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Leucocyte ascorbic acid and pregnancy

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1. Leucocyte ascorbic acid concentrations have been measured in 1147 females during early pregnancy and in smaller numbers of women before conception, throughout pregnancy and at 6 months post partum.

2. The leucocyte concentration in the 1st trimester was found to be affected by season, social class and smoking. Selecting individuals by extremes of social class, season and smoking produced two small populations with almost separate ascorbic acid distributions and mean concentrations of 21·7 and 45·1 $\mu g/10^8$ leucocytes.

3. Early pregnancy had little effect on leucocyte ascorbic acid concentrations but values decreased in the second trimester. However, this was associated with a leucocytosis so that the total leucocyte ascorbic acid content of blood was unchanged.

4. Low ascorbic acid concentrations during the 1st trimester were not associated with subsequent spontaneous abortions, still-births or neonatal deaths, but there was an increased frequency of low values in women who gave birth to infants smaller than 3250 g.

5. The adequacy of ascorbic acid reserves in early pregnancy is discussed.

Although recent work has shown that the ascorbic acid intake of a proportion of the population in the UK is below that recommended by the Department of Health and Social Security (1969) (Andrews, Brook & Allen, 1966; Allen, Brook & Broadbent, 1968; Eddy, 1968; Milne, Lonergan, Williamson, Moore, McMaster & Percy, 1971; Smithells, Ankers, Carver, Lennon, Schorah & Sheppard, 1977), the dietary allowance suggested by the Department is well above that intake which is believed to produce clinical scurvy (Hodges, Hood, Canham, Sauberlich & Baker, 1971). In addition, the effects of a moderate reduction in dietary ascorbic acid are largely unknown (Burr, Elwood, Hole, Hurley & Hughes, 1974). However, changes associated with a moderate depletion of cellular ascorbic acid have been reported (Brocklehurst, Griffiths, Taylor, Marks, Scott & Blackley, 1968; Kinsman & Hood, 1971; Sulkin & Sulkin, 1975) and such a depletion might be of disadvantage to the developing foetus, the long-stay hospital subject and the surgical patient. In these groups requirements may be increased to facilitate rapid growth or repair of tissue (Hume, Weyers, Rowan, Reid & Hillis, 1972; Chen & Raisz, 1975), combat infection (Hume & Weyers, 1973) and aid drug metabolism (Wilson, 1975; Zannoni & Sato, 1975). Inappropriately, 'at risk' populations, such as pregnant women and patients in hospital, rather than having an increased consumption, more frequently have poor ascorbic acid intakes (Eddy, 1968; Smithells *et al.* 1977). However, although intake has been assessed, little is known of the ascorbic acid status of these subjects.

In old people leucocyte ascorbic acid is found to be moderately decreased when intakes are below those recommended (Bowers & Kubik, 1965; Andrews *et al.* 1966; Milne *et al.* 1971; Burr *et al.* 1974).

In general there is a relationship between leucocyte and plasma ascorbic acid levels (Andrews & Brook, 1968), although the extent of correlation may depend on the age and sex of the subject (Loh & Wilson, 1971). Leucocyte levels are, however, believed to reflect total body ascorbic acid better than plasma levels (Andrews & Brook, 1968; Gerson, 1968) and are less affected by change in recent dietary intake (Sauberlich, 1975) such as that which

could accompany vomiting or adjusted intake in early pregnancy. Hence leucocyte ascorbic acid was chosen as a simple index of ascorbic acid reserves in pregnancy and this paper reports the levels found in women, primarily during early pregnancy, but throughout pregnancy on a smaller number of subjects. Other reports in preparation will consider surgical and institutionalized patients.

MATERIALS AND METHODS

Leucocyte ascorbic acid was measured in over a thousand pregnant women as part of a large-scale prospective study of nutrition in early pregnancy. Mothers volunteered through general practitioners and hospital antenatal clinics and were therefore not a representative population sample. No restriction was made on age or parity. Women were seen and blood samples were taken within 13 completed weeks from the start of the last menstrual period. In addition, a much smaller group also had blood samples taken 6 months post partum and a group of mothers who had previously given birth to children with malformations of the central nervous system (CNS) had blood analyses before pregnancy and during each trimester.

Mothers were interviewed at home when a comprehensive questionnaire was completed which incorporated sections describing social environment, smoking habit and details of all drugs, including vitamin supplements, taken since the beginning of pregnancy. Mothers were asked to attend for venepuncture in the morning either fasting or after eating no more than a light breakfast of tea and toast and a record was made of any pharmaceutical preparation taken since the completion of the questionnaire. For leucocyte ascorbic acid estimation a 4 ml sample of blood was dispensed into 1 ml sterilized acid–citrate–dextrose (ACD) consisting of 7·9 g citric acid monohydrate, 22·0 g trisodium citrate dihydrate and 22·2 g glucose/l. Samples were prepared for analysis within 3 h. A portion (3 ml) of the blood–ACD mixture was mixed with 1 ml of separating fluid using a rolling action. The separating fluid consisted of Dextraven 150 (a dextran of average molecular weight 150 000 in saline (9 g sodium chloride/l); Fisons Ltd, Loughborough)–EDTA (50 g/l) (2:1, v/v) autoclaved at 103 kN/m^2 (15 lb/in^2) for 20 min before storage at 4°. The mixture of blood and separating fluid was left for 30 min to sediment the red cells. A sample of the supernatant fraction was taken for cell counting and the remainder, containing approximately 45% of the white cells and some of the platelets in the original blood sample, was centrifuged and the cell plug resuspended in 1·3 ml trichloroacetic acid solution (50 g/l) and homogenized with a glass rod. The resulting suspension was frozen and analysed within 3 weeks by the 2,4-dinitrophenylhydrazine technique of Denson & Bowers (1961). The average 'between-batch' coefficient of variation of control samples run with each analysis throughout the survey period was ± 11·4%.

Results are expressed as μg/10^8 leucocytes (μg/10^8 L) and also as μg in leucocytes/ml blood (μg L/ml blood). The latter values were calculated by the formula:

$$\frac{\text{ascorbic acid } (\mu\text{g}/10^8\text{ L}) \times \text{no. of leucocytes/ml blood}}{10^8}.$$

White cell counts were measured using a Coulter counter (model S; Coulter Electronics Ltd, Harpenden, Herts) on sequestrene samples (1·5 g EDTA/l blood) collected at the same time as the 'ACD' sample. This method of expressing leucocyte ascorbic acid was used when there were significant differences in white cell numbers between the population groups under examination as it has been reported that the leucocyte ascorbic acid concentration varies inversely with the number of circulating white cells (Griffiths, 1968; MacLennan & Hamilton, 1976). These values will not be considered in the text when mean leucocyte counts do not differ significantly.

Table 1. *Leucocyte ascorbic acid levels in pregnant women* and the effect of the preceding meal*

(Mean values and standard deviations)

		No. of women	Ascorbic acid (μg/10⁸ leucocytes) Mean	SD
Total population		1147	34·5	12·8
Preceding meal	Tea and toast or none	929	34·4	12·9
	More substantial meal	218	34·8	12·2

* For details, see p. 140.

Table 2. *Seasonal changes in leucocyte ascorbic acid levels of pregnant women*, pooled by month, during a 2 year period*

(Mean values and standard deviations)

	Month	No. of women	Ascorbic acid μg/10⁸ leucocytes Mean	SD	Ascorbic acid μg leucocytes /ml blood Mean	Leucocytes (no./mm³ blood) Mean	SD
1970–1	June	79	35·1	11·3	2·79	7945	1784
	July	70	†39·2 39·2	12·6	3·00	7648	1735
	August	39	†39·2 39·6	14·4	2·92	7384	1591
	September	79	†39·2 38·9	13·1	3·02	7750	1680
	October	58	†34·2 36·9	14·3	2·58	6996	1821
	November	70	†34·2 31·7	10·3	2·51	7923	1781
	December	68	†34·2 34·4	15·6	2·33	6774	1960
1971–2	January	84	†35·8 37·2	13·5	2·72	7311	1619
	February	108	†35·8 34·3	12·9	2·56	7475	1518
	March	113	†35·8 36·1	13·5	2·63	7286	1645
	April	86	†30·2 28·6	10·5	2·05	7149	1570
	May	65	†30·2 27·5	8·8	2·21	8038	2003
	June	68	†30·2 34·9	11·9	2·56	7348	1684

* For details, see p. 140.
† Quarterly groupings.

RESULTS

Table 1 shows the mean values and standard deviations of the leucocyte ascorbic acid levels in the study population. Women taking ascorbic acid supplements in the week before venepuncture have not been included. Probability plots of the population indicate a distribution skewed towards the upper limit with a 95% range of 15–66 μg/10⁸ L.

Subjects were asked to attend either fasting or after a light breakfast (see p. 140), but some forgot to follow these instructions and others were able to attend only in the early afternoon after a light lunch. A comparison between leucocyte ascorbic acid of those women who had eaten a light breakfast as requested and those who had eaten more substantially showed no significant difference (Table 1).

Table 3. *Leucocyte ascorbic acid levels of pregnant women* relative to social class*

(Mean values and standard deviations)

Class†	No. of women	Ascorbic acid: μg/10⁸ leucocytes Mean	Ascorbic acid: μg/10⁸ leucocytes SD	Ascorbic acid: % decrease below class I	Ascorbic acid: μg leucocytes /ml blood Mean	Ascorbic acid: % decrease below class I	Leucocytes (no./mm³ blood) Mean	Leucocytes (no./mm³ blood) SD
I	126	‡37·1	12·6	—	2·71	—	7303	1642
II	173	‡36·8	12·4	0·8	2·71	0·0	7355	1587
IIIN	177	35·3	12·7	4·9	2·60	4·1	7375	1842
IIIM	441	33·4	12·9	10·0	2·54	6·3	7611	1717
IV	130	33·6	12·5	9·4	2·56	5·5	7623	1787
V	51	§29·6	11·9	20·2	2·19	19·2	7407	1855
VIII	17	30·8	11·9	17·0	2·30	15·1	7467	1639

* For details, see p. 140.

† Based on the Registrar General's classification (Office of Population Censuses and Surveys, 1970): I, professional; II, intermediate occupations; IIIN, skilled, non-manual; IIIM, skilled, manual; IV, partly skilled; V, unskilled. Group VIII is the authors' grouping, economically inactive for ≥ 6 months.

‡ Values for classes I and II were significantly higher than: class IIIM, $P < 0.01$; class IV, $P < 0.05$; class V, $P < 0.001$.

§ Value for class V was significantly lower than: classes I and II, $P < 0.001$; class IIIN, $P < 0.01$; classes IIIM and IV, $P < 0.05$.

Season

For 25 months of the study sufficient samples were collected to allow monthly mean values to be determined accurately. Four of the five lowest mean values occurred in the months of April and May of each year. No change in quality-control was noted which could have explained this finding on the grounds of changes in accuracy of the method. The mean values over this 2 year period (June 1970–June 1972 inclusive), pooled by month, are shown in Table 2. For clarity, pooled quarterly values are also shown. The mean values for both April and May were significantly lower ($P < 0.01$) than those of all other months except November. Indeed, of the seventy-eight possible pair-combinations for the thirteen monthly values, significant differences were present in twenty-six instances ($P < 0.01$) and twenty of these included either April or May as half of the pair (see p. 148).

Converting the mean values for leucocyte ascorbic acid to leucocyte ascorbic acid on a per ml blood basis using the mean leucocyte counts for each month did not alter the trends (Table 2), indicating that low white cell concentrations in April and May were not due to increased leucocyte numbers.

Social class

In a subsection of this pregnant population we found that social class groups based on the Registrar General's classification (Office of Population Censuses and Surveys, 1970) had different dietary intakes (Smithells *et al.* 1977). Table 3 shows that these dietary differences were reflected by leucocyte ascorbic acid stores, there being a gradual and in many instances significant decrease in the mean leucocyte ascorbic acid as the social class number increased. Social class VIII (our classification for those who were economically inactive for periods in excess of 6 months before the study) had an ascorbic acid level closest to class V.

Within each of the social groups I+II and IV+V (individual classes were paired to increase numbers) the seasonal difference persisted (Table 4). This was also true for the social class differences at specific times of the year, although these differences were not as great as those produced by season (Table 4). This indicated separate effects of season and social class on leucocyte ascorbic acid.

Table 4. *The effects of season and social class on leucocyte ascorbic acid levels of pregnant women**

(Mean values and standard deviations)

Class†	Season	No. of women	Ascorbic acid (μg/10^8 leucocytes) Mean	SD
I+II	July, Aug, Sept.	39	43·7	11·8
I+II	April, May	50	30·8	9·1
IV+V	July, Aug, Sept.	36	38·2	12·9
IV+V	April, May	25	25·0	10·2

Value for group I+II, July, Aug, Sept. was significantly higher than: group I+II, April, May, $P < 0.001$; group IV+V, July, Aug, Sept., $P < 0.05$ (one tailed t test).

Value for group IV+V, April, May was significantly lower than: group I+II, April, May, $P < 0.01$; group IV+V, July, Aug, Sept., $P < 0.001$ (one tailed t test).

* For details, see p. 140.

† Based on the Registrar General's classification (Office of Population Censuses and Surveys, 1970): I, professional; II, intermediate occupations; IV, partly skilled; V, unskilled.

Table 5. *The effect of smoking on leucocyte ascorbic acid levels of pregnant women**

(Mean values and standard deviations)

Smoking habit (no. of cigarettes/d)	No. of women	Ascorbic acid μg/10^8 leucocytes Mean	SD	% decrease below non-smokers	μg leucocytes /ml blood Mean	% decrease below non-smokers	Leucocytes (no./mm³ blood) Mean	SD
None (0)	760	36·1	12·8	—	2·60	—	7190	1629
Light (1–9)	126	33·8	13·0	6·4	2·61	0	7721	1691
Moderate (10–20)	243	†30·2	11·4	16·3	2·47	5·0	8192	1736
Heavy (>20)	15	‡28·3	11·8	21·6	2·33	10·4	8230	2374

* For details, see p. 140.

† Value for moderate smokers was significantly different from that of non-smokers ($P < 0.001$) and light smokers ($P < 0.01$).

‡ Value for heavy smokers was significantly different from that of non-smokers ($P < 0.05$).

Smoking habit

Smokers had a mean leucocyte ascorbic acid concentration that was significantly lower than that of non-smokers, and those smoking moderately had a lower average value than those smoking between 1 and 9 cigarettes/d (Table 5). However, in this study, smokers also had an increased number of white cells in circulation. In consequence, although there was a percentage decrease in the leucocyte ascorbic acid concentration of smokers compared with non-smokers, this decrease was less when values were expressed as ascorbic acid μg L/ml blood (Table 5).

Smoking became significantly more common with decreasing social status (χ^2 analysis). Those smoking more than 9 cigarettes/d in the paired social class groupings I+II, IIIN+IIIM and IV+V were 10, 26 and 34% of the total respectively. How much does smoking habit contribute to the social class differences in leucocyte ascorbic acid? Reference to Table 6 suggests that this was insignificant. Here the effect of smoking on ascorbic acid levels within the social classes was examined. Again classes were paired to increase numbers of women. It can be seen that differences between social classes remained significant for the

Table 6. *The effects of smoking and social class on leucocyte ascorbic acid levels of pregnant women**

(Mean values and standard deviations)

Class†	No. cigarettes/d	No. of women	Ascorbic acid (μg/10^8 leucocytes) Mean	SD
I+II	0	248	37·6	12·9
	10–20	30	31·8	9·1
IIIN+IIIM	0	379	35·9	13·0
	10–20	144	30·4	11·8
IV+V	0	99	32·9	11·2
	10–20	61	29·7	11·8

Values for non-smokers were significantly higher than those for smokers (10–20 cigarettes/d) in group I+II ($P < 0{\cdot}01$), group IIIN+IIIM ($P < 0{\cdot}001$) and group IV+V ($P < 0{\cdot}05$) (one tailed t test). Value for non-smokers in group IV+V was significantly lower than for non-smokers in group I+II ($P < 0{\cdot}001$) and group IIIN+IIIM ($P < 0{\cdot}05$) (one tailed t test). Differences in values for smokers between the social class groups were not significant.

* For details, see p. 140.

† Based on the Registrar General's classification (Office of Population Censuses and Surveys, 1970): I, professional; II, intermediate occupations; IIIN, skilled, non-manual; IIIM, skilled, manual; IV, partly skilled; V, unskilled.

Table 7. *Leucocyte ascorbic acid levels in extreme groupings of pregnant women**

(Mean values and standard deviations)

Class†	Season	No. cigarettes/d	No. of women	Ascorbic acid (μg/10^8 leucocytes) Mean	SD	95% range
I+II	July, Aug, Sept.	0	33	45·1	12·1	29–70
IV+V	April, May	10–20	11	21·7	6·6	14–34

* For details, see p. 140.

† Based on the Registrar General's classification (Office of Population Censuses and Surveys, 1970): I, professional; II, intermediate occupations; IV, partly skilled; V, unskilled.

non-smoking group but were non-significant in those smoking moderately. Smoking reduced white cell ascorbate within each class grouping with the possible exception of social classes IV+V. However, heavy smokers were not included because of small numbers, but results on fourteen subjects in group IIIN+IIIM smoking more than 20 cigarettes/d showed a further decrease to 27·2 μg/10^8 L.

Extreme groupings

For each factor (smoking, season and social class) mean differences in leucocyte ascorbate concentrations, although significant, were not large. It was, however, possible to consider extreme situations which could have arisen at different times of the year in the various social classes. Table 7 shows that the distributions of the values in these extreme populations were almost completely separated producing large differences in their mean values.

Table 8. *Effect of pregnancy on leucocyte ascorbic acid levels*

(Mean values and standard deviations)

		Ascorbic acid							
		μg/10^8 leucocytes			μg leucocytes /mg blood			Leucocytes (no./mm^3 blood)	
	No. of women	Mean	SD	% decrease below NP	Mean	SD	% decrease below NP	Mean	SD
Not taking OC post partum		(A) Post partum and 1st trimester							
1st Trimester	26	‡31·9	11·0	—	2·46	0·72	—	*7926	1388
Post partum	26	‡30·7	9·8	—	2·08	0·72	—	6942	1548
Taking OC post partum									
1st Trimester	19	*37·2	15·4	—	2·75	1·25	—	7363	1626
Post partum	19	27·3	8·8	—	2·18	0·90	—	8053	2565
		(B) Before and during pregnancy							
Non-pregnant	28	31·8	12·3	—	2·18	0·82	—	†7030	1780
1st Trimester	9	29·6	12·8	6·9	2·25	0·77	increase	8070	1890
2nd Trimester	15	22·3	10·7	29·9	1·90	0·77	12·8	8900	2600
3rd Trimester	11	24·0	11·1	24·5	2·20	1·07	0·0	9230	1900

NP, non-pregnant.
OC, oral contraceptives.
* Values were significantly different from post partum values ($P < 0·01$) (paired t test).
† Value was significantly different from that for 2nd trimester ($P < 0·05$) and 3rd trimester ($P < 0·01$).
‡ Correlation coefficient between values in the two groups 0·56.

Effect of pregnancy

A group of mothers whose ascorbic acid was estimated during the 1st trimester had repeat analyses made 6 months post partum (Table 8A). The mean leucocyte ascorbic acid concentration for these two groups differed little, although the significantly higher leucocyte count in early pregnancy increased the total leucocyte ascorbate above the post partum level. A smaller group of women who were taking oral contraceptives post partum had values which at that time period were significantly lower than during pregnancy. It is, however, well documented that oestrogen therapy lowers circulating ascorbic acid (Briggs & Briggs, 1973; Rivers & Devine, 1975).

A number of women were also followed throughout pregnancy with samples taken before they conceived and during the three trimesters. This group was specially selected as having had a previous child with a CNS malformation and thus was not representative of the general population. Results are reported in Table 8B. Despite reduced numbers, because of failure to re-attend or ascorbic acid supplementation in the later stages of pregnancy, a marked reduction in ascorbic acid was noted predominantly in the 2nd trimester. The difference between the mean value of the non-pregnant plus 1st trimester values ($31·2 \pm 12·3$ μg/10^8 L) and the mean value for later pregnancy ($23·0 \pm 10·7$ μg/10^8 L) was significant. This change would appear, as with smoking, to be associated with a leucocytosis. The recalculation of each individual value as ascorbic acid μg L/ml blood showed that total body white-cell ascorbic acid altered little during pregnancy.

Table 9. *Observations on birth weight (% of mothers† with children < or ⩾ 3250 g birth weight who had a leucocyte ascorbic acid < 20 μg/10⁸ L, a leucocyte ascorbic acid < 16 μg/10⁸ L, or who were from social class IV or V‡ or smoked ⩾ 10 cigarettes/d)*

(Values in parentheses exclude women with gestation < 38 weeks or > 42 weeks)

	Birth weight < 3250 g (% total)	Birth weight ⩾ 3250 g (% total)
Ascorbic acid (< 20 μg/10⁸ L)	***14·3 (15·1)	***6·7 (6·8)
Ascorbic acid (< 16 μg/10⁸ L)	*5·1 (5·4)	*1·8 (1·8)
Social class IV and V‡	16·4	13·8
Smoking ⩾ 10 cigarettes/d	**26·3	**16·3

$2 \times 2\chi^2$ analyses were significant: $*P < 0{\cdot}05$, $**P < 0{\cdot}005$, $***P < 0{\cdot}001$.

† For details, see p. 140.

‡ Based on the Registrar General's classification (Office of Population Censuses and Surveys, 1970): IV, partly skilled; V, unskilled.

Outcome of pregnancy

Our findings showing the effect of general nutrition in early pregnancy on the outcome of that pregnancy will be considered elsewhere. However, some of the ascorbic acid findings will be reported here. One could predict that an ascorbate depletion in the 1st trimester, if of consequence, would influence the early events of pregnancy. Induction of foetal malformations is one example and the possible effect of a reduced ascorbic acid on the incidence of CNS malformations is reported elsewhere (Smithells, Sheppard & Schorah, 1976). Abortions are another early occurrence, but sixty-seven women who aborted spontaneously had a mean ascorbic acid value that was not significantly different from the 1080 who did not, $36{\cdot}7 \pm 13$ and $34{\cdot}4 \pm 12{\cdot}8$ μg/10⁸ L respectively.

Death near to term occurred in a heterogeneous group of twenty-nine foetuses and neonates, but their mothers' 1st trimester ascorbic acid mean value was not significantly different from that of the total population ($32{\cdot}5 \pm 8{\cdot}4$ μg/10⁸ L). However, an examination of birth weight did suggest that mothers of lighter children had a higher incidence of low ascorbic acid levels in early pregnancy and that ignoring gestations less than 38 weeks or greater than 42 weeks had little effect on these frequencies (Table 9). There were also more smokers in the light-birth-weight group but surprisingly little increase in the proportion of social classes IV + V. Mean values of maternal leucocyte ascorbic acid for the light- (< 3250 g), medium- (3250–3750 g) and heavy- (> 3750 g) birth-weight groups of children, although showing a slight increase with increasing birth weight ($34{\cdot}1 \pm 13{\cdot}2$, $34{\cdot}7 \pm 12{\cdot}5$, $35{\cdot}6 \pm 12{\cdot}7$ μg/10⁸ L respectively), were not significantly different. Probability plots of the values showed that the distribution differences seen at low ascorbic acid values were not maintained at values greater than 30 μg/10⁸ L, i.e. moderate or increased levels of ascorbic acid did not predominate in the mothers of larger children in the way that lower values predominated in the mothers of light-birth-weight babies.

DISCUSSION

Seasonal changes in body ascorbic acid reserves could be predicted from dietary intake (National Food Survey Committee, 1973–5) and have been reported in geriatric subjects (Andrews *et al.* 1966; Milne *et al.* 1971). In all these publications, however, it was the winter months which provided the lowest intakes and the poorest body stores. Our results from the pregnant population suggest that the lowest body reserves occur in the spring, a finding supported by Davidson, Passmore & Brock's (1972) observation that the occurrence of

scurvy is commonest in Edinburgh during the spring and early summer. This could result from the consumption of old potatoes low in ascorbic acid at this time of year (McCance & Widdowson, 1960). In addition, in 1971 the lowest consumption of ascorbic acid was recorded in March and April (D. H. Buss, personal communication) and the mean value published by the National Food Survey Committee (1973–5) for the spring quarter is thus an average of the initial low intakes and the much higher ones seen in June. The highest leucocyte ascorbic acid values we recorded were in mid-summer and coincide with the period of peak dietary intake of this vitamin, an effect which is largely due to consumption of the new potato (National Food Survey Committee, 1973).

Our findings of social class differences in leucocyte ascorbate are supported by further reference to the values published by the Ministry of Agriculture, Fisheries and Food (1970–72) which show that the lower the income group the smaller the intake of ascorbic acid.

Smoking induces a leucocytosis (Friedman, Siegelaub, Seltzer, Feldman & Collen, 1973) and is also known to reduce leucocyte and serum ascorbic acid concentrations (Brook & Grimshaw, 1968; Pelletier, 1975; Burr *et al.* 1974). We have further confirmed the report of Andrews & Bonsnes (1951) that advancing pregnancy also increases the number of white cells and have demonstrated a concurrent reduction in leucocyte ascorbic acid concentrations, a change which might be predicted from decreases in plasma ascorbic acid reported by others (Mason & Rivers, 1971; Vobecky, Vobecky, Shapcott & Munan, 1974). However, because of the leucocytosis it is possible that the changes we observed in leucocyte ascorbic acid content may, partially in smokers and more completely in pregnancy, reflect a redistribution of ascorbic acid amongst a greater number of leucocytes rather than a decrease in body ascorbic acid status. Alternatively, it is known that platelets contaminating the simple dextran-leucocyte preparation contain significant quantities of ascorbic acid (Attwood, Robey, Ross, Bradley & Kramer, 1974). Expressing the results on a $\mu g/10^8$ L basis will artificially reduce the platelet contribution to the leucocyte ascorbic acid concentration to an extent which increases with increasing leucocyte number. Changes in white cell numbers do not appear to contribute to seasonal and social class differences in leucocyte ascorbic acid concentration.

The results reported in this paper show how an apparently homogeneous population of pregnant women is composed of definable subgroups which may differ greatly in their leucocyte ascorbate reserves. The variables which isolate these groups largely affected leucocyte ascorbic acid independently (Tables 4, 6), but a low ascorbic acid induced by season, smoking or social class limited the ability of the other factors to further reduce the value, as though larger leucocyte reserves are more easily depleted by a reduction in intake than lower levels.

It is difficult to assess the adequacy of the levels of ascorbic acid found in this pregnant population. A survey of other publications using buffy-layer ascorbic acid to assess body ascorbate status in general populations, suggests that levels of $> 20\ \mu g/10^8$ L are adequate, those between 8 and 20 $\mu g/10^8$ L are low and concentrations of $< 8\ \mu g/10^8$ L are deficient (Brocklehurst *et al.* 1968; Kinsman & Hood, 1971; Sauberlich, 1975). In comparison with these levels, the majority in this pregnant population is adequately supplied. Only 9·5% of the women had values of $< 20\ \mu g/10^8$ L with 2·5 % of women having values of $< 15\ \mu g/10^8$ L. However, the fact that women in this study were pregnant complicates comparisons with a general population. Higher reserves may be appropriate to ensure an adequate supply to the rapidly developing foetus and give sufficient maternal reserves to prevent depletion of both mother and foetus as pregnancy progresses. Nevertheless, this consideration aside, almost 50% of social class IV and V mothers who smoke and who are pregnant during spring have a leucocyte ascorbate content which would be considered unsatisfactory in the general population (Table 7).

It must be emphasized however, that the effect on the foetus of any extent of maternal ascorbic acid depletion is largely unknown, although animal studies indicate both a decreased litter frequency and an increased number of abortions even in marginal deficiency (Pye, Taylor & Fontanares, 1961; Rivers & Devine, 1975). In the human it has been suggested that decreased ascorbic acid levels could be associated with increased neonatal mortality and decreased birth weight (Wideman, Baird & Bolding, 1964), whilst other publications have shown no association between low ascorbic acid and the outcome of pregnancy (Vobecky *et al.* 1974; Rivers & Devine, 1975). We have no measure of fertility and in agreement with Vobecky *et al.* (1974) we could show no relationship between low ascorbic acid in early pregnancy and neonatal mortality or still-births, but our numbers are small. Our observation of a prevalence of lower ascorbic acid concentrations in mothers of lighter-birthweight children could be associated with the known involvement of ascorbic acid in bone and connective tissue growth (Chen & Raisz, 1975). Alternatively, an increased incidence of smoking by mothers who gave birth to children lighter than 3250 g could account for the decreased birth weights in this group (Table 9). However, as lower leucocyte ascorbic acid levels are also observed in smokers, an increased incidence of smoking in the lower-birthweight group does not necessarily argue against ascorbic acid as a factor in determining birth weight. On the other hand, nutritional status in later pregnancy should arguably be important in determining the size of the child and multinutritional deficiencies are more likely to be disadvantageous to the foetus than ascorbic acid depletion alone (Dobbing, 1970; Gordon, 1975; Smithells *et al.* 1976).

In conclusion, whilst the effect of a moderate ascorbic acid depletion is uncertain, results presented here suggest that in the light of available evidence most women in early pregnancy do have adequate ascorbic acid reserves. Any concern should be directed towards social classes IV and V especially when pregnancy or illness coincides with early spring. Inadequacy in this group is becoming an increasing problem for we have recently seen the first marked decrease in ascorbic acid intake for several years due to the increase in cost of the potato (National Food Survey Committee, 1976). If this decreased intake is reflected in body reserves, then leucocyte levels would have been lower in the spring of 1976 than they were in the years of comparative plenty when this survey was completed.

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Statistical note

An examination of the results indicated that distributions of values were not strictly Gaussian. However, tests of significant difference between the population means were analysed by Student's t test provided that variances of the populations were similar, as assessed by variance ratios. Variances differed significantly between months (Table 2), but these differences were not present when values were represented on log scales, a transformation which produced distributions closer to normal. Significances of the difference between the means of monthly log values were, however, little changed from those calculated on linear values.

All probability values in the tables refer to Student's t test with the exceptions of Table 8 A where analyses on the same individual were compared by paired t tests and in Table 9 where values were subjected to χ^2 analysis.

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