The Ciba Foundation is an international scientific and educational charity. It was established in 1947 by the Swiss chemical and pharmaceutical company of CIBA Limited—now CIBA-GEIGY Limited. The Foundation operates independently in London under English trust law.

The Ciba Foundation exists to promote international cooperation in biological, medical and chemical research. It organizes about eight international multidisciplinary symposia each year on topics that seem ready for discussion by a small group of research workers. The papers and discussions are published in the Ciba Foundation symposium series. The Foundation also holds many shorter meetings (not published), organized by the Foundation itself or by outside scientific organizations. The staff always welcome suggestions for future meetings.

The Foundation's house at 41 Portland Place, London, W1N 4BN, provides facilities for meetings of all kinds. Its Media Resource Service supplies information to journalists on all scientific and technological topics. The library, open seven days a week to any graduate in science or medicine, also provides information on scientific meetings throughout the world and answers general enquiries on biomedical and chemical subjects. Scientists from any part of the world may stay in the house during working visits to London.

Silicon biochemistry

Ciba Foundation Symposium 121

A Wiley–Interscience Publication

1986
JOHN WILEY & SONS
Chichester · New York · Sydney · Toronto · Singapore

Silicon as an essential trace element in animal nutrition

EDITH MURIEL CARLISLE

School of Public Health, University of California, Los Angeles, California 90024, USA

Abstract. Within the last decade silicon has been recognized as participating in the normal metabolism of higher animals and as being an essential trace element. Silicon is found to perform an important role in connective tissue, especially in bone and cartilage. 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. Silicon's primary effect in bone and cartilage is on the matrix, with formation of the organic matrix appearing to be more severely affected by silicon deficiency than the mineralization process. Additional support for silicon's metabolic role in connective tissue is provided by the finding that silicon is a major ion of osteogenic cells and is present in especially high concentrations in the metabolically active state of the cell; furthermore, silicon reaches relatively high levels in the mitochondria of these cells. Further studies also indicate that silicon participates in the biochemistry of the subcellular enzyme-containing structures. Silicon also forms important interrelationships with other elements. Although it is clear from the body of recent work that silicon performs a specific metabolic function, a structural role has also been proposed for it in connective tissue. A relationship established between silicon and ageing probably relates to glycosaminoglycan changes.

1986 Silicon biochemistry. Wiley, Chichester (Ciba Foundation Symposium 121), p 123–139

Within the last decade, silicon has been recognized as an essential trace element participating in the normal metabolism of higher animals. We have shown that silicon is required in bone, cartilage and connective tissue formation as well as participating in several other important metabolic processes. 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 & Belt 1938), emphasis has been placed until recently on the toxicity of silicon, its effect on forage digestibility, on urolithiasis and especially on silicosis (caused by dust inhalation). This paper is mainly concerned with information that has extended the physiological significance of silicon in nutrition. This results in emphasis being placed on silicon's role in connective tissue metabolism.

Essentiality

A series of experiments has contributed to the establishment of silicon as an essential element. The first experiments were *in vitro* studies in which we 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 demonstrated (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 (Carlisle 1972). During the same year Schwarz & 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.

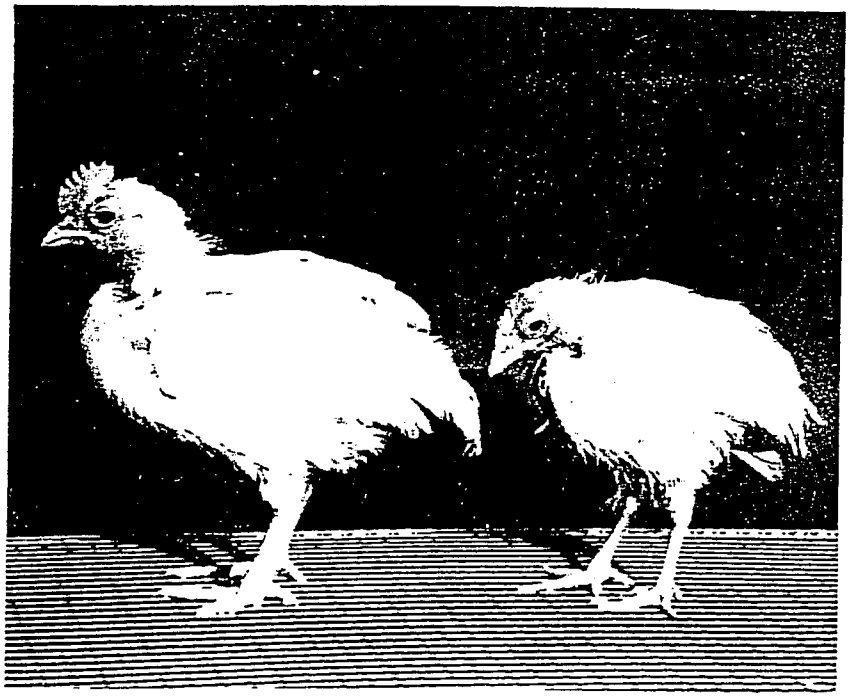

FIG. 1. Four-week-old chicks on silicon-supplemented diet (left) and low-silicon basal diet (right) (Carlisle 1972).

Tissue silicon

Earlier findings on the silicon content of living tissues 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 μg/dl (Carlisle 1986a); 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 been found, although the level increased when silicon compounds were specifically administered.

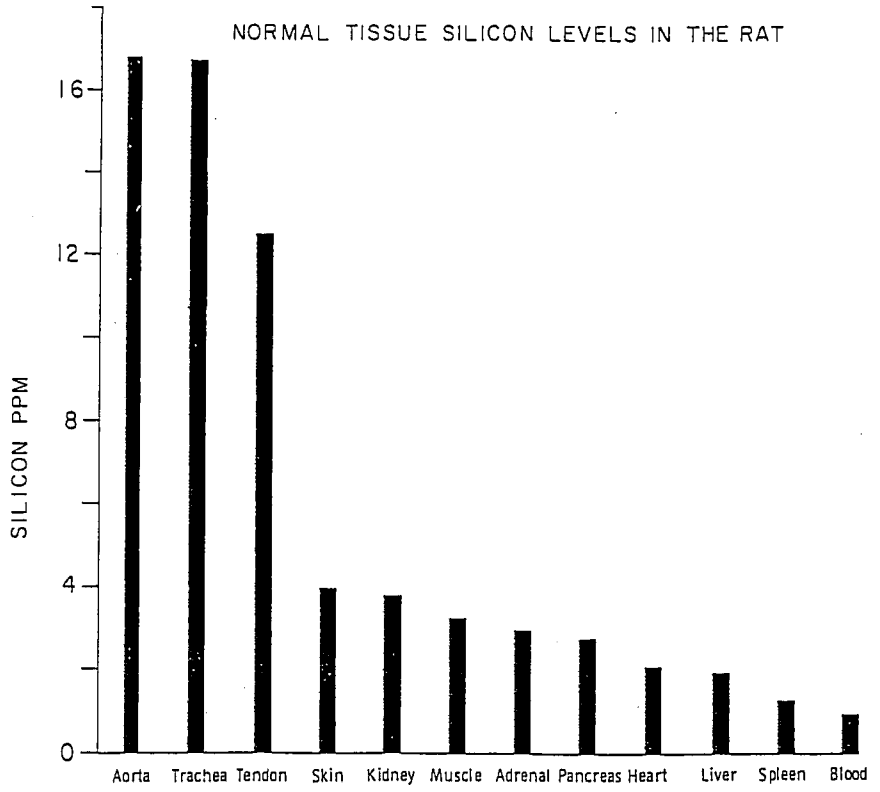

FIG. 2. Normal tissue silicon levels in the adult male rat. Values represent mean silicon levels in 20 animals (4 months of age) expressed as parts per million wet weight of tissue. (From Carlisle 1974.)

Connective tissues such as aorta, trachea, tendon, bone and skin and its appendages are unusually rich in silicon, as shown by studies of several animal species (Carlisle 1974). In the rat, for example (Fig. 2), the aorta, trachea and tendon are four to five 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 consistently low concentrations of silica in most organs do not appear to vary appreciably during life. Parenchymal tissues such as heart and muscle, for example, range from 2 to 10 μg of silicon/g dry weight (Carlisle 1986). The lungs are an exception. Similar levels of silicon have been reported in rat and rhesus monkey tissues, where soft tissue levels in both species varied from 1 to 33 μg of silicon/g dry weight, except in the primate lung and lymph nodes, which averaged 942 p.p.m. and 101 p.p.m. respectively. High levels in human lymph nodes have been associated with the presence of clusters and grains of quartz (Carlisle 1986a).

Silicon deficiency and function

Calcification

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. In electron microprobe studies (Carlisle 1970) silicon was shown to be uniquely localized in active growth areas in young bone of mice and rats (Fig. 3). 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 the silicon and calcium contents of the osteoid tissues were both 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, such 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 below 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.

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

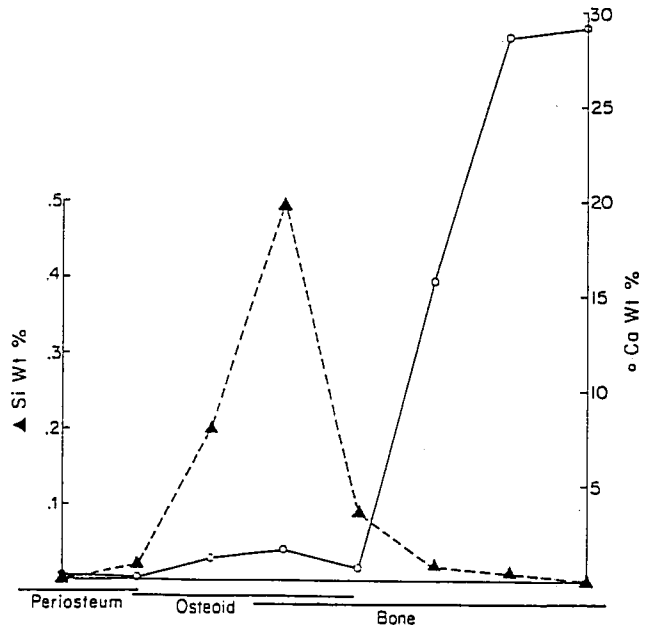

FIG. 3. A spatial relation between silicon (▲) and calcium (O) composition (% by weight) in a typical traverse across the periosteal region of young rat tibia (cross-section) using microprobe techniques. (Reproduced from Carlisle 1970 with permission.)

were maintained on diets containing three levels of calcium (0.08, 0.40, 1.20%) at three levels of silicon (10, 25, 250 p.p.m.). An increase in silicon in the low calcium diet resulted in a highly significant (35%) increase in the percentage of ash in the tibia during the first three weeks of the experiment. Silicon was found to hasten the rate of bone mineralization. The 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 development. In the chick, this is evidenced by the reduced circumference, thinner cortex and reduced flexibility of the 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 & 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, on 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 lacked 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 introducing three different levels of vitamin D, I showed 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; 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 that in the groups receiving adequate vitamin D, being considerably less calcified and more transparent, so enabling the cells and underlying structure to be seen more easily. The deficient chicks appeared to have markedly fewer osteoblasts than 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 its effect on 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

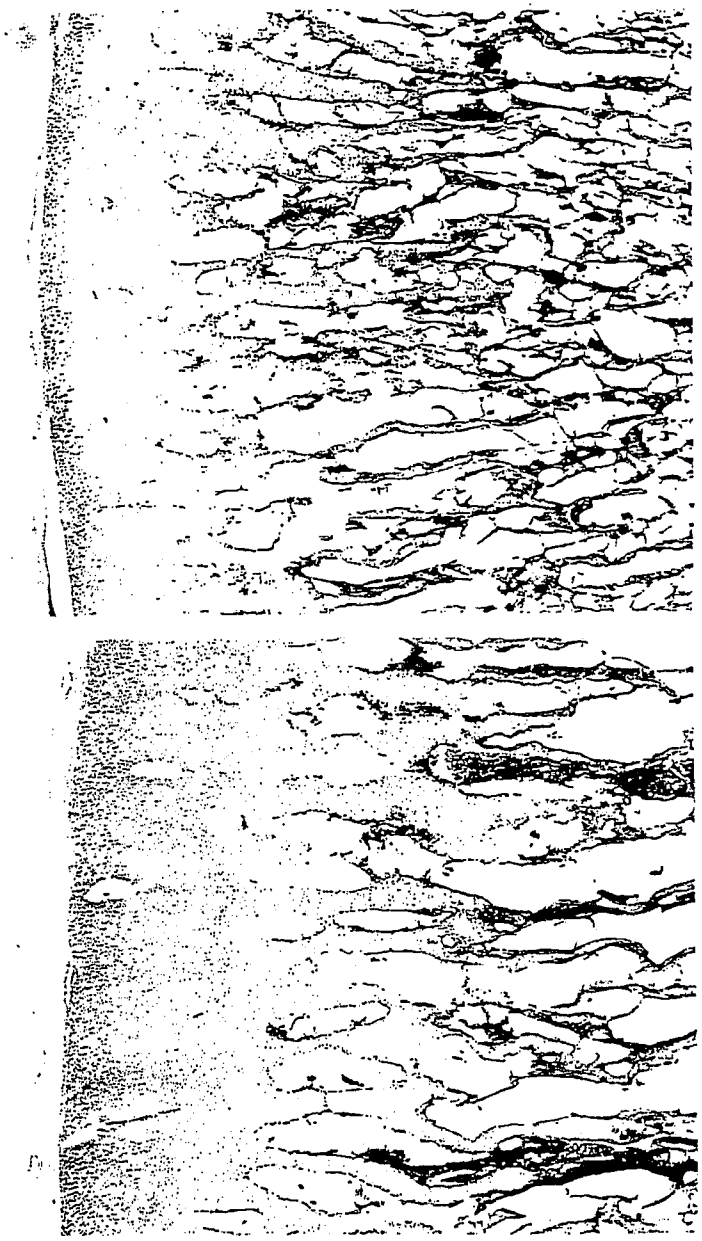

FIG. 4. Longitudinal section through the proximal end of the tibia from 4-week-old chicks fed a silicon-supplemented diet (left) and low-silicon diet (right). In the silicon-deficient chick note the great reduction in width of the epiphyseal cartilage lying below the cartilaginous epiphyses, especially striking in the narrow zone of proliferating cartilage cells. The proliferative zone of silicon-deficient chicks is seven to eight times narrower than that of silicon-supplemented chicks. Also

TABLE 1 Effect of silicon intake on articular cartilage composition[a]

Diet	Tissue (mg wet wt)	Total hexosamine (mg wet wt)	Percentage 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] Twelve chicks per group. All values reported as mean ± SD.
[b] Significantly different from the supplemented animals at $P < 0.001$.

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 given a diet containing a natural protein in place of the crystalline amino acids used in the earlier studies (Carlisle 1980b). Tibia from silicon-deficient chicks had significantly less glycosaminoglycan and collagen, the difference being greater for glycosaminoglycans than collagen. Tibia from silicon-deficient chicks also showed rather marked histological changes, profound changes being demonstrated in epiphyseal cartilage (Fig. 4). 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.

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.

Studies in which embryonic skull bones were grown in culture (Table 2) further demonstrate the dependence of bone growth on the presence of silicon (Carlisle & 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 compared with 10-day and 12-day embryos (Carlisle & Alpenfels 1980). Silicon's effect on collagen formation was also especially striking in cartilage from 14-day

TABLE 2 Effect of silicon on rate of synthesis of bone matrix components

Days in culture	Bone chondroitin sulphate[a]		Bone collagen[b]		Bone non-collagenous protein[c]	
	Silicon		Silicon		Silicon	
	Low	Suppl.	Low	Suppl.	Low	Suppl.
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] Hexose nitrogen × 2.56.
[b] Hydroxyproline = 7.46.
[c] Leucine = NH_2 nitrogen corrected for collagen and hexose nitrogen.
* Significantly different from the supplemented media at $P < 0.05$.

embryos (Fig. 5), 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 to apply also to cartilage.

An interaction between silicon and ascorbate (Carlisle & Suchil 1983) has also been shown in cartilage. Silicon's effect on cartilage formation was investigated 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 effect on hexosamine content was greater than that on proline. Furthermore, silicon and ascorbate interact to give maximal production of hexosamines. Silicon also appears to increase hydroxyproline, total protein and non-collagenous protein independently of the effects of ascorbate.

An effect of silicon on the formation of extracellular cartilage matrix components by connective tissue cells has also been demonstrated (Carlisle & 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; the 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.

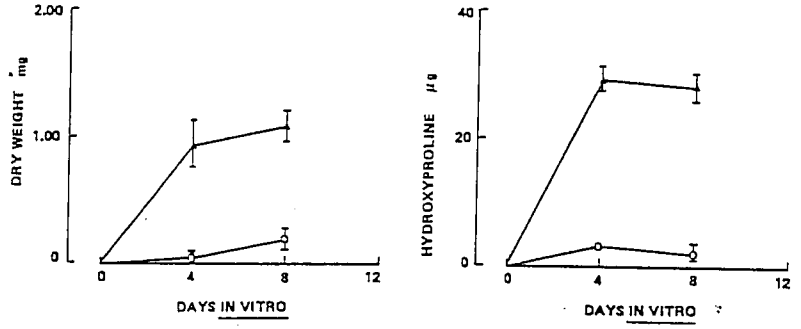

FIG. 5. Rate of growth (left) and collagen synthesis (right), measured as hydroxyproline, in chick tibial epiphyseal cartilage from 14-day-old embryos in culture (Carlisle & Alpenfels 1980). ○, low Si; ▲, Si-supplemented.

We have also shown that maximal prolyl hydroxylase activity depends on silicon (Carlisle et al 1981). Prolyl hydroxylase obtained from the frontal bones of 14-day-old chick embryos incubated for four or eight days under low silicon conditions with 0, 0.2, 0.5, or 2.0 mM-Si added to the media showed lower activity in low-silicon bones, with increasing activity in 0.2, 0.5 and 2.0 mM 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 rates of collagen biosynthesis.

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

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 sulphates and keratan sulphate—are found to be linked covalently to proteins as components of the extracellular amorphous ground substance that surrounds the collagen, elastic fibres and 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 p.p.m. compared to 13 p.p.m. 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, Schwarz 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 our 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 results indicate that silicon is not merely involved in glycosaminoglycan formation but that, in animal glycosaminoglycans at least, and quite probably in plant polysaccharides, it is also 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 p.p.m.) had a plasma silicon level 348% lower than chicks on a casein diet (Mo 1 p.p.m.). Molybdenum supplementation also reduced silicon levels in those tissues examined. Conversely, plasma molybdenum levels are also markedly and inversely affected by the inorganic silicon intake. Silicon also reduced molybdenum retention in tissues. The interaction occurs within normal dietary levels of these elements. Although a copper–molybdenum–sulphate interrelationship has been shown in animal species, this is the first work demonstrating a silicon–molybdenum interaction.

Aluminium is another element with which silicon is shown to form an interrelationship. Since the establishment of silicon as an essential trace element (Carlisle 1972), all tissues analysed for silicon have been analysed simultaneously for aluminium and a number of other elements. From the many analyses of tissues we have done in several animal species (E.M. Carlisle, unpublished work 1985) we have established a relationship between silicon and aluminium which may have relevance for Alzheimer's disease in humans (Carlisle 1986b). Other papers in this symposium deal with the silicon–aluminium interrelationship (Birchall 1986, this volume, and Edwardson 1986, this volume).

Ageing

Because connective tissue changes are prominent in ageing, it is not surprising to find a relationship between silicon and ageing in certain tissues. The silicon content of the aorta, other arterial vessels and skin was found to decline with age, in contrast with other analysed 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 was seen in several animal species.

In human beings, the silicon content of the skin 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; furthermore, 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 et al 1978, Dawson et al 1978). It is of possible significance here that 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 & Peres 1971).

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 ageing process. In humans, it was shown in an earlier study (King & Belt 1938) that silicon levels gradually increase with age in the human peribronchial lymph nodes, even in subjects who have no history of unusual exposure to dust. 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 in silicon has been reported in the cores and rims of the senile plaques. The precise relationship of silicon with the ageing process remains to be determined.

Conclusion

Silicon is one of the most recent trace elements in nutrition to be established as 'essential' for higher animals, and a mechanism and site of action have been identified. Silicon has been demonstrated to perform an important role in connective tissue, especially in bone and cartilage. It is clear from the body of recent work that silicon performs a specific metabolic function. However, a structural role has also been proposed for silicon in connective tissue. A relationship has been established between silicon and ageing which is probably related to glycosaminoglycan changes. The precise relationship of silicon with the ageing process remains to be determined.

Acknowledgement

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

REFERENCES

Birchall JD, Espie AW 1986 Biological implications of the interaction (via silanol groups) of silicon with metal ions. This volume, p 140–153

Carlisle EM 1970 Silicon: a possible factor in bone calcification. Science (Wash DC) 167:179–280

Carlisle EM 1972 Silicon an essential element for the chick. Science (Wash DC) 178:619–621

Carlisle EM 1974 Silicon as an essential element. Fed Proc 33:1758–1766

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

Carlisle EM 1979 A silicon–molybdenum interrelationship *in vivo*. Fed Proc 38:553

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

Carlisle EM 1980b Biochemical and morphological changes associated with long bone abnormalities in silicon deficiency. J Nutr 10:1046–1056

Carlisle EM 1981a Silicon: a requirement in bone formation independent of Vitamin D. Calc Tiss Int 33:27–34

Carlisle EM 1981b Silicon in bone formation. In: Simpson TL, Volcani BE (eds) Silicon and siliceous structures in biological systems. Springer-Verlag, New York, p 69–94

Carlisle EM 1982 The nutritional essentiality of silicon. Nutr Rev 40:193–198

Carlisle EM 1986a Silicon In: Mertz W (ed) Trace elements in human and animal nutrition, 5th edn. Academic Press, Orlando, Florida

Carlisle EM 1986b Effect of dietary silicon and aluminum on silicon and aluminum levels in rat brain. Alzheimer Dis Assoc Dis 1:

Carlisle EM, Alpenfels WF 1978 A requirement for silicon for bone growth in culture. Fed Proc 37:1123

Carlisle EM, Alpenfels WF 1980 A silicon requirement for normal growth of cartilage in culture. Fed Proc 39:787

Carlisle EM, Alpenfels WF 1984 The role of silicon in proline synthesis. Fed Proc 43:680

Carlisle EM, Garvey DL 1982 The effect of silicon on formation of extracellular matrix components by chondrocytes in culture. Fed Proc 41:461
Carlisle EM, Suchil C 1983 Silicon and ascorbate interaction in cartilage formation in culture. Fed Proc 42:398
Carlisle EM, Berger JW, Alpenfels WF 1981 A silicon requirement for prolyl hydroxylase activity. Fed Proc 40:866
Charnot Y, Peres G 1971 Contribution à l'étude de la régulation endocrinienne du métabolisme silicique. Ann Endocrinol 32:397–402
Dawson EB, Frey MJ, Moore TD, McGanity 1978 Relationship of metal metabolism to vascular disease mortality rates in Texas. Am J Clin Nutr 31:1188–1197
Edwardson JA, Klinowski J, Oakley AE, Perry RH, Candy JM 1986 Aluminosilicates and the ageing brain: implications for the pathogenesis of Alzheimer's disease. This volume, p 160–173
King EJ, Belt TH 1938 The physiological and pathological aspects of silica. Physiol Rev 18:329–365
Loeper, J. Loeper J, Fragny M 1978 The physiological role of silicon and its anti-atheromatous action. In: Bendz G, Lindqvist I (eds) Biochemistry of silicon and related problems. Plenum Press, New York p 281–296
Muller SA, Posner HS, Firschein HE 1966 Effect of vitamin D-deficiency on the crystal chemistry of bone mineral. Proc Soc Exp Biol Med 121:844–846
Nikaido T, Austin J, Trueb L, Rinehart R 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 USA 70:1608–1612
Schwarz K 1978 Significance and functions of silicon in warm-blooded animals. In: Bendz G, Lindqvist I (eds) Biochemistry of silicon and related problems. Plenum Press, New York, p 207–230
Schwarz K, Milne DB 1972 Growth-promoting effects of silicon in rats. Nature (Lond) 239:333–334

DISCUSSION

Daniele: Have you looked at collagen metabolism in fibroblasts?

Carlisle: No, we had planned to do this using 3T3 cells. I suspect that it is similar to what we have found in chondroblasts.

Richards: We looked at the effects of mineral dusts including silica on lung fibroblasts (Richards & Hunt 1983). The results are a little different from yours. Growth (as measured by time-lapse cinematography [Absher & Sylwester 1981] or by the DNA content of the culture) is promoted in the same way as in chondroblasts, but when collagen (hydroxyproline) deposition is expressed in terms of DNA, control and quartz-treated cultures are much the same. So there seems to be an initial growth-promoting effect of silica and then a later extra deposition of collagen and increased glycosaminoglycan metabolism. I was therefore surprised that in your chick silicon deficiency studies *in vivo* there were enormous differences between chick tibia in the numbers of chondroblasts, yet when your data are expressed in terms of DNA, one almost expects some of your collagen synthetic rates to go down, rather than up, after silicon supplementation.

Carlisle: Fig. 4 showed that the supplemented chick tibia did have a considerably greater number of chondroblasts in the proliferative zone of the articular cartilage. Analyses showed that the shaft which includes the epiphyseal cartilage at both ends did not contain significantly more collagen but did have a significantly greater amount of glycosaminoglycans. However, in the *in vitro* study when chondrocytes from embryonic chick cartilaginous epiphyses were grown in culture there was considerably more collagen and glycosaminoglycans, both total and expressed per milligram of DNA.

Richards: Which cell is making the collagen?

Carlisle: Chondroblasts in the growth plate and also osteoblasts in other parts of the tibia.

Henrotte: We have also studied the effect of silicon on cellular growth, looking at human peripheral blood lymphocytes in culture, using monomethylsilanetriol salicylate at a concentration of 5 mg silicon per litre. The salicylate was present to stabilize the molecule and prevent its polymerization. The incorporation of [^{3}H]thymidine gave us an estimate of cell proliferation. Our preliminary results show a significant enhancement of cell proliferation by the silicon compound (J.G. Henrotte and S. Claverie-Benureau, unpublished results). When a known mitogen, either concanavalin A (Con A) or pokeweed mitogen, was added to the cell culture, silicon had no additional effect on [^{3}H]thymidine incorporation. We obtained the same kind of results when salicylate was replaced by glucose. These results suggest that silicon and the mitogen compete for the same receptor on the cell surface. Since the receptors for Con A and pokeweed mitogen are glycopeptides, this observation would fit with the known properties of silicon in binding to glycans.

Carlisle: We measured DNA content along with the matrix components in our chondroblast culture studies and found that at higher silicon concentrations there was an inhibitory effect of silicon on DNA synthesis.

Richards: I think it is true that you reach a toxic level, where the cells are killed. At a level of silicon just below that you may stimulate DNA synthesis, or growth; at a lower level still, you may affect collagen or glycosaminoglycan metabolism (Richards & Curtis 1984).

Henrotte: In our experiments on lymphocytes, if we use a silicon concentration 10 times greater (50 mg/l rather than 5 mg/l), there is no stimulatory effect on cell proliferation. In some cases, there is even an inhibitory effect. At these higher concentrations, we may reach a toxic level.

Williams: Are you suggesting that in silicon deficiency, proline is a very susceptible synthesis?

Carlisle: Yes; silicon appears to be involved in the synthesis of proline necessary for the synthesis of deoxycollagen, supplying more substrate for the hydroxylation reaction.

Williams: If silicon is involved in proline synthesis, one would expect it to affect a large number of proteins, because there is a range of proline-rich proteins. Several proteins in saliva, for instance, are very rich in proline. So one

would expect them to be affected by silicon and the ratio of proline to all other amino acids in the total protein should be reduced.

Carlisle: I haven't checked that, but the non-collagenous protein is also affected by lack of silicon.

Williams: It needs a reference point, to establish that it is an effect on proline rather than something to do with collagen, because the total proline content would apparently go down if only collagen synthesis failed at any stage.

Carlisle: When 'deoxycollagen' is formed, proline is needed for that. Also, for hydroxylation, you need α-ketoglutarate, a proline precursor. So one is affecting collagen synthesis at two different places.

Williams: Then you are almost into the Krebs cycle, and that affects all amino acids; so the question is whether you have a baseline for saying that proline itself is affected rather than that the cells have to produce a lot of proline because they have to produce collagen. This might simply be indicative of a general synthetic problem, not just of proline.

Hench: Do you see any effects of silicon on matrix vesicles that would correlate with these extracellular effects?

Carlisle: We have just finished isolating matrix vesicles and are now performing the chemical analyses. Just previous to this we fractionated epiphyseal cartilage from growing chicks into microsomes, lysosomes and mitochondria. We found very high silicon levels in microsomes, and in an enriched lysosomal fraction.

Sullivan: Do you have any model or working hypothesis for the molecular action of silicon in any of these processes? Is silicon acting as a small-molecule effector, or is it covalently bonded or hydrogen-bonded in some way that is influencing a number of processes, as you have shown?

Carlisle: Unfortunately, I cannot supply a satisfactory answer at present. However, I think that in bone formation, for example, silicon is acting as a small-molecule effector.

Hench: Have you looked at the effects of silicon on dentition in the rat model?

Carlisle: Dr K. Schwarz has noticed an effect of silicon on pigmentation in the rat, but other elements, such as fluoride and tin, had the same effects, so it wasn't specific. I haven't noticed any gross effects of silicon on dentition in the silicon-deficient rat, but I have not looked at the microscopic level.

Werner: You discussed the interaction between molybdenum and silicon. These molecules are rather different. Molybdate biochemistry is linked to a number of enzymes—nitrate reductase, xanthine oxidase and nitrogenase, for instance. What exactly is the point of interaction of these two trace elements?

Carlisle: Molybdenum appears to reduce the absorption of silicon from the diet, resulting in reduced plasma silicon levels and a reduction in tissue levels, as mentioned in my paper. Nearly all the colorimetric methods for measuring silicon in tissues are modifications of the silicon–molybdate method, indicating that they must react very readily.

REFERENCES

Absher M, Sylwester D 1981 Effects of silica on human lung fibroblasts: survival data analysis of time-lapse cinematography data. Environ Res 26:438–452

Richard RJ, Curtis CG 1984 Biochemical and cellular mechanisms of dust-induced lung fibrosis. Environ Hlth Perspect 55:393–416

Richards RJ, Hunt J 1983 Reaction of mineral dusts with primary lung fibroblast cultures. Environ Hlth Perspect 51:61–65