# Ascorbic Acid Metabolism In Preeclampsia

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IF ONE WERE to select a single tissue the damage to which characterizes eclampsia, one would have to select the vascular endothelium: while abnormalities are found in several organs and tissues, endothelial damage seems to be the common denominator. For example, the changes in the kidney in preeclampsia were studied in renal biopsies with the electron microscope by Hopper *et al.;* the characteristic change was found to be pronounced swelling of the glomerular endothelium associated with subendothelial and interendothelial fibrinoid deposition.

One of the factors affecting the endothelium and the intercellular cement is ascorbic acid, which is particularly important for man and the primates, who have an inborn error in metabolism which renders them incapable of synthesizing ascorbic acid. (Most other mammals can synthesize ascorbic acid and do not need it in their diets.) It is therefore pertinent in a study of preeclampsia, a disorder of pregnancy peculiar to human beings and possibly other primates, to ascertain whether there is an associated derangement of ascorbic-acid metabolism.

Lund and Kimble in 1943 stated that they found no relationship between plasma ascorbic-acid levels and the occurrence of toxemia, but they did not present the figures obtained in toxemia. Martin *et al.,* in a much larger study, also found no significant difference between the incidence of preeclampsia in women on a consistently high dietary ascorbic-acid intake as compared with women on a diet consistently low in this substance; nor did they find any significant difference in incidence between women with consistently high or consistently low serum-ascorbic-acid levels. However, their figures suggested some influence in that the incidence of preeclampsia was 6.1% in 442 women with consistently low serum-ascorbic-acid levels, and only 3% in 198 women with consistently high serum-ascorbic-acid levels. The incidence of abruptio placentae was found to be significantly different, being 2% in 442 women with consistently low serum-ascorbic-acid levels, and 0% in 198 women with consistently high levels. Nine of 10 cases of abruptio occurred in women in the consistently low group, 1 in the intermediate group, and none in the women with consistently high serum-ascorbic-acid levels. The authors' use of the word "consistently" in this context meant that the ascorbic acid levels of these women had been found to be similar when tested earlier in pregnancy. Thus 9 out of 10 women with abruptio placentae had low ascorbic-acid levels before the abruptio occurred. Martin *et al.* also found increased incidence of premature birth in those mothers with the lowest dietary ascorbic-acid intakes and the lowest serum-ascorbic-acid levels.

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Pankamaa and Räihä showed a close negative correlation between the seasonal fluctuations of the stillbirth rate and the Vitamin-C content of fetal tissues. Among 116,700 deliveries at the Women's Clinic of the University of Helsinki, the lowest percentage incidence of stillbirths occurred in September and the highest in January. This was also true for the country as a whole; there were 90,553 deliveries in Finland in 1956, of which 1657 were stillbirths, and Räihä reported that the frequency of stillbirths was highest (2.03%) in December and January, and lowest (1.50%) in September.

Wideman *et al.* reported a correlation between ascorbic acid deficiency and premature rupture of the fetal membranes in a study conducted in Birmingham, Ala.

Most studies of Vitamin-C levels in pregnancy have estimated only the reduced form of ascorbic acid and did not measure the oxidized or dehydro form which is commonly known as dehydroascorbic acid; this substance is a neutral trione and has been named ascorbone by Lloyd and Sinclair. However, Mukherji and Banerjee estimated both ascorbic acid and ascorbone by the method of Banerjee and Belavady, as well as the blood glutathione by the method of Woodward and Fry. They found that the blood levels of ascorbic acid and glutathione were significantly lower in women with preeclamptic toxemia; ascorbone, which was absent from the blood of normal pregnant women, appeared in the blood of a few toxemic patients.

The work presented here reports a study of the amounts of reduced and oxidized ascorbic acid in the plasma in normal and toxemic pregnancy, using a spectrophotometric method instead of the titration method used by Mukherji and Banerjee.

## METHOD

Fasting blood samples were drawn from 19 normal pregnant women and from 11 women with mild to moderate preeclamptic toxemia near term. No severe preeclamptic or eclamptic patients were available. Samples were also obtained from 4 women within 1 or 2 days after abruptio placentae in the third trimester of pregnancy. Analyses were performed by a method similar to that used by Stewart, Horn, and Robson. Each 30-ml. sample of blood was slowly ejected from a syringe into a tube containing 0.3 ml. of heparin solution (1000 Toronto U./ml.) and was centrifuged to separate the plasma within 15 min. of collection.

The plasma was removed with care to avoid disturbance of the buffy coat and was placed in a tube, covered with parafilm and inverted to insure mixing, before pipetting 3 4-ml. aliquots, *A*, *B*, and *C*, for analysis. The plasma residue after removal of aliquots was tested for hemolysis with Hemastix.* Samples *A* and *B* were each analyzed in triplicate for reduced ascorbic acid by a buffered indophenol photometric method, approximately 1 hr. and 3 hr. after the blood had been drawn, so that the rate of oxidation of the ascorbic acid in the plasma at room temperature could be determined. Sample *C* was analyzed in duplicate by the Roe and Kuether method for total ascorbic acid—comprising ascorbic acid, ascorbone, and diketogulonic acid, and using standards prepared from the same stock ascorbic-acid solution as was used for the indophenol method. The difference between the results obtained by the indophenol method and by the Roe and Kuether method represents ascorbone plus diketogulonic acid, but mainly ascorbone, since only traces of diketogulonic acid are normally present in plasma.[29]

## RESULTS

None of the plasma samples from normal or toxemic women showed hemolysis detectable with Hemastix. Two of the 4 plasma samples taken from women after abruptio

* Ames Company, Inc., Elkhart, Ind.

placentae showed hemolysis which was both visible and gave a definite green color when tested with Hemastix. However, visible hemolysis is not uncommon after abruptio placentae, so this was considered to be an in-vivo phenomenon rather than an artefact; consequently the results of analysis of the four samples have been grouped together.

Fasting total ascorbic-acid levels were found to be lower in 11 samples from toxemic women than in 19 samples from normal pregnant women; this difference just reaches a significant level ($p<.05$).

Plasma-reduced ascorbic-acid levels were also found to be lower in the toxemic than in the normal group, and this difference was more significant ($p<.02$).

The total and reduced ascorbic-acid levels of plasma samples obtained from women after abruptio placentae (2 showing hemolysis) were distinctly lower than those of the normal pregnant women, but were too few for statistical analysis.

The percentage of the plasma ascorbic acid in the oxidized forms was found to be higher in the toxemic (20%) than in the normal pregnant women (13%), and this difference was highly significant ($p<.01$).

Ascorbic acid reduced / oxidized ratios have been calculated from the mean values for each group and are found to be 7.0 to 1 in the normal, 4.2 to 1 in the toxemic, and 1.6 to 1 in the abruptio placentae group, as shown in Table 1 and Fig. 1.

Knowing the exact times of drawing blood and of performing the first and second series of indophenol analyses has enabled us to calculate the percentage of ascorbic acid lost per hour by oxidation at room temperature between 1 and 3 hr. after drawing blood (Table 1). The percentage loss of substrate (reduced ascorbic acid) per hour was found to be higher in the toxemic and abruptio groups, but this difference was not significant. If we assume a linear rate of oxidation of ascorbic acid during the first 3 hr., we can extrapolate the amounts and proportions of

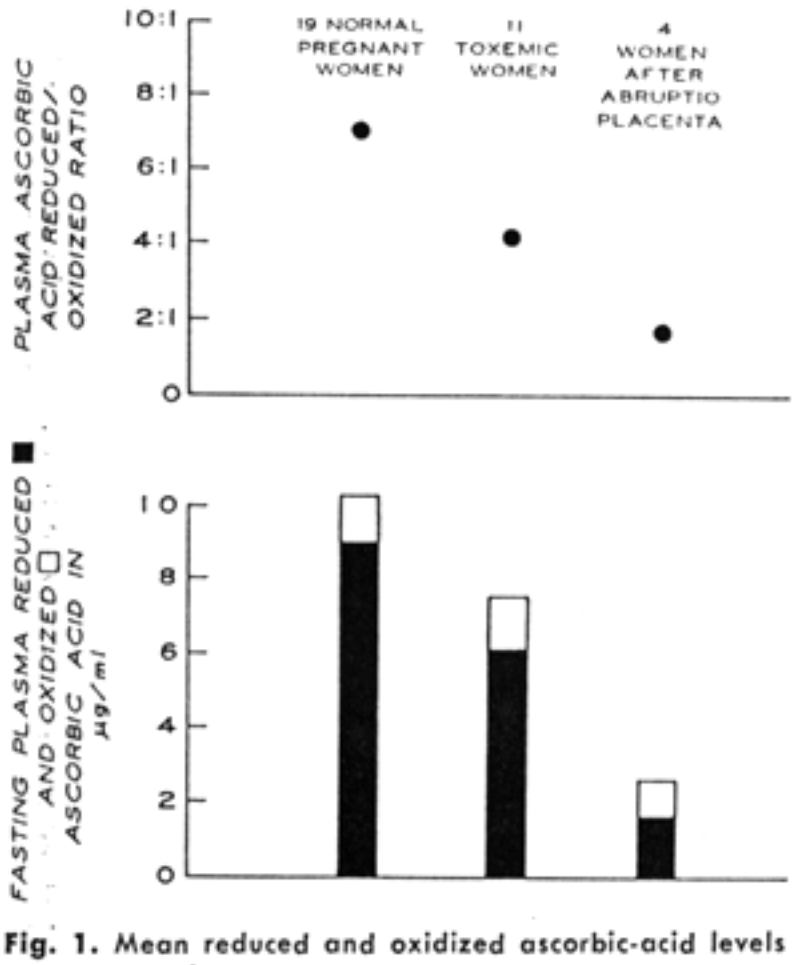


**Fig. 1.** Mean reduced and oxidized ascorbic-acid levels and reduced/oxidized ratios in fasting plasma samples obtained from pregnant women (analyzed 1 hr. after collection).

reduced and oxidized ascorbic acid which might have been present at the time of drawing blood. These calculations show a slightly higher level of the oxidized forms and a markedly lower ratio of reduced to oxidized ascorbic acid in the toxemic group, as compared with that of the normal pregnant women. However, these extrapolations are only approximations and should not be considered as definite values, for the spontaneous oxidation of ascorbic acid in plasma shows a slight acceleration with time that is detectable even during the first 3 hr. after drawing blood.

## DISCUSSION

The reversible equilibrium between ascorbic acid and ascorbone at pH 7.4 in vitro is influenced by several factors (Fig. 2), oxidation occurring in the presence of traces of copper and reduction in the presence of reduced glutathione or other thiols.

Plasma copper levels are raised by estrogen administration, as shown by Russ and

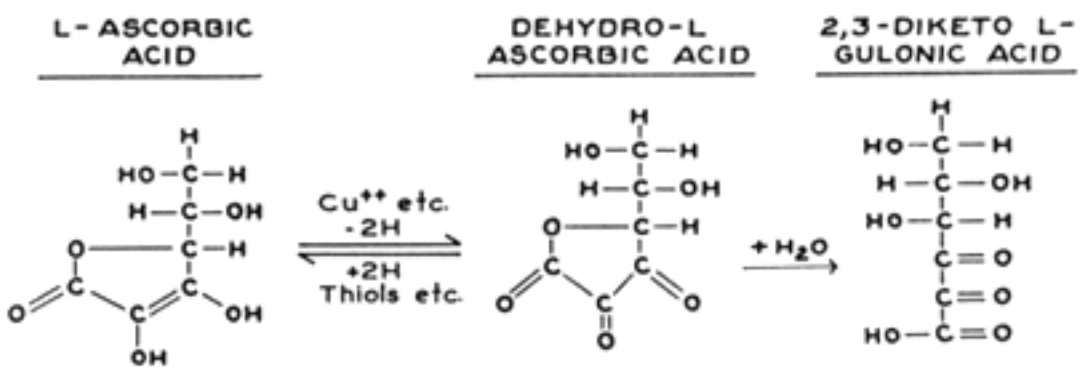


**Fig. 2.** Some factors affecting reversible equilibrium between ascorbic acid and dehydroascorbic acid (ascorbone) in vitro. Intermediate mono-dehydro compounds postulated but not shown. Spontaneous hydrolysis of ascorbone to diketogulonic acid indicated.

Raymunt, and are markedly increased in pregnancy—as was first shown by Krebs and confirmed by Thompson and Watson and by Lahey *et al.*, who found mean values of 109 μg./100 ml. in nonpregnant and 222 μg./100 ml. in pregnancy.

Thompson and Watson found a significant increase of serum copper in preeclampsia, giving mean values of 106 μg./100 ml. for normal nonpregnants, 230 for pregnant normals near term, and 277 for 12 women with preeclampsia. They did not, however, find a good correlation of serum copper with the severity of the toxemia. No significant difference was found between the nonpregnant and pregnant sera relative to the percentages of copper appearing in the protein fraction precipitated by 15%, 19.6%, or 26.8% $Na_2SO_4$, so there was presumably a proportional increase of both the bound and unbound copper in late pregnancy.

Mukherji and Banerjee found a significantly decreased level of reduced glutathione in the blood of women with preeclampsia.

It seems therefore that two factors affecting the ascorbic acid–ascorbone ratio in vitro, namely the copper and glutathione levels, are also disturbed in preeclampsia (in vivo). It is possible that the disturbance of these factors may be responsible for the alteration of the ratio of reduced to oxidized ascorbic acid found in this study.

The question as to what effects such a change in this ratio might produce in the body would depend on how much of the nonreducing ascorbic acid was ascorbone, and how much diketogulonic acid, which we cannot detect by this method.

Patterson and Mastin have shown that ascorbone is highly toxic when given intravenously to rats, causing hypertension in doses as low as 5 mg./kilo (5 μg./gm.) and temporary or permanent atrophy of the islets of Langerhans, atrophic changes in the fur, development of fatty liver, and even death when given in larger doses. These toxic effects of ascorbone can be prevented by a prior injection of glutathione, as shown by Patterson and Lazarow. Both ascorbone and alloxan are diabetogenic, and Patterson has pointed out that a common feature of their chemical structures is the presence of 3 adjacent carbonyl groups(Fig. 3).

The factors to be considered as possible causes of such a decrease in the ratio of reduced to oxidized ascorbic acid in the plasma of women with preeclampsia include dietary insufficiency of sulphydril compounds or ascorbic acid, or possibly an excess of absorbable copper compounds in the diet.

Dietary factors which might counteract such a change and increase the reduced/oxidized ascorbic acid ratio in the plasma would include ascorbic acid itself, but more

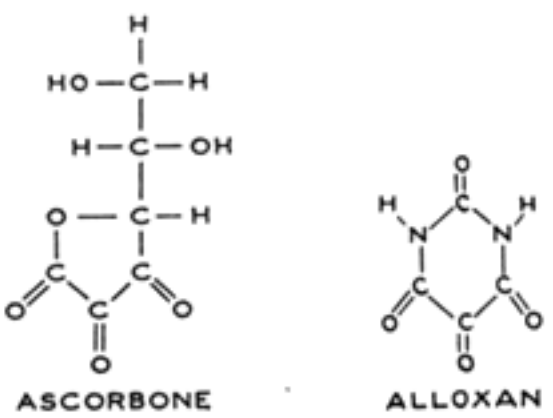


**Fig. 3.** Chemical structure of synthetic substance alloxan and of ascorbone (natural dehydro form of ascorbic acid), showing the 3 adjacent carbonyl groups they have in common.

important would be sulphydril compounds and also orthodiphenols such as the capillary active flavonoids which act as antioxidants for ascorbic acid in vitro and in vivo, as shown by Clemetson and Andersen. The bioflavonoids may act by chelating copper. The tocopherols are also antioxidants, but it is not known whether they affect this ratio.

Many authors such as Utheim-Toverud have found a fall in the incidence of preeclampsia, stillbirths, and prematurity to result from improvement in the diet of a population, but it has always been difficult to ascertain which of many factors may have been responsible.

It seems unlikely that dehydroascorbic acid itself or a disturbance of the ratio of reduced to oxidized ascorbic acid could be the chemical mediator of the hypertension of preeclampsia, because we have found similar changes in some women with menorrhagia, and Banerjee and Belavady have found similar changes in typhoid fever—neither of which conditions is associated with hypertension.

There is considerable evidence which suggests that uteroplacental ischemia, arising from any of several causes, may be a precipitating factor in the causation of preeclampsia, and that in many instances the cause of the ischemia is maternal arterial disease. The disturbance of ascorbic-acid metabolism may be a result of such disease, and it is also possible that it may be a potent predisposing factor.

A synergism between uteroplacental ischemia and a disturbance of ascorbic-acid metabolism can be envisaged if one accepts the supposition that the health of the vascular endothelium is dependent on a normal ratio of reduced to oxidized ascorbic acid in the plasma and that a lowered ratio causes endothelial damage. This seems probable since in scurvy the ratio is reversed—reduced ascorbic acid being absent and only oxidized ascorbic acid being found in the plasma.

The petechial hemorrhages of hypoxia would be more likely to occur in ischemic decidua if the endothelium of affected capillaries were already weakened by a disturbance of ascorbic-acid metabolism. Subsequent hemolysis of extravasated red cells might lead to further oxidation of ascorbic acid, as Kellie and Zilva have shown that hemolysis does in vitro. This further disturbance of ascorbic-acid metabolism might cause either further hemorrhage in the decidua, leading on to abruptio placentae, or damage to the endothelial intima of the arterioles, resulting in thrombosis and further ischemia.

Most experimental studies of uterine ischemia in pregnancy have been carried out on dogs or rabbits, which synthesize their own ascorbic acid. Such experiments might be more comparable to the human situation if carried out on monkeys or guinea pigs on various diets, for these animals, like man, are dependent on exogenous ascorbic acid.

A detraction from the value of this study is the fact that most of the preeclamptic patients had received treatment with a variety of drugs—including diuretics, sedatives, and anticonvulsants—on the day before the blood samples were drawn, which the normal pregnant women had not; it is therefore impossible to know whether the changes found are characteristic of preeclampsia or whether they are the result of the drugs. However, the report of similar findings by Mukherji and Banerjee working in Calcutta and our own finding of even greater changes in the same direction after abruptio placentae, do suggest that a fall in the ratio of reduced to oxidized ascorbic acid in the plasma may be a characteristic of the disease.

Moreover, the controlled-study findings of Clemetson and Andersen that dietary supplements of citrus bioflavonoids (Duo-C.V.P.*), which increased the ratio of re-

* U. S. Vitamin and Pharmaceutical Corp., New York, N.Y.

duced to oxidized ascorbic acid in the plasma also resulted in a significant increase in the birth weight of babies born to these women, suggest that a disturbance of the ratio may be of profound significance in pregnancy. Feeding of ascorbic acid to women already on a good diet did not affect birth weight.

An association between megaloblastic anemia of pregnancy and abruptio placentae was postulated by Hourihane *et al.* when they found that abruptio placentae had occurred in 13 out of 95 patients with megaloblastic anemia, while Coyle and Geohegan in 1962 found megaloblastic erythropoeisis on marrow biopsy in 35 out of 77 patients with abruptio placentae. This association was confirmed by Hibbard and Hibbard, who found evidence of megaloblastic erythropoeisis in 46% of 73 cases of abruptio placentae as compared with 5% of 121 controls. They also found folic-acid deficiency, as evidenced by positive formiminoglutamic acid excretion tests, in 98.6% of patients with abruptio placentae and in only 10.7% of controls.

An increased incidence of preeclampsia (23% as compared to 12%) was found by Goodall in women with megaloblastic anemia of pregnancy.

May *et al.*[16-18] produced megaloblastic anemia in immature monkeys by prolonged feeding of ascorbic-acid-deficient milk diets, and their work suggests that folic-acid deficiency, both in monkeys and in human infants, is in some way conditioned by a lack of ascorbic acid, for the anemia was found to respond to either folic acid or ascorbic acid; it did not respond to treatment with Vitamin $B_{12}$, but was promptly cured by very small quantities of citrovorum factor (folinic acid).

Nichol and Welch found that ascorbic acid has a stimulating effect on the conversion of folic to folinic acid in vitro by enzymes in rat liver, and Nichol has shown that under aerobic conditions ascorbic acid protects folinic acid from destruction. It may be that the correlations of abruptio placentae with a low plasma ascorbic-acid level (found by Martin *et al.*), a markedly reduced ratio of reduced to oxidized ascorbic acid in the 4 patients presented here, and a folic acid deficiency are in fact all related to a common disturbance of an oxidation–reduction system.

The present study suggests that there is a similar but less severe disturbance of the ascorbic acid reduced/oxidized ratio in the plasma of women with preeclampsia. Endothelial damage is seen in the glomerular capillaries in renal biopsies from women with preeclampsia and is suggested by the generalized edema of these patients. Hemorrhages under the capsule of the liver, hemorrhagic necrosis about the portal spaces, thrombosis of the portal veins or their branches, and cerebral hemorrhage are characteristic postmortem findings in eclampsia which also suggest endothelial damage. It may be that this damage does not show itself as a capillary fragility state because of a superimposed arteriolar spasm, which can give rise to false negative results to capillary fragility tests, as described by Kramar *et al.*

Lee has shown that in scurvy, where reduced ascorbic acid is absent from the blood, arterioles become incapable of constriction—but it would seem that in the disturbance of ascorbic-acid metabolism found in preeclampsia and in abruptio placentae, where reduced ascorbic acid is present, vasoconstriction can and does occur. It is not known whether this disturbance of ascorbic-acid metabolism is a contributory cause or a result of preeclampsia, but it does seem to exist. The most obvious cause of such a disturbance might seem to be an ascorbic-acid-deficient diet, but this is not necessarily so.

Just as folic-acid deficiency can occur as a result of ascorbic-acid deficiency in children on an otherwise sufficient but borderline folic-acid intake, so as a disturbance of ascorbic-acid metabolism such as we have found in preeclampsia might occur, even on an adequate ascorbic-acid intake, as a result

of factors which tend to increase the rate of oxidation of ascorbic acid to its toxic dehydro form—factors such as trauma, hemolysis, infection, or a dietary deficiency of sulphydril compounds that seem to play an important part in keeping ascorbic acid in the reduced form. Also, an excess of copper over copper-chelating substances in the diet might affect the equilibrium.

It is well established that arterial disease predisposes to preeclampsia. Willis has shown that typical atherosclerosis can be produced in more than 50% of guinea pigs by feeding scorbutic diets, and that if cholesterol is added to such a diet, the incidence of atherosclerosis rises to 100%. Moreover, Willis and Fishman have demonstrated ascorbic-acid deficiency in human arteries affected by atherosclerosis. It is therefore possible that a disturbance of ascorbic-acid metabolism, even in the nonpregnant state, may influence the incidence of preeclampsia in a subsequent pregnancy.


## SUMMARY

Analysis of fasting blood samples from 19 normal pregnant women and 11 women with mild-to-moderate preeclampsia showed a significantly lower ratio of reduced to oxidized ascorbic acid in the plasma of women with preeclamptic toxemia. Four women whose plasma was analyzed after abruptio placentae showed an even greater change in the same direction.



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