54), mothers of premature [4] infants had lower serum vitamin C levels and lower intakes of vitamin C during pregnancy,

[3] Tuberculosis, venereal disease, endocrine disorders, heart disease, genito-urinary diseases, and miscellaneous other conditions (McGanity et al., '54).

[4] Premature infants are those having a birth weight of 2500 gm or less.

the lowering being significant in both the second and third trimesters. The increased frequency of occurrence of premature birth is limited to the group with intakes of vitamin C below 20 mg. Table 7 shows the results for single live-born infants for the third trimester. In the case of serum vitamin C levels, differences are not as large but it appears that the percentage of premature infants is higher for the group with the lowest serum levels. These differences are not necessarily indicative of a causal relation between vitamin C and premature birth since the group with low intakes and serum levels of vitamin C may have been different in other respects from the rest of the study group. The data do suggest an area for further study.

It is of interest to note that mothers of twins had significantly lower serum vitamin C levels in the second and third trimesters, while vitamin C intakes were not significantly different from those of the total study group. Cases of pre-eclampsia had generally lower intakes of all nutrients, including vitamin C, than the total study group. These patterns are believed to be reflections of the illness. There was no tendency for a difference in either vitamin C intakes or serum levels during pregnancy between mothers who later breast-fed their infants and those who did not. As previously noted there were differences in serum levels postpartum. Serum vitamin C levels postpartum tended to be high in those conditions, such as diabetes, where the mother did not nurse her infant.

Lund and Kimble ('43) found no relation of plasma vitamin C levels to the occurrence of toxemia, puerperal morbidity, or duration of labor in 197 cases. They concluded that low values found among cases of hyperemesis probably occurred as a result of the illness. We concur with this position.

It has been reported (Greenblatt, '53; and Javert, '54) that women who were habitual aborters responded favorably to a regime including supplements of vitamin C in the diet along with other measures. In the present study there was no indication of lowered vitamin C nutriture in the 40 women who aborted. Twenty-three of these had serum vitamin C determinations in the first trimester, and 18 in the second trimester. The distributions did not differ from those for the total study group. Although but a few had diet records, there was no indication of any unusual intake.

Consistent dietary intake groups. As noted earlier, individuals having high (or low) intakes on two or more diet records probably represent a group whose level of intake was generally high (or low). Study of such groups might be expected to show relationships not apparent when classification is based on a single diet record. Consequently, cases having two or more diet records were divided into subgroups as follows:

(1) "consistently low group"—166 cases having intakes under 40 mg on at least two diet records;

(2) "consistently intermediate group"—262 cases having intakes of 40 to 80 mg on at least two diet records;

(3) "consistently high group"—193 cases having intakes of 80 mg or more on at least two diet records:

(4) cases not falling into any of the above groups.

Cases for whom three diet records were available were included in the above groups (1), (2), or (3) if two out of three records satisfied the necessary conditions.

Variation in serum levels of vitamin C for the consistent intake groups. For each of the "consistent" intake groups the median values of serum vitamin C were determined by weeks of gestation in each of two broad seasons, February through June, a period of low blood levels, and July through January, a period of high serum vitamin C levels. Postpartum values were further subdivided according to whether or not the mother was breast-feeding her infant. These are shown in table 8. The small size of the group of cases at 34 weeks gestation and over accounts for the irregularities in this group.

Differences by weeks gestation and weeks postpartum. The group with consistently high intakes maintained essentially the same average serum vitamin C levels throughout pregnancy but exhibited lowering of the concentrations during the postpartum period.

The group with consistently intermediate intakes showed a decrease in serum concentration between early and late pregnancy. It appears that their intake was sufficient to maintain their average level at about the same value as that of the high intake groups during the first trimester, but was not adequate to maintain these levels throughout pregnancy.

TABLE 8

Median levels of serum vitamin C by weeks gestation and postpartum within consistent intake groups

SEASON	LEVEL OF VITAMIN C INTAKE	WEEKS GESTATION					POSTPARTUM	
		13 and under	14–19	20–26	27–33	34 and over	Not nursing	Nursing
		Median serum vitamin C (*mg/100 ml*)						
Feb.–June	Low	0.18	0.20	0.28	0.14	0.12	0.14	0.12
	Medium	0.54	0.38	0.30	0.34	0.16	0.24	0.12
	High	0.46	0.68	0.58	0.54	0.80	0.36	0.22
July–Jan.	Low	0.40	0.38	0.58	0.30	0.54	0.24	0.16
	Medium	0.52	0.60	0.56	0.40	0.44	0.36	0.18
	High	0.52	0.76	0.56	0.64	0.44	0.48	0.28
		Number of cases						
Feb.–June	Low	24	41	39	71	7	19	20
	Medium	43	52	58	98	20	33	55
	High	28	29	40	85	8	36	43
July–Jan.	Low	32	42	44	77	10	31	55
	Medium	66	75	75	129	11	58	63
	High	41	58	63	85	13	34	42

Values in the postpartum period showed a further decline, significant only for the lactating group.

The group with consistently low intakes had low serum levels early in pregnancy, and a further decrease as pregnancy advanced. Postpartum values were still lower, although in the February-June season 70% of the cases already fell into the lowest grouping interval during the third trimester, so that there was not much opportunity for observing a further decline.[5]

While differences between third trimester and postpartum values of non-lactating women were not statistically significant within all the individual subgroups, the differences were in the direction of lower values in the postpartum period, and the combined effect of all intake groups in both seasons is significant.

Lactating women had lower serum C levels than non-lactating women in the same intake group, except for those with the lowest intakes where serum C levels of both lactating and non-lactating women were low.

In the consistently high intake group, there were 80 cases who had intakes of 100 mg or over on two or more diet records. During pregnancy their levels were not different from the remainder of the consistently high intake group, but 32 cases who were not nursing their infants at the time of the postpartum laboratory determination had median serum vitamin C levels of 0.70 mg/100 ml. This was significantly higher than the postpartum level of non-lactating women in the remainder of the consistently high intake group. The women who had had intakes of 100 mg or more and who were lactating at the time of the postpartum check-up had median levels of 0.30 mg/100 ml, which is not significantly different from the levels at somewhat lower intakes. Moreover, a group of 18 lactating women whose intakes had been

[5] Differences in median serum C levels between consistent intake groups were not statistically significant in the first trimester except that women with low intakes had lower serum C levels than those with medium and high intakes during the period February through June. In the second trimester, differences were definitely significant in the period February through June, and in the period July through January, those on low intakes had significantly lower serum C levels than those with medium and high intakes. Differences between all intake groups were clearly significant in the third trimester. During the postpartum period differences between intake groups were significant for both lactating and non-lactating women, except that there was little difference between low and medium intake groups among lactating women, both groups having very low serum C levels.

120 mg or more on at least two diet records during pregnancy had median postpartum levels of 0.27 mg/100 ml.

Within each intake group there was considerable individual variation in serum level about the median values, which may be due in part to individual differences in requirements. It is therefore difficult to interpret the results in terms of individual needs, but the figures indicate that on the average a dietary intake level of less than 40 mg was inadequate to support what might be regarded as acceptable concentrations of serum ascorbic acid during pregnancy or lactation. The intermediate daily intake range of 40 to 80 mg did support high levels of serum ascorbic acid early in pregnancy, but failed to do so during the latter part of gestation or during lactation. High serum concentration of vitamin C was supported throughout pregnancy by the consumption of 80 to 100 mg, but during the immediate postpartum period these levels were not attained unless the intake of vitamin C exceeded 100 mg per day. Finally, it appears that intakes in excess of even 120 mg per day did not maintain the average serum level of lactating women above about 0.3 mg.

With the knowledge available at present, it is not possible to say what levels of vitamin C in the serum are optimal, or whether any impairment of health results from lower levels. There is apparently a considerable margin of safety. In deprivation studies (Medical Research Council, '53) about 100 days elapsed between the virtual disappearance of vitamin C from the plasma and the appearance of the first clinical signs of scurvy.

Course and outcome of pregnancy in the consistent low, intermediate and high intake groups. Differences between the three intake groups for each of the following special conditions were within the limits of chance variation: hyperemesis, eclampsia and pre-eclampsia, puerperal fever, stillbirth, neonatal death, birth of single premature [6] infants, and congenital malformation (table 9). There were more cases of

[6] See footnote 4, page 211.

disease conditions [7] in the low intake group, somewhat fewer in the medium intake group, and the smallest number in the high intake group. Low, medium and high groups were also compared for weeks gestation at delivery, labor complications, length of labor, various types of operative deliveries, lacerations occurring at delivery, placenta weight, birth weight of the infant, and type of infant feeding, whether breast or artificial. No significant difference was found. These findings for the "consistent" groups are confirmatory of the analysis

TABLE 9

Occurrence of special conditions in consistent vitamin C intake groups

SPECIAL CONDITIONS	VITAMIN C INTAKE GROUP					
	Low		Medium		High	
	No.	*%*	*No.*	*%*	*No.*	*%*
Disease complicating pregnancy	23	13.9	25	9.5	11	5.7
Hyperemesis	2	1.2	5	1.9	1	0.5
Eclampsia	1	0.6	3	1.1	1	0.5
Pre-eclampsia	12	7.2	14	5.3	9	4.7
Puerperal fever	8	4.8	21	8.0	8	4.1
Stillbirth	1	0.6	3	1.1	0	0
Neonatal death	3	1.8	4	1.5	3	1.6
Live-born premature, excluding twins	8	4.8	5	1.9	8	4.1
Congenital malformations	5	3.0	9	3.4	4	2.1
Total number in group	166		262		193	

of the whole study group (McGanity et al., '54), except for findings relating to premature birth.

Failure to find differences in the number of premature births was surprising in view of the increased incidence of this condition among mothers with intakes under 20 mg, reported above. However, in the present broader classification most of the low group had intakes above the 20 mg level, where no relationship was found. There were 23 women who had intakes of under 20 mg in both the second and third trimesters, and three of these delivered premature infants.

Relation to hematologic values. In view of the lower vitamin C intakes and serum levels among women with anemia

[7] See footnote 3, page 211.

during pregnancy, it is of interest to compare hematologic values for the consistent intake groups, and at the same time to consider associated differences in iron intake.

In the low vitamin C intake group the average hemoglobin, packed cell volume, mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration, but not the red cell count, were lower than in the other two groups. There was also a difference of about 4 mg in the daily intake of iron between the low and high vitamin C intake groups. In order to judge whether or not differences in hematologic values were attributable to associated differences in iron intake, those women with iron intakes of 10 mg or more in both second and third trimesters were selected within each of the vitamin C intake groups. Mean hematologic values in the third trimester for each of these groups are shown in table 10. Comparison of low and high groups showed significant differences in the second trimester for hemoglobin levels ($P = 0.03$), mean cell hemoglobin ($P = 0.02$) and for mean cell hemoglobin concentration ($P = 0.01$). The only difference which was significant in the third trimester was packed cell volume ($P = 0.05$). No significant differences existed postpartum. We are not able to say whether or not the small observed differences in hematological values are directly associated with vitamin C nutriture. They are of interest in view of the recent reports of Steinkamp, Dubach and Moore ('55) and of Moore ('55) on the effect of ascorbic acid on the absorption of radioiron. Interpretation of our observations is complicated by the possibility that the general abundance of the dietary of women in the high intake category is greater.

Consistently low, intermediate and high serum vitamin C groups. Cases were divided into low, medium and high serum vitamin C groups on the basis of second and third trimester determinations:

(1) "consistently low group" — 442 cases having serum vitamin C levels under 0.40 mg/100 ml in both the second and third trimesters or between 0.40 and 0.60 mg/100 ml in the second trimester and under

TABLE 10

Hematologic values in consistent vitamin C intake groups and in consistent serum vitamin C groups, third trimester

(Cases with iron intakes of 10 mg/day or more second and third trimesters)

CONSISTENT GROUP	NO. OF CASES	HEMOGLOBIN	RED BLOOD COUNT	PACKED CELL VOLUME	MEAN CELL VOLUME	MEAN CELL HEMOGLOBIN	MEAN CELL HEMOGLOBIN CONCENTRATION
		gm/100 ml	*million/mm*^{3}	%	μ^3	$\mu\mu g$	%
Vitamin C intake							
Low	67	11.44 ± 0.16	3.89 ± 0.05	34.6 ± 0.4	88.6 ± 1.0	29.24 ± 0.41	32.94 ± 0.25
Medium	199	11.55 ± 0.10	3.92 ± 0.03	35.0 ± 0.2	90.2 ± 0.6	29.76 ± 0.24	33.06 ± 0.14
High	164	11.72 ± 0.10	3.93 ± 0.03	35.5 ± 0.3	90.5 ± 0.7	30.02 ± 0.26	33.04 ± 0.16
Serum vitamin C							
Low	242	11.44 ± 0.09	3.92 ± 0.03	34.6 ± 0.2	88.8 ± 0.5	29.32 ± 0.22	32.98 ± 0.13
Medium	139	11.61 ± 0.11	3.96 ± 0.04	35.0 ± 0.3	89.3 ± 0.7	29.54 ± 0.29	33.08 ± 0.17
High	128	11.69 ± 0.12	3.87 ± 0.04	35.3 ± 0.3	91.4 ± 0.7	30.40 ± 0.30	33.12 ± 0.18

0.20 mg/100 ml in the third trimester; those having serum levels under 0.20 mg/100 ml in both the second and third trimesters were later separated out from this "low" group and designated as the "very low" group (162 cases);

(2) "consistently intermediate group" — 249 cases having serum levels of vitamin C between 0.40 and 0.80 mg/100 ml in the second trimester and between 0.20 and 0.80 mg/100 ml in the third trimester;

(3) "consistently high group" — 198 cases with serum vitamin C levels of 0.80 mg/100 ml or more in either second or third trimester and 0.60 mg/100 ml or more in the other trimester.

The occurrence of various special conditions within the three groups is shown in table 11. The following conditions did not differ between the groups: hyperemesis, eclampsia and pre-eclampsia, stillbirth, neonatal death or congenital

TABLE 11

Occurrence of special conditions in consistent serum vitamin C groups

SPECIAL CONDITIONS	CONSISTENT SERUM C GROUPS									
	Very low		Medium low		Total low		Medium		High	
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Disease complicating pregnancy	23	14.2	29	10.4	52	11.8	30	12.0	12	6.1
Hyperemesis	3	1.9	2	0.7	5	1.1	2	0.8	3	1.5
Eclampsia	2	1.2	1	0.4	3	0.7	3	1.2	1	0.5
Pre-eclampsia	10	6.2	17	6.1	27	6.1	12	4.8	6	3.0
Premature separation of placenta	2	1.2	7	2.5	9	2.0	1	0.4	0	0
Puerperal fever	6	3.7	23	8.2	29	6.6	15	6.0	5	2.5
Stillbirth	2	1.2	3	1.1	5	1.1	5	2.0	1	0.5
Neonatal death	3	1.9	7	2.5	10	2.3	2	0.8	4	2.0
Live-born premature, excluding twins	13	8.0	9	3.2	22	5.0	10	4.0	4	2.0
Congenital malformations	1	0.6	14	5.0	15	3.4	2	0.8	7	3.5
Total number in group	162		280		442		249		198	

malformation. Puerperal fever was significantly lower in the group with highest serum levels, allowance being made for differences by season and parity in both serum vitamin C and in the occurrence of puerperal fever. No relation of puerperal fever to intake of vitamin C was observed, nor was any relation to serum levels apparent when data for single trimesters were studied. We conclude that ascorbic acid nutriture is not a major factor in the occurrence of puerperal fever.

Differences in per cent of premature[8] single births and disease conditions complicating pregnancy[9] were not statistically significant on the basis of the initial division of cases; however, when the low group was further subdivided so as to separate out 162 cases having values of less than 0.20 mg/100 ml in both trimesters, both the percentage of cases of premature single live births and percentage of cases having complicating diseases were significantly higher in the low group. The disease conditions showing higher than average frequency in this group were (a) venereal disease or a history of syphilis (9 cases), and (b) pyelitis, cystitis, pyelonephritis, and pyonephrosis (5 cases).

Among labor complications there was a significant difference for premature separation of the placenta (table 11). Serum vitamin C levels were not related to the gestation time at delivery. However, within the group of women who gave birth to premature infants (on the basis of birth weight) there was a tendency for the cases in the group with low serum vitamin C to have shorter gestations. Sixteen of 22 single live-born prematures in the low group had gestations of less than 38 weeks. For the medium group the corresponding numbers were 5 of 10, and for the high group none of 4. (Premature single live-birth was associated with premature separation of the placenta in only two cases.) Consistent serum vitamin C groups were also compared for length of labor, various types of operative deliveries, lacerations oc-

[8] See footnote 4, page 211.
[9] See footnote 3, page 211.

curring at delivery, placenta weight, birth weight of the infant, and type of infant feeding. No significant difference was found.

Hematologic values varied with serum vitamin C levels in a manner similar to that discussed above for intake values. Comparison of low and high groups (with iron intake of 10 mg or more in both second and third trimesters) showed significant differences for packed cell volume in all trimesters, and for hemoglobin, mean cell volume and mean cell hemoglobin in the third trimester (table 10 shows data for the third trimester).

In evaluating these results it should be noted that a causal relationship cannot be established from data of this type. Groups of cases selected on the basis of one characteristic, e.g. serum levels of vitamin C, will be expected to differ in other characteristics. In interpreting the data, we have allowed for differences recognized to exist, such as those for parity or season of the year, but it is obvious that other differences may be present. Such possibilities cannot be ruled out in an observational study.

SUMMARY

Data on intakes and serum levels of vitamin C in 2,129 pregnant women are studied in relation to many factors, including the course and outcome of pregnancy.

In general, serum levels decreased during pregnancy except in the group at a high level of intake. Values were further decreased postpartum, and were lower for lactating than for non-lactating women. Evidence is presented that on the average intakes of 80 to 100 mg daily supported high levels of ascorbic acid in the serum during pregnancy. The serum levels of non-lactating mothers averaged 0.7 mg per 100 ml during the puerperium on intakes (during pregnancy) of 100 mg or over per day; the serum concentration of lactating mothers did not average greater than 0.3 mg even on intakes exceeding 120 mg daily.

Analysis of findings relative to the health of the mother and baby revealed only 5 categories which may possibly be associated with ascorbic acid nutriture: hematologic findings, gingivitis, premature separation of the placenta, premature birth, and puerperal fever. Increased frequency of premature birth was limited to the lowest intake levels and lowest serum concentrations. In none of the conditions was there a strong relation to both intakes and serum levels. Hence we believe that ascorbic acid nutriture is at most a contributary factor in any of these.

LITERATURE CITED

ANDERSON, R. K., W. D. ROBINSON, J. CALVO AND G. C. PAYNE 1946 Nutritional status during pregnancy and after delivery of a group of women in Mexico City. J. Am. Diet. Assoc., *22:* 588.

DARBY, W. J., ET AL. 1953a The Vanderbilt cooperative study of maternal and infant nutrition. I. Background. II. Methods. III. Description of the sample and data. J. Nutrition, *51:* 539.

——— 1953b The Vanderbilt cooperative study of maternal and infant nutrition. IV. Dietary, laboratory and physical findings in 2,129 delivered pregnancies. Ibid., *51:* 565.

FOOD AND NUTRITION BOARD 1953 Recommended Dietary Allowances. National Research Council, Publication No. 302, Washington, D. C., p. 19.

GREENBLATT, R. B. 1953 Habitual abortion, possible role of vitamin P in therapy. Obstet. and Gynecol., *2:* 530.

HOCH, H., AND J. R. MARRACK 1948 The composition of blood of women during pregnancy and after delivery. J. Obstet. Gynaecol. Brit. Empire, *55:* 1.

INGALLS, T. H., R. DRAPER AND H. M. TEEL 1938 Vitamin C in human pregnancy and lactation. II. Studies during lactation. Am. J. Diseases Children., *56:* 1011.

JAVERT, C. T. 1954 Repeated abortion, results of treatment in 100 patients. Obstet. and Gynecol., *3:* 420.

LINGHORNE, W. J., W. G. MCINTOSH, J. W. TICE, F. F. TISDALL, J. F. MCCREARY, T. G. H. DRAKE, A. V. GREAVES AND W. M. JOHNSTONE 1946 The relation of ascorbic acid intake to gingivitis. Can. Med. Assoc. J., *54:* 106.

LUND, C. J., AND M. S. KIMBLE 1943 Some determinants of maternal and plasma vitamin C levels. Am. J. Obstet. Gynecol., *46:* 635.

MCGANITY, W. J., ET AL. 1954 The Vanderbilt cooperative study of maternal and infant nutrition. V. Description and outcome of obstetric sample. VI. Relationship of obstetric performance to nutrition. Am. J. Obstet. Gynecol., *67:* 491.

Medical Research Council, Vitamin C Subcommittee of the Accessory Food Factors Committee 1953 Vitamin C requirement of human adults. Spec. Rept. Ser., No. 280.

Moore, C. V. 1955 The importance of nutritional factors in the pathogenesis of iron-deficiency anemia. Am. J. Clin. Nutrition, *3:* 3.

Moyer, E. Z., H. J. Kelly, I. G. Macy, H. C. Mack, P. C. DiLoreto and J. P. Pratt 1954 Nutritional status of mothers and their infants. Children's Fund of Michigan, Detroit, Michigan.

Munks, B., M. Kaucher, E. Z. Moyer, M. E. Harris and I. G. Macy 1947 Metabolism of women during the reproductive cycle. XI. Vitamin C in diets, breast milk, blood and urine of nursing mothers. J. Nutrition, *33:* 601.

Snelling, C. E., and S. H. Jackson 1939 Blood studies of vitamin C during pregnancy, birth, and early infancy. J. Pediat., *14:* 447.

Steinkamp, R., R. Dubach and C. V. Moore 1955 Studies in iron transportation and metabolism. VIII. Absorption of radioiron from iron-enriched bread. Arch. Int. Med., *95:* 181.

Teel, H. M., B. S. Burke and R. Draper 1938 Vitamin C in human pregnancy and lactation. I. Studies during pregnancy. Am. J. Diseases Children., *56:* 1004.

Young, J., E. J. King, E. Wood, and I. D. P. Wootton 1946 A nutritional survey among pregnant women. J. Obstet. Gynaecol. Brit. Empire, *53:* 251.