The Science of the Total Environment, 73 (1988) 95–106
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

SILICON AS A TRACE NUTRIENT

EDITH MURIEL CARLISLE
School of Public Health, University of California, Los Angeles, CA 90024 (U.S.A.)

ABSTRACT

Silicon performs an important role in connective tissue, especially in bone and cartilage. Silicon's primary effect in bone and cartilage appears to be on formation of the organic matrix. Bone and cartilage abnormalities are associated with a reduction in matrix components, resulting in the establishment of a requirement for silicon in collagen and glycosaminoglycan formation. Additional support for silicon's metabolic role in connective tissue is provided by the finding that silicon is a major ion of osteogenic cells, especially high in the metabolically active state of the cell. Further studies also indicate that silicon participates in the biochemistry of subcellular enzyme-containing structures. Silicon also forms important relationships with other elements. Although it is clear from the body of recent work that silicon performs a specific metabolic function, a structural role has been proposed for silicon in connective tissue. A relationship established between silicon and aging probably relates to glycosaminoglycan changes.

INTRODUCTION

The discovery of the "newer" essential trace elements in recent years has encouraged reconsideration of elements that were previously thought to be environmental contaminants. Silicon is one of these elements. Because of silicon's abundance in the earth's crust, to label it a trace element may require an adjustment in thinking for some people. Only within the last decade has it been recognized that silicon actively participates in the normal life processes of higher animals (Carlisle, 1972; Schwarz and Milne, 1972). We have shown silicon to be required in bone, cartilage, and connective tissue formation as well as participating in several other important metabolic processes (Carlisle, 1978). Although interest in the silicon content of animal tissues and the effect of siliceous substances on animals was expressed over half a century ago (King and Belt, 1938), emphasis has been placed until recently on the toxicity of silicon, its effect upon forage digestibility, urolithiasis and especially silicosis (caused by dust inhalation).

A series of experiments has contributed to the establishment of silicon as an essential element (Carlisle, 1974, 1982). The first of these were in vitro studies which showed that silicon is localized in active growth areas in bones of young mice and rats, suggesting a physiological role of silicon in bone calcification processes. These were followed by in vivo studies showing that silicon affects the rate of bone mineralization. Of critical importance, we subsequently

0048-9697/88/$03.50

demonstrated in 1972 (Fig. 1) that silicon deficiency is incompatible with normal growth and skeletal development in the chick and that these abnormalities could be corrected by a silicon supplement. During the same year Schwarz and Milne showed that silicon deficiency in the rat results in depressed growth and skull deformations. Later studies, both in vitro and in vivo, emphasize silicon's importance in bone formation and connective tissue metabolism and confirm the postulate that silicon is involved in an early stage of bone formation. Some of these studies are discussed in this presentation.

SILICON IN TISSUES

Earlier data on the silicon content of living tissues has varied greatly, and in general, reported values were considerably higher before the advent of plastic laboratory ware and the development of suitable methods. Even with more recent methods, considerable variance still exists in reported tissue concentrations of silicon (Schwarz, 1978).

Normal human serum has a narrow range of silicon concentration averaging 50 $\mu g\, dl^{-1}$ (Carlisle, 1986); the range is similar to that found for most of the other well-recognized trace elements in human nutrition. The silicon is present almost entirely as free soluble monosilicic acid. No correlations of age, sex, occupation or pulmonary condition with blood silicon concentrations have

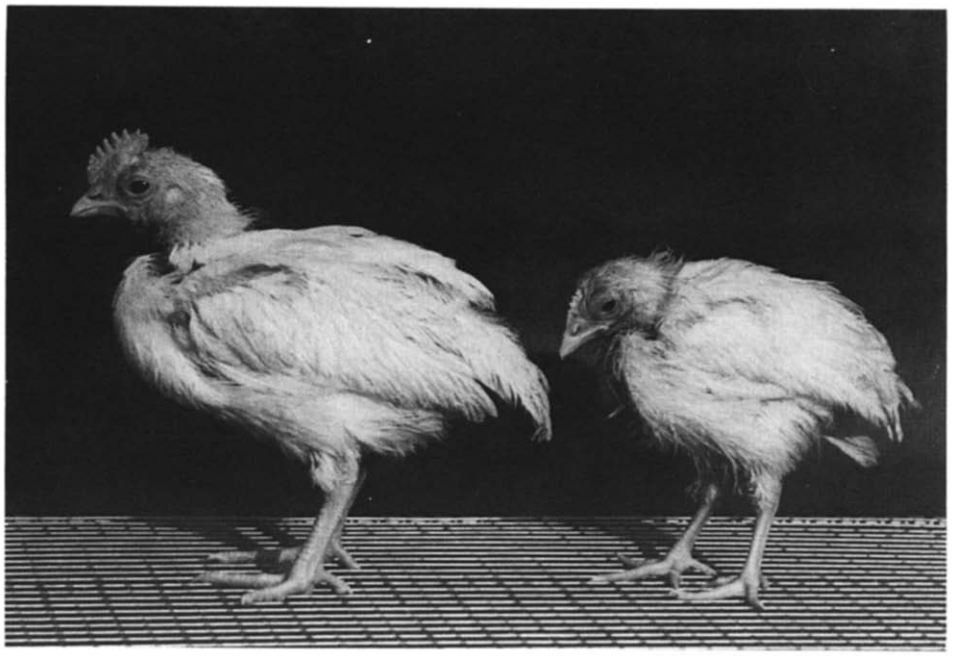

Fig. 1. Photograph of 4-week old chicks on silicon-supplemented diet (left) and low-silicon basal diet (right) (Carlisle, 1972).

been found, although the level increased when silicon compounds were specifically administered.

Connective tissues such as aorta, trachea, tendon, bone, skin and its appendages are unusually rich in silicon, as shown by studies in several animal species (Carlisle, 1974). In the rat, for example (Fig. 2), the aorta, trachea and tendon are 4–5 times richer in silicon than liver, heart, and muscle. The high silicon content of connective tissues appears to arise mainly from its presence as an integral component of the glycosaminoglycans and their protein complexes which contribute to the structural framework of this tissue. Fractionation procedures reveal that connective tissues such as bone, cartilage and skin yield complexes of high silicon content. Silicon is also found as a component of glycosaminoglycans isolated from these complexes.

The consistent low concentrations of silica in most organs do not appear to vary appreciably during life. Parenchymal tissue such as heart and muscle, for example, range from 2 to 10 μg of silicon/g dry weight (Carlisle, 1982). The lungs are an exception. Similar levels of silicon in rat and rhesus monkey tissues have been reported, where soft tissue levels in both species varied from 1 to 33 μg of silicon/g dry weight, excepting the primate lung and lymph nodes, which averaged 942 and 101 ppm, respectively. High levels in human lymph nodes have been associated with the presence of clusters and grains of quartz (Carlisle, 1986).

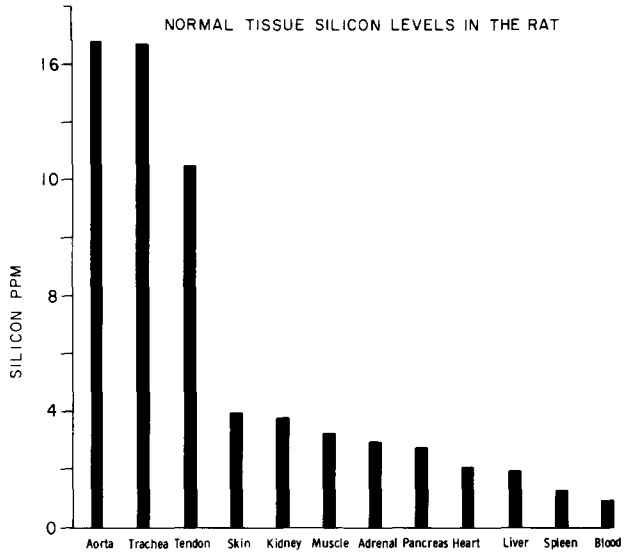

Fig. 2. Normal tissue silicon levels in the adult male rat. Values represent mean silicon levels of 20 animals (4 months of age) expressed as parts per million wet weight of tissue (Carlisle, 1974).

Calcification

In vitro studies

The first indications of a physiological role for silicon were from this laboratory, reporting that silicon is involved in an early stage of bone calcification. Using electron microprobe studies (Carlisle, 1970) silicon was shown to be uniquely localized in active growth areas in young bone of mice and rats. The amount present in specific very small regions within the active growth areas appeared to be uniquely related to "maturity" of the bone mineral. In the earliest stages of calcification in these regions both the silicon and calcium contents of the osteoid tissue were found to be very low, but as mineralization progressed the silicon and calcium contents rose congruently. In a more advanced stage the amount of silicon fell markedly so that as calcium approached the proportion present in bone apatite, the silicon was present only at the detection limit. In other words, the more "mature" the bone mineral, the smaller the amount of measurable silicon. Further studies of the Ca/P ratio in silicon-rich regions gave values < 1.0 compared with a Ca/P ratio of 1.67 in mature bone apatite. These findings suggested strongly that silicon is involved in an organic phase during the series of events leading to calcification.

In vivo studies

Subsequent in vivo experiments showed that silicon has a demonstrable effect on in vivo calcification (Carlisle, 1974); that is, a relationship between the level of dietary silicon and bone mineralization was established. Weanling rats were maintained on diets containing three levels of calcium (0.08, 0.40, 1.20%) at three levels of silicon (10, 25, 250 ppm). Increasing silicon in the low calcium diet resulted in a highly significant (35%) increase in the percentage ash contained in the tibia during the first 3 weeks of the experiment. Silicon was found to hasten the rate of bone mineralization. Calcium content of the bone also increased with increased dietary silicon, substantiating the theory of a relationship between mineralization and silicon intake. The tendency of silicon to accelerate mineralization was also demonstrated by its effect on bone maturity, as indicated by the [Ca]/[P] ratio. The concept of an agent that affects the speed of chemical maturity of bone is not new. Muller et al. (1966) found that the chemical maturity of vitamin D-deficient bone, although inferior to control bone during the period of maximum growth, approaches the control level at the end of the experiment.

Bone formation

The earliest studies suggesting a role for silicon in bone formation were those mentioned above. Most significant, however, was the establishment of a silicon deficiency state incompatible with growth and normal skeletal develop-

ment. In the chick, this is evidenced by reduced circumference, thinner cortex and less flexible leg bones as well as by smaller and abnormally shaped skulls with the cranial bones appearing flatter (Carlisle, 1972). Silicon deficiency in rats was also shown to result in skull deformations (Schwarz and Milne, 1972).

Recent studies further emphasize the importance of silicon in bone formation. Skull abnormalities associated with reduced collagen content have been produced in silicon-deficient chicks under conditions promoting optimal growth using a diet containing a natural protein in place of the crystalline amino acids used in earlier studies (Carlisle, 1980a). An additional finding was the striking difference in the appearance of the skull matrix between the silicon-deficient and silicon-supplemented chicks, the matrix of the deficient chicks totally lacking the normal striated trabecular pattern of the control chicks. The deficient chicks showed a nodular pattern of bone arrangement, indicative of a primitive type of bone.

Using the same conditions and by introducing three different levels of vitamin D, it has been shown that the effect exerted by silicon on bone formation is substantially independent of the action of vitamin D (Carlisle, 1981a). All chicks on silicon-deficient diets, regardless of the level of dietary vitamin D, had gross abnormalities of skull architecture, and, furthermore, the silicon-deficient skulls showed considerably less collagen at each vitamin D level. As in the previous study, the bone matrix of the silicon-deficient chicks totally lacked the normal striated trabecular pattern of the control chicks. In the rachitic groups of chicks, the appearance of the bone matrix was quite different from the groups receiving adequate vitamin D, being considerably less calcified and more transparent, enabling the cells and underlying structure to be seen more easily. The deficient chicks appeared to have a marked reduction in number of osteoblasts compared with the controls. In these two studies, the major effect of silicon appears to be on the collagen content of the connective tissue matrix and this is independent of vitamin D.

Cartilage and connective tissue formation

In addition to bone, silicon-deficiency is manifested by abnormalities involving articular cartilage and connective tissue (Carlisle, 1976). Chicks in the silicon-deficient group had thinner legs and smaller combs in proportion to their size. Long bone tibial joints were markedly smaller and contained less articular cartilage than those of silicon-supplemented chicks. The deficient chicks also revealed a significantly lower hexosamine content in their articular cartilage (Table 1). In cock's comb also, a smaller amount of connective tissue, a lower total percentage of hexosamines and a lower silicon content were found in the silicon-deficient group. These findings point clearly to an involvement of silicon in glycosaminoglycan formation in cartilage and connective tissue.

In more recent studies, long bone abnormalities similar to those reported above have been produced in silicon-deficient chicks using a diet containing a

TABLE 1

Effect of silicon intake on articular cartilage composition[a]

Diet	Tissue (mg wet wt.)	Total hexosamine (mg wet wt.)	Percent hexosamine (% wet wt.)
Low silicon	63.32 ± 8.04	0.187 ± 0.23	0.296 ± 0.009[b]
Supplemented	86.41 ± 4.82	0.310 ± 0.031	0.359 ± 0.011

[a] There were 12 chicks per group. All values reported as mean ± SD.
[b] Significantly different from the supplemented animals at $P < 0.001$.

natural protein in place of crystalline amino acids used in the earlier studies (Carlisle, 1980b). Tibia from silicon-deficient chicks had significantly less glycosaminoglycans and collagen, the difference being greater for glycosaminoglycans than collagen (Fig. 3). Tibia from silicon-deficient chicks also showed rather marked pathology, profound changes being demonstrated in epiphyseal cartilage. The disturbed epiphyseal cartilage sequences resulted in defective endochondral bone growth, indicating that silicon is involved in a metabolic chain of events required for normal growth of bone.

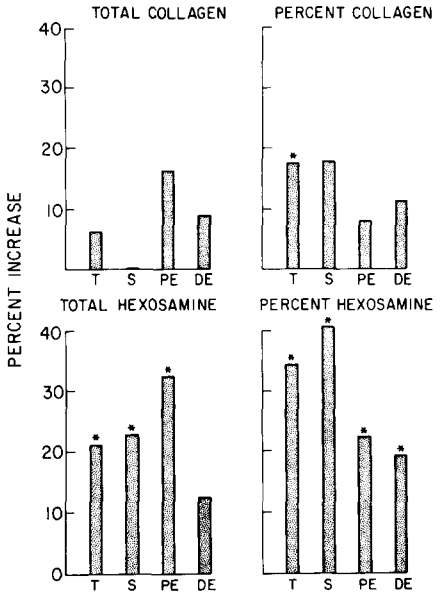

Fig. 3. Effect of silicon intake on tibial composition illustrated by increase (%) of collagen and hexosamines in tibia of silicon-supplemented chicks over silicon-deficient chicks. *Significantly different from supplemental animals at $p < 0.05$. T, tibia; S, shaft; PE, proximal epiphies; DE, distal epiphies (Carlisle, 1980b).

Connective tissue matrix

The preceding in vivo studies have shown silicon to be involved in both collagen and glycosaminoglycan formation. Silicon's primary effect in bone and cartilage appears to be on formation of the matrix, although silicon may also participate in the mineralization process itself. The in vivo findings have been corroborated and extended by studies of bone and cartilage in organ and cell culture.

Organ culture

Studies growing embryonic skull bones in culture (Table 2) further demonstrate the dependence of bone growth on the presence of silicon (Carlisle and Alpenfels, 1978). Most of the increase in growth appears to be due to a rise in collagen content; silicon-supplemented bones showed a 100% increase in collagen content over silicon-low bones after 12 days. Silicon is also shown to be required for formation of glycosaminoglycans; at day 8, the increase in hexosamine content of supplemented bones was nearly 200% more than in silicon-low bones, but by day 12 it was the same in both groups.

A parallel effect has been demonstrated in the growth of cartilage in culture and is especially marked in cartilage from 14-day embryos as compared with 10- and 12-day embryos (Carlisle and Alpenfels, 1980). Silicon's effect on collagen formation was also especially striking in cartilage from 14-day embryos, appearing to parallel the rate of growth. Similarly, matrix hexosamines (glycosaminoglycans) were formed more rapidly by silicon-supplemented cartilage, the most striking difference in this case being in cartilage from 12-day embryos. The requirement for silicon in collagen and glycosaminoglycan formation thus proves not to be limited to bone matrix but applies also to cartilage.

An interaction between silicon and ascorbate (Carlisle and Suchil, 1983) has also been shown in cartilage. Silicon's effect on cartilage formation was inves-

TABLE 2

Effect of silicon on rate of synthesis of bone matrix components

Days in culture	Bone chondroitin sulfate[a] silicone		Bone collagen[b] silicon		Bone non-collagenous protein[c] silicon	
	Low	Suppl.	Low	Suppl.	Low	Suppl.
0						
4	0.51	7.42*	0	62.7*	241	236
8	3.58	10.50*	64.2	117.9*	158	102
12	6.14	5.90	89.5	176.1*	200	188

[a] By hexose nitrogen × 2.65.

[b] By hydroxyproline × 7.46.

[c] As leucine by NH_2 nitrogen corrected for collagen and hexose nitrogen.

* Significantly different from the supplemented media at $P < 0.05$.

tigated both in the presence and absence of ascorbate. No significant effect on hexosamine content occurred in the absence of ascorbate. However, silicon supplementation resulted in significant increases in wet weight, hexosamine and proline content in the presence of ascorbate. The greatest effect was on hexosamine content. Furthermore, silicon and ascorbate interact to give maximal production of hexosamines. Silicon also appears to increase hydroxyproline, total protein, and noncollagenous protein beyond the effects of ascorbate.

Recent studies suggest a mitochondrial role for silicon in the synthesis of proline precursors (Carlisle and Alpenfels, 1984). Studies growing epiphyseal cartilage from 12- and 14-day embryos were continued on a larger scale and proline was determined in addition to analyses for hexosamine, hydroxyproline and noncollagenous protein as previously. In 12-day cultures, by far the most obvious difference found was with proline synthesis, large differences resulting at days 4 and 8 between deficient and silicon-supplemented media suggest the possibility of a role for silicon in the proline synthetic pathway.

Cell culture

An effect of silicon on formation of extracellular cartilage matrix components by connective tissue cells has also been demonstrated (Carlisle and Garvey, 1982) in chondrocytes isolated from chick epiphyses cultured under silicon-low and silicon-supplemented conditions. The major effect of silicon appeared to be on collagen. Silicon-supplemented cultures demonstrated a 243% ($p < 0.01$) increase in collagen measured as hydroxyproline over low-silicon cultures. Silicon also had a pronounced stimulatory effect on matrix polysaccharides; matrix polysaccharide content of silicon-supplemented cultures increased 152% ($p < 0.01$) more than that of low-silicon cultures. Silicon's effect on collagen and glycosaminoglycan formation was not due to cellular proliferation but to some system in the cell participating in their formation.

Enzyme activity

We have also shown a dependence on silicon for maximal prolyl hydroxylase activity (Carlisle et al., 1981). Prolyl hydroxylase obtained from frontal bones of 14 day-old chick embryos incubated for 4 or 8 days with 0, 0.2, 0.5, or 2.0 m*M* Si added to a basic, low silicon media show lower enzyme activity in low-silicon bones with increasing enzyme activity in 0.2, 0.5 and 2.0 m*M* cultures. The results support the in vivo and in vitro findings of a requirement for silicon in collagen biosynthesis, the activity of prolyl hydroxylase being a measure of the rate of collagen biosynthesis.

Connective tissue cellular component

Additional support for silicon's metabolic role in connective tissue at the cellular level is provided by evidence of its presence in connective tissue cells

(Carlisle, 1982). X-ray microanalysis of active growth areas in young bone and isolated osteoblasts show silicon to be a major ion of osteogenic cells, the amounts of silicon being in the same range as that of calcium, phosphorus and magnesium. Moreover, silicon appeared to be especially high in the metabolically active state of the cell, the osteoblast. Clear evidence that silicon occurs in the osteoblast and is localized in the mitochondria adds strong support to the proposition that silicon is required for connective tissue matrix formation.

Structural component

Although the discussion above indicates that silicon plays an important metabolic role in connective tissue, a structural role has also been proposed, mainly supported by the finding that in connective tissue silicon is a component of animal glycosaminoglycans and their protein complexes. In higher animals, the glycosaminoglycans, hyaluronic acids, chondroitin sulfates and keratan sulfate, are found to be linked covalently to proteins as components of the extracellular amorphous ground substance that surrounds the collagen, elastic fibers and the cells. By extraction and purification of several connective tissues, we have shown silicon to be chemically combined in the glycosaminoglycan fraction. The silicon content of the glycosaminoglycan protein complex extracted in this laboratory from bovine nasal septum, for example, is 87 ppm compared with 13 ppm in the original dried cartilaginous tissue (Carlisle, 1976). From this complex, smaller molecules considerably richer in silicon were isolated. Silicon was found to be associated with the larger, purer polysaccharide and smaller protein moieties.

Similar results on isolated glycosaminoglycans, which included some reference research standards, have been reported by Schwarz (1973). More recently, however, he has reported (Schwarz, 1978) that many of his earlier observations on the occurrence of bound silicon in glycosaminoglycans were in error because they were based partially on results obtained with materials contaminated by silica or polysilicic acid. Work in this laboratory shows that silicon is indeed a component of the glycosaminoglycan–protein complex; however, the amount of silicon in these complexes is less than the values reported by Schwarz (1973) for isolated glycosaminoglycans.

The preceding data indicate the silicon is not merely involved in glycosaminoglycan formation but that, in animal glycosaminoglycans at least and quite probably in plant polysaccharides, silicon is a structural component.

INTERACTION WITH OTHER ELEMENTS

An interrelationship between silicon and molybdenum has recently been established (Carlisle, 1979). Plasma silicon levels were strongly and inversely affected by molybdenum intake; silicon-supplemented chicks on a liver-based diet (Mo 3 ppm) had a 348% lower plasma silicon level than chicks on a casein diet (Mo 1 ppm) (Fig. 4). Molybdenum supplementation also reduced silicon

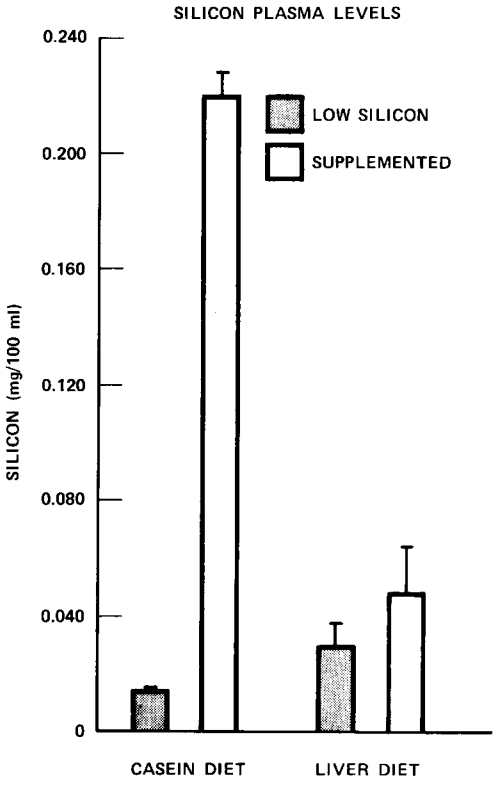

Fig. 4. Effect of silicon supplementation on plasma silicon levels of chicks fed a low-silicon basal diet with casein as the protein source and with liver as the protein source. Total duration of experiment, 4 weeks; 12 animals per group. Plasma silicon values of silicon-supplemented diets significantly different at $P < 0.001$ (Carlisle, 1979).

levels in those tissues examined. Conversely, plasma molybdenum levels are also markedly and inversely affected by the inorganic silicon intake. Reduction in molybdenum tissue retention by silicon also occurred. The interaction occurs within dietary levels of these elements. Although a copper–molybdenum–sulfate interrelationship has previously been shown in animal species, this is the first work demonstrating a silicon–molybdenum interaction.

Aluminum is another element with which silicon is shown to form an interrelationship. Since the establishment of silicon as an essential trace element in 1972 by this laboratory (Carlisle, 1972), all tissues analyzed for silicon have been analyzed simultaneously for aluminum and a number of other elements. From the many analyses of tissues we have done in several animal species, we have established a relationship between silicon and aluminum which may have relevance to Alzheimer's disease in humans (Carlisle, 1986).

AGING

Connective tissue changes are prominent in aging, so that it is not surprising to find a relationship between silicon and aging in certain tissues. The silicon content of the aorta, other arterial vessels and skin was found to decline with age in contrast to other analyzed tissues, which showed little or no change (Carlisle, 1974). The decline in silicon content was significant and was particularly dramatic in the aorta commencing at an early age. This relationship occurred in several animal species.

Similarly, in human skin, the silicon content of the dermis has been reported to diminish with age. In contrast with an earlier finding, French investigators (Loeper et al., 1978) reported that the silicon content of the normal human aorta decreases considerably with age, and furthermore, that the level of silicon in the arterial wall decreases with the development of atherosclerosis. The potential involvement of silicon in atherosclerosis has been suggested by others (Schwarz, 1978; Dawson et al., 1978). Of possible significance here, a relationship has been reported between silicon, age and endocrine balance and it is suggested that the decline in hormonal activity may be responsible for the changes in silicon levels in senescence (Charnot and Peres, 1971).

The precise relationship of silicon with the aging process remains to be determined. In contrast to the decrease in silicon content with age found in certain connective tissues, the accumulation of silicon in certain other tissues, mainly due to environmental influences, raises the possibility that a failure to dispose of silicon may also affect the aging process. In humans, it was shown in an earlier study (King and Belt, 1938) that silicon levels gradually increase with age in the human peribronchial lymph nodes, even in subjects who do not have a history of unusual dust exposure. More recently, in Alzheimer's disease (Nikaido et al., 1972), a presenile condition characterized pathologically by the presence of glial plaques in the brain, an unexpectedly high increase of silicon has been reported in the cores and rims of the senile plaques.

ACKNOWLEDGEMENT

This research was supported by a grant from the USPHS National Institute of Health, AM 16611.

REFERENCES

Carlisle, E.M., 1970. Silicon: A possible factor in bone calcification. Science, 167: 179–280.

Carlisle, E.M., 1972. Silicon an essential element for the chick. Science, 178: 619–621.

Carlisle, E.M., 1974. Silicon as an essential element. Fed. Proc., 33: 1758–1766.

Carlisle, E.M., 1976. In vivo requirement for silicon in articular cartilage and connective tissue formation in the chick. J. Nutr., 106: 478–484.

Carlisle, E.M., 1978. Essentiality and function of silicon. In: G. Bendz and I. Lindquist (Eds), Biochemistry of Silicon and Related Problems. Plenum Press, New York, pp. 231–253.

Carlisle, E.M., 1979. A silicon-molybdenum interrelationship in vivo. Fed. Proc., 38: 553.

Carlisle, E.M., 1980a. A silicon requirement for normal skull formation. J. Nutr., 10: 352–359.

Carlisle, E.M., 1980b. Biochemical and morphological changes associated with long bone abnormalities in silicon deficiency. J. Nutr., 10: 1046–1056.
Carlisle, E.M., 1981a. Silicon: a requirement in bone formation independent of Vitamin D. Calcif. Tissue Int., 33: 27–34.
Carlisle, E.M., 1982. The nutritional essentiality of silicon. Nutr. Rev., 40: 193–198.
Carlisle, E.M., 1986. Silicon. In: W. Mertz (Ed.), Trace Elements in Human and Animal Nutrition, 5th edn. Academic Press Inc, New York.
Carlisle, E.M. and W.F. Alpenfels, 1978. A requirement for silicon for bone growth in culture. Fed. Proc., 37: 787.
Carlisle, E.M. and W.F. Alpenfels, 1980. A silicon requirement for normal growth of cartilage in culture. Fed. Proc., 39: 787.
Carlisle, E.M. and W.F. Alpenfels, 1984. The role of silicon in proline synthesis. Fed. Proc., 43: 680.
Carlisle, E.M. and D.L. Garvey, 1982. The effect of silicon on formation of extracellular matrix components by chondrocytes in culture. Fed. Proc., 41: 461.
Carlisle, E.M. and C. Suchil, 1983. Silicon and ascorbate interaction in cartilage formation in culture. Fed. Proc., 42: 398.
Carlisle, E.M., J.W. Berger and W.F. Alpenfels, 1981. A silicon requirement for prolyl hydroxylase activity. Fed. Proc., 40: 866.
Charnot, Y. and G. Peres, 1971. Contribution a l'etude de la regulation endocrinienne du metabolisme silicique. Anal. Endocrinol., 32: 397–402.
Dawson, E.B., M.J. Frey, T.D. Moore and W.J. McGanity, 1978. Relationship of metal metabolism to vascular disease mortality rates in Texas. Am. J. Clin. Nutr., 31: 1188–1197.
King, E.J. and T.H. Belt, 1938. The physiological and pathological aspects of silica. Physiol. Rev., 18: 329–365.
Loeper, J., J. Loeper and M. Fragny, 1978. The physiological role of silicon and its anti-atheromatous action. In: G. Bendz and I. Lindquist (Eds), Biochemistry of Silicon and Related Problems. Plenum Press, New York, pp. 281–296.
Muller, S.A., H.S. Posner and H.E. Firschein, 1966. Effect of vitamin D-deficiency on the crystal chemistry of bone mineral. Proc. Soc. Exp. Biol. Med., 121: 844–846.
Nikaido, T., J. Austin, L. Trueb and R. Rinehart, 1972. Studies in aging of brain. II. Microchemical analysis of the nervous system in Alzheimer patients. Arch. Neurol., 27: 549–554.
Schwarz, K., 1973. A bound form of silicon in glycosaminoglycans and polyuronides. Proc. Natl. Acad. Sci., 70: 1608–1612.
Schwarz, K., 1978. Significance and functions of silicon in warm-blooded animals. In: G. Bendz and I. Lindquist (Eds), Biochemistry of Silicon and Related Problems. Plenum Press, New York, pp. 207–230.
Schwarz, K. and D.B. Milne, 1972. Growth-promoting effects of silicon in rats. Nature, 239: 333–334.