INVESTIGATION ON THE SEASONAL FLUCTUATION OF VITAMIN C EXCRETION IN PALESTINE

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(With 4 Figures in the Text)

Since Harris *et al.* (1933) first showed the significance of urinary vitamin C excretion, numerous papers have been published on vitamin C excretion by man. Few of these investigations deal with excretion of vitamin C by normal individuals under natural nutritional and climatic conditions. v. Euler & Malmberg (1935) carried out a comprehensive study of the vitamin C excretion of the inhabitants of north Sweden, south Sweden and Stockholm. Their observations, however, are based on but a few random samples obtained during the winter months. Hamel (1937) investigated the daily vitamin C excretion of different classes of the population of Rostock, during a small part of the year. The investigations of van Eekelen & Wolff (1936) dealt with thirty-three Dutch families and were intended mainly to yield information on the vitamin C nutrition of the Dutch population, rather than on the seasonal fluctuation in vitamin C excretion in urine.

The aim of the present investigations was twofold: (1) to follow the vitamin C excretion in man under prevailing nutritional conditions, and (2) to ascertain the influence of the specific natural nutritional conditions of Palestine on the seasonal variations in the excretion of vitamin C.

Technique

The vitamin C excretion in the urine of thirty adult residents of Jerusalem of both sexes (mostly staff members of the Department of Hygiene and Bacteriology or students of the Hebrew University) was followed over a period of one year from November 1936 to November 1937. The urine samples were obtained at intervals of 8–10 days, mostly in the morning hours. In all, over 1000 urine samples of this class were examined.

Once a month samples of the morning urine were analysed from each of twenty-five children attending the day nursery of the Straus Health Centre at Jerusalem. These children, aged 2–5 years, are kept in the nursery throughout the day and, as they are fed there, constitute a group of fairly uniform nutrition.

Investigations were also conducted at two places which represent climatic and nutritional extremes; the workers at Kalliah at the Dead Sea, and of the

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settlements Givath-Brenner and Dederah in the Coastal Plain. Kalliah is 390 m. below the sea-level, and is distinguished by its low rainfall and high temperature—a dry tropical climate. The examinations were made once in the summer and once in winter; the summer investigation comprised fifty-eight samples and the winter investigation forty-seven. Gederah and Givath-Brenner lie in the centre of the Palestine citrus culture and enjoy a Mediterranean climate. The citrus crop ripens in the winter months, and consequently here too the tests were carried out once in winter (sixty-two tests) and once in summer (sixty-one tests). The subjects consisted of the agricultural workers and their families; the common kitchen at Givath-Brenner and Gederah provided a group of people under approximately uniform nutrition.

We limited ourselves to determinations of the vitamin C content of samples of the urine, since under the conditions of our work it was impossible to obtain 24 hr. samples of urine. From a few subjects we were able to obtain at different times of the year the 24 hr. output of urine, and these outputs furnish a general idea of the average daily excretion of urine. In adult persons the quantity varied from 1250 to 1500 c.c. during the winter months and 600 to 800 c.c. during the summer months.

Although the urine samples were collected into dark bottles and as a rule analysed the same day the addition of vitamin C stabilizers was found to be necessary. The problem of stabilization of vitamin C in various solutions is still obscure. Leibowitz & Guggenheim (1938) investigated the question of stabilization of vitamin C by acids and showed that the different acids tested provided different degrees of protection, according to the substances present in the solutions. For urine examination sulphuric acid proved to be most suitable; hence, 2 c.c. concentrated sulphuric acid were added to 100 c.c. in the dark brown bottle used for collecting the urine samples.

The vitamin C determinations were made with dichlorphenol-indophenol by Tillmans' method. The titre of the indicator was standardized against a known solution of ascorbic acid, and the vitamin C in the urine determined by the amount of urine required to bring a given amount of the dichlorphenol-indophenol solution to a faint pink. The results are expressed as mg./litre of urine.

Results

The results show that there is a regular and constant seasonal fluctuation in the vitamin C excreted in the urine; it is consistently high in winter and low in summer. The data relating to the different groups are summarized below.

(*a*) *Findings on thirty adult residents in Jerusalem*

Table I and Fig. 1 present the vitamin C values found. The figures represent the monthly averages of the analyses of all adult samples of urine.

The high order of values found in November to May and the low order of values found in June to October are notable. If we assume on the basis of

data available in the literature that the standard vitamin C excretion is 15–35 mg./l. (Harris & Ray, 1935; Ippen, 1935; v. Euler & Burstroem, 1936), it is apparent that the average values for November to May are well above the

Table I. *Urinary excretion of vitamin C in Jerusalem during one year (average of samples tested)*

	30 adults		25 children	
Month	Range, mg./l.	Average, mg./l.	Range, mg./l.	Average, mg./l.
January	11–470	61	14–237	57
February	12–540	56	14–185	44
March	11–330	69	11–340	64
April	11–350	55	10– 78	41
May	11–590	61	8–144	31
June	10–100	28	10– 67	25
July	10– 62	27	10– 69	22
August	7– 60	25	8– 34	22
September	7– 50	23	11– 31	19
October	5–129	27	9– 72	23
November	13–120	42	11– 84	31
December	11–286	47	9– 74	34
January–April	—	—	11–340	51
May, November, December	—	—	8–144	31
June–October	5–129	28	8–72	22
November–May	11–590	54	—	—

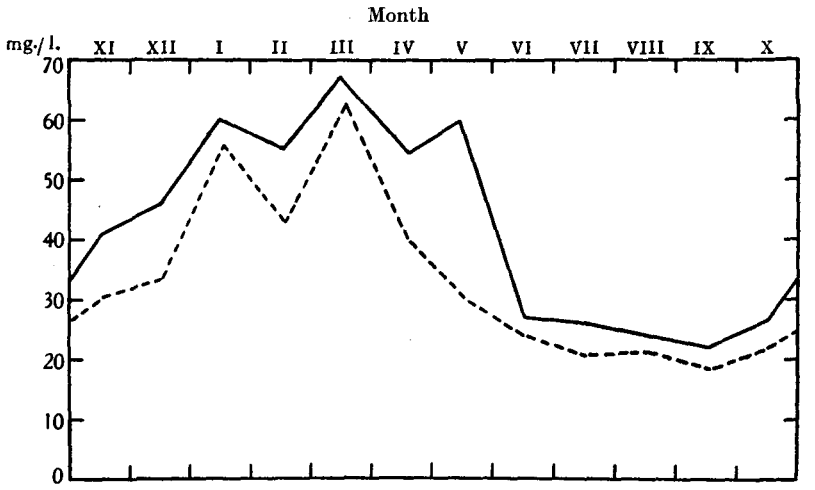

Fig. 1. Seasonal fluctuation in the monthly average urinary vitamin C excretion in 30 adults and 25 children in Jerusalem.

——— Adults. – – – – – Children.

standard. During these months the vitamin C excretion often reaches the high values of 400–600 mg./l. Although instances occurred during these months where less than 15 mg./l. were excreted, these were on the whole extremely rare, and were found only in persons who consumed very small quantities of citrus fruits. Taking the accepted standard values as a basis, 46–71 % of the

analyses during November to May yielded superstandard values, while between June to October only 8–21 % of the analyses were above standard. On the other hand, substandard values were found in only 2–5 % of the cases between November and May, and in 9–18 % of the analyses between June to October. The relationship is shown graphically in Fig. 2.

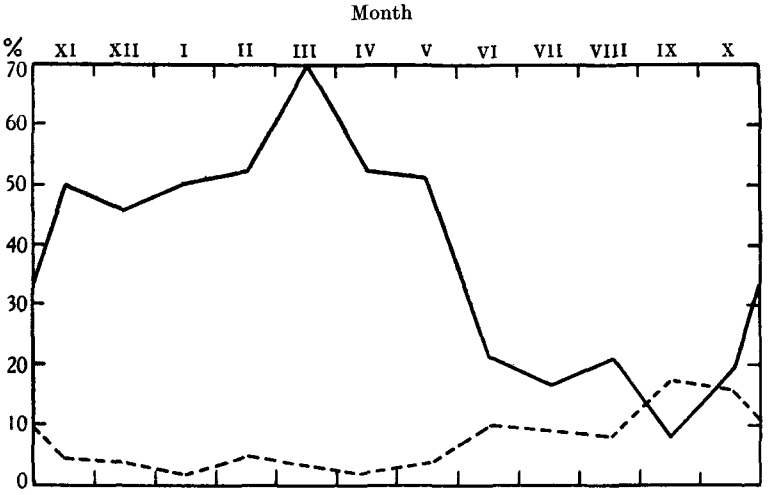

Fig. 2. Percentage distribution of super- and substandard urinary vitamin C excretion in 30 adults in Jerusalem.

——— Superstandard: excretion above 34 mg./l.
– – – – – Substandard: excretion below 15 mg./l.

The frequence distribution of the substandard, standard and superstandard values obtained during the periods November to May and June to October, respectively, gives striking curves which are shown in Fig. 3.

It will be noted that between November and May only 4 % of the values were below 15 mg./l., whereas between June and October 15 % were below this figure. On the other hand, between November and May 53 % if the values were over 34 mg./l., and between June and October only 17 % were over this figure.

(*b*) *Findings on twenty-five children, aged* 2–5 *years, in Jerusalem*

The results obtained among children resemble in many respects those among adults (Table I, Fig. 1), but there are certain deviations. The correspondence is evident in the high excretion during the winter and spring months and the low values found in summer. The curves for children are, however, consistently lower than those for adults. Among the children three distinct periods may be distinguished: January to April, when the average was 41–64 mg./l., May, November and December, when the average was 31–34 mg./l., and June to October, when the average was 19–25 mg./l. The percentage distribution of vitamin C excretion during the different periods of the year are shown in Fig. 4 and corresponds with curves for adults shown

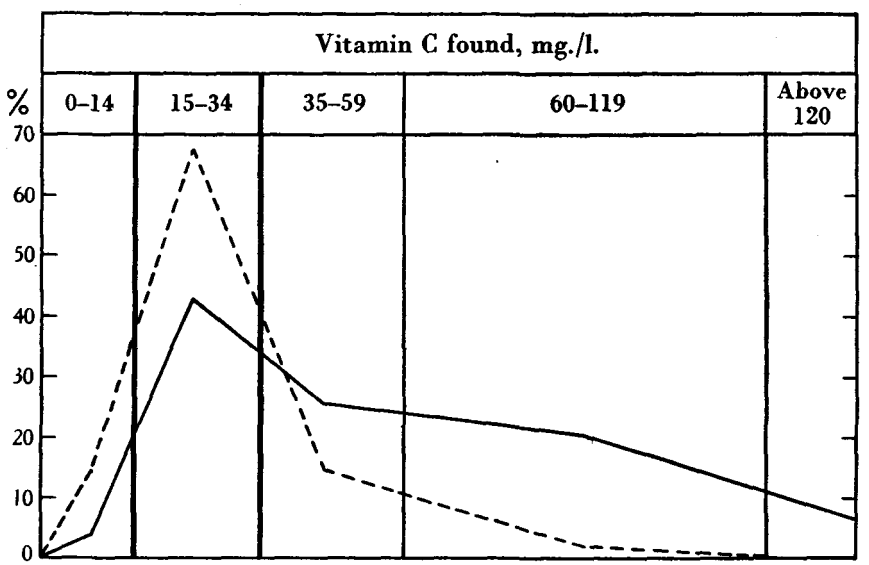

Fig. 3. Frequency distribution of quantity of vitamin C excreted in urine by 30 adults in Jerusalem (expressed in percentage of total).

——— November to May. – – – – – June to October.

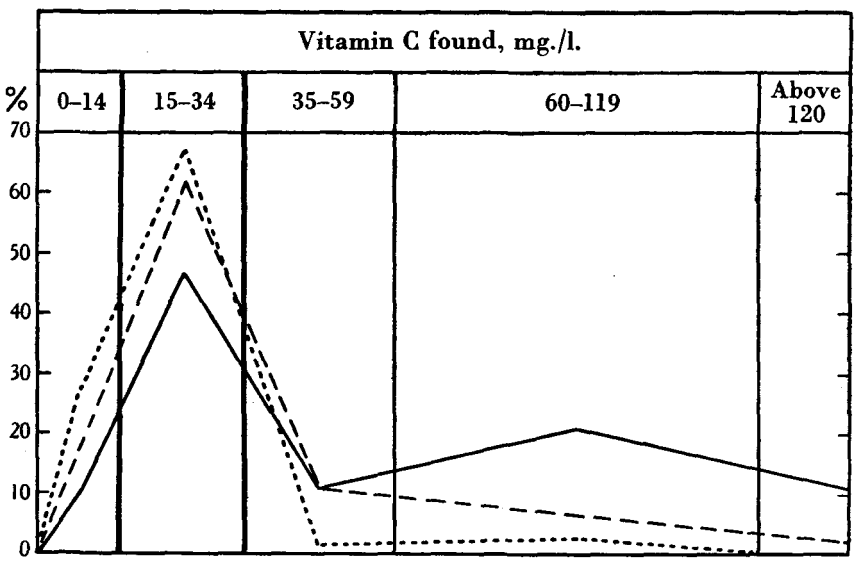

Fig. 4. Frequency distribution of quantity of vitamin C excreted in urine by 25 children in Jerusalem (expressed in percentage of total).

——— January to April. – – – – – May, November, December.
. . . . . June to October.

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in Fig. 3. During the winter months (January to April) 43 % of the urine samples gave values above 34 mg./l. and 10 % below 15 mg./l.; during the summer months (June to October) only 5 % of the samples contained over 34 mg./l. and 27 % below 15 mg./l. May, November and December seem to constitute transition months giving wide divergence in either direction: 21 % of the samples contained over 34 mg./l. and 17 % less than 15 mg./l.

There are two possible explanations for the somewhat lower vitamin C concentrations found in the urine samples of children both in summer and winter. One is a relatively smaller vitamin C intake; the other a higher vitamin C requirement by the growing body. Since citrus fruits, which constitute the most important source of vitamin C in Palestine, are so cheap that they form a staple food of even the poorest classes, we may reject the assumption of a lesser intake of vitamin C. It seems much more probable that the lower excretion is due to a higher vitamin C requirement by the growing body. A similar conclusion, it may be recalled, was reached by Everson & Daniels (1936). This explanation accounts for the slow rise in the vitamin C excretion during November and December, the initial months of the citrus season and the rapid fall in May at the close of the season.

(*c*) *Findings in Kalliah at the Dead Sea*

The winter survey was carried out on 18 January 1937, the summer survey on 28 June 1937. The population was divided on a nutritional basis into two groups: (1) workers who live together and eat in the common kitchen, and (2) workers who live with their families and take their meals at home. The results are shown in Table II. The pronounced difference between the vitamin C

Table II. *Urinary vitamin C excretion at Kalliah near the Dead Sea*

		18. i. 1937		
Subjects investigated	Age	No. of subjects	Range, mg./l.	Average, mg./l.
Workers, taking their meal in the common kitchen	17–63	12	14– 75	38
Police	21–48	5	36– 188	77
Workers and their wives, taking their meals at home	21–56	17	23– 330	93
Children	3–13	12*	142–1000	392
Total—adults	17–63	34	14– 330	69
		28. vi. 1937		
Subjects investigated	Age	No. of subjects	Range, mg./l.	Average, mg./l.
Workers, taking their meal in the common kitchen	17–63	27	22–48	28
Police	21–48	3	13–34	25
Workers and their wives, taking their meals at home	21–56	18	13–48	24
Children	3–13	10	16–54	30
Total—adults	17–63	48	13–48	26

* The extremely high values found in the urine of children are attributable to the orange juice meal which they received 2 hr. before the urine samples were taken.

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excretion in winter of canteen members and of workers who take their meals at home and receive a diet richer in citrus fruit is striking. In the summer, when the vitamin C content of the diet is smaller, this difference between the two groups tends to disappear. The extremely high concentrations found in the urine of the children at winter survey must be regarded as exceptional and are attributable to the meal of orange juice which they received 2 hr. before the urine samples were taken. No influence of climate on vitamin C excretion is indicated. The data tend rather to show the all-important role of the form of nutrition, i.e. of the amount of the citrus consumption. This conclusion is even more obvious from the figures obtained in the investigation of a group of inhabitants of the citrus zone.

(*d*) *Findings in the citrus zone*

As has been pointed out above, the investigation on the agricultural workers and their families in the citrus zone was carried out in two adjacent settlements, Givath-Brenner and Gederah. The results obtained at these points in the winter (4 January 1937) and summer (8 July 1937) are set forth in Table III. The extraordinary large amounts of vitamin C excreted during

Table III. *Urinary vitamin C excretion at Givath-Brenner and Gederah*

		4. i. 1937			8. vii. 1937		
Subjects investigated	Age	No. of subjects	Range, mg./l.	Average, mg./l.	No. of subjects	Range, mg./l.	Average, mg./l.
Man	17–38	26	15–920	375	30	13–126	30
Women	17–51	20	31–194	96	23	12– 50	31
Children	1–11	16	22–157	68	8	7– 13	11

the winter are outstanding. Whereas at Jerusalem and Kalliah no differences were noted in the vitamin C excretion of males and females, in the citrus zone there is a pronounced difference between male and female during the winter but not in the summer. The values for the females are not appreciably higher than those found in Jerusalem, but the values for the males are markedly higher. This difference is due to the fact that most of the men work in citrus plantations, where they consume large quantities of citrus fruits, whereas few of the women are occupied in this manner. The same circumstance explains also why the difference between males and females is absent in summer. The low vitamin C excretion of children in summer is notable, but the data available seem insufficient as a basis for any conclusion.

Our survey shows the significance of the consumption of citrus fruits for the vitamin C supply of Palestine's population. The citrus season begins in October, is in its full swing in December and does not end until May of the succeeding year. Among the different population groups the form of the excretion curves was similar. The minimum shown towards the close of summer (September) is characteristic, and coincides with the time of the lowest fresh fruits supply. As citrus fruits are of such great importance for the vitamin C

supply of the Palestine population the possible sources of vitamin C supply in that time of the year when citrus fruits are not available here, i.e. from June to September, seemed of interest. Accordingly we made a few analyses of the popular fruits and vegetables eaten fresh. The vitamin C content of some Palestinian fruits has been determined already by Robinson (1937); his examinations, however, were limited to bananas, chestnuts, milk and the

Table IV. *Vitamin C content of some fruit and vegetables grown in Palestine*

Food stuff	mg./100 c.c. or g.	Average values according to Eddy & Dalldorf (1937)	
		Units/oz.	mg./100 c.c. or g.
Oranges	33·0–64·0	230–235	38·0–39·0
Grapefruit	48·0–72·0	185–200	31·0–33·0
Apricots	4·4–6·3	10–70	1·7–12·0
Figs	1·1–2·3	10	1·7
Plums	0·6–1·0	30	5·1
Grapes	0·9–3·9	8–20	1·3–3·4
Pomegranate juice (iodine titration)	5·0–13·0	—	—
Melon	16·7–22·0	—	—
Watermelon	2·3–5·0	40	6·8
Cucumbers	14·1–14·9	70	12·0
Cucumbers (pickles)	2·5	—	—
Tomatoes	8·3–38·0	78–140	13·0–23·0
Egg plant	1·1–4·3	20	3·4
Endive	13·0–19·0	56	9·3
Peppers, green	70·0–84·0	155–310	25·5–52·0

juice of citrus fruit, grapes and tomatoes. The values found by us using the Tillmans' reagent are shown in Table IV. The comparison with the standard values given by Eddy & Dalldorf (1937) shows that there is no essential difference between the vitamin C content of Palestinian and foreign fruit and vegetables. The great fluctuations in vitamin C content of several kinds (grapes, tomatoes) are, however, remarkable. It remains to ascertain whether season or plant nutrition have any influence on the vitamin C content.

Discussion

As mentioned above, the only existing comparative investigations on the vitamin C excretion of different population groups are those by v. Euler & Malmberg (1935) and by Hamel (1937). v. Euler & Malmberg examined by titrating against indophenol the vitamin C concentration of urine samples sent to the laboratory during the winter months. The average values found were:

	mg./l.
North Sweden	5
Rural population of Sweden	9
South Sweden	11
Stockholm	25

Hamel (1937) investigated the total daily excretion of people in Rostock by titrating against methylene blue after treatment with oxidase. He, there-

fore, got much lower values than with the Tillmans' reagent. His results were as follows:

	mg.
Students, warm meals twice weekly, fruit rare	1·0– 4·5
Students, mensa meals	4·5– 8·0
Normal family food (often warmed meals)	2·2– 9·6
Owners of vegetable gardens	14·0–21·0

Our results cannot be compared with those found by Hamel, but a comparison with the Swedish data shows that even the low average values in Jerusalem are as high as the Stockholm values. These figures are as high as the average vitamin C concentration of the urine given by v. Euler & Burstroem (1936) (15–25 mg./l.), Ippen (1935) (10–35 mg./l.) and by Harris & Ray (1935) (20–30 mg./l.).

It is generally assumed that deficiency in vitamin C is subject to seasonal fluctuations and is greater in winter and spring months than during the rest of the year. This view is supported by a limited number of investigations. Forsgren (1932) examined 500 patients of a Swedish sanatorium by the Goethlin capillary method, and found that about 10–20 % of the patients were subscorbutic. Stocking (1933) also found a lowered capillary resistance in the spring and attributed it to vitamin C subnutrition. Demole (1936) and Ippen (1935) found by means of the saturation tests that most people have a certain degree of vitamin C deficiency in the winter and at the beginning of spring. On the basis of this finding Demole considered the spring tiredness with the accompanying psychoneuroses was due to a prescorbutic condition. Degeller (1936) also found a seasonal fluctuation in the vitamin C content of the blood. He found the minimum during January to March and the maximum from June to October. Our investigations, however, lead us to believe that a reduced excretion is not necessarily a consequence of an increased requirement during the winter and spring months; it seems more likely that the low values found by the European investigators are indications of a lower vitamin C intake. Our results are completely the reverse of those reported in the literature due to the high intake of vitamin C during these months.

It may be asked whether the vitamin C supply of the Palestinian population is adequate in the summer, namely, between May and October. On the whole the average values (23–28 mg./l.) found among adults of Jerusalem during this time are not subnormal. It is of interest, however, that while 17 % of all samples showed a high vitamin C concentration (above 34 mg./l.), 15 % of the samples give values below 15 mg./l. The conditions were worst in September, when only 8 % of the adults gave values above 34 mg./l., and 18 % below 15 mg./l. It is apparent, therefore, that during the summer months an appreciable number of people are below standard in vitamin C.

SUMMARY

During the period November 1936–November 1937 the vitamin C excreted in the urine by adults and children was investigated in a number of places in Palestine. The pronounced influence of the citrus season (winter and spring) with its greater consumption of citrus fruit was noted in all the population groups investigated. In the summer, however, when the supply of fresh vegetable sources of vitamin C is remarkably poor, much lower, partly subnormal, excretion values are to be found.

The lower average excretion of vitamin C in the urine samples taken from children probably indicate a higher requirement by the growing than by the adult body.

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NOTE. Since this paper has been submitted for publication two studies of a similar character have appeared. Trier (*Klin. Wschr.* 1938, **17**, 976) studied the seasonal variation in the vitamin C in serum and Mueller (*Z. Vitaminforsch.* 1938, **7**, 311) studied the variation in vitamin C excreted in the urine. Both authors worked in Europe and obtained curves which are the mirror image of those obtained by us. The results emphasize the importance of the fluctuating seasonal supply of vitamin C in the diet.