Histamine and Ascorbic Acid in Human Blood

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ABSTRACT Analysis of 437 human blood samples has shown that when the plasma-reduced ascorbic acid level falls below 1 mg/100 ml, the whole blood histamine level increases exponentially as the ascorbic acid level decreases. When the ascorbic acid level falls below 0.7 mg/100 ml, there is a highly significant increase in the blood histamine level. Oral administration of ascorbic acid (1 g daily for 3 days) to 11 selected volunteers resulted in a reduction of the blood histamine level in every instance. J. Nutr. *110:* 662–668, 1980.

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Studies of guinea pigs by Chatterjee et al. (1) have shown that the blood histamine level starts to rise on the third day of receiving a scorbutogenic diet, at a stage when the plasma ascorbic acid level has only just begun to fall below 1 mg/100 ml; the histamine level was found to be markedly elevated after 14 days when the animals began to develop signs of scurvy.

Moreover, Chatterjee et al. (2) have shown that ascorbic acid could be responsible for the breakdown of histamine to hydantoin acetic acid, and they suggest that the accumulation of histamine in the blood and tissues in scurvy may be due to a lack of this chemical change. These authors also suggest that the vasodilating action of histamine on the small blood vessels may be responsible for some of the manifestations of scurvy. There are several ways in which inflammation has seemed to resemble local scurvy; now it is apparent that this may be more than a coincidence, for it is possible that scurvy may be partly due to histamine intoxication.

The present study was undertaken in an attempt to answer three physiological questions: 1) Do persons with low plasma ascorbic acid levels have elevated whole blood histamine levels?; 2) If so, at what ascorbic acid level does the histamin level begin to be elevated?, and 3) Does oral ascorbic acid-loading cause a decrease in the blood histamine level of persons with suboptimal ascorbic levels?

METHODS AND MATERIALS

Permission was obtained from the Research Committee and from the Ethical Review Committee of The Methodist Hospital to obtain venous blood samples for research purposes from consenting women in the middle and third trimesters of pregnancy. All of these women had been given a prescription for the usual prenatal vitamin supplement (Stuart Prenatal, Stuart Pharmaceuticals Division of I.C.I., Wilming, DE) providing 60 mg of ascorbic acid with other vitamins and had been advised to take one a day.

Blood was also obtained from male and non-pregnant female members of the hospital staff. Eleven non-pregnant subjects, who were found to have suboptimal ascorbic acid levels or higher than average histamine levels, volunteered to take 0.5 g ascorbic acid tablets twice daily for 3 days and then have another blood sample drawn, without taking ascorbic acid, on the morning of the fourth day.

Random non-fasting blood samples (20 ml each) were obtained from women attending the prenatal clinic at The Meth-

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odist Hospital and from the other volunteers between 0800 and 1000 hours 5 days a week. The blood samples were placed in heparinized tubes and were taken to our department laboratory for analysis without delay. Addition of acid for both analyses was always carried out within 1 hour of collecting the blood. Avoidance of delay in analysis for ascorbic acid is essential, as reduced ascorbic acid in blood plasma is lost by oxidation at a rate of 5–8%/hour at room temperature (3). For this and other reasons no more than six blood samples were analyzed in any one day. At the time of writing, 437 blood samples obtained from 400 people have been analyzed, both for reduced ascorbic acid and for histamine; 240 of these came from 223 pregnant women attending the prenatal clinic.

Whole blood histamine analysis. As soon as each 20 ml blood sample arrived in the laboratory, 10 ml was pipetted off and added to 1 ml of 12 N perchloric acid and 9 ml of distilled water, as the first step in the analysis for histamine by the method of Shore, Burkhalter and Cohn (4) using a spectrofluorometer (Model 430, Turner Associates, Palo Alto, CA). In this method, histamine is extracted and then condensed with ortho-phthalaldehyde to yield a relatively stable fluorescent compound. Duplicate analyses were obtained for all specimens.

Plasma reduced ascorbic acid analysis. The remainder of the blood was centrifuged to obtain clear plasma with no evidence of hemolysis; any hemolyzed specimen was discarded since hemolysis causes rapid oxidation of ascorbic acid. Turbidity of plasma may also cause a problem in analysis, but this becomes evident in the blank correction; care was taken to avoid the buffy coat of leukocytes and platelets as these are rich in ascorbic acid. Only glass tubes and pipettes that had been soaked in acid and then washed three times in demineralized distilled water were used.

Four milliliters of the plasma was drawn off using a rubber bulb aspirator with a finger pressure control valve and was added to 6 ml of 5% metaphosphoric acid as the first step in the analysis for ascorbic acid by the 2,6-dichloroindophenol method of Roe (5). The tube was then covered by a paraffin wax film (Parafilm M, American Can Co., Marathon Products, Neenah, WI), shaken to mix the contents and centrifuged at 1,000 rpm for 10 minutes. The supernatant fluid was decanted and filtered twice using the same filter paper (Whatman No. 50, Whatman Inc., Clifton, NJ) to obtain a clear extract for analysis. A grating spectrophotometer with a 25 cm linear-log recorder (Model DBG, Beckman Instruments, Fullerton, CA) was used to obtain a graphic record of all analyses.

An indophenol dye solution was prepared as follows: 100 mg of 2,6-dichloroindophenol was placed in a 1 liter flask. This was then filled to the mark with 1.6% sodium citrate, mixed, filtered and stored in a refrigerator. A stock ascorbic acid solution (100 mg/100 ml) was prepared in 0.5% oxalic acid using fresh refrigerated and dessicated ascorbic acid powder. Standard ascorbic acid solutions (1 mg/100 ml) were prepared daily as needed from this stock in 0.5% metaphosphoric acid. The blank solution consisted of 0.5% metaphosphoric acid. The spectrophotometer was set at 520 nm, the recorder was set to run at 2.5 cm/minute and 1 cm light path rectangular glass cells were used. A 2 ml sample of standard, blank or plasma filtrate was placed in the sample cell; 1 ml of indophenol dye was rapidly injected into the cell using an automatic pipette (Eppendorf, Brinkmann Instruments Inc., Westbury, NY), just as the pen of the recorder passed one of the minute lines on the paper.

The optical density (OD) for zero time (when the buffered indophenol dye is added) was calculated by plotting backwards from the OD values for 30 seconds and for 1 minute. Correction for turbidity was made by decolorizing the dye with a pinch of ascorbic acid after each reaction had been completed. The OD, after complete decolorization, was substracted from the OD zero time for each sample to get the corrected OD zero time. Three blank analyses and three analyses for each sample were carried out on all occasions. The OD change, due to reduction of the dye by ascorbic acid in the sample, was calculated

TABLE 1

Relationship between plasma ascorbic acid and blood histamine[1]

	Plasma ascorbic acid	Number of samples	Mean whole blood histamine	Statistical significance of differences	Mean age of group
	mg/100 ml		*ng/ml*		*years*
A	0.00–0.19	14	59.1±10.1[2]	A versus B $P < 0.001$	27.2
B	0.20–0.39	35	36.5± 6.2	B versus C $P < 0.001$	28.9
C	0.40–0.59	61	27.9± 1.4	C versus D $P < 0.001$	28.1
D	0.60–0.79	101	21.0± 1.0	D versus E NS	26.8
E	0.80–0.99	99	19.2± 1.0	E versus F NS	27.6
F	1.00–1.19	61	16.8± 0.9	F versus G NS	30.7
G	1.20–1.39	30	18.1± 2.4	G versus H NS	28.5
H	1.40–1.59	17	17.1± 1.7		31.8
I	1.60–1.79	5	13.8		27.2
J	1.80–1.99	5	16.0		31.8
K	2.00–2.19	6	12.0		29.0
L	2.20–2.39	1	13.0		31.0
M	2.40–2.59	2	17.0		36.0

[1] Results of analysis of 437 blood samples are divided into even-numbered 0.2 mg/100 ml plasma ascorbic acid groups. [2] Mean±SEM.

from the difference between the mean zero time corrected OD sample and the mean zero time corrected OD blank. Including the correction procedure for the blanks as well as the samples obviates any concern about variability in the optical density of the cells.

RESULTS

The results of analysis of all 437 blood samples from 400 people are shown in table 1, where it is evident that persons with low plasma ascorbate levels have high histamine levels. It may be noted that the histamine level rose gradually when the ascorbic acid level fell below 1 mg/100 ml; statistical analysis using Student's *t*-test showed that this rise became highly significant when the ascorbic acid level fell below 0.7 mg/100 ml. Plasma reduced ascorbic acid levels between 0.50 and 0.69 mg/100 ml were associated with a significantly higher mean blood histamine level than were ascorbic acid levels between 0.70 and 0.89 mg/100 ml ($P < 0.001$).

The relationship between whole blood histamine in ng/ml (y) and plasma reduced ascorbic acid in mg/100 ml (x) is closely approximated by the formula

$$y = 2.5\ x^{-2} + 17.5$$

Figure 1 shows the results of analysis of 240 blood samples obtained from 223 pregnant women attending the prenatal clinic. The same relationship between low ascorbic acid and high histamine levels is seen, but the mean histamine levels for each ascorbic acid group were somewhat lower in the pregnant women than in the non-pregnant women (fig. 2). The non-pregnant women used for comparison in figure 2 were those who stated they were not taking birth control pills or other medications. However, the mean age of the pregnant women (24.8 years) was younger than that of the non-pregnant women (36.4 years), so one does not know whether their lower histamine levels were due to their pregnant state or to their relative youth.

Table 2 shows the results of the oral ascorbic acid-loading tests which were performed on 11 volunteers who had been

found to have suboptimal ascorbic acid levels or somewhat elevated blood histamine levels. The whole blood histamine level was markedly decreased in 10 of the 11 subjects after receiving 1 g ascorbic acid daily for 3 days. There was even a slight decrease in the blood histamine level of the other subject R.A.M. listed second in table 2. One of the most interesting observations was that subject S.G.F., a 31-year-old male resident obstetrician, was found to have the highest blood histamine level in the study after he had been up all night and working in the delivery room for 24 hours. His histamine level was 180 ng/ml and ascorbic acid 0.14 mg/100 ml. When tested again after a night of sleep, his histamine level had fallen to 82 ng/ml, which was still higher than all but one other subject in the study, and his ascorbate level was still low. After taking 1 g of ascorbic acid daily by mouth for 3 days, his plasma ascorbic acid level had risen to 1.15 mg/100 ml and his histamine level had fallen to 17 ng/ml. He smokes cigarettes and this is a known cause of ascorbic acid depletion, so smoking may have been partly responsible for his low plasma ascorbic acid level; it would seem that the stress of having worked long hours may also have contributed to his very high histamine level.

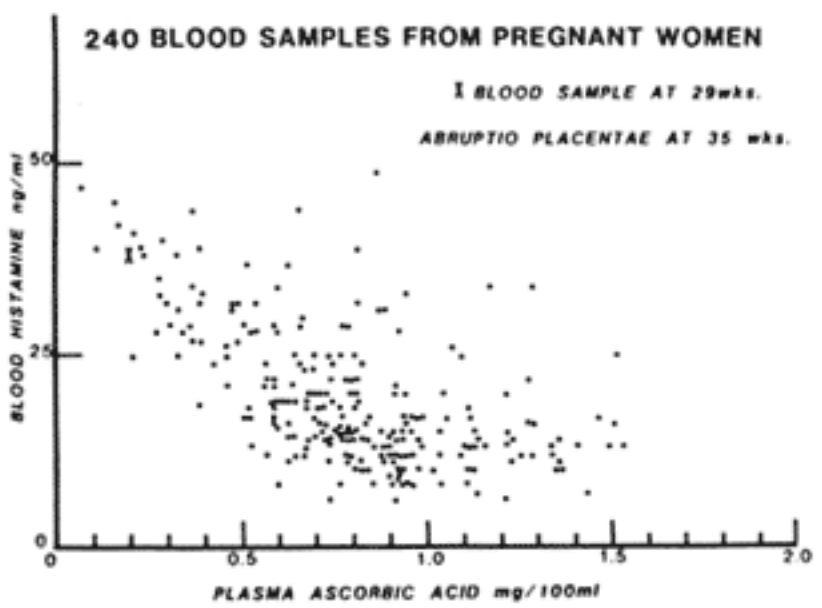

Fig. 1 The results of analysis of all blood samples obtained from pregnant women before the onset of labor are shown here. Only one of these women subsequently developed abruptio placentae; the ascorbic acid and histamine levels of the blood sample obtained from her in the 29th week of pregnancy were 0.19 mg/100 ml and 38 ng/ml and are marked by an X on the graph.

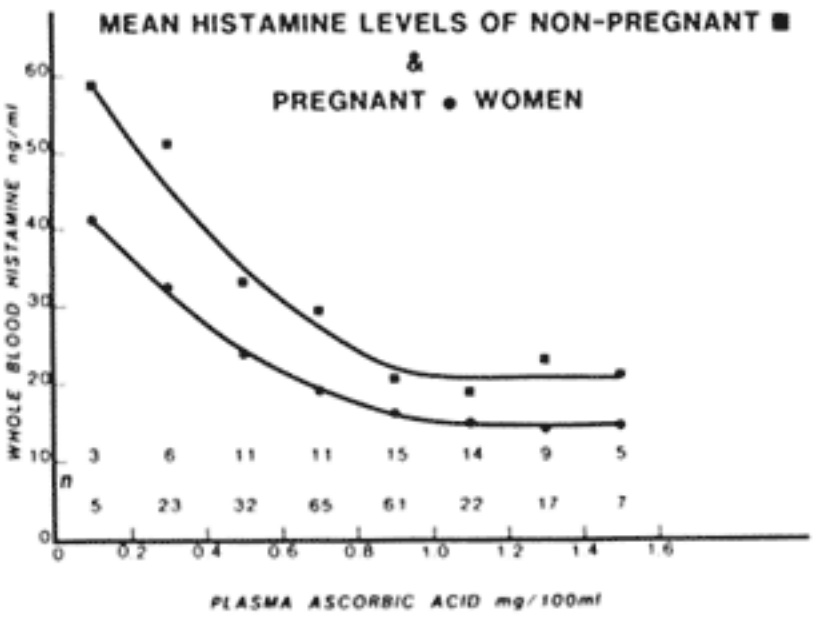

Fig. 2 Comparison of the mean blood histamine levels for each ascorbic acid group in the pregnant and non-pregnant women of this study showed lower histamine levels in the pregnant women. However, it is not known whether this difference was due to pregnancy or to the younger age of the pregnant women.

DISCUSSION

It is evident that persons with low plasma ascorbic acid levels do have elevated blood histamine levels, but some persons have high blood histamine levels without having low ascorbate levels. Clearly, there can be many reasons for a person's elevated blood histamine level. One of these may be stress or simply being overtired. Thus, we can see how smoking, malnutrition, stress and other factors can contribute to elevated blood histamine levels. This may perhaps give some insight as to why various forms of hardship hasten the development of symptoms in scurvy, as reported by James Lind (6) in 1753.

It would seem that ascorbic acid deficiency is one of the most common causes for an elevated blood histamine level, as all 11 of the volunteers given 1 g of ascorbic acid daily for 3 days showed a reduction in blood histamine. Clearly, ascorbic acid loading can be used as a test to determine the cause of an elevated blood histamine level. If the blood histamine level falls as a result of ascorbic acid administration, we may reasonably conclude that it was elevated as a result of ascorbic acid deficiency. Statistical analysis has shown that plasma reduced ascorbic acid levels below 0.7 mg/100 ml are associated with significantly elevated blood histamine levels, so perhaps

TABLE 2

Histamine and ascorbic acid in morning blood samples

Initials and condition of of volunteer	Date	Sex	Age	Smoker or non-smoker	Before ascorbic acid supplement: Plasma ascorbic acid	Before ascorbic acid supplement: Duplicate whole blood histamine levels	After oral ascorbic acid 1 g daily for 3 days: Plasma ascorbic acid	After oral ascorbic acid 1 g daily for 3 days: Duplicate whole blood histamine levels
					mg/100 ml	*ng/ml*	*mg/100 ml*	*ng/ml*
S. G. F. (M.D.)								
After day, night on duty	7/28/78	M	31	S	0.14	180 and 180		
After night of sleep	7/31/78				0.20	80 and 84		
After night of sleep	8/4/78						1.15	15 and 18
R. A. M. (M.D.)								
After day, night on duty	8/15/78	M	29	S	0.57	28 and 29		
After night of sleep	8/16/78				0.65	29 and 31		
After night of sleep	8/23/78						1.54	22 and 25
R. S. (medical student)								
After night of sleep	8/15/78	M	28	NS	0.31	24 and 22		
After night of sleep	8/23/78						1.71	14 and 16
R. S. (M.D.)								
After night of sleep	8/7/78	F	31	NS	0.86	34 and 33		
After day, night on duty	8/21/78				0.95	45 and 46		
After day, night on duty	9/19/78						1.82	20 and 21
E. T. (registered nurse)								
After night on duty	3/19/79	F	23	S	0.85	27 and 28		
After night on duty	3/23/79						1.92	10 and 10
C. F. (ward clerk)								
After night of sleep	3/19/79	F	34	NS	0.73	24 and 24		
After night of sleep	3/23/79						2.18	6 and 7
R. S. (medical student)								
After night of sleep	3/20/79	M	23	S	0.55	26 and 26		
After night of sleep	3/26/79						1.56	11 and 12
A. F. (medical student)								
After night of sleep	3/27/79	M	28	NS	0.54	22 and 22		
After night of sleep	4/3/79						1.23	7 and 8
J. M. (subintern)								
After night of sleep	3/30/79	M	28	S	0.63	23 and 26		
After night of sleep	4/6/79						1.46	10 and 9
G. S. (subintern)								
After night of sleep	3/30/79	M	44	NS	0.43	28 and 28		
After night of sleep	4/6/79						1.87	11 and 7
E. H. (patient with purpura simplex)								
After night of sleep	4/5/79	F	24	S	0.44	28 and 27		
After night of sleep	4/9/79						1.41	13 and 13

this could be used as a dividing line. If so, persons with plasma reduced ascorbic acid levels below 0.7 mg/100 ml would be considered ascorbic acid deficient. In the present study this included 137 of 400 volunteers or 34% of the population.

Previously, it was thought that ascorbic acid deficiency caused only one problem—scurvy, which can begin to develop when the plasma reduced ascorbic acid level falls below 0.2 mg/100 ml but only becomes fully developed when all reduced ascorbic acid has disappeared from the blood. However, it is now evident that a toxic metabolite, namely histamine, begins to accumulate in the blood long before the plasma ascorbic acid level falls to 0.2 mg/100 ml and that any level below 0.7 mg/100 ml should be considered deficient. This is of particular interest because ascorbic acid deficiency has been reported to affect cholesterol metabolism (7) and seems to predispose one to atherosclerosis (8–10) and to heart attacks (11). The question of

the relationship between ascorbic acid deficiency and vascular disease has recently been reviewed (12). It arouses additional interest in view of the present findings.

In the Vanderbilt University cooperative study of maternal and infant nutrition in Nashville, TN (13), it was found that 9 of 10 cases of premature separation of the placenta occurred in women who had consistently low serum ascorbic acid levels (below 0.4 mg/100 ml) in the second and third trimesters of pregnancy. Moreover, four patients in another study (3), who had blood samples taken after developing premature separation of the placenta, or abruptio placentae, had plasma reduced ascorbic acid levels ranging from 0.03 to 0.35 mg/100 ml and a mean level of 0.16 mg/100 ml.

In the present study, only one patient had blood drawn before abruptio placentae (fig. 1). She had a low ascorbic acid level of 0.19 mg/100 ml and a high histamine level of 38 ng/ml in the 29th week of pregnancy, which was 6 weeks before the placental separation. She developed partial separation of the placenta associated with vaginal bleeding, of about 300 ml at 35 weeks. After hospital admission, the placental position was determined by ultrasound B scan. Her ascorbic acid level was now found to be 0.27 mg/100 ml and her histamine level was 35 ng/ml. She was given plain white 500 mg ascorbic acid tablets and instructed to take two a day, but she refused to continue taking them because she said they upset her stomach. Later she was persuaded to take green tablets containing 333 mg sodium ascorbate and 20 mg rutin three times a day. She had no more bleeding and was delivered of a healthy 4,167 g male child at 40 weeks.

Two other patients in this study, who had blood drawn only after abruptio placentae, had ascorbic acid levels of 0.38 and 0.25 mg/100 ml and histamine levels of 44 and 55 ng/100 ml, respectively. Clearly, many more blood samples must be obtained from pregnant women and analyzed for histamine and ascorbic acid to provide enough data concerning the ascorbate and histamine status of women before they develop abruptio placentae. The present evidence is scant but it suggests that such women may have low ascorbate and high histamine levels. This is interesting in view of the old report by Hofbauer (14) in 1926 that injection of histamine into guinea pigs and cats caused premature placental separation.

Kapeller-Adler (15) showed that the blood of pregnant women possesses histaminase activity due to synthesis of this enzyme by the placenta and that this enzyme activity is decreased in women with pre-eclampsia. We need blood samples from age and race-matched groups of pregnant and non-pregnant women to find out whether the histamine levels of pregnant women are actually lower in comparable ascorbic acid groups; if so, this could be due to placental histaminase. This enzyme might be considered as partial protection against histamine intoxication in pregnancy and could be responsible for reducing the risk of premature placental separation.

In the Vanderbilt nutrition study, there was little correlation between dietary ascorbic acid intake levels and premature separation of the placenta, but this event was definitely associated with low serum ascorbate levels. So it would seem that the problem may be a disturbance of ascorbic acid metabolism rather than a simple dietary deficiency of ascorbic acid. If so, ascorbic acid supplements should perhaps be combined with natural plant polyphenol antioxidants (16), which act by chelating copper and iron in tap water, to prevent losses of ascorbic acid by oxidation and subsequent hydrolysis in the stomachs of achlorhydrics and in the jejunum. Moreover, this vitamin supplement should be given in the morning and any iron supplement should not be given until later in the day when the ascorbic acid has been absorbed.

Ascorbic acid deficiency would account for the folic acid deficiency and megaloblastic anemia sometimes found in association with abruptio placentae (17–19). Ascorbic acid deficiency causes a disturbance of folic acid metabolism (20) and megaloblastic anemia (21), which can be corrected by administration of folic acid or by ascorbic acid alone (22).

If ascorbic acid deficiency and histamine intoxication do predispose to abruptio placentae, there might be some hope of reducing both its incidence and the incidence of megaloblastic anemia of pregnancy by giving larger amounts of ascorbic acid to pregnant women.

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LITERATURE CITED

1. Chatterjee, I. B., Das Gupta, S., Majumder, A. K., Nandi, B. K. & Subramanian, N. (1975) Effect of ascorbic acid on histamine metabolism in scorbutic guinea-pigs. J. Physiol. *251*, 271–279.
2. .Chatterjee, I. B., Majumder, A. K., Nandi, B. K. & Subramanian, N. (1975) Synthesis and some major functions of vitamin C in animals. Second conference on vitamin C. Ann. N.Y. Acad. Sci. *258*, 24–47.
3. Clemetson, C. A. B. & Andersen, L. (1964) Ascorbic acid metabolism in pre-eclampsia. Obst. Gynec. *24*, 774–782.
4. Shore, P. A., Burkhalter, A. & Cohn, V. H. (1959) A method for the fluorometric assay of histamine in tissues. J. Pharmacol. Exp. Ther. *127*, 182–186.
5. Roe, J. H. (1954) Photometric indophenol method for determination of ascorbic acid. In: Methods of Biochemical Analysis (Glick, D., ed.), vol. 1, pp. 121–124, Interscience Publishers, New York.
6. Lind, J. (1753) A Treatise of the Scurvy, 1st ed., Millar, London. Reprinted by Edinburgh University Press in 1953.
7. Ginter, E. (1975) Ascorbic acid in cholesterol and bile acid metabolism. Ann. N.Y. Acad. Sci. *258*, 410–421.
8. Willis, G. C. (1953) An experimental study of the intimal ground substance in atherosclerosis. Can. Med. Assoc. J. *69*, 17–22.
9. Shaffer, C. F. (1970) Ascorbic acid and atherosclerosis. Am. J. Clin. Nutr. *23*, 27–30.
10. Ginter, E. (1974) Vitamin C in lipid metabolism and atherosclerosis. In: Vitamin C. Technological & Nutritional Aspects of Ascorbic Acid, Applied Science Publishers, London.
11. Krebs, H. A. (1953) The Sheffield experiment on the vitamin C requirement of human adults. Proc. Nutr. Soc. *12*, 237–246.
12. Clemetson, C. A. B. (1979) Some thoughts on the epidemiology of cardiovascular disease. Role of ascorbic acid. Med. Hypotheses *5*, 825–834.
13. Martin, M. P., Bridgforth, E., McGanity, W. J. & Darby, W. J. (1957) The Vanderbilt Cooperative Study of maternal and infant nutrition. J. Nutr. *62*, 201–224.
14. Hofbauer, J. (1926) Experimental studies on the toxemias of pregnancy. Can histamine poisoning be regarded as the etiologic factor? Am. J. Obstet. Gynec. *12*, 159–189.
15. Kapeller-Adler, R. (1949) Histamine metabolism in pregnancy. Lancet *2*, 745–747.
16. Clemetson, C. A. B. & Andersen, L. (1966) Plant polyphenols as antioxidants for ascorbic acid. Ann. N.Y. Acad. Sci. *136*, 339–378.
17. Hourihane, B., Coyle, C. V. & Drury, M. I. (1960) Megaloblastic anemia of pregnancy. J. Irish Med. Assoc. *47*, 1–6.
18. Coyle, C. & Geoghegan, F. (1962) The problem of anemia in a Dublin maternity hospital. Proc. Roy. Soc. Med. *55*, 764–765.
19. Hibbard, B. M. & Hibbard, E. D. (1963) Aetiological factors in abruptio placentae. Br. Med. J. *2*, 1430–1436.
20. Nichol, C. A. (1953) The effect of ascorbic acid on the enzymatic formation of the citrovorum factor. J. Biol. Chem. *204*, 469–475.
21. May, C. D., Nelson, E. N., Lowe, C. J. & Salmon, R. J. (1950) Pathogenesis of megaloblastic anemia in infancy. An interrelationship between pteroylglutamic acid and ascorbic acid. Am. J. Dis. Child. *80*, 191–206.
22. May, C. D., Sundberg, R. D., Schaar, F., Lowe, C. U. & Salmon, R. J. (1951) Experimental nutritional megaloblastic anemia; relation of ascorbic acid and pteroylglutamic acid; nutritional data and manifestations in animals. Am. J. Dis. Child. *82*, 282–309.