

THE STATISTICAL ASSOCIATION BETWEEN THE DIET RECORD OF ASCORBIC ACID INTAKE AND THE BLOOD CONTENT OF THE VITAMIN IN SURVEYED POPULATIONS¹

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I. A STATISTICAL RECONNAISSANCE

INTRODUCTION

EARLY in 1939 the International Health Division of The Rockefeller Foundation entered the field of nutrition with a grant to the Vanderbilt University School of Medicine. Later field studies were inaugurated in North Carolina under the auspices of the State Board of Health and Duke University. In 1942 similar studies, also supported by the International Health Division, were undertaken in Mexico under the auspices of the Ministry of Public Health and Welfare.

These field investigations were designed to furnish a picture of the individual's food consumption and of his nutritional and physical status at a given time. One objective was to determine whether a relationship existed between consumption, as derived from the diet record, the biochemical measure of the nutritional status of the individual, and his physical well-being. In other words, would persons in groups with poor diets have a lower average rating in the laboratory examinations and exhibit signs of clinical deficiency with greater frequency than would individuals with more adequate diets? Such an eventuality may be expected only if the diet record affords a reasonably accurate statement of the amount of the nutrient ingested, if the amount ingested during the week of the survey is similar to that customarily consumed, if the laboratory findings do in fact reflect its concentration in the body, and if there exists a

¹The data analyzed were obtained in surveys made (1) under the auspices of the North Carolina State Board of Health and Duke University, and (2) the Ministry of Public Health and Welfare of Mexico and its subdivisions, with the assistance of the International Health Division of The Rockefeller Foundation.

direct relationship between physical well-being and the quantity of the nutrient consumed.

The frequently observed lack of association between nutritional status and recorded dietary intake has been disappointing to investigators, and attempts have been made to explain the phenomenon. Kruse (1) calls attention to the time element involved in the development of tissue evidence of deficiency states. Blood plasma level of some nutrients may vary from week to week. It may be below normal for some time before tissue lesions appear and may return to normal long before they heal. Nutrition Reviews (2) makes the following comment on the inadequacy of the instantaneous field survey:

It would appear that there is a fundamental fallacy inherent in the short-term nutrition survey in which dietary history or careful records of food intake, physical examinations, biochemical and microbiologic determinations are carried out in so short a span. . . . The clinical signs which are diagnostic of deficiency disease usually result from protracted deficiencies; the biochemical status at an instant in time is a composite of underlying tissue stores or deficits and the balance between recent losses and recent accruals from intake; and the dietary story is accurate only for the particular time when records are kept.

Dann and Darby (3) define five zones of nutriture: (1) saturation; (2) unsaturated, but functionally unimpaired; (3) potential deficiency disease; (4) latent deficiency disease, and (5) clinically manifest deficiency disease. They comment:

A deduction frequently made in the past is that for every manifest case of deficiency disease caused by severe dietary deficiency, many persons must consume diets deviating less widely from completeness and therefore be suffering either from mild, or latent, or potential deficiency disease. Many attempts have therefore been made (a) to refine the methods of clinical diagnosis of mild and latent deficiency disease; (b) to devise biochemical or physiological tests which will detect potential deficiency disease. Unfortunately these attempts have met with little success.

Examination of the data collected in the various population surveys in which the International Health Division was associated revealed relatively few individuals with recognizable deficiency states of a serious character. Although intake as measured by the diet record may have been unsatisfactory in many instances, any untoward effect upon the nutriture of the individual lay chiefly within the realm of unsaturation or of potential or latent deficiency disease which cannot be adequately assessed by present-day clinical methods suitable for application in large population groups.

THE PRESENT OBJECTIVE

Dietary, biochemical, and clinical findings have been presented in the published reports of the various field surveys, but no previous report has described an attempt to explore the statistical association between the amount of a nutrient consumed, as given by the diet record, and its level in blood serum or plasma as revealed by the laboratory examination in these surveyed populations. The object of this paper is to report the results obtained from the application of statistical method to the data from these surveys to determine the existence, the amount and the form of such a relationship.

THE INITIAL APPROACH TO THE PROBLEM

A preliminary analysis was made of the data provided by the diet records and the laboratory tests for persons in white families who were included in the surveys of Wayne and Alamance Counties of North Carolina to determine their interrelationships. Data pertaining to persons surveyed in each of the four seasons were kept separate and the population was further subdivided into three age and sex groups, males and females under 15 years of age, males of 15 years and over and females of 15 years and over. There were thus twelve population groups for each county or twenty-four in all.

For each of these twenty-four groups three series of correlation tables were set up. In the first, the results of the laboratory

tests were compared, e.g., the blood content of ascorbic acid with that of vitamin A, vitamin A with carotene, etc. Correlation coefficients for seven pairs of variables were computed. The results will not be reported in detail, but significant correlation was obtained with sufficient frequency to warrant the conclusion that individuals with a satisfactory rating biochemically in respect to one nutrient might be expected to rate well for at least some of the others. An instance of this was the significant correlation found for fourteen of the twenty-four population groups between plasma vitamin C and carotene.

In the second series of correlations a comparison was made between fourteen pairs of nutrients in the food consumed, as given by the diet records. Many of these comparisons yielded significant correlation of a fairly high order. This was to be expected, however, since many foods, e.g., milk or leafy vegetables, are rich in more than one nutrient, and if consumed in large quantities, will raise the intake level of each of their constituents.

The crucial series of correlations, however, was that in which the nutrients in the diet were paired with those in the blood. Here the significant coefficients were few in number. The only comparison giving consistently significant correlation (fifteen of twenty-four population groups) was that between ascorbic acid intake and blood content. This result was not unexpected, for although vitamin C is stored in the body tissues, it must be frequently ingested if the blood levels are to be maintained.

The present analysis will, therefore, attempt to describe the various aspects of this relationship. Means and standard deviations have been computed for the two variables (ascorbic acid in the diet and in the blood) together with the correlation coefficients which indicate the amount of association between them. Chi-square tests have been applied to the data arranged in fourfold tables. Finally, regression equations have been calculated and straight lines plotted to the data for populations grouped on an area basis. The objective has been not only to determine the existence and nature of the relationship but to

show in some detail the steps in the statistical approach to this problem.

SURVEY MATERIAL AND METHODS

The data for Wayne County, North Carolina, used in this analysis were collected in 1942–1943 and pertain to persons in 120 white families from the rural sections of eleven of twelve townships. In the summer of 1943 a similar survey was begun of 160 white families in Alamance County. Procedures and results in respect to dietary intake of nutrients and to clinical manifestations of deficiency have been reported for the surveys in both counties (4 and 5a, b). The 1943–1944 Mexican survey for which data have been analyzed was that of the Otomi Indians, living in four villages in the Mezquital Valley about 75 miles from Mexico City (6).

The diet records of persons included in this study covered a seven-day period from which average daily amounts of calories, and specific nutrients consumed by each person were computed with the use of food tables. The ascorbic acid content of food consumed by persons surveyed in the North Carolina counties was computed for raw foods only because of the belief that the customary prolonged cooking of vegetables destroyed most of the vitamin, while in the Mexican survey no correction was made for cooking losses since the most common foods containing vitamin C were either eaten raw or seldom cooked excessively. The amount of ascorbic acid consumed by the North Carolinians was, therefore, understated while that estimated for the Mexican Indians was perhaps overstated. The Otomi Indians obtained 48 per cent of their ascorbic acid from pulque, the fermented juice of the century plant (from 3 to 5 per cent alcohol), which is consumed in large quantities by persons at all ages but particularly adults.

Standard sources were used in North Carolina in preparing tables for converting food consumed into its component nutrients, but the dietary calculations in Mexico were based largely on the analysis of foods collected locally during the period of the survey (7). For the data collected in Mexico and in Wayne

Table 1. Means of ascorbic acid blood plasma content and of recorded daily food intake, with standard deviations and correlation coefficients for surveyed white population groups in North Carolina.

POPULATION GROUP	ASCORBIC ACID						CORREL. COEFF.
	NO. PERSONS	PLASMA (MGM %)		INTAKE (MGM)		CORREL. COEFF.	
		MEAN	S. D.	MEAN	S. D.		
WAYNE CO. 1942-1943							
<i>Summer</i>							
M, F - 15	35	0.89	0.29	50.6	54.4	+ 0.592*	
M 15 +	23	0.60	0.42	38.0	46.2	+ 0.365	
F 15 +	26	0.54	0.30	23.5	22.6	+ 0.248	
TOTAL	84	0.70	0.42	39.3	45.9	+ 0.432*	
<i>Fall</i>							
M, F - 15	37	0.90	0.38	30.9	28.0	+ 0.481*	
M 15 +	28	0.40	0.30	30.4	32.3	+ 0.028	
F 15 +	41	0.70	0.36	28.1	21.1	+ 0.338*	
TOTAL	106	0.68	0.40	29.8	26.8	+ 0.267*	
<i>Winter</i>							
M, F - 15	49	0.61	0.40	31.9	22.6	+ 0.180	
M 15 +	30	0.41	0.15	24.0	21.7	+ 0.572*	
F 15 +	30	0.48	0.30	28.0	18.9	+ 0.622*	
TOTAL	109	0.49	0.37	28.3	21.4	+ 0.390*	
<i>Spring</i>							
M, F - 15	18	0.61	0.40	26.1	26.4	+ 0.714*	
M 15 +	15	0.42	0.46	16.8	11.8	+ 0.449	
F 15 +	31	0.35	0.36	19.8	16.0	+ 0.371*	
TOTAL	64	0.44	0.41	21.9	17.9	+ 0.521*	
GRAND TOTAL							
M, F - 15	139	0.76	0.40	35.4	35.6	+ 0.426*	
M 15 +	96	0.43	0.37	28.1	32.1	+ 0.310*	
F 15 +	128	0.53	0.37	23.7	20.0	+ 0.404*	
TOTAL	363	0.59	0.41	30.2	30.4	+ 0.395*	
ALAMANCE CO. 1943-1944							
	40	1.01	0.34	46.0	25.7	+ 0.738*	
	36	0.68	0.46	46.9	30.1	+ 0.397*	
	52	0.68	0.37	40.2	23.8	+ 0.390*	
	128	0.78	0.42	43.9	26.5	+ 0.394*	
	42	1.04	0.48	47.1	28.0	+ 0.031	
	31	0.52	0.32	31.1	25.5	+ 0.206	
	44	0.70	0.32	27.2	17.3	+ 0.284	
	117	0.78	0.45	35.6	25.4	+ 0.246*	
	64	0.72	0.42	43.6	28.7	+ 0.303*	
	20	0.34	0.31	47.5	38.1	+ 0.151	
	42	0.48	0.31	41.2	33.4	+ 0.478*	
	126	0.58	0.40	43.4	32.0	+ 0.285*	
	35	0.76	0.35	48.1	28.6	+ 0.511*	
	34	0.31	0.25	40.6	23.7	+ 0.451*	
	51	0.52	0.41	43.2	23.4	+ 0.471*	
	120	0.53	0.39	43.9	25.3	+ 0.466*	
	181	0.87	0.43	45.8	27.9	+ 0.279*	
	121	0.48	0.39	41.2	29.6	+ 0.276*	
	189	0.59	0.37	38.2	25.7	+ 0.336*	
	491	0.66	0.43	41.7	27.7	+ 0.416*	

County each food was evaluated separately, but certain similar foods were grouped in the tables used for Alamance County and an average value was assigned to each of the nutrients contained in the foods of the group.

Ascorbic acid in the blood was determined by the macro-method described by Mindlin and Butler (8). In North Carolina the determinations were based on plasma, in Mexico on serum. Here reference should be made to the discussion by Wiehl and Kantorovitz (9) of the errors in the photometric method of determining ascorbic acid in the plasma. These authors obtained a standard deviation of approximately 0.04 mgm per cent for estimates of the plasma content when the amount of ascorbic acid in the standard employed was 1.5 mgm per cent when K of the formula was computed. In North Carolina and Mexico an average value for K was computed daily from the results obtained from three concentrations of the standard, 1.0, 1.5 and 2.0 mgm per cent.

Records included in this study pertain to individuals with data on both dietary intake and blood examination. Because of this requirement records of children under two years of age have been omitted. Records of pregnant and lactating women are included, although it has been shown (10) that the blood content of ascorbic acid of such persons is reduced. Consequently the members of the various groups may not be strictly homogeneous in respect to intake necessary to maintain a given blood content.

STATISTICAL ATTRIBUTES OF THE POPULATION GROUPS

North Carolina. Table 1 contains the statistics pertaining to ascorbic acid intake and plasma content for each of the twenty-four population groups in Wayne and Alamance Counties. In this table are included the number of individuals in each group, the mean plasma content of ascorbic acid and standard deviation, the mean intake and standard deviation, together with the coefficient of correlation between intake and plasma level.

Among families surveyed in any specified season, the mean

ascorbic acid content of the plasma was higher among children than it was among adults. Indeed, this is the notable difference among the plasma means, since differences between those for adult males and females were not conspicuous. The summer and fall surveys revealed higher mean plasma levels than did those conducted in winter and spring. In general, Wayne County means were lower than the corresponding ones for Alamance County.

The standard deviations of the distributions contained in Table 1 measure the degree of dispersion of the observed values given by the individuals in the various groups. In respect to plasma ascorbic acid they indicate that persons were similarly distributed in each county. The standard deviations for individual groups varied from 0.15 to 0.48 mgm per cent and were all above the 0.04 expected minimum error in the laboratory determination (9).

The average daily intakes of ascorbic acid ranged widely. Those for Wayne County were lower, giving a final average for all persons in the study of only 30.2 mgm, as compared with one of 41.7 for Alamance County. In general, the average intakes of children were somewhat higher than those for adults. The summer intake means for Wayne County, particularly that for children, were above those of the other seasons.

For Wayne County the standard deviations of intake values of individual groups varied from 11.8 to 54.4 mgm, with an overall average of 30.4 mgm. Those for Alamance varied from 17.3 to 38.1 mgm, with an average for the county of 27.7 mgm. Much of the difference between the two counties, however, lies in the wider dispersion of intake values among children in the two summer surveys. It seems unlikely, therefore, that the greater variability in the Wayne County data is attributable to the fact that foods were not grouped when the amount of the nutrient was calculated.

Significant correlation ($P < 0.05$) between ascorbic acid content of plasma and recorded intake was obtained for six of the eight groups of children and ranged from +0.303 to +0.738.

Significant correlation was also found for six of the eight groups of females of 15 years and over. There were only three instances of significant correlation among adult males, although two of the coefficients, those for Wayne County summer and spring surveys, might have been significant had the groups been larger. When age and sex were disregarded and the data summarized by season, higher correlation was obtained for data collected in summer and spring surveys in both counties. When season was disregarded and the data summarized by age and sex, significant coefficients were obtained. Finally, when the data for each county were assembled in a single table, the coefficients were still significant: + 0.395 for Wayne and + 0.416 for Alamance County.

The significance of a correlation coefficient depends not only on its size but also on the number of persons in the group to which it pertains. Hence coefficients for seasonal and age-sex subtotals are significant, although of a lower order than some of those obtained for the small subgroups which were not significant. It is noteworthy that the highest coefficients were obtained when sex, age and season were simultaneously considered.

Mexico. Corresponding statistics for the three age and sex groups of Otomi Indians are contained in Table 2, with season-

Table 2. Means of ascorbic acid blood serum content and of recorded daily food intake with standard deviations and correlation coefficients for surveyed groups of Otomi Indians, Mexico, 1943-1944.

POPULATION GROUP	NUMBER OF PERSONS	ASCORBIC ACID				Correl. Coef.
		Serum (mgm %)		Intake (mgm)		
		Mean	S.D.	Mean	S.D.	
M,F, - 15	267	1.21	0.41	51.0	38.1	+ 0.218*
M 15 +	183	1.20	0.46	164.5	102.5	+ 0.123
F 15 +	249	1.19	0.38	107.4	66.8	+ 0.147*
TOTAL	699	1.20	0.41	100.8	83.2	+ 0.110*

* P < 0.05.

ality omitted. Here the average ascorbic acid content of the blood serum was 1.21 mgm per cent for children and similarly high for adults. The standard deviation for all persons surveyed was 0.41 mgm per cent and thus well above the variation inherent in laboratory procedures.

The mean daily intake of ascorbic acid for the Indian children was 51.0 mgm, while that for adult males was 164.5 and for females 107.4 mgm, both means well above the National Research Council's recommended daily allowances of from 70 to 75 mgm.

In view of the high blood content and intake of vitamin C it is not surprising that little correlation was obtained. With serum level at or near the renal threshold much of the ingested vitamin may have been excreted. The two significant coefficients, those for children and adult females, were too low to have any real meaning.

Summary. Since it is known that the administration of large amounts of ascorbic acid will increase the amount of the vitamin in the blood, the discovery of some statistical correlation between intake, as measured by the diet record, and plasma or serum content is not surprising and must be accepted as an indication that the diet records do furnish some measure of the amount of vitamin C consumed. Confirmation of this appeared recently in a paper by Kaser *et al.* (11) in which the correlation between calculated amounts of ascorbic acid in diets from a local field survey and those determined in the laboratory for 80 sample diets containing the same foods was found to be + 0.7.

Several factors must affect the amount of correlation observed: (a) inaccuracies in the diet records and in their conversion into values of the nutrient consumed; (b) the exclusion of cooked foods from the computations in North Carolina and their inclusion without adjustment in Mexico; (c) the lag in time between ingestion of the nutrient and its appearance or disappearance from the blood; (d) the inclusion of records for a few pregnant and lactating women; and (e) especially with

the Otomi Indians, the high serum and intake levels of the vitamin which probably induced excretion of the excess.

A FOURFOLD TABLE ANALYSIS

Persons engaged in nutritional surveys are familiar with the chi-square test as applied to a fourfold table. In such a table subjects with clinical symptoms of a deficiency are divided into two groups, one containing those with a laboratory rating with respect to the nutrient concerned at or above a certain level, and the other containing persons with ratings below this level. Persons without symptoms are similarly divided for comparison in making the test. The chi-square calculated from such a table indicates whether or not association may be said to exist between the presence of symptoms and the laboratory rating as defined. The test does not indicate the amount or form of the association.

An examination of the survey data used in this analysis has been made by this method. The records were subdivided into those with intake of ascorbic acid at 40 mgm or above and those with intakes below this level. On the blood content scale a division was made at 0.6 mgm per cent. One fourfold table for each area is contained in Table 3.

The large chi-square obtained for each North Carolina group indicates that a significantly greater proportion of persons with low ascorbic acid intakes are found in the low blood content group and also that a larger proportion of individuals with low blood level falls into the low intake group, so there is significant association between intake and plasma content of the vitamin.

A comparison of the fourfold table arrangement of the data for the Mexican Otomi Indians with those for the North Carolinians indicates that only 8.2 per cent of the Indians had intakes of less than 40 mgm as compared with 75.8 per cent of the persons surveyed in Wayne County and 55.6 per cent of Alamance County residents. The proportion of persons in the Indian group with low blood level of the vitamin (36.6 per cent) was also less than the corresponding percentages for the

BLOOD LEVEL (mgm %)	ASCORBIC ACID INTAKE (MGM)							
	0-39	40 +	Total	Per Cent 0-39	0-39	40 +	Total	Per Cent 0-39
	WAYNE CO., N. C.				ALAMANCE CO., N. C.			
Under 0.6	181	27	208	87.0	165	78	243	67.9
0.6 +	94	61	155	60.6	108	140	248	43.5
TOTAL	275	88	363	75.8	273	218	491	55.6
Per Cent -0.6	65.8	30.7	57.3		60.4	35.8	49.5	
	$X^2 = 33.6 P < 0.01$				$X^2 = 29.5 P < 0.01$			
	OTOMI INDIANS, MEXICO							
Under 0.6	30	226	256	11.7				
0.6 +	27	416	443	6.1				
TOTAL	57	642	699	8.2				
Per Cent -0.6	52.6	35.2	36.6					
	$X^2 = 6.85 P < 0.01$							

Table 3. A fourfold table analysis of data pertaining to ascorbic acid blood level and intake, from population surveys in three areas.

North Carolinians. The chi-square of 6.85 for the Indians indicates some significant association between intake and blood levels.

Since vitamin C deficiency is likely to occur among persons with low intake and low blood content, the fourfold table analysis furnishes a ready method of determining the possible magnitude of the deficiency problem and its relation to intake in a surveyed population. The wide dispersion of the observed ascorbic acid intakes and blood levels pertaining to the individuals in these population groups is eliminated, however, when the data are distributed in this manner. Furthermore, the variability in individual observations may reduce the amount of correlation and limit its use statistically. We shall now return to a consideration of the correlation coefficients and of the regression equations to be derived from them.

THE REGRESSION EQUATIONS

A significant positive correlation coefficient indicates that,

on the average, blood content of the vitamin rises as intake increases. It also signifies that intake may be expected to increase as the blood level rises. Both of these relationships must be considered and may be expressed in regression equations which are computed from the standard deviations, the correlation coefficient and the mean values of blood level and intake. The form of the equation is as follows:

$$y = a + bx \quad (1)$$

in which y = the average or expected blood content

x = a specified intake value

a = the blood content when intake is zero

and b = the rise in blood level per milligram increase in intake, i.e., the slope of the line

This equation may be modified to express the regression of intake on blood level, thus:

$$x = a' + b'y \quad (2)$$

in which a' = the intake when the blood level is zero

and b' = the rise in intake per milligram per cent increase in blood level, i.e., the slope

These regressions are in the form of straight lines and if the correlation is of a high order may, within limits, have a prediction value when groups of persons with similar attributes are considered. The first equation enables one to compute the expected blood level of the vitamin for any specified intake value, the other the expected intake for a particular blood level. The low order of the correlation coefficients obtained for the area-wide population groups described here will not permit the application of the equations for prediction purposes to other population groups, but they will be presented and discussed briefly with reference to the observed data.

“GOODNESS OF FIT” OF THE REGRESSION EQUATIONS

North Carolina. The raw data for all persons included in the surveys of Wayne and Alamance Counties are contained in the correlation tables in Figures 1 and 2, with the equations entered and the regression lines plotted. Examination of these tables

BLOOD PLASMA CONTENT (MG/M PER CENT) = y	ASCORBIC ACID INTAKE (MG/M) = x											TOTAL	
	0	20	40	60	80	100	120	140	160	180	200		220
1.8				1									1
1.6		1		1	1								3
1.4		2	1				1					1	5
1.2	5	3	4	3	2	1			2				20
1.0	13	12	10		2		1						41
.8	18	10	8	2	3	1			1	1			44
.6	19	11	7	3	1								41
.4	32	25	6	2	1			1					67
.2	34	18	4	6									62
0	60	12	6		1								79
TOTAL	181	94	46	21	11	2	2	1	3	1		1	363

$\text{EXPECTED } y = 0.4305 + 0.005264 x \quad r = +0.395 \pm 0.053$
 $\text{EXPECTED } x = 12.74 + 29.57 y$

Fig. 1. Wayne County, North Carolina. Distribution of persons surveyed, according to diet record of ascorbic acid intake and blood plasma level.

indicates that the large proportion of the observations falls into the low intake-low plasma content categories, a fact that was obvious when the fourfold tables were analyzed. However, the observations in both tables extend over a range of plasma categories extending from 0 to 1.8 mgm per cent. Observations on intake are also concentrated at the lower end of the scale with only a few entries in either table of intakes of 100 mgm or over. Obviously, these North Carolinians were not distributed symmetrically with respect either to intake or to plasma level of ascorbic acid.

The raw data presented in Figures 1 and 2 emphasize "poor-ness" rather than "goodness of fit" inasmuch as there is little apparent drift of the observations along the regression lines. The actual trend of the data is best described when the means

	ASCORBIC ACID INTAKE (MG M) = x										TOTAL
	0	20	40	60 x	80	100	120	140	160	180	
1.8			2	1							3
1.6		3	2	1	1	1					y 8
1.4		5	2	3	1	2					13
1.2	6	5	12	10	4	1					38
1.0	7	15	12	15	6		1			1	57
.8	9	19	18	10	8	2	1				67
.6	13	26	10	8	3	2					62
.4	13	23	22	10	4						72
y											
.2	36	39	14	2		2	1				94
0	28	26	15	4	2		1			1	77
TOTAL	112	x 161	109	64	29	10	4			2	491

EXPECTED $y = 0.3950 + 0.006469x$ $r = +0.416 \pm 0.045$

EXPECTED $x = 23.94 + 26.78y$

Fig. 2. Alamance County, North Carolina. Distribution of persons surveyed, according to diet record of ascorbic acid intake and blood plasma level.

of the rows and columns in the tables are plotted with the regression lines, as has been done in Figures 3 and 4. The "goodness of fit" can now be examined.

The Wayne County diagram in Figure 3 indicates that the observed means of plasma ascorbic acid content for successive intake categories lie along the regression line ($y-y$) for intakes under 60 mgm. At higher intake levels the plasma means are dispersed over a wide range of values which are actually based on observations from a very few persons. At intakes of 140 mgm and over all plasma means lie below the regression

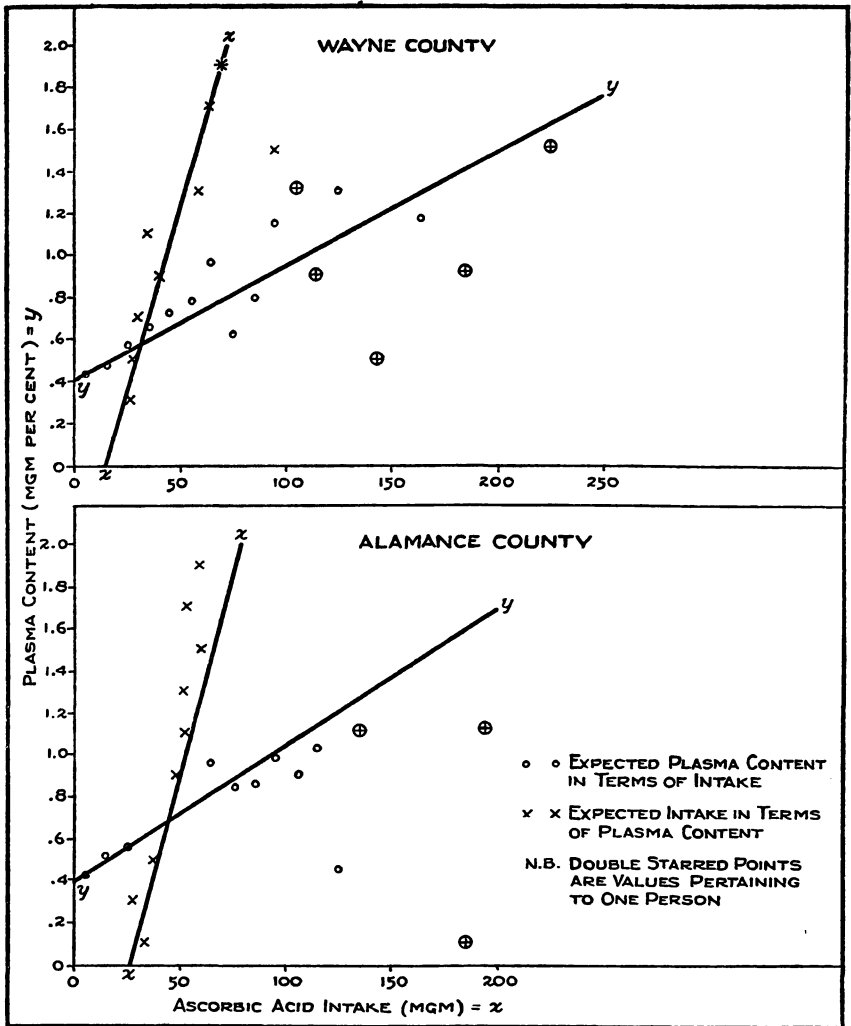


Fig. 3. North Carolina. Comparison of the trend of observed means with that of the corresponding rectilinear regression line for all surveyed persons.

line instead of on either side of it. In other words, the actual rise in plasma ascorbic acid content per milligram increase in intake diminishes at higher intake levels so that the trend of the means is curvilinear not rectilinear. The plasma means for Alamance County reveal this nonlinearity more clearly, inasmuch as all values for intakes of 70 mgm and over fall below the computed line.

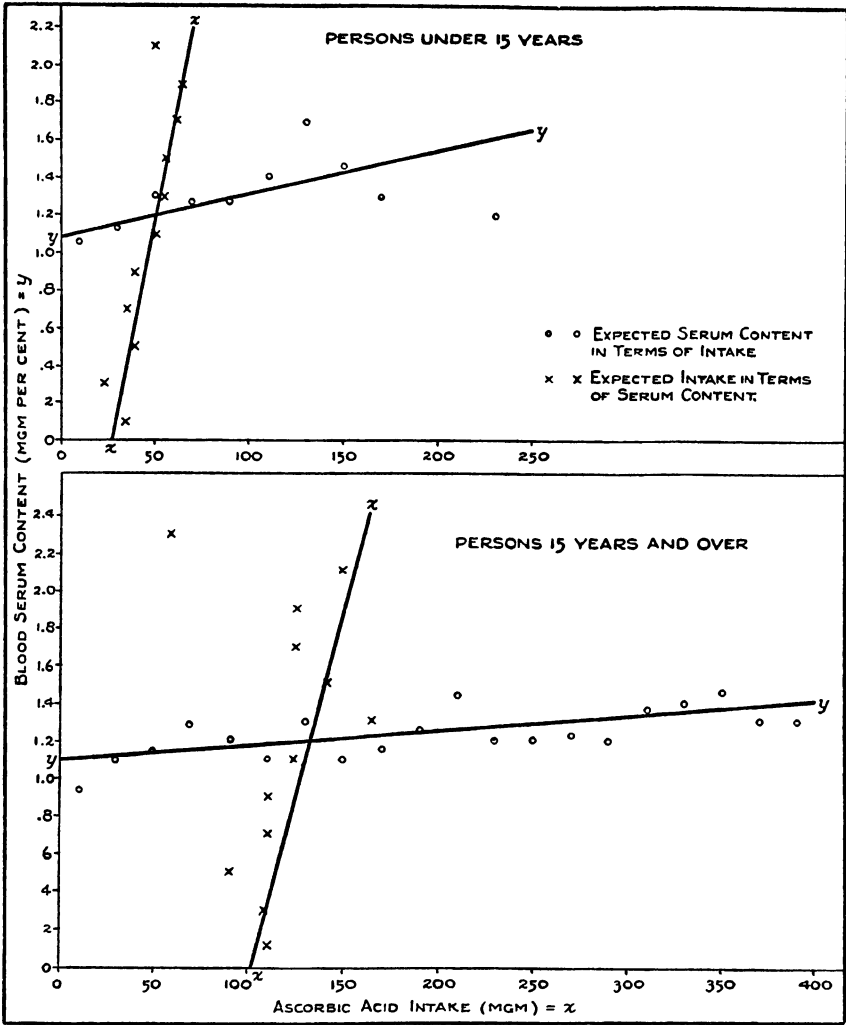


Fig. 4. Mexican Otomi Indians. Comparison of the trend of observed means with that of the corresponding rectilinear regression line.

The departure from linearity of the mean intakes in terms of plasma content is not as pronounced as that of the plasma means, and the intake means are less variable. Those for Alamañe County, however, rise very little for plasma levels of 1.0 mgm per cent and over.

Ascorbic acid potential or latent deficiency states may be expected more frequently among persons with low intake and low

plasma level of the vitamin. Figure 3 indicates that over this range of values the regression lines follow the observed means closely.

Mexico. The diagrams pertaining to the Mexican Otomi Indians in Figure 4 afford a sharp contrast with those for the North Carolinians. The amount of correlation, although significant, was negligible, being $+0.218$ for children and only $+0.124$ for adults. The reason lies in the high mean ascorbic acid level of both population groups together with the excessive intakes among the pulque-drinking adults. The mean serum level of 1.2 mgm per cent must have approximated the renal threshold so that little further rise for the group as a whole could be expected whatever the intake. Interestingly enough, the mean intake of adults reached its peak when serum content attained 1.2 mgm per cent and then dropped, suggesting that appetite may have been sated.

There is some curvilinearity in the trend of the serum content means of the children but none for those of adults. A straight line is probably also the best-fitting one for the mean intakes, although their trend in the adult group is definitely not linear.

The correlation tables for the Otomi Indians are not shown. The distributions of both children and adults were symmetrical with respect to serum ascorbic acid but were asymmetrical with respect to intake.

II. THE RELATIONSHIP DEFINED THE OBJECTIVE

It has been demonstrated that a simple correlation technique does not adequately describe the form of the association existing between ascorbic acid intake, as measured by the diet record, and its level in blood serum or plasma. Furthermore, the intake distributions for all the population groups presented were asymmetric in that the peaks were left of center. A technique is needed, therefore, that will describe the curvilinear trend of the observed means and render the intake distributions more symmetrical.

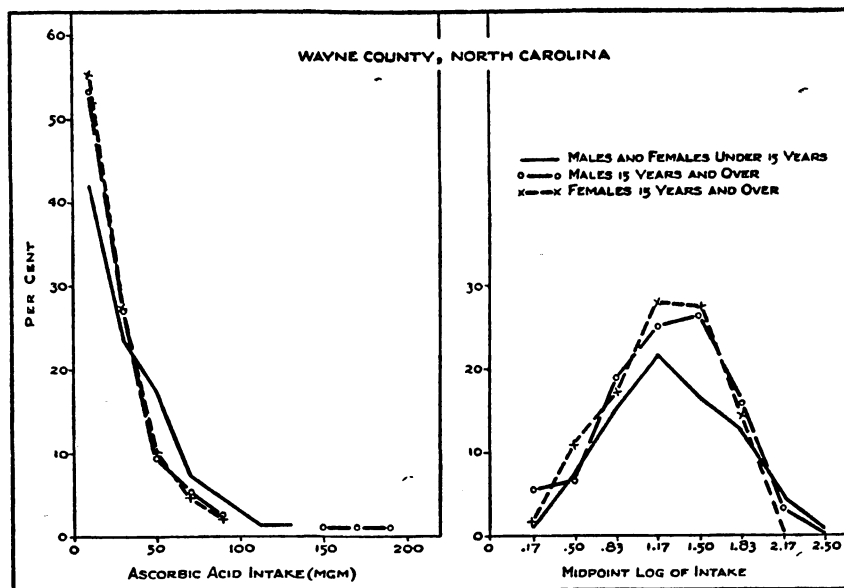


Fig. 5. Percentage distribution of persons surveyed according to ascorbic acid intake when plotted to arithmetic and logarithmic scales.

Both objectives may be realized by setting up the correlation tables in terms of the logarithms of the intake values, leaving the blood content scale in arithmetic progression. This realignment will push the peaks of the intake distributions to the right by extending the scale for low values and compressing that for higher ones. Figure 5 illustrates the effect of this redistribution upon the intake frequencies for persons included in the Wayne County survey.

Nine population groups have been retained for the present analysis with three for each area: children under 15 years, and males separate from females for persons of 15 years and over. A correlation table was set up for each group and new correlation coefficients and regression equations were computed. The relationship described by these calculations is that existing between the observed blood levels and the logarithms of the intakes. It is similar to that obtained from the biological assay in which the response to a drug is measured in terms of the logarithm of the dose except that here we have the distributions of persons

Table 4. Constants of regression equations describing the association between ascorbic acid in blood serum or plasma and the logarithm of the intake.

POPULATION GROUP		No. OF PER-SONS (3)	EXPECTED ASCORBIC ACID IN BLOOD SERUM OR PLASMA (mgm per cent) = y y = a + b(log x)		EXPECTED GEOMETRIC INTAKE (mgm) = x Log x = Log a' + y(Log b')			PER CENT INCREASE (9)	CORREL. COEFF. (10)
SEX (1)	AGE (2)		a (4)	b (5)	Log a' (6)	Anti-log a' (7)	Log b' (8)		
WAYNE CO., N. C.									
M, F	- 15	139	0.3515 ± 0.0314	+ 0.3023 ± 0.0680	1.0811 ± 0.0367	10.7	+ 0.4121 ± 0.0927	158.3	+ 0.353*
M	15 +	96	0.0869 ± 0.0358	+ 0.2760 ± 0.0761	1.0459 ± 0.0450	11.1	+ 0.4364 ± 0.1203	173.1	+ 0.347*
F	15 +	128	0.0994 ± 0.0300	+ 0.3541 ± 0.0720	0.9737 ± 0.0364	9.4	+ 0.4491 ± 0.0913	181.2	+ 0.399*
ALAMANCE CO., N. C.									
M, F	- 15	181	0.2512 ± 0.0307	+ 0.3915 ± 0.0952	1.3810 ± 0.0229	24.0	+ 0.2182 ± 0.0531	65.3	+ 0.292*
M	15 +	121	0.0029 ± 0.0344	+ 0.3143 ± 0.0981	1.3895 ± 0.0305	24.6	+ 0.2482 ± 0.0775	77.1	+ 0.279*
F	15 +	189	0.0833 ± 0.0258	+ 0.3433 ± 0.0803	1.3926 ± 0.0223	21.5	+ 0.2566 ± 0.0601	80.6	+ 0.297*
OTOMI INDIANS, MEXICO									
M, F	- 15	267	0.7081 ± 0.0240	+ 0.3197 ± 0.0680	1.2711 ± 0.0213	18.7	+ 0.2526 ± 0.0522	78.9	+ 0.284*
M	15 +	183	0.9282 ± 0.0337	+ 0.1283 ± 0.1181	2.0580 ± 0.0337	114.3	+ 0.0539 ± 0.0475	13.2	+ 0.083
F	15 +	249	0.8816 ± 0.0239	+ 0.1605 ± 0.0715	1.7843 ± 0.0212	60.9	+ 0.1237 ± 0.0551	33.1	+ 0.141*
OTOMI INDIANS, MEXICO, WITH WEIGHT AT THE MEAN ¹									
M, F	- 15	267	0.7665	+ 0.2827 ± 0.0695	1.3270	21.2	+ 0.2065 ± 0.0508	60.9	+ 0.242*
M	15 +	183	0.9940	+ 0.0973 ± 0.1198	2.0784	119.8	+ 0.0368 ± 0.0455	8.9	+ 0.060
F	15 +	247	0.8728	+ 0.1659 ± 0.0736	1.7862	61.1	+ 0.1216 ± 0.0539	32.8	+ 0.142*

* P < 0.05.

¹ These are coefficients for log intake versus blood content with weight held constant.

in each population both with respect to response (blood level) and dose (intake). The problem may well be considered from this viewpoint, since, if the diet records furnish an adequate measure of actual intake, the relationship should resemble that observed when known doses of ascorbic acid are administered.

If the new equations afford a better "fit" for the observed data, the correlation coefficients should be higher than those contained in Tables 1 and 2 for corresponding population groups. Actually some were a trifle higher and others not as good (Table 4); and since the differences were not significant, nothing has been gained by the application of the more elaborate technique. We shall, however, continue with the analysis since it offers a method which may be of value in other studies of a similar character.

THE SEMILOGARITHMIC REGRESSION EQUATIONS

Expressed in the symbols used in equations (1) and (2) the new regression equations are:

$$\text{Expected blood content: } y = a + b(\log x) \quad (3)$$

$$\text{Expected log intake: } \log x = \log a' + y(\log b') \quad (4)$$

The b in equation (3) is the increase or decrease in milligrams per cent of blood content per integer change in log intake. The antilog of b' in equation (4) signifies the rate or per cent of change in x (intake) for each milligram per cent increase or decrease in the observed y (blood content). Since b and $\log b'$ are constants of figures computed from the regression equations plot as straight lines on a semilogarithmic scale.

Constants of the regression equations with their standard errors for each of the nine population groups are contained in Table 4. Antilog values of the constants in equation (4) are also given as well as the new correlation coefficients. In Figures 6 and 7 the lines indicating the rise in the expected blood content of ascorbic acid (response) in terms of increasing log intake (dose) are shown in the upper drawings and the complementary lines showing the regression of log intake upon blood

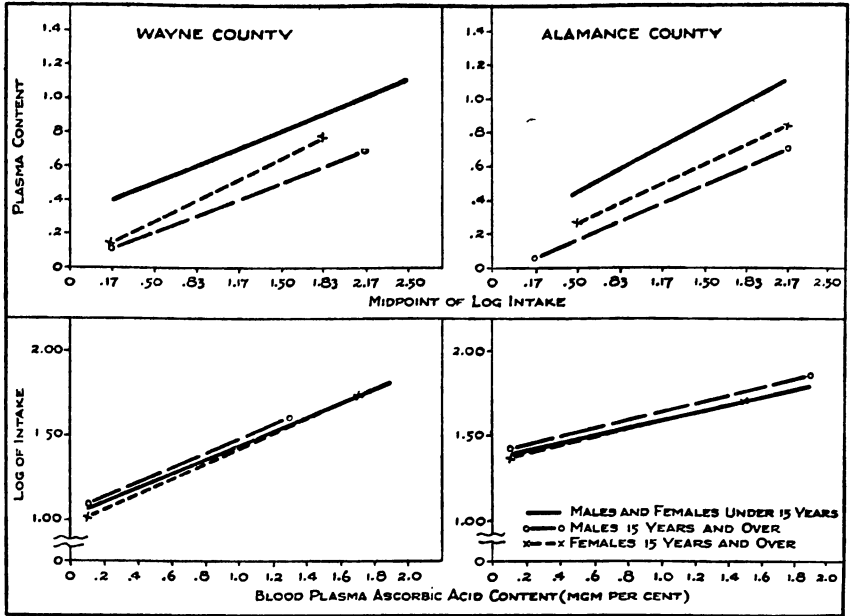


Fig. 6. North Carolina. The regressions of (a) blood plasma ascorbic acid content on log intake and (b) log intake on plasma level.

level are contained in the lower diagrams. The lines are drawn from the midpoint of the first scale interval to that of the last for which data were available.

North Carolina. Increases in plasma ascorbic acid content (b constants, column 5 in Table 4) for rising values of log intake, obtained from data for the three population groups in Wayne County, do not differ significantly, with the result that the slopes of the lines (Figure 6) may be considered essentially parallel. The same is true of the slopes of the corresponding lines computed from data for Alamance County. Evidently similar responses were given by these six population groups as dosage increased. The heights of the lines above the bases of the diagrams, as determined by the a constants, column 4 of Table 4, do differ significantly, however, when that for children in each county is compared with the levels for adults.

The two diagrams in the lower part of Figure 6 show the regression of log intake of ascorbic acid on plasma level as computed from equation (4) for each of the six North Carolina

groups. Here, not only the slopes but also the levels of log intake are similar for the three groups in each county.

An explanation of these phenomena may lie in the differing physiological requirements of children and adults. The family was the unit of observation and the food consumed was derived largely from the family table and was available to all its members. Apparently the plasma content of ascorbic acid attained under a common intake regimen was appreciably less for adults than for children in these two North Carolina counties.

Mexico. The constants for equations (3) and (4) computed from data for the three groups of Otomi Indians are also contained in Table 4 but the lines plotted in Figure 7 are derived from the constants in the last set of equations in the table in which weight has been held constant at the mean for each group. The effect of weight upon the correlation between ascorbic acid intake and blood content will be discussed presently. When its effect is considered the a and $\log a'$ constants in the last set of equations, with one exception, are raised somewhat and the b and $\log b'$ constants, also with one exception, are lowered slightly.

Among the Indians, as among the North Carolinians, the computed ascorbic acid serum levels (Table 4, column 4) differ significantly while the slopes (b constants) do not. The computed log intake values for the Indians, on the other hand, differ both as to level and slope. (Figure 7.) The regression line depicting intake for adult males lies high above the others and does not rise significantly, while that for children starts from a low level and rises rapidly.

Unlike the North Carolinians differences between the Indian groups are greater with respect to ascorbic acid intake than as to serum content. The varying quantities of pulque drunk probably account for intake differences. If so, the net effect of dietary differences has been to raise serum content to essentially similar levels. The question raised by these comparisons is what the physiological character may be that enables children to attain a blood ascorbic acid level as high or higher than that

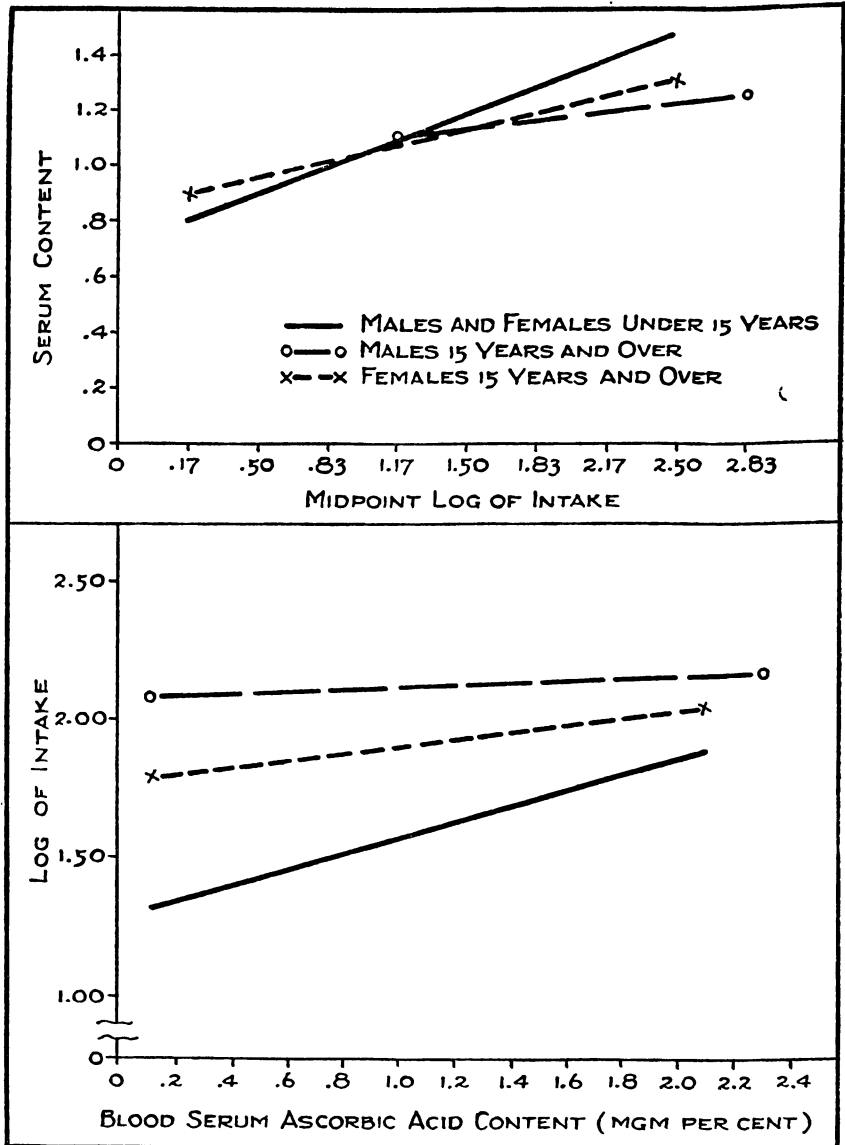


Fig. 7. Mexican Otomi Indians. The regressions, with weight at the mean, of (a) blood serum ascorbic acid on log intake and (b) log intake on serum level.

of adults on smaller intakes. One obvious group difference is that of weight.

THE EFFECT OF WEIGHT

A consideration of weight is important for two reasons. Ob-

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viously weight of children under 15 years is associated with normal growth, while for persons of 15 years and over increase in weight is not necessarily a function of growth. A second reason is that the increase in the total amount of ascorbic acid in body tissues accompanying growth is not reflected in the unit of measurement employed in the laboratory which is the amount contained in 100 cc of blood. If the effect of growth is not included in one of the variables in the correlation it should be excluded from the other.

Presumably total food consumption increases with growth so that the average caloric intake of adults is probably greater than that of children from 2 to 14 years of age. As a corollary

Table 5. Correlation coefficients indicating the amount of association for each population group between (a) total calories and weight, (b) total calories and ascorbic acid intake, and (c) log ascorbic acid intake and weight.

POPULATION GROUP		TOTAL CALORIES VERSUS WEIGHT	TOTAL CALORIES VERSUS ASCORBIC ACID INTAKE ¹	LOG ASCORBIC ACID INTAKE VERSUS WEIGHT
Sex	Age			
OTOMI INDIANS, MEXICO				
M,F	-15	+0.506*	+0.370*	+0.326*
M	15+	+0.368*	+0.696*	+0.321*
F	15+	+0.363*	+0.566*	+0.233*
WAYNE CO., N. C.				
M,F	-15	+0.243*	+0.220*	-0.092
M	15+	-0.010	+0.342*	-0.108
F	15+	-0.015	+0.230*	-0.047
ALAMANCE CO., N. C.				
M,F	-15	+0.291*	+0.070	+0.005
M	15+	-0.015	+0.292*	-0.116
F	15+	+0.033	+0.183*	-0.161*

¹ Distributions on an arithmetic scale.

* P < 0.05.

one might also expect ascorbic acid intake to increase with growth i.e., weight, and hence with total food consumption. Table 5 contains the correlation coefficients indicating the degree of association between total calories and weight, total calories and ascorbic acid intake, and that between log ascorbic acid intake and weight, for each population group.

For the Otomi Indians significant positive correlation was obtained for each age and sex group between total calories and weight. The coefficient for children (Table 5) is highest as might be expected for the reason given above. The correlation between total calories and ascorbic acid intake is also positive and significant and is higher for adults than children. The consumption of pulque enters the picture again since 12 per cent of the calories consumed came from the protein and alcohol in pulque (6). As much as 2 per cent of the caloric intake was attributed to alcohol in pulque drunk by children from 1 to 3 years of age and the proportion increased to 15 per cent for males in the 21 to 50 year age group. A significant correlation between total calories and vitamin C consumed is, therefore, not unexpected.

Among North Carolinians the picture is conspicuously different. A small significant correlation between total calories and weight is indicated for children only. All three Wayne County groups exhibit some significant positive correlation between total calories and ascorbic acid intake, while data for adults only in Alamance County give small significant positive coefficients.

Logarithms of ascorbic acid intakes were used for calculating the correlation with weight. Again data for the Indians give significant positive correlation but only one small negative coefficient, that for adult females in Alamance County, is significant for the North Carolinians.

Under the circumstances no attempt has been made to add weight to the variables considered in the analysis of the data for North Carolina. From data pertaining to the Otomi Indians, however, a third set of equations was computed by a

partial correlation technique by which the regression of blood content upon log intake and weight, as well as the regression of log intake upon blood content and weight, could be computed. These equations will not be presented or discussed in detail but the effect of weight at the mean upon blood content and log intake for each population group has been added to the *a* and log *a'* values in the final set of equations in Table 4, from which computed points are plotted in Figure 7.

The coefficient signifying the regression of blood content on weight was not significant in any of the three newly computed equations. That indicating the regression of log intake on weight was significant and, interestingly enough, was virtually the same for the three Indian groups in that the rate of increase

Table 6. Relative adequacy of the mean ascorbic acid intake (geometric) in each population group in terms of average weight and blood vitamin C level.

POPULATION GROUP		GEOMETRIC MEAN ASCORBIC ACID INTAKE (mgm)	MEAN WEIGHT (kgm)	MEAN BLOOD ASCORBIC ACID CONTENT (mgm Per Cent)
Sex	Age			
OTOMI INDIANS, MEXICO				
M,F	- 15	37.79 ± 1.93	21.81 ± 0.52	1.21 ± 0.025
M	15+	132.66 ± 6.73	54.96 ± 0.56	1.20 ± 0.034
F	15+	85.35 ± 4.23	47.28 ± 0.44	1.19 ± 0.024
WAYNE CO., N. C.				
M,F	- 15	22.04 ± 1.99	32.32 ± 0.95	0.76 ± 0.034
M	15+	17.07 ± 1.89	67.73 ± 1.44	0.43 ± 0.038
F	15+	16.25 ± 1.38	61.49 ± 1.25	0.53 ± 0.033
ALAMANCE CO., N. C.				
M,F	- 15	37.15 ± 2.05	32.35 ± 0.84	0.87 ± 0.032
M	15+	32.20 ± 2.36	69.53 ± 1.00	0.48 ± 0.036
F	15+	30.53 ± 1.64	61.31 ± 0.90	0.59 ± 0.027

in ascorbic acid intake per kilogram of weight was 2.8 per cent for each. Mean weight differences between the groups, therefore, call for an average intake among adult females of twice that of children, while the intake of adult males should be 2.5 times that of children.

Table 6 contains the mean values for each population group for (a) ascorbic acid intake (geometric), (b) weight, and (c) blood content. Among the Otomi Indians the observed geometric mean intakes of adults more than fulfill the weight requirements indicated by the regression coefficients, while the mean serum levels attained are 1.2 mgm per cent for each group. Among persons surveyed in North Carolina the geometric mean intakes of children are actually higher than those of adults in spite of the differences in weight. If the 2.8 per cent rise in intake per kilogram of weight may be applied to data for the North Carolina groups, the geometric mean intakes of children should be multiplied by the following factors to take care of the differences in weight:

	<i>Wayne County</i>	<i>Alamance County</i>
Adult Males	2.7	2.8
Adult Females	2.2	2.2

We may not be justified in applying these correction factors to data for the North Carolinians. Obviously, however, the geometric mean intakes for adults in North Carolina could not be expected to achieve plasma levels equivalent to those attained by children.

TISSUE RESERVES OF VITAMIN C

Since theoretical blood ascorbic acid means computed from the semilogarithmic equations given in Table 4 pursue a curvilinear course when plotted to an arithmetic scale, the trend of observed and theoretical means with respect to tissue stores of the vitamin may be investigated. Although these equations do not approach finite limits, within the range of observed values, blood levels rise more slowly as intake is increased.

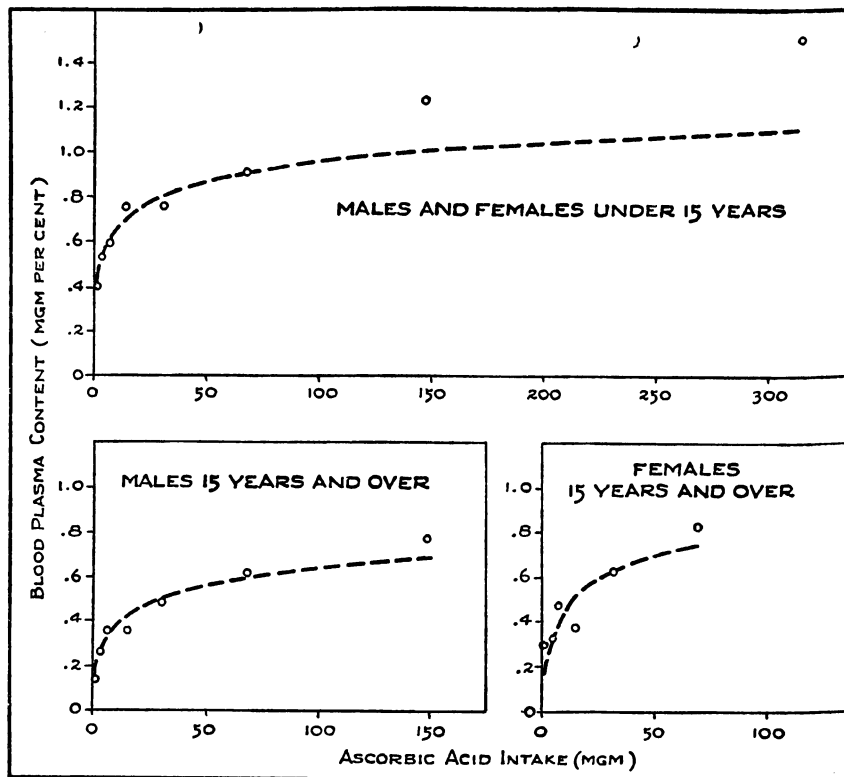


Fig. 8. Wayne County, North Carolina. Tissue stores of ascorbic acid as indicated by the level of response (plasma content) to equivalent intakes.

Figures 8 to 10 contain the observed blood content means with the corresponding regression lines plotted to an arithmetic scale for the various population groups, exclusive of that for adult male Indians for whom there was no significant correlation between ascorbic acid intake and blood level. The observed means are plotted at the midpoints of the logarithmic intervals from the correlation tables with antilogs: 1.47, 3.16, 6.81, 14.7, 31.6, 68.1, 147 mgm, etc. On an arithmetic scale the distance between these points widens rapidly.

For comparing the observed and computed ascorbic acid mean blood levels of the various groups, those pertaining to an intake range of from 47 to 99 mgm have been selected, with a geometric intake midpoint of 68.1 mgm. At this point the regression lines lie close to the observed means and the number of ob-

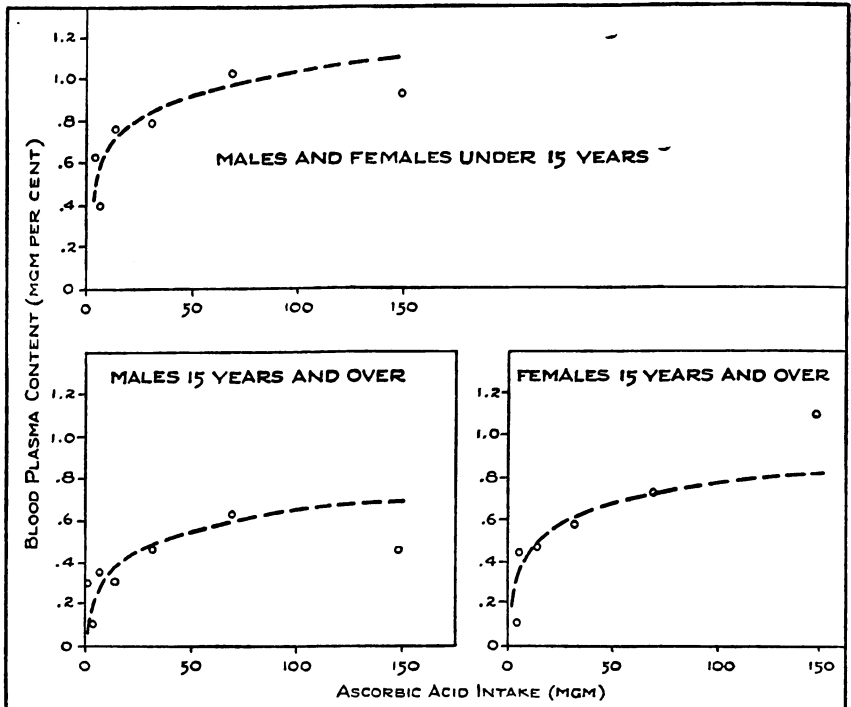


Fig. 9. Alamance County, North Carolina. Tissue stores of ascorbic acid as indicated by the level of response (plasma content) to equivalent intakes.

servations in each group was large enough to afford fairly stable means. Table 7 contains the observed and computed mean blood levels at geometric intake midpoints of 68 and 147 mgm.

If the individuals in these nine population groups had been selected at random, similar blood levels of ascorbic acid for the same intake values might be expected. The groups are known to differ, however, as to age, sex, race and weight but none of these factors will explain why adult Indians with intakes ranging from 47 to 99 mgm attained blood levels of 1.25 and 1.22 mgm per cent, while the same intakes give corresponding values for North Carolina adults of from 0.62 to 0.84 mgm per cent. When the regression lines are extended to the midpoint of the next log intake interval, antilog 147 with a range of from 100 to 213 mgm, amounts which should provide enough ascorbic acid to supply any weight requirements, the discrepancies in blood

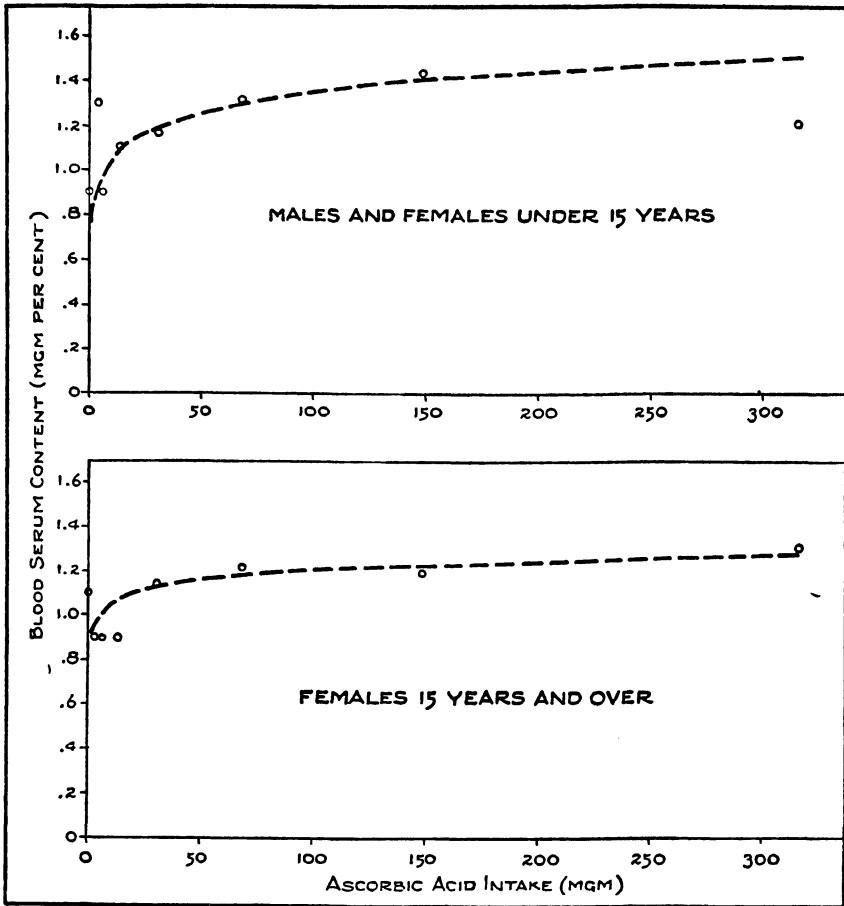


Fig. 10. Mexican Otomi Indians. Tissue stores of ascorbic acid as indicated by the level of response (serum content) to equivalent intakes.

levels between adult Indians and North Carolinians are still not met. Something else enters the picture.

If we look back along the regression lines in Figure 10 pertaining to adult Indians to a midpoint of 32 mgm we find blood levels of 1.12 mgm per cent for both males and females, indicating that they were in a state approximating tissue saturation at a time when ascorbic acid intake was below anything considered adequate for normal nutrition. Blood plasma content for North Carolinians, on the other hand, fell below this amount on intakes as high as 147 mgm daily. Obviously, the recorded

intake figures do not tell the entire story about ascorbic acid nutriture.

Basic nutriture evidently differed for these groups when the diet surveys were made and the blood samples drawn. Examination of the *a* constants, column 4 in Table 4, indicates this. On an arith-log scale these constants represent the theoretical blood ascorbic acid content when intake was equal to 1 mgm. Among the North Carolina adults these constants were

Table 7. Varying response (blood ascorbic acid content) achieved by population groups receiving the same dose (log intake).

POPULATION GROUP		ASCORBIC ACID LOG INTAKE INTERVAL					
		Geometric Midpoint 68.1 mgm, Interval Range 47-99 mgm			Geometric Midpoint 147 mgm, Interval Range 100-213 mgm		
Sex	Age	Number of Persons	Mean Blood Content (mgm Per Cent)		Number of Persons	Mean Blood Content (mgm Per Cent)	
			Observed	Computed		Observed	Computed
WAYNE CO., N. C.							
M,F	- 15	31	0.85	0.91	6	1.23	1.01
M	15+	15	0.62	0.59	3	0.77	0.69
F	15+	19	0.84	0.75	0	—	—
ALAMANCE CO., N. C.							
M,F	- 15	69	1.02	0.97	9	0.92	1.10
M	15+	42	0.63	0.58	4	0.45	0.68
F	15+	54	0.72	0.71	3	1.10	0.83
OTOMI INDIANS, MEXICO							
M,F	- 15	99	1.30	1.29	22	1.44	1.40
M	15+	31	1.25	1.16	94	1.19	1.21
F	15+	86	1.22	1.18	110	1.20	1.23

less than 0.1 mgm per cent while among Indian adults they were approximately 1.0 mgm per cent. The *b* constants were similar for the six North Carolina groups as well as for the Indian children, indicating a similar rise in blood level per log integer increase in intake. Obviously, therefore, the level of blood ascorbic acid for the North Carolina adults would never rise to that of the children with equivalent intakes.

Tissue reserves of the vitamin were evidently low among North Carolina adults, and weight requirements (for saturation) as to intake were not being supplied. While average daily intakes varied among individuals and although mean plasma levels were higher for persons with larger intakes, among adults the impact of the larger consumption was not sufficient to raise the level of tissue reserves of the groups to the saturation point. Among the Indians, on the other hand, both children and adults, tissue reserves of ascorbic acid were virtually at saturation level when the diet survey was made, and although individuals varied as to their consumption, the period of observation was too short to permit low intakes to affect the nutritional status of the groups adversely.

It should be noted that the blood specimens were drawn just preceding, during, or shortly after the week the diet was recorded. This analysis has been based on the assumption that the recorded diet did not deviate significantly from that of any week covered by the observations on individuals of a given family. We may not know intake on the day just before the blood sample was drawn, but it seems unlikely that the picture of ascorbic nutriture afforded by these data would be altered appreciably by some discrepancy between the recorded intake and that just prior to the taking of the blood specimen.

SUMMARY AND CONCLUSIONS

A statistical analysis has been performed to determine the existence, degree, and form of association between the average daily intake of ascorbic acid, as given by the seven-day diet record, and the amount found in blood serum or plasma in the

laboratory examinations. The data were obtained from nutrition surveys of white families in the rural sections of Wayne and Alamance Counties of North Carolina in 1942-1944 and from a survey of Otomi Indians in four villages about 75 miles from Mexico City in 1943-1944. Ascorbic acid was chosen for specific investigation.

The statistical techniques employed in the initial stage of the analysis have included the computation of means, standard deviations, and correlation coefficients for population groups specific for age, sex, season of survey, and area. Fourfold tables for the data by area were set up for the chi-square test. Rectilinear regression equations were computed for populations on an area basis and their goodness of fit examined. Later, because of the slight curvilinearity of the existing relationship and because of the asymmetry of the intake distributions, new correlation coefficients and regression equations were computed from the logarithms of ascorbic acid intakes and the blood levels in arithmetic progression. The effect of weight was analyzed by a partial correlation technique. The object has been to ascertain whether by these statistical maneuvers the existing relationships could be adequately described.

I. *The Preliminary Reconnaissance.* The first phase of the analysis revealed significant correlation between ascorbic acid intake and plasma content for fifteen of the twenty-four North Carolina groups examined. When data were assembled on a county basis the coefficients were: +0.395 for Wayne and +0.416 for Alamance County. Significant coefficients were higher when season, age and sex as well as county were considered simultaneously. Data pertaining to the Mexican Otomi Indian children and females of 15 years and over gave significant correlation of a low order.

This significant association between ascorbic acid intake, as measured by the diet record, and blood content indicated (a) that higher blood levels on the average accompanied higher intakes and *vice versa*, and (b) that higher intakes were likely to be found among persons with higher blood content and *vice*

versa. Factors operating against a closer association were probably (a) inaccuracies in the estimation of ascorbic acid consumed, (b) the lag in time between ingestion of the vitamin and its appearance or disappearance from the blood stream, (c) the lack of homogeneity within the population groups, particularly those containing pregnant and lactating women, and (d) the excretion of the vitamin when tissue stores and blood levels are high, as in the case of the Otomi Indians, with the result that blood levels do not increase in accordance with increasing intakes.

The association of a state of unsaturation among the North Carolinians with low intakes was demonstrated by the fourfold table analysis. Fewer persons with such conditions were observed among the Otomi Indians.

A comparison of the trend of blood content means at successive ascorbic acid intake levels with that of the regression line indicated that the true relationship was curvilinear. In other words, the rise in mean blood level of the vitamin was greater when intake was low than when it was high. In the range where unsaturation occurs, however, with blood content below 0.6 mgm per cent and intake below 40 mgm, the rectilinear regression equation satisfactorily defines the relationship.

Examination of the mean intake values for successive categories of ascorbic acid blood content reveals that this relationship is generally rectilinear at lower blood levels. If and when, however, a state of tissue saturation is attained, mean intake, too, may drop or tend to rise more slowly.

II. *The Relationship Defined*. Although the goodness of fit of the regression lines was not significantly improved by the substitution of log intake values for those in arithmetic progression, differences between groups specific for age, sex, and area with respect to their response (blood content) to the logarithm of the dose (ascorbic acid intake) have been examined and discussed.

Among persons in the six North Carolina groups the rise in blood content per log integer rise in intake was similar, so that

the regression lines pursued essentially parallel courses. Their levels differed, however, with the line for children lying above the lines for adults in each county. Increases in log intake among adults and children for each milligram per cent rise in blood content were also similar, while the differences in level of the regression lines were negligible.

Among the Mexican Otomi Indians wide group differences in log intake levels were associated with similar serum content of ascorbic acid.

Weight was positively associated with total calories and with log ascorbic acid intake among the Indians. Among the North Carolinians there was some correlation between weight and total calories for children only and there was no positive association between weight and log intake of ascorbic acid.

The lower level of response (blood content) to similar dosage (log intake of the vitamin) among the adult North Carolinians is believed to have been due to low tissue reserves. The high blood ascorbic acid content among the Indians at low recorded intake values suggest that they had attained a tissue saturation that persisted at least during the period of the survey.

The interpretation of the results of this analysis must be accepted with reservation in view of the low order of statistical correlation found. The diet record does afford some measure of ascorbic acid intake, however, and a demonstration of various statistical methods for describing the inherent relationships seemed of value.

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