

HYPOTHESIS: THE ROLE OF VITAMIN C IN DIABETIC ANGIOPATHY*

GEORGE V. MANN, Sc.D., M.D.†

The optimal daily intake of vitamin C for human beings is uncertain. Pauling's contention that human beings have high requirements for vitamin C is based upon inconclusive trials and inferences from evolutionary evidence [1]. His argument has been discounted by many nutritionists because they have not seen signs of classical scurvy among persons taking 10–100-mg quantities of ascorbic acid (AA) daily, nor are they persuaded that clinical trials have shown any health advantages for subjects taking 10–50 times larger quantities [2]. There is another possible explanation for high and variable requirements of AA which can be derived from the known facts about its metabolism and the nature of diabetes mellitus.

The primates and a variety of other organisms require dietary AA because they lack gulonolactone oxidase, which most other organisms use to make AA from hexoses in liver cells [3]. This circumstance puts the dietary-dependent species in a kind of metabolic double jeopardy. They must have a dietary source, and they must be able to transport vitamin C across cell membranes.

The transport of many different sugars is facilitated by insulin [4]. These include D-glucose, D-mannose, D-galactose, D-xylose, L-arabinose, and D-lyxose. Both pyranose and furanose structures are included in this group of insulin-facilitated sugars. Attempts have been made to generalize the molecular structure required by this insulin-sensitive transport system [5].

Crane, Field, and Cori [6] found that the Goldstein-Levine [7] structural hypothesis for transport of sugars according to their configuration at carbons 1, 2, and 3 cannot be true. This admits the possibility of insulin facilitation of such a 2-3 diketose as dehydroascorbic acid (DHA), the transportable form of AA.

*Paper submitted to *Perspectives* June 25, 1973.

†Nutrition Division, Vanderbilt University School of Medicine, Nashville, Tennessee 37232. Career Investigator, National Heart Institute, Bethesda. 5KO6 HL-08288-12.

Another suggestive feature of sugar transport is its phylogenetic peculiarity. Widdas [8] found that the erythrocytes of fetal pigs, rabbits, guinea pigs, sheep, and deer are all hexose permeable at birth, but this sensitivity soon begins to disappear so that by the eighth day of life only the primates, of all the species examined, retain facilitated transport. There are thus three unusual features of facilitated transport of sugars: it is structurally selective for certain molecules; it is tissue specific, the dependency on insulin tending to be prominent in the eye, in muscle cells, and in adipocytes; and it is phylogenetically specific, tending to persist into adult life in the red blood cells of primates, which happen also to require dietary AA.

Facilitated transport of sugars also shows competitive inhibition by structurally related molecules. This is interpreted to mean that these molecules are competing for a common carrier. Thus impairment of AA utilization could have two possible mechanisms. Insulin lack may impair transport of AA if the latter is an insulin-dependent process, or hyperglycemia, of whatever cause, might impair transport of AA if the two sugars compete for a common carrier.

The prevalence of carbohydrate intolerance and diabetes mellitus varies among ethnic groups, being common among people in technically advanced countries [9]. The prevalence of carbohydrate intolerance increases strikingly with age, coming to affect 40 percent of persons past the age of 50 years [10, 11]. The disorder is often associated with accelerated vascular disease [12]. This diabetic angiopathy consists of thickening of basement membranes and fragility of capillary vessel walls. These changes impair transport and tissue nutrition and lead to aneurysmal dilatation and hemorrhage. In some patients with overt diabetes, the angiopathy is manifested as retinopathy causing blindness or as a nephropathy with uremia. The hallmark of chronic diabetes is accelerated vascular disease, and conventional therapy with insulin has had only limited preventive success. These vascular complications of diabetes are not well correlated with elevation of levels of cholesterol and triglycerides in the blood, with age at diagnosis, with severity, or even with degree of control, although there is argument about the last point [13]. They are correlated with resistance to the action of insulin and with duration of diabetes.

A number of experimental trials have been made to produce diabetic angiopathy in animals. These experiments have mainly used rats [14, 15] and dogs [16, 17], but there are few such attempts with primates. Our trials with *Cebus* monkeys made diabetic with alloxan were unsuccessful, but they were fed diets with high levels of AA (25–50 mg/kg body weight) [G. V. Mann, unpublished data].

Gibbs, Wilson, and Gifford [18] have followed *Rhesus* monkeys made diabetic with alloxan for periods up to 133 months. Those animals also

were fed diets containing abundant vitamin C. The low-fat diet used contained 90 mg of vitamin C per 1,000 kcal; the high-fat diet contained 45 mg/1,000 kcal. The animals showed some of the early changes of diabetic microangiopathy in the kidney, and five of 14 developed retinal microaneurysms. However, the devastating disorders seen in human beings were not reproduced. It would be of great interest to determine whether these would result if the animals were kept on marginal intakes of ascorbate.

For the present argument the report of Creutzfeld et al. [19] is relevant. Using similar methods, they could *not* demonstrate the thickening of basement membranes in diabetic mice which Siperstein, Unger, and Madison [20] had described in human diabetics. Nor did Siperstein et al. see such thickening in diabetic hamsters, mice, or sand rats, none of which is an AA-requiring species. Bloodworth et al. did find basement membrane thickening in alloxan diabetic dogs, and they describe diabetic retinopathy in three of 10 dogs [17].

The classical lesion of scurvy is venular extravasation of blood leading to small and large hematomata. This results from a weakened capillary or venule wall [21]. The biochemical function of AA is not known, but the evidence suggests it is involved in the conversion of soluble tropocollagen to collagen fibrils and the hydroxylation of proline at positions C3 or C4 after that amino acid is assembled in the tropocollagen molecule [22]. It can be shown that ascorbic acid is concentrated in areas of inflammation and collagen growth [23] and that the turnover rate of acid mucopolysaccharides and the rate of healing are impaired in both diabetic [24] and scorbutic animals [25]. One of the strange observations in human scurvy is the reddening and tenderizing—even rupture—of old scars [26].

Following Szent-Gyorgyi's lead [27] a class of compounds involved with vascular integrity was described, at first called vitamin P "in recognition of paprika and permeability." These were thought to be distinct from vitamin C. Szent-Gyorgyi thought scurvy might be a combined deficiency of vitamins C and P. The P compounds are flavones [28], abundant in citrus rind, yellow in color, and often poorly absorbed from the intestine. They can open the pyrone ring to form chalcones which are powerful reducing agents, as is AA. For a time these compounds were marketed, and there were some encouraging reports of their clinical value, usually for bleeding disorders, but no one was able to show that they were useful in controlling diabetic retinitis [29]. There have been no adequate clinical trials of large doses of AA in diabetic angiopathy. Plasma levels of AA were found not to be unusual in diabetes [30].

The observations by Ralli and Sherry in dogs [31] and Haid in human subjects [32] that insulin lowers the level of AA in both blood and urine

support the idea that insulin affects the distribution of AA among tissue compartments. In subsequent work Sherry and Ralli [33] showed that the effect was associated in the dog with a shift of AA from plasma to white blood cells. In the dog, there was no measurable increase of the AA content of red blood cells after insulin. While Sherry also demonstrated a lowering of AA in plasma by insulin in both diabetic and normal human beings, the critical measurements of an insulin effect on uptake of AA were not reported for human beings.

The facilitation of sugar transport by insulin has been extensively studied in muscle, ocular, and adipose tissue [4]. The phenomenon also occurs in cardiac muscle. Wertheimer and Bentor [34] showed with rat aorta that the uptake of D-glucose, D-galactose, D-xylose, and L-arabinose is facilitated by insulin and is impaired in alloxan diabetes. Insulin had no effect on the uptake of either D-fructose or D-arabinose, thus showing the familiar pattern of structural specificity seen in muscle. The authors also describe an interesting age effect in the rat, the uptake of the facilitated sugars being less in old rats than in young. Thus the uptake of sugars by aorta was diminished by insulin lack, by age, and by hypothyroidism. These are regular associates of vascular disease in man.

Another unexplained complication of diabetes is cataract. Young diabetics are especially susceptible, but all diabetics show accelerated development of cataracts. Because these changes in the lens can be seen in 65 percent of all persons 50–60 years of age and in 95 percent of persons over 65 years of age, ophthalmologists accept lens changes as usual accompaniments of aging. In the Western world cataract causes from 15 to 25 percent of all blindness and is the most common ophthalmologic disease. Every year one person in 2,000 in the United Kingdom over the age of 20 years seeks treatment for cataract [35, pp. 84 ff.].

The mechanisms of cataract formation are well described but poorly understood. There are legions of causes including many classes of cataractogenic chemicals [36]. For the present argument the most relevant facts are these: *Three common sugars, D-glucose, D-galactose, and D-xylose, can cause cataracts when fed to animals. In human diabetes and hereditary galactosemia cataracts are commonly seen. The accumulation of xylose in pentosuria does not produce cataracts, perhaps because those subjects seem in time to adapt and control the xylosemia. These three sugars act synergistically in producing experimental cataracts in animals, as though they may be acting by some common mechanism.*

It is reported that either insulin treatment or exercise prevents or delays the cataractogenic action of glucose and galactose. [37]. Both of these treatments facilitate sugar transport, since the hypoxia caused by exercise has an insulin-like effect on transport. Corticoid hormones augment the cataractogenic action of these sugars, and those hormones

are well-known insulin inhibitors [38]. Deficiency of AA augments the cataractogenic action of these sugars and also that of naphthalene, tyrosine, and adrenaline. Indeed, in the guinea pig some of these agents will produce cataract *only* when the animal is fed an AA-deficient diet [39].

The correlation between the levels of plasma and ocular AA and the presence of cataract is confusing. The ocular tissues of primates maintain a 20:1 aqueous:plasma level of AA, but it has not been clearly shown that persons with cataract have lower levels of AA in either the eye or the plasma [40]. It is of passing interest that in South India, where cataract is endemic, the dietary staple is cereal grain, which is low in AA content. All these relationships suggest that human cataracts may be a late consequence of marginal intake of AA, the diabetic being especially prone to cataracts because he also lacks insulin to facilitate its transport through the ciliary epithelium.

These facts and inferences lead to the formulation of a hypothesis with two variations of mechanisms: *Human beings, and perhaps other species requiring vitamin C, need insulin for the transport of the vitamin into the cells of certain tissues. Impairment of insulin function, whether by its absence, as in juvenile diabetes, or by its inhibition, as in adult-onset diabetes, will lead to impaired transport of vitamin C. Certain insulin-sensitive tissues, lacking AA, are prone to develop a kind of "local scurvy" with faulty collagen formation. This leads to fragility of vascular walls, hemorrhage, and thickening of basement membranes. In the eye, lenticular opacities are another result. A combination of a large intake of AA and adequate insulin will prevent these lesions. A variation of this hypothesis is also to be considered: If dehydroascorbate and glucose share the same transport mechanism and if hyperglycemia will compromise the transport of ascorbate into cells, this may lead to faulty collagen formation and microangiopathy. A large intake of ascorbate may correct this effect of hyperglycemia.*

Discussion

There are a number of relevant questions which challenge this hypothesis and its mechanisms:

1. Why do human requirements for AA vary [41]? Is this caused by a variable supply of insulin? A diminishing insulin effect with age may account for the rising prevalence of angiopathy, cataract, and scurvy with age.

2. Why do diabetics have microangiopathy affecting especially the eye and the kidney and not classical scurvy, if they are AA deficient? Is it because sufficient AA can, with conventional intakes, reach certain tissues which are not insulin dependent, including the skin, gums, and bone, but cannot reach those insulin sensitive tissues so vulnerable in

diabetes? The mural cells of the capillaries, called pericytes by the ophthalmologists, may be especially vulnerable.

3. Why do a few diabetics escape microangiopathy? Is it because some optimal combination of insulin therapy and a high intake of AA will transport enough of the vitamin by mass action to prevent vascular lesions? Experienced clinicians have long contended that good control of diabetes will prevent vascular disease.

4. Why has it not been possible to produce diabetic angiopathy in experimental animals? Is it because the attempts have used the mouse, rat, hamster, and dog, which are not AA-requiring species? They synthesize AA and are less dependent on insulin for transport. When primates were used they were flooded with ascorbic acid.

Deficiency of AA has been produced experimentally a number of times in human beings [42, 43]. There are several instructive features. The disorder produced has not matched natural scurvy, perhaps because investigators could not ethically proceed to extreme deprivation. Hood and Hodges [44] observed both ocular lesions and neuropathy in human subjects during AA deficiency, but the ocular lesions were conjunctival, not retinal.

The clinical trials necessary to evaluate the validity of this hypothesis, or of any role of AA in preventing angiopathy, infection, poor healing, or ill health, are bound to be difficult because of the obscurity of the end points. In the diabetic with early retinopathy the vascular beds in the eyegrounds may be the best index. In nondiabetics some general measure of muscle perfusion such as physical fitness with a treadmill test [45] may, by indirection, be the best measure. The most crucial initial experiment would be the examination of whether DHA uses the glucose carrier system, whether the two sugars interfere, and whether insulin facilitates DHA transport into muscle, adipose tissue, and the eye of primates. If these trials are supportive for the hypothesis, the next step might be an attempt at producing diabetic microangiopathy in experimental primates by *combining* diabetes and marginal AA deficiency.

If this hypothesis is correct in either of its variants, Pauling's contention could be correct [46]. If the current official recommendations for daily intakes of 35–60 mg of AA per person are followed, the manifestations of Lind's scurvy may be prevented. But if, with age, we become insulin insensitive and hyperglycemic, and if certain tissues in the primates are impaired in AA transport by hyperglycemia or require a hormonal assist for AA transport, it may follow that high intakes of AA could, by mass action, force entry of the vitamin despite either of these impairments. If either mechanism set forth is correct, a way is open to prevent some of the disabling accompaniments of aging. At any rate we could agree with Darwin [47]: "*False facts* are highly injurious

to the progress of science for they often endure long, but *false views* if supported by some evidence do little harm, for everyone takes a salutary pleasure in proving their falseness—and when this is done, one path toward error is closed, and the road to truth is often at the same time opened.”

REFERENCES

1. L. PAULING. Proc. Natl. Acad. Sci. USA, **67**:1643, 1970.
2. R. V. LEE, R. PASSMORE, F. J. STARE, and C. F. ENLOE, JR. Nutr. Today, **6**:16, 1971.
3. J. J. BURNS. Am. J. Med., **26**:740, 1959.
4. M. S. GOLDSTEIN. Ann. N.Y. Acad. Sci., **82**:378, 1959.
5. P. G. LEFEVRE. Pharmacol. Rev., **13**:39, 1961.
6. R. K. CRANE, R. A. FIELD, and C. F. CORI. J. Biol. Chem., **224**:649, 1957.
7. M. S. GOLDSTEIN, W. L. HENRY, B. HUDDLESTON, and R. LEVINE. Am. J. Physiol., **173**:207, 1963.
8. W. F. WIDDAS. J. Physiol. (Lond.), **127**:318, 1955.
9. Editorial. Diabetes mellitus: disease or syndrome. Lancet, **1**:583, 1971.
10. N. S. HAYNER, M. O. KJELSBERG, F. H. EPSTEIN, and T. FRANCIS, JR. Diabetes, **14**:413, 1965.
11. J. L. PROZEFESKY, J. L. COLKER, H. M. LANGS, and R. ANDRES. Ann. Intern. Med., **63**:988, 1965.
12. C. KILO, N. VOGLER, and J. R. WILLIAMSON. Diabetes, **21**:881, 1972.
13. R. S. ELKELES, C. LOWY, A. D. H. WYLLIE, J. L. YOUNG, and T. R. FRASER. Lancet, **1**:880, 1971.
14. V. G. FOGLIA, R. E. MANCINI, and A. F. CARDEZA. Arch. Pathol., **50**:75, 1950.
15. G. V. MANN, J. W. GODDARD, and L. ADAMS. Am. J. Pathol., **27**:857, 1951.
16. H. T. RICKETTS, C. E. TEST, E. S. PETERSEN, H. LINTS, N. TUPIKOVA, and P. E. STEINER. Diabetes, **8**:298, 1959.
17. J. M. B. BLOODWORTH, JR., R. L. ENGERMAN, and K. L. POWERS. Diabetes, **18**:455, 1969.
18. G. E. GIBBS, R. B. WILSON, and H. GIFFORD. Diabetes, **15**:258, 1966.
19. W. CREUTZFELD, D. MENDE, B. WILLMS, and D. SÖLING. Diabetologia, **6**:356, 1970.
20. M. D. SIPERSTEIN, R. H. UNGER, and L. L. MADISON. J. Clin. Invest., **47**:1973, 1968.
21. I. GORE, M. WALLA, and M. L. GOODMAN. Arch. Pathol., **85**:493, 1968.
22. S. UDENFRIEND. Science, **152**:1335, 1966.
23. W. B. ROBERTSON. Ann. N.Y. Acad. Sci., **92**:159, 1961.
24. H. WAGNER. Diabetologia, **6**:412, 1970.
25. R. E. HUGHES and E. KODICEK. Biochem. J., **77**:3P, 1960.
26. Lind's treatise on scurvy. Edinburgh: Edinburgh Univ. Press, 1953.
27. L. ARMENTANO, A. BENTSATH, T. BERES, S. RUSZNYAK, and A. SZENT-GYORGYI. Dtsch. Med. Wochenschr., **62**:1325, 1936.
28. Editorial. Br. Med. J., **1**:235, 1969.
29. R. RODRIQUEZ and H. F. ROOT. N. Engl. J. Med., **238**:391, 1948.
30. L. B. OWENS, J. WRIGHT, and E. BROWN. Am. J. Med. Sci., **201**:636, 1941.
31. E. P. RALLI and S. SHERRY. Proc. Soc. Exp. Biol. Med., **43**:669, 1940.
32. H. HAID. Z. Klin. Med., **139**:435, 1941.
33. S. SHERRY and E. P. RALLI. J. Clin. Invest., **27**:217, 1948.

34. H. E. WERTHEIMER and V. BENTOR. *Diabetes*, **11**:422, 1962.
35. S. DUKE-ELDER (ed.). *Diseases of the lens and vitreous*, vol. **11**, Glaucoma and hypotony. System Ophthalmol. Ser. Saint Louis: Mosby, 1969.
36. H. DAWSON. *The eye*, vol. **1**, Vegetative physiology and biochemistry. 2d ed. New York: Academic Press, 1969.
37. H. S. MITCHELL and G. M. COOK. *Arch. Ophthalmol.*, **19**:22, 1938.
38. J. W. BETTMAN, W. E. FUNG, R. A. WEBSTER, P. P. NOYES, and N. J. VINCENT. *Am. J. Ophthalmol.*, **65**:581, 1968.
39. Y. UYAMA and S. OGINO. *Med. J. Osaka Univ.*, **6**:813, 1956.
40. E. F. PURCELL, L. H. LERNER, and V. E. KINSEY. *Arch. Ophthalmol.*, **51**:1, 1954.
41. R. J. WILLIAMS and G. DEASON. *Proc. Natl. Acad. Sci. USA*, **57**:1638, 1967.
42. J. H. CRANDON, C. C. LUND, and D. B. DILL. *N. Engl. J. Med.*, **223**:353, 1940.
43. W. BARTLEY, H. A. KREBS, and J. R. P. O'BRIEN. Vitamin C requirements of human adults. *Med. Res. Council. Spec. Rep. Ser. (Lond.)*, no. 280, 1953.
44. J. HOOD and R. E. HODGES. *Am. J. Clin. Nutr.*, **22**:559, 1969.
45. H. L. GARRETT, J. MACBETH, and G. V. MANN. *J. Chronic Dis.*, **23**:559, 1971.
46. L. PAULING. *Vitamin C and the common cold*. San Francisco: Freeman, 1970.
47. C. DARWIN. *Descent of man and selection in relation to sex*. London: Murray, 1871.

AFRICAN GENESIS

You think in blood and call it *game*,
 You know the glassy eye you will not see,
 You know when short hair stands,
 You know the flared nostril and the clammy hands,
 But do you wonder why?
 Young hunter, teach me why
 I cannot love a gun
 Or want a frantic heart to die.

Imprisoned in the freedoms others forged,
 You laugh in lies
 And speak your words from other mouths.
 Swinging from ancient tree-limbs
 Rooted in your heart,
 Teach me young hunter,
 The beast that can't be learned,
 The pattern of archaic kill,
 This virgin rage politely turned.

HARRY P. KROITOR