*In vitro* studies indicated that glutathione (GSH) can protect ascorbic acid from oxidation to dehydroascorbic acid. Hopkins and Morgan (44) showed that in plant oxidase system GSH protects ascorbic acid and no dehydroascorbic acid is formed until all the GSH is converted to GSSH. Certain enzymes of the body depend on-SH groups for their activity, and, if the -SH groups are inactivated, the enzymes become inactive (31). The activity could be restored in most cases by the addition of glutathione (45). The toxic action of dehydroascorbic acid in rats could be prevented by a prior injection of glutathione (52). These observations indicated interrelations of glutathione, ascorbic acid, and dehydroascorbic acid. In patients suffering from cholera, small pox, pyogenic meningitis and gonorrhoea there had been a fall in the glutathione and L-ascorbic acid (33) (Table II).

TABLE II: Glutathione, dehydroascorbic acid and ascorbic acid contents of blood (*mg*/100 *ml*) in normal and diseased subjects (Bhaduri and Banerjee, 1960).

| *Subjects* | *Glutathione* | *Ascorbic acid* | *Dehydroascorbic acid* |
|---|---|---|---|
| Normal (16) | 58±2 | 0.89±0.20 | 0.06±0.01 |
| Cholera (21) | 48±2 | 0.62±0.03 | 0.37±0.02 |
| Small pox (16) | 46±2 | 0.51±0.05 | 0.56±0.03 |
| Meningitis pyogenic (16) | 31±2 | 0.32±0.02 | 0.48±0.02 |
| Meningitis tubercular (16) | 35±2 | 0.55±0.03 | 0.36±0.02 |
| Gonorrhoea (16) | 41±2 | 0.53±0.02 | 0.26±0.02 |

Figures are mean ±SE. Figures in parenthesis indicate the number of subjects.

In thyrotoxic patients there was also a decrease in total ascorbic acid along with an appreciable increase in dehydroascorbic acid content of plasma. Analysis of the surgically removed thyroid tissue also revealed a decrease in total ascorbic acid with an appreciable increase in dehydroascorbic acid content of the thyroid tissue (59). Thyroxin also increased the concentration of circulating dehydroascorbic acid (37). Corticotropin and cortisone facilitated the oxidation of ascorbic acid to dehydroascorbic acid (38). The accumulation of dehydroascorbic acid, therefore, might be due to liberation of adrenal cortical hormone as a result of stress produced in the diseased conditions (57). In man the greatest ability to reduce dehydroascorbic acid to ascorbic acid was shown by the liver (48). In the infectious diseases the liver function is likely to be disturbed which may contribute to the increase accumulation of dehydroascorbic acid. Glutathione is absolutely necessary for the growth of certain bacteria (42, 53). Glutathione is a necessary growth factor in tissue culture (1). An increase in the glutathione content of the media results after the addition of penicillin to a bacterial culture (49). These factors might be responsible for the low values of glutathione in patients suffering from infectious diseases.

It is, however, a paradox that dehydroascorbic acid which behaves essentially like ascorbic acid in giving protection from or curing scurvy accumulates in the blood and tissues of scorbutic monkeys (11) and scorbutic guinea pigs (18). Glutathione content diminished in most tissues of the scorbutic guinea pigs along with appearance of dehydroascorbic acid and the diminution of total ascorbic acid (Table III). Apart from the reduced protection of ascorbic acid from oxidation as a result of the decreased glutathione content of the scorbutic tissues, the tendency of oxidation may be increased due to extensive haemorrhage in scurvy. The extent of dehydroascorbic acid formation may perhaps be related to the intensity of the haemorrhagic state in scurvy. The higher dehydroascorbic acid value in scurvy might be explained on this basis.

TABLE III: Glutathione, dehydroascorbic acid and ascorbic acid values of tissues of normal and scorbutic guinea pigs (Modified from Banerjee, Deb and Belavady, 1952).

| | *Blood* | *Adrenals* | *Pancreas* | *Spleen* | *Intestine* | *Kidney* | *Liver* |
|---|---|---|---|---|---|---|---|
| *Glutathione* | | | | | | | |
| Normal guinea pigs | 58±8 | 204±12 | 237±11 | 216± 7 | 206±15 | 172±6 | 351±12 |
| Scorbutic guinea pigs | 31±7 | 142±2 | 123±12 | 175±11 | 174± 9 | 165±8 | 352±11 |
| *Dehydroascorbic acid* | | | | | | | |
| Normal guinea pigs | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Scorbutic guinea pigs | | 2.9±0.39 | 1.26±0.28 | 1.04±0.34 | 1.01±0.07 | 1.23±0.53 | 1.92±0.56 |
| *Ascorbic acid* | | | | | | | |
| Normal guinea pigs | | 67.67±4.83 | 12.90±0.91 | 30.81±1.65 | 13.01±0.91 | 14.90±1.22 | 16.98±1.62 |
| Scorbutic guinea pigs | | 10.11±1.25 | 2.93±0.18 | 3.65±0.55 | 3.65±0.29 | 4.87±0.41 | 7.31±0.35 |

Values are means (*mg*/100 *gm*) of 12 observations ±SE.

Scurvy is associated with abnormal carbohydrate, lipid and protein metabolism as well as a derangement in the insulin production (2-30, 32, 55). Glutathione not only keeps ascorbic acid in the reduced condition but also protects the sulphydryl enzymes which are necessary for the synthesis of insulin (45). Glutathione may also supply cystine which is an important constitutent of insulin. Decreased glutathione content of the pancreas and other tissues in scurvy (11, 18) might be related to the decreased insulin content of the pancreas (29, 9) and to the occurrence of dehydroascorbic acid in the tissues of the scorbutic animal.

Due to structural similarity to alloxan, injection of which led to destruction of islets of Langerhans of the pancreas and diabetes (5,6,8,14), dehydroascorbic acid was used to produce diabetes. Intravenous injection of a very large dose, 1 *gm* per *kg* body weight, was necessary to produce destruction of islets of Langerhans and diabetes in rats (50). Diabetogenic action of dehydroascorbic acid, however, could not be demonstrated in any other species of animal (13).

Accumulation of dehydroascorbic acid in blood and tissues under diversified conditions possibly indicate increased requirement of L-ascorbic acid under those conditions. In patients suffering from pulmonary tuberculosis urinary excretion of ascorbic acid was found greatly diminished as compared to normal controls and a proportionately more dehydroascorbic acid to ascorbic acid appeared in the urine (28). When the patients were fed ascorbic acid, 700 *mg* per 10 stone body weight per day for several days, there was progressive increase in the urinary excretion of ascorbic acid with diasappearance of dehydroascorbic acid (Table IV). Thus in a condition

TABLE IV: Dehydroascorbic acid and ascorbic acid in urine (*mg*/24 hrs) of patients suffering from pulmonary tuberculosis fed ascorbic acid, 700 *mg* per stone body weight per day, for several days. (Modified from Banerjee and Guha, 1941).

| *Subjects* | | *Days after ascorbic acid was fed* | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 3 | 5 | 7 | 9 |
| 1 | Ascorbic acid | 10.00 | 5.62 | 17.78 | 35.33 | 56.39 |
| | Dehydroascorbic acid | 0.43 | 0 | 0 | 1.50 | 0 |
| 2 | Ascorbic acid | 2.36 | 2.51 | 2.71 | 3.77 | 40.00 |
| | Dehydroascorbic acid | 0 | 0.56 | 0 | 0 | 2.88 |
| 3 | Ascorbic acid | 6.39 | 12.90 | 10.94 | 7.37 | |
| | Dehydroascorbic acid | 1.48 | 1.93 | 0 | 0 | |
| 4 | Ascorbic acid | 3.07 | 48.04 | 157.27 | | |
| | Dehydroascorbic acid | 0 | 54.03 | 42.23 | | |
| 5 | Ascorbic acid | 6.81 | 5.00 | 50.89 | | |
| | Dehydroascorbic acid | 1.32 | 1.77 | 28.10 | | |
| 6 | Ascorbic acid | 4.31 | 86.69 | 165.15 | | |
| | Dehydroascorbic acid | 3.80 | 0 | 0 | | |
| 7 | Ascorbic acid | 4.17 | 6.28 | 65.95 | 80.76 | |
| | Dehydroascorbic acid | 3.22 | 0.72 | 0 | 0 | |
| 8 | Ascorbic acid | 7.39 | 24.57 | 35.13 | 15.18 | |
| | Dehydroascorbic acid | 2.63 | 0 | 2.53 | 3.51 | |
| 9 | Ascorbic acid | 4.18 | 33.84 | 277.88 | | |
| | Dehydroascorbic acid | 5.10 | 31.39 | 0 | | |
| 10 | Ascorbic acid | 1.01 | 47.09 | 73.14 | | |
| | Dehydroascorbic acid | 0 | 0 | 0 | | |
| 11 | Ascorbic acid | 1.54 | 69.50 | 89.21 | | |
| | Dehydroascorbic acid | 0 | 0 | 0 | | |
| 12 | Ascorbic acid | 14.53 | 49.52 | 222.47 | | |
| | Dehydroascorbic acid | 0 | 0 | 0 | | |
| 13 | Ascorbic acid | 1.84 | 10.40 | 9.46 | | 65.61 |
| | Dehydroascorbic acid | 0.52 | 0 | 0 | | 0 |
| 14 | Ascorbic acid | 5.25 | 6.77 | 104.73 | | 120.28 |
| | Dehydroascorbic acid | 0.28 | 0.73 | 16.78 | | 0 |

where dehydroascorbic acid accumulated administration of ascorbic acid not only did not increase the dehydroascorbic acid but diminished it (23). In normal human subjects and in normally-fed guinea pigs, administration of ascorbic acid led to disappearance of dehyproascorbic acid in urine, if present (Tables V, VI). It will thus be seen that whenever dehydroascorbic acid appears in the tissues there is simultaneous ascorbic acid deficiency leading to abnormal body metabolism. The administration of ascorbic acid corrects the abnormality and as a consequence dehydroascorbic acid disappears.

TABLE V: Dehydroascorbic acid and ascorbic acid in urine (*mg*/24 hrs) of normal humans fed ascorbic acid 700 *mg* per stone body weight per day, for several days. (Modified from Banerjee and Guha, 1941).

| *Subjects* | | *Days after ascorbic acid was fed* | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 |
| 1 | Ascorbic acid | 30.66 | 490 | 379 | | |
| | Dehydroascorbic acid | 3.46 | 0 | 0 | | |
| 2 | Ascorbic acid | 3.42 | 13.3 | 170 | 342 | 111 |
| | Dehydroascorbic acid | 0 | 11.2 | 23.5 | 0 | 0 |
| 3 | Ascorbic acid | 8.68 | 176 | 216 | 260 | |
| | Dehydroascorbic acid | 2.65 | 30.8 | 0 | 0 | |
| 4 | Ascorbic acid | 9.60 | 17.1 | 300 | 196 | |
| | Dehydroascorbic acid | 0 | 0 | 0 | 5.4 | |
| 5 | Ascorbic acid | 5.59 | 10.0 | 19 | 118 | 278 |
| | Dehydroascorbic acid | 2.97 | 1.8 | 0 | 0 | 0 |
| 6 | Ascorbic acid | 17.07 | 136 | 198 | | |
| | Dehydroascorbic acid | 0 | 0 | 0 | | |

TABLE VI: Dehydroascorbic acid and ascorbic acid in urine (*mg*/24 hrs) of normally fed guinea pigs injected with ascorbic acid, 100 *mg* per animal of 400 *gm* average weight per day for two weeks. (Modified from Banerjee and Guha, 1941).

| *Animal No.* | | *Before injection of ascorbic acid* | *After injection of ascorbic acid* |
|---|---|---|---|
| 1 | Ascorbic acid | 0.47 | 49.1 |
| | Dehydroascorbic acid | 0.06 | 0 |
| 2 | Ascorbic acid | 0.57 | 20.8 |
| | Dehydroascorbic acid | 0 | 3.38 |
| 3 | Ascorbic acid | 0.52 | 30.9 |
| | Dehydroascorbic acid | 0.04 | 0.56 |
| 4 | Ascorbic acid | 0.77 | 34.5 |
| | Dehydroascorbic acid | 0.97 | 0 |

The extreme sensitivity of the ascorbate system to physiological changes is suggestive of a major biochemical role for this redox system but its exact role has yet to be ascertained. Evidences have accumulated to indicate that dehydroascorbic acid controls cell division (40). High concentration of dehydroascorbic acid prepare quiscent cells for division by releasing and activating lysosomal hydrolytic enzymes while inhibiting mitosis (39). Adrenal cortical function is preceded by the conversion of ascorbic acid to dehydroascorbic acid (56). Highest mitotic activity in the adrenal cortex is observed when adrenocortical activity is at a minimum and the concentration of dehydroascorbic acid is lowest (54). Lutenizing hormone-stimulation of the ovaries is also preceded by a decrease in ascorbic acid (41). Thyroxine increases the concentration of circulating dehydroascorbic acid (37). Szent-Gyorgyi (61) believes that cell division is controlled by an electron acceptor containing a glyoxal grouping. Dehydroascorbic acid is an electron acceptor, structurally similar to a glyoxal and thus might have an inhibitory role on mitosis. Because of its non-ionic character and lipid solubility dehydroascorbic acid is considered to be the transporatable form of ascorbate (47). The action of dehydroascorbic acid thus may not be confined to the cells directly affected.

Various stimuli which activate mitotically dormant cells may initially increase the concentration of dehydroascorbic acid which activate hydrolytic enzymes. The enzymes destroy or inhibit mitotic repressors (35) and prepare quiscent cells for division. Glutathione is commonly associated with the reduction of dehydroascorbic acid (4) and its appearance in high concentration coincides with mitotic activity (60). The initial decrease in the concentration of dehydroascorbic acid may provoke the formation of glutathione and the increase in glutathione may lead to decrease of dehydroascorbic acid and eventually to cell division.

## REFERENCES

1. Astrup, T. and A. Fisher. Protein metabolism in tissue cell *in vitro*. II. Properties of malt extracts and glutathione as necessary growth substances. *Acta Physiol. Scandinav.*, **11** : 187-189, 1946.
2. Bandyopadhyay, A. and S. Banerjee. Effect of scurvy on serum proteins of rhesus monkeys. *Proc. Soc. Exptl. Biol. Med.*, **117** : 771-773, 1964.
3. Banerjee, S. Vitamin C and carbohydrate metabolism. *Nature*, **152** : 329, 1943.
4. Banerjee, S. Relation of scurvy to histological changes in the pancreas. *Nature*, **153** : 344, 1944.
5. Banerjee, S. Alloxan diabetes in monkeys. *Lancet*, **2** : 658, 1944.
6. Banerjee, S. On the hypoglycemic action of alloxan. *J. Biol. Chem.*, **158** : 547-550, 1945.
7. Banerjee, S. Relation of scurvy to the adrenaline content of the adrenals of guinea pigs. *J. Biol. Chem.*, **159**: 327-331, 1945.
8. Banerjee, S. Effect of certain substances on the prevention of diabetogenic action of alloxan. *Science*, **106**: 128-130, 1947.
9. Banerjee, S. and A. Bandyopadhyay. Plasma lipids in scurvy : effect of ascorbic acid supplement and insulin treatment. *Proc. Soc. Exptl. Biol. Med.*, **112** : 372-374, 1963.
10. Banerjee, S. and A. Bandyopadhyay. Plasma lipids in ascorbic acid-deficient rhesus monkeys. *Am. J. Physiol.*, **208** : 329-333, 1965.
11. Banerjee, S. and H. Bal. Biochemical changes in scorbutic rhesus monkeys. *Indian J. Med. Res.*, **47** : 663-667, 1959.
12. Banerjee, S. and B. Belavady. Dehydroascorbic acid level of blood in health and typhoid fever. *Lancet*, **2**: 912-913, 1953.
13. Banerjee, S., B. Belavady and A. K. Mukerjee. Effect of dehydroascorbic acid in rabbits. *Proc. Soc. Exptl. Biol. Med.*, **83** : 133-135, 1953.

14. Banerjee, S. and G. Bhattacharyya. Studies on the mechanism of alloxan hypoglycemia. *J. Biol. Chem.*, **175** : 923-928, 1948.

15. Banerjee, S. and D.K. Biswas. Urinary excretion of pyruvic acid, a-ketoglutaric acid and oxaloacetic acid in scurvy. *J. Biol. Chem.*, **234** : 3094-3096, 1959.

16. Banerjee, S., D.K. Biswas and H.D. Singh. Studies on carbohydrate metabolism in scorbutic guinea pigs. *J. Biol. Chem.*, **230** : 261-270, 1958.

17. Banerjee, S., D.K. Biswas and H.D. Singh. Dehydrogenase activity of tissues in scurvy. *J. Biol. Chem.*, **234** : 405-408, 1959.

18. Banerjee, S., C. Deb and B. Belavady. Effect of scurvy on glutathione and dehydroascorbic acid in guinea pig tissues. *J. Biol. Chem.*, **195** : 271-276, 1952.

19. Banerjee, S. and E. R. Divakaran. Utilization of glucose, fructose and galactose by scorbutic guinea pigs. *Am. J. Physiol.*, **195** : 291-294, 1958.

20. Banerjee, S. and N. C. Ghosh. Relation of scurvy to glucose tolerance test, liver glycogen and insulin content of pancreas of guinea pigs. *J. Biol. Chem.*, **168** : 207-211, 1947.

21. Banerjee, S. and P.K. Ghosh. Metabolism of acetate in scorbutic guinea pigs. *Am. J. Physiol.*, **195** : 1064-1066, 1960.

22. Banerjee, S. and P.K. Ghosh. Effect of insulin on serum protein in scurvy. *Proc. Soc. Exptl. Biol. Med.*, **106** : 63-64, 1961.

23. Banerjee, S. and B.C. Guha. The role of vitamin C in infection : Effect of administration of ascorbic acid on the urinary excretion of combined ascorbic acid by normal and tubercular subjects and by guinea pigs. *Ann. Biochem. Exp. Med., India.* **1** : 239-256, 1941.

24. Banerjee, S. and W. K. Kawishwar. Studies on the operation of the tricarboxylic acid cycle in scurvy. *J. Biol. Chem.*, **234** : 1347-1349, 1959.

25. Banerjee, S. and W. K. Kawishwar. Studies on the metabolism of butyrate in scurvy. *J. Sci. Ind. Res., India*, **20C** : 141-144, 1961.

26. Banerjee, S. and W. K. Kawishwar. Operation of hexose monophosphate oxidative pathway of glucose metabolism in scorbutic guinea pigs. *Indian J. Exptl. Biol.*, **1** : 105-107, 1963.

27. Banerjee, S. and K.S. Rohatgi. Different fractions of serum proteins in normal and scorbutic guinea pigs. *Proc. Soc. Expll. Biol. Med.*, **97** : 234-235, 1958.

28. Banerjee, S., P. B. Sen and B.C. Guha. Urinary excretion of combined ascorbic acid in pulmonary tuberculosis. *Nature*, **145** : 706, 1940.

29. Banerjee, S. and H.D. Singh. Adrenal cortical activity in scorbutic monkeys and guinea pigs. *Am. J. Physiol.*, **190** : 265-267, 1957.

30. Banerjee, S. and H. D. Singh. Cholesterol metabolism in scorbutic guinea pigs. *J. Biol. Chem.*, **233** : 336-339, 1958.

31. Barron, E. S. G. and T. P. Singer. Studies on the biological oxidations. XIX. Sulfhydryl enzymes in carbohydrate metabolism. *J. Biol. Chem.*, **157** : 221-224, 1945.

32. Belavady, B. and S. Banerjee. Metabolism of cholesterol in scorbutic guinea pigs. *J. Biol. Chem.*, **209** : 641-645, 1954.

33. Bhaduri, J. N. and S. Banerjee. Ascorbic acid, dehydroascorbic acid and glutathione levels in blood of patients suffering from infectious diseases. *Indian J. Med. Res.*, **48** : 208-211, 1960.

34. Boyer, P.D., H. Lardy and K. Myrback, Ed. The Enzymes. Academic Press, Inc., New York, 1960, Vol. 2, p—264.

35. Bullough, W.S. and T. Rytomaa. Mitotic homeostasis. *Nature*, **205** : 573–578, 1965.

36. Chakrabarti, B. and S. Banerjee. Dehydroascorbic acid level on blood of patients suffering from various infectious diseases. *Proc. Soc. Exptl. Biol. Med.*, **88** : 581–583, 1955.

37. Chandra, R. The effect of thyroxine on phosphatase, ascorbic acid, tocopherol content in the blood and milk of cow and buffalo. *Current Sci.*, **27** : 102–104, 1958.

38. Cornforth, J.M. and D.A. Long. Influence of organic phosphates on tuberculin sensitivity in B.C.G. infected guinea pigs. *Lancet*, **1** : 950–952, 1952.

39. Edgar, J.A. Is dehydroascorbic acid an inhibitor in the regulation of cell division in plants and animals? *Experientia*, **25** : 1214–1215, 1969.

40. Edgar, J.A. Dehydroascorbic acid and cell division. *Nature*, **227** : 24–26, 1970.

41. Goldstein, D.P. and S.H. Sturgis. Leutinizing hormone induced depletion of ascorbic acid in the rat ovary. *Am. J. Physiol.*, **201** : 1053–1056, 1961.

42. Gould, R.S. Glutathione as an essential growth factor for certain strains of *Neisseria gonorrhoeae*. *J. Biol. Chem.*, **153** : 143–146, 1940.

43. Hellman, L. and J.J. Burns. Metabolism of L-ascorbic acid-1-$C^{14}$. *J. Biol. Chem.*, **233** : 923–924, 1958.

44. Hopkins, F.G. and E.J. Morgan. Some relations between ascorbic acid and glutathione. *Biochem. J.*, **30** : 14446–14448, 1936.

45. Hopkins, F.G., E.J. Morgan and C. Lutwakmann. The influence of thiol groups in the activity of dehydrogenases. II. with an addendum on the location of dehydrogenases in muscle. *Biochem. J.*, **32** : 1829–1833, 1938.

46. Kanfer, J., G. Ashwell and J.J. Burns. Formation of L-lyxonic acid and L-xylonic acids from L-ascorbic acid in the rat kidney. *J. Biol. Chem.*, **235** : 2518–2521, 1960.

47. Martin, G.R. Studies on the tissue distribution of ascorbic acid. *Ann. N.Y. Acad. Sci.*, **92** : 141–147, 1961.

48. Matusis, I.I. Reduction of dehydroascorbic acid by human and animal tissues. *Biokhimiya*, **16** : 420–422, 1951.

49. Mosonyi, L. Recent penicillin researches. *Hungarian Acad. Med.*, **1** : 68–70, 1949.

50. Oser, B.L. Hawk's Physiological Chemistry. McGraw-Hill Book Company, New York, 14th Ed. 1965. p-694–695.

51. Patterson, J.W. The diabetogenic effect of dehydroascorbic acid and dehydroisoascorbic acid. *J. Biol. Chem.*, **183** : 81–85, 1950.

52. Patterson, J.W. and A. Lazarow. Sulfhydryl protection against dehydroascorbic acid in diabetes. *J. Biol. Chem.* **186** : 141–143, 1950.

53. Rane, L. and Y. Subbarow. The nutritional requirement of hemolytic streptococci. *Proc. Soc. Explt. Biol. Med.*, **38** : 837–839, 1938.

54. Rinne, U.K. and O. Kytomaki. Diurnal rhythm in the adrenal ascorbic acid concentration of the rat. *Experientia*, **17** : 512–513, 1961.

55. Rohatgi, K.S., H. Roy and S. Banerjee. Protein metabolism in scorbutic monkeys. *Indian J. Med. Res.*, **46** : 661–668, 1958.

56. Salomon, L.L. Ascorbic acid metabolism in guinea pigs. *Texas Rep. Biol. Med.*, **15** : 925–928, 1957.

57. Sayers, G., M.A. Sayers, H.K. Lewis and C.N.H. Long. Effect of adrenotropic hormone on ascorbic acid and cholesterol contents of adrenals. *Proc. Soc. Explt. Biol. Med.*, **55** : 238–242, 1944.

58. Seberell, W.H. and R.S. Harris, Ed. The Vitamins. Academic Press, Inc., New York, 1954. Vol. I., p-211.

59. Singh, R.P., S.S. Dubey and R.C. Gupta. Ascorbic acid in thyrotoxicosis. *Q.J. Surg. Sci.*, **10** : 178–182,1974.

60. Stern, H. Sulfhydryl groups and cell division. *Science*, **124** : 1292–1293, 1956.

61. Szent-Gyorgyi, A. Bioelectronics. *Science*, **161** : 988 : 990, 1968.