Tissue Silicon: A Study of the Ethanol-soluble Fraction, using ^{31}Si

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(Received 4 September 1952)

The view that silicon in animal tissues occurs in combination with organic groups has appeared repeatedly in the literature. As early as 1897 Drechsel & Winogradow described the extraction from goose feathers of an orthosilicate of the formula $\text{Si}(\text{OC}_4\text{H}_9\text{O})_4$, stated to be an ester of a dihydric alcohol homologous with cholesterol. Isaacs (1924) inferred the presence of silicolipins in brain. Holzapfel (1943*a*), investigating the composition of lung tissue in silico-tuberculosis, found organosilicates, 'compounds in which the free valencies of the silica framework, instead of being satisfied with inorganic elements, such as calcium and magnesium, are satisfied with organic radicals'. She further reported both nitrogen-free and nitrogenous organic silicon compounds as derivatives of carbohydrates and proteins.

Holzapfel (1942) reported that silicon compounds could be extracted from silicotic lungs by alcohol and ether, and that Debye-Scherrer photographs of these extracts and of substances similarly extracted from goose feathers showed similarities, but she gave no experimental evidence of this. Holzapfel (1943*b*) found that ether removes only fats and cholesterol from blood, but that subsequent extraction with a mixture of ether and ethanol gives a fraction containing silicon, together with phosphatides, cholesterol esters and soaps, and assumed that compounds similar to the phosphatides exist in which one or more phosphorus atoms are replaced by silicon. Assuming that the whole of the blood silicon is in organic combination, the insolubility of the greater portion was explained by the suggestion that compounds of polysilicic acids possess a very low solubility. Similar extraction of lung tissue showed that it behaved differently from blood (Holzapfel, 1947); the phosphatide fractions were free from silicon. As it was found in the ethereal extract containing fats and esters, Holzapfel suggested that silicon also occurred in combination with glycerol esters.

Olmeyer & Olpp (1944) assumed that esters of silicic acid are present in the tissues and compared the ethanol-ester soluble silicon levels in several tissues. They found no significant variation in the 'organic silicon', although the urine gave a high value for non-extractable (inorganic) silicon.

Although silicon has been reported as occurring in so many different types of organic compounds in

biological material, there is no case recorded in which a compound has been isolated, and this work was undertaken in a further attempt to elucidate the problem. It soon became apparent that the usual chemical methods of analysis for the determination of silicon were inadequate to deal with the very small amounts present in the fractions. For this reason much of this investigation has been carried out with isotopically labelled silicon.

Preliminary work (Holt, Yates & Tomlin, 1951) had shown that radioactive silicon, injected as silicic acid, is absorbed and is distributed over the tissues generally. A more extensive survey has confirmed that there is no large accumulation in any organ except the kidney, although the more vascular tissues give rather higher values. The liver, being the largest vascular organ, was chosen as the source of material for this investigation into the nature of the silicon complexes.

Earlier workers have used ethanol or ether-ethanol mixtures to remove 'organic silicon' compounds from tissue. Ethanol also removes water and it is possible that a part of the silicon removed may be silicic acid which is associated with this water. For this reason tissues were usually dried by azeotropic distillation before extraction. As there is no evidence that ethanol is the most efficient solvent and particularly because it has been shown that ethyl silicate may be formed by some extraction procedures (Holt & Yates, 1950), other solvents have also been used.

Fractionation of the ethanol-soluble extracts by the usual methods of fractional distillation and solvent partition have been attempted with little success; indirect methods have therefore been used. Attempts made to form the complexes *in vitro* have indicated their probable nature, and have explained why the usual methods of fractionation are unsuccessful.

METHODS

Preparation of isotopically marked silicate solution. The silicate solution used for injection was prepared from pure fused silica which was powdered, sealed in a fused silica tube and placed in the Harwell pile (BEPO) for 12 hr. A weighed amount of irradiated material was fused with three times its weight of Na_2CO_3 in a platinum crucible and the melt was dissolved in water to prepare a solution containing 1.0 mg. SiO_2/ml . Injections were made intraperitoneally into adult rats in the quantities indicated.

Procedure for counting. A Geiger counter of the annular type (Veall, 1948) was used. Variations in density among solutions were too small to necessitate self-absorption corrections. The proportion of injected silicate reaching the tissues was determined by comparing counts made on solutions prepared from the tissues with counts on known dilutions of the injected solution.

Decay curves plotted from measurements of the radioactivity of one of the stock solutions, and of a portion of kidney removed from an animal after injection showed only the 170 min. half-life characteristic of ^{31}Si and proved that impurity activities were negligible.

On account of the short half-life of ^{31}Si all operations were performed, of necessity, as rapidly as possible. The activated sample was received within 45 min. of its removal from the pile and injections were made within a further 30 min.

Method of assay of total marked tissue silicon. The tissue was washed to remove unabsorbed silicate which might contaminate the surface, chopped, dried at 100° , ignited in a platinum crucible and fused with Na_2CO_3 . The melt was dissolved in water and the whole or a suitable portion was transferred to the Geiger counter.

Method for the extraction and assay of ethanol-soluble silicon complexes. The tissue was washed, chopped and dried by the Dean & Stark (1920) procedure using benzene to produce an azeotrope with the tissue water. The dried tissue was refluxed with ethanol for several hours. A part of the extract was filtered, its volume measured and, when necessary, reduced by evaporation. This solution was transferred to a Geiger counter for assay as above. Any deviations from these procedures are mentioned.

EXPERIMENTAL AND RESULTS

Extraction of tissues of ^{31}Si -injected mice

Distribution of injected silicate in several tissues of the body. The radioactive solution was injected into the rats in 5 ml. doses (5 mg. SiO_2) and the rats were killed in the several experiments 3 hr. after injection. Heart, lung, liver, spleen, kidney, muscle and bone tissue was removed for analysis.

The ^{31}Si content of the several tissues are given in Table 1. No large differences are revealed between liver, spleen, lung and bone tissue. Muscular tissue (heart and gastrocnemius)

have the lowest ^{31}Si content whilst the ^{31}Si content of the kidney tissue is some ten times greater than any other.

Effect of the tissue/solvent ratio on the quantity of silicon extracted by ethanol. Four rats were each injected with 8 ml. of the labelled silicate solution. They were killed and dissected after 3 hr. The liver tissue was pooled and a small sample taken for the assay of the total ^{31}Si .

One 8 g. sample and one 4 g. sample were dried and extracted with 50 ml. ethanol for 5 hr. Portions (10 ml.) were removed for assay at hourly intervals.

A part of the injected silicon can be extracted in ethanol. The extraction procedure adopted as standard involved the extraction of 4 g. tissue with 50 ml. of ethanol. The amount of silicon extracted is not increased when twice this volume of solvent is used.

Rate of extraction from and distribution of ethanol-soluble ^{31}Si in several tissues. Seven rats were each injected with 8 ml. of the solution of labelled silicate and were killed 3 hr. after. Spleen, heart, liver, kidney and lung tissues were separately collected and similar tissues were pooled. Part of each type was assayed for total labelled silicon, the rest being dried and refluxed with ethanol for 5 hr. At hourly intervals, samples of the extracts (10 ml.) were removed and assayed, the volume of the extracting liquid being kept constant by the addition of ethanol.

The extraction of the ethanol-soluble fraction of ^{31}Si from any of the tissues is almost complete under the conditions used after 2-3 hr. and the amount extracted after 1 hr. is, in most cases, some 70-80% of the total ethanol-soluble ^{31}Si (Fig. 1).

The fraction of the tissue ^{31}Si which is ethanol-soluble is highest for the heart and liver tissue (over 50%), and is very low for the kidney tissue (about 3%). Spleen and lung tissue give values of about 30% (Fig. 1).

Comparison of different organic liquids as solvents for tissue silicon. Several rats, each injected with 8 ml. of the solution of labelled silicate, were killed 3 hr. after injection and dissected. The livers were pooled and a portion was taken for direct assay. One portion of the remainder was dried and extracted with ethanol for 5 hr. Three similar portions were treated in an identical manner, but dioxan, ethyl acetate and ether were used as solvents. Samples of the extract (10 ml.) were removed at hourly intervals for direct assay.

Table 1. *Silicon content of tissues following the intraperitoneal injection of labelled silicate into rats and mice*

(Each rat received 5 mg., each mouse 1 mg. labelled SiO_2 . Silicon found as $\mu\text{g. SiO}_2$.)

Tissue	Silicon in 1 g. undried tissue		Total silicon in organ	
	Rat	Mouse*	Rat	Mouse
Kidney	368 $\pm$ 89 (4)†	138	720 $\pm$ (4)†	685
Liver	39 $\pm$ 12 (4)	55	282 $\pm$ (4)	347
Spleen	34 $\pm$ 6 (4)	48	25 $\pm$ (4)	68
Lung	26 $\pm$ 5 (4)	23	52 $\pm$ (4)	39
Heart	21 $\pm$ 4 (3)	15	24 $\pm$ (3)	22
Muscle (gastrocnemius)	7 $\pm$ 1 (2)		29 $\pm$ (2)	
Bone (femur)				
Total	26		22	
Distal epiphyses	34		11	
Axial epiphyses	27		8	
Shaft	13		3	

* Tissues from eight mice pooled.

† Mean $\pm$ s.e. and, in parenthesis, number of experiments.

Solvents other than ethanol will extract silicon from the tissues but they are less efficient. From liver tissue, in 5 hr. ether or ethyl acetate extract 10–15% of the total ^{31}Si , dioxan about 25% and ethanol up to 50%.

Fig. 1. Rate of extraction from and distribution of ^{31}Si in several tissues.

Fig. 2. Total and ethanol-soluble ^{31}Si in liver at different intervals after injection.

Comparison of the rates of increase in (a) total and (b) ethanol-soluble marked silicon in the liver after injection. Labelled silicate solution (8 ml.) was intraperitoneally injected into several rats. The rats were killed at intervals in batches of three. Pilot experiments indicated that, because of the rapid decay in the activity of the silicon, experiments could not be usefully carried out in which the silicon remained in the animal for more than 5 hr. A part of the pooled liver tissue from each batch was assayed for total marked silicon. The remainder was dried and extracted with ethanol for 3 hr. The ethanol extract was assayed.

The total ^{31}Si in the liver rapidly rises to a maximum 1–2 hr. after the injection, then slowly falls. The ethanol-

soluble ^{31}Si follows a similar course, but reaches a much smaller maximum value and falls only slightly after the second hour (Fig. 2).

Formation in vitro of ethanol-soluble silicon complexes

In these experiments the silicate, formed by fusion of irradiated silica with sodium carbonate as previously, was neutralized to phenol red, then diluted with phosphate buffer, pH 6.5, to a concentration of 0.1 mg. $\text{SiO}_2/\text{ml.}$ of solution.

Addition of silicate to fresh liver. Labelled silicate solution (4 ml.) was added to sliced, freshly dissected rat liver (about 10 g.). After 3 hr. the mixture was treated by the procedure for the extraction of ethanol-soluble complexes. The extraction was continued for 5 hr. A portion of the ethanol extract (10 ml.) was removed and assayed after every hour.

Fig. 3. Ethanol extraction of ^{31}Si added to fresh and to dried liver tissue.

The addition of silicate to excised liver tissue results in the formation of ethanol-soluble substances containing ^{31}Si . Over 50% of the added silicon can be extracted in this way (Fig. 3).

Addition of silicate to dried liver. The last experiment was repeated using liver tissue which was previously dried by azeotropic distillation.

Dried liver tissue is somewhat less effective in producing ethanol-soluble silicon complexes (Fig. 3).

Addition of silicate to ethanol-extracted liver. Rat liver was dried and given a preliminary extraction with ethanol for 6 or 24 hr. Silicate solution (4 ml.) was added to the extracted tissue which, after 3 hr., was treated by the procedure for extracting ethanol-soluble silicon complexes. At hourly intervals during 5 hr. portions of the extract (10 ml.) were removed for assay.

When the tissue is extracted with ethanol prior to the addition of silicate, the amount of silicon which is subsequently removed in ethanol is considerably diminished and the degree of the reduction varies according to the efficiency of the initial ethanol extraction. When the initial ethanol extraction is continued for 6 hr., the proportion of

the added silicon which can be subsequently extracted by ethanol is reduced to 15%; an initial 24 hr. extraction reduces the fraction to 7%.

Addition of silicate to an ethanol extract of liver. Rat liver (20 g.) was subjected to the procedure for the extraction of ethanol-soluble silicon complexes, continuing the extraction for 5 hr. Ethanol was removed from the extract by distillation under reduced pressure. Silicate solution (4 ml.) was added and, after 5 hr., the mixture was dried by azeotropic distillation. Benzene was finally removed by distillation under reduced pressure. The residue was refluxed for 5 hr. with ethanol (50 ml.), samples (10 ml.) being removed for assay at hourly intervals.

The addition of silicate to an ethanol extract of liver tissue results in the formation of ethanol-soluble complexes so that, after extraction for 5 hr., some 60% of the added silicon can be extracted (Fig. 4).

Fig. 4. Ethanol extraction of ^{31}Si added to an ethanolic extract of liver tissue.

Addition of silicate and certain other substances to ethanol-extracted liver tissue. Extraction of tissue with ethanol was shown to remove substances which acted upon added silicate. Among the substances removed by the extraction of tissue with ethanol are cholesterol, reported as forming esters with silicic acid, and phosphatides. Cholesterol and lecithin were, therefore, added to samples of ethanol-extracted tissue in an attempt to restore its property of rendering the silicate soluble in ethanol. Similar additions were made to non-protein material (cotton wool) for comparison.

Labelled silicate solution (4 ml.) was added to the ethanol-extracted rat liver (4 g.) and to cotton wool (1 g.). Cholesterol (0.4 g.), lecithin (0.4 g.), or choline chloride (0.2 g.) was added as a solution or suspension in water (8 ml.), except in the case of the blank experiments. After standing for 3 hr. the tissue was treated by the method for the extraction of ethanol-soluble silicon complexes and the ethanol extract was assayed.

Both cholesterol and lecithin added to ethanol-extracted liver tissue partially restore its ability to form ethanol-

soluble silicon compounds. Cholesterol is most effective (Table 2). Replacement of the tissue by cotton wool in these experiments has little qualitative effect on the extraction except that lecithin is found to be rather more effective than cholesterol. Choline chloride is more active than either, but none of these substances is as active as the residue obtained by evaporating a benzene extract of liver tissue (Table 2).

Table 2. ^{31}Si extracted by ethanol after the addition of ^{31}Si and organic substances to ethanol-extracted liver tissue and to cotton wool

(Time allowed for reaction before drying, 3 hr. Extraction time, 3 hr. 400 μg . SiO_2 added to 4 g. extracted liver or 1 g. cotton wool.)

Substance added	Silicon extracted (μg .)	^{31}Si (% of total ^{31}Si)
(a) Addition to ethanol-extracted liver tissue		
Lecithin (0.4 g.)	67.5	16.9
Cholesterol (0.4 g.)	202.4	50.6
No addition	25	6.3
(b) Addition to cotton wool		
Lecithin (0.4 g.)	134.7	33.7
Cholesterol (0.4 g.)	69.7	17.4
Choline chloride (0.2 g.)	139	34.8
Benzene extract from 20 g. liver	180	45.0
No addition	5.8	1.5

DISCUSSION

After the introduction of labelled silicate into the peritoneal cavity of a rat, part of the silicon which reaches the tissues can be extracted with ethanol or, less effectively, with other organic solvents such as dioxan, ethyl acetate or ether. It is not extracted by benzene. The extraction is almost complete in 3 hr.

The low proportion of ethanol-soluble silicon in the kidney (about 3%) suggests that the greater part of the silicon in the kidney is in a different chemical form from that in other tissues. This silicon is presumably present as inorganic silicate, concentrated by tubular re-absorption of water from the glomerular filtrate.

The solubility in ethanol of a large part of the silicon present in other tissues supports the view that it is in some form of organic combination. The fraction of ethanol-soluble ^{31}Si in the liver reaches an approximately constant value after injection in about 2 hr. (Fig. 2), and in 1 hr. it has reached about half this value, indicating either that the organic ^{31}Si complex is rapidly synthesized or that the silicon normally present in organic combination readily interchanges with ^{31}Si .

Other experiments have shown, however, that combination of silicate with organic compounds can take place outside the body. It occurs when silicate solutions are left in contact with excised tissues, even after the tissue is dried. When tissue is extracted

with ethanol prior to the addition of silicate the quantity of 'organic silicon' produced is considerably reduced, but the ethanolic extract of the tissue itself combines with silicate (Fig. 4). These results show that the organic silicon complex is formed by a simple interaction between inorganic silicate and one or more substances present in tissues; it does not require biological activity.

It is apparent that the 'organic silicon' fraction can be increased by the addition of substances such as lecithin, choline, or cholesterol. Moreover, the tissue is not necessary for the interaction; even higher yields are obtained when cotton wool is used as the carrier. Up to 30% of added silicate is converted into 'organic silicon' by this means (Table 2).

It is interesting that the substance which is extracted from the tissue by benzene during the azeotropic drying procedure, which was repeatedly shown to contain no silicon, will produce an ethanol-soluble silicon complex (Table 2). This indicates that, although benzene will not dissolve organic silicon complexes, the organic part of a potential complex is removed by this solvent.

Orthosilicic acid, $\text{Si}(\text{OH})_4$, has, under normal conditions, only a transitory existence, polymerization commencing as soon as it is formed (Willstätter, Kraut & Lobinger, 1929). The silicic acid sols used in these experiments must be regarded as consisting of polymers of various molecular weights, the distribution of the particle sizes depending on the history of the solution. The structure of the silicic acid polymers is considered to be similar to that of quartz itself (Carman, 1940), and the surface consists of hydroxyl groups (Carman, 1940; Weyl & Hauser, 1951).

The rapid excretion of silicic acid following its administration by any route indicates that polymers which appear in the blood mostly pass through the wall of the kidney glomeruli and are removed. Polymers too large to be removed by this mechanism and possibly a proportion of the smaller polymers also, must be regarded as taking part in the reaction which produces ethanol-soluble complexes. The fact that such complexes can be formed *in vitro* from silicic acid and cholesterol, choline or lecithin indicates their nature.

The rapidity of the formation of the ethanol-soluble complexes, even when choline hydrochloride or cholesterol react in neutral solution, suggests that the mechanism involves no attack on the main silica framework. Combination may occur by hydrogen-bond formation between hydroxyl groups at the surface of the polymers and hydroxyl groups of the organic molecules. Complexes will also be formed by the production of polar linkages between the silicic acid, known to have a high surface potential of 300–400 mV., and ions of an opposite charge or

oriented dipoles. These two types may be represented thus:

Polar linkage will be expected between silicic acid and the highly polar structures of choline and with lecithin and cholesterol. The resulting micelle will have an internal, negatively charged silicate structure surrounded by a positively charged layer of adsorbed organic ions; this layer is again surrounded by a negative, diffuse, 'gegenion' layer. The solubility of these complexes in organic solvents will depend on the proportion of (hydrophilic) hydroxyl groups of the silicic acid polymer which are blocked by the (hydrophobic) organic residues.

The formation of this type of micelle may be expected between silicic acids and all soluble biological substances which have a strongly polar group or a group which can co-ordinate with hydroxyl by hydrogen-bond formation. The multiplicity of the types of organic compounds which have been previously reported as occurring in the tissues can then be readily understood. It seems doubtful whether any reaction of silicic acid with organic molecules occurs in stoichiometric proportions, and there is no evidence that silicon takes part in any biosynthetic process. This conception is in agreement with the observation (King & Stantial, 1933), that the total and soluble silicon values for blood are almost equal showing 'the absence of any insoluble particulate silica and of any organic compound of silicon in the blood, unless such a compound reacts directly with molybdate or is extremely susceptible to hydrolysis'. Such micelles, in the presence of strong acid, do react with molybdate.

Micelles of this type will be unable to produce the tissue damage which is caused by the action of quartz, since the outer surface of the micelle consists of organic groups and effectively isolates the silicic acid—an unusual type of detoxication. Neither a silicon-enriched diet nor large doses of injected silica sol have the deleterious effects on tissue which are produced by silica particles of the order of 1 μ . in diameter.

Silicon occurs in the tissues, then, in three forms. Smaller silicic acids exist as inorganic ions which

readily pass through the walls of the glomeruli and account for the high excretion rate when silica is ingested or injected. Large polymers of silicic acid form adsorption complexes with polar and hydroxylic organic molecules which may be soluble in organic solvents such as ethanol and dioxan. Silicon also occurs in the tissues in a form which is not excreted and is not soluble in organic solvents. The nature of these complexes is under investigation.

SUMMARY

1. The silicon in tissues has been studied by injecting into rats silicate solutions containing ^{31}Si . Muscular tissue retains the least ^{31}Si , kidney tissue the most.

2. After drying the tissues, a part of the ^{31}Si can be extracted in ethanol, dioxan or (less effectively) ethyl acetate and ether. The ethanol-soluble silicon is probably present in micelles having organic groups adsorbed on silicic acid polymers.

3. The greater part of the ^{31}Si in the kidney occurs as inorganic silicate which is not extracted by organic solvents.

4. A part of the ^{31}Si is associated with the tissue, but is not soluble in ethanol.

The authors are indebted to the British Steel Founders' Association for a grant towards the cost of this research, and to Dr D. H. Tomlin for his assistance in assaying the solutions.

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Induced Loss in Cerebral Tissues of Respiratory Response to Electrical Impulses, and its Partial Restoration by Additional Substrates

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(Received 15 November 1952)

Cerebral tissues are the most susceptible of those in the animal body to lowered blood levels of glucose. Cerebral activity rapidly fails with low glucose and if severe hypoglycaemia is maintained for periods between a few minutes and an hour, recovery may not be complete when glucose is again supplied. In the present study we have sought to reproduce in separated cerebral tissues a comparable loss of excitability in absence of glucose, a loss which was not restored on adding glucose alone. These conditions having been found, restoration of excitability by other means has been sought.

Experiments of this type have become possible through the development of metabolic means of measuring response by such tissues to their electrical excitation (McIlwain, 1951a). Applied impulses

induce various metabolic changes in cerebral tissues (McIlwain, 1952). Of these changes, the increase in respiration and lactic acid formation were chosen for the present study for the following reasons. They are large and relatively easy to determine. They give a measure of the main energy-yielding reactions of the tissue. It is understandable that failure of these reactions should lead to irreversible loss of function in the tissue.

EXPERIMENTAL

Cerebral cortex. Cerebral cortex mainly of guinea pigs was prepared and mounted for electrical stimulation as described previously, using vessels A^1 and the tissue-holding electrodes type D (McIlwain, 1951a; McIlwain & Gore, 1951). The vessels were fitted to Warburg type manometers and changes in O_2 or CO_2 were followed at 37° by the usual