CXXXII. THE BIOCHEMISTRY OF SILICIC ACID.
IV. RELATION OF SILICA TO THE GROWTH OF PHYTOPLANKTON[1].

BY EARL JUDSON KING AND VIOLA DAVIDSON.

From the Department of Medical Research, Banting Institute, University of Toronto.

(*Received May 30th, 1933.*)

IT was shown by Miquel [1892] that either fresh- or salt-water diatoms could be grown in the laboratory under artificial conditions if certain nutrient salts were added to the water in which they grew. Through the use of these salts the elements potassium, sodium, calcium, iron, nitrogen, phosphorus and chlorine were introduced in amount sufficient to give the optimum growth. Miquel also mentioned magnesium, sulphur, bromine, iodine and silicon as being favourable to growth and included them all, with the exception of silicon, in his nutrient solution. Houghton Gill [quoted by Van Heurck, 1893] used nutrient solutions of essentially the same composition as those of Miquel but included silicon, as calcium silicate, in the list of salts recommended.

Numerous other workers [*e.g.* Allen and Nelson, 1910; Allen, 1914; Fritz, 1918; Peach and Drummond, 1924] have cultured diatoms in natural and artificial sea-waters, using modifications of Miquel's solutions for nutrient purposes. In almost all these investigations no addition of silicon as a nutrient element was made or its presence was considered as being assured through dissolution of the glass.

The possibility of silica being a limiting factor in the production of diatoms was considered by Richter [1904], who obtained luxuriant growths in a medium as free as possible from silica when the culture was made in glass vessels, but very poor development in paraffined containers. Coupin [1922] also found silica to be essential for the growth of *Nitzschia linearis*, a fresh-water form. When cultivated in Knopf medium in Petri dishes very poor development occurred in the absence of added silica or when silica was present in an insoluble form such as washed sand or gelatinous silica. If, however, the silica were added in the form of a slightly soluble silicate, such as orthoclase, felspar or kaolin, healthy growth of the diatom occurred. Curiously enough, potassium or sodium silicate led to very confusing results. In small amounts no advantage could be observed from the addition of either of these salts, while when large amounts were used a definitely toxic action became apparent.

Coupin does not mention the quantities he used, and it is possible that his small amounts were insufficient to give any more than a small temporary increase in the amount of growth. Large amounts of either potassium or sodium silicate, on the other hand, would make the reaction markedly alkaline. Coupin

[1] This investigation was commenced and carried out in part at the Atlantic Biological Station, St Andrews, New Brunswick, in the summer of 1931.

apparently made no attempt to control the p_H of his culture media, and it seems possible that the toxic effect he mentioned was only due to the high p_H resulting from the addition of these alkaline silicates.

It was with the purpose of obtaining more information on the part played by silica in the production of diatoms that the present experiments were begun.

Experimental.

One of the greatest obstacles to studying the effect of silica in the growth of marine organisms is the fact that the experiments must, almost of necessity, be carried out in glass or quartz vessels of siliceous composition. Recourse was had to coating the inside of glass vessels with a non-siliceous material in the hope of being able more effectively thus to study the effect of silica in cultures of marine diatoms.

Spar varnish was first tried, but while it gave a fine, hard, transparent and firmly adhering coat on the inside of the jar, it appeared to contain some toxic substance, as a culture of mixed forms rapidly died out when transferred to the varnished jar. Through the kindness of Mr J. E. Buchan, of the Toronto branch of the Bakelite Corporation, we received a sample of bakelite air-drying varnish, which was tested with some success. The first attempts to use this material were with pint "Gem" jars, the insides of which were painted with a thin coat of the bakelite varnish which was then baked in the oven at a little over 100° until quite hard. This appeared to give an excellent coating, but after a dozen jars had been made up with a Miquelised sea-water containing various amounts of silica and planted with *Nitzschia closterium*, the coating of bakelite slowly began to flake and peel off, and after a few days the cultures in most of the jars appeared to be dead, apparently through the action of something of a toxic nature contained in the varnish.

With large museum jars of 4-litre capacity, however, our success was better. The bakelite was applied to the thoroughly dried glass by means of a fine brush and was allowed to dry in the air exposed to the hot rays of the sun for a week before using. Applied in this way the bakelite showed no tendency to peel off the glass when the water was added, and the toxic substance appeared to have been either lost through evaporation or to have been trapped in the hard coating, as the cultures grew readily in these jars.

About 10 litres of strained sea-water were treated with the modified Miquel solutions of Allen and Nelson [1910][1]. The clear solution was siphoned from the precipitated calcium iron phosphate after standing for a day in a large cylindrical jar. The silica content of this water, estimated by the colorimetric method of King and Lucas [1928], was 0·92 mg. SiO_2 per litre. The solution was divided into three portions of 3·5 litres each and each portion placed in one of the large bakelite-coated jars. To each were added 10 cc. of a fresh plankton containing several species of *Chaetoceros*, *Nitzschia closterium*, *Thalassiosira balticum*, *Thalassiothrix nitzschoides* and many others.

The jars were labelled A, B and C. Jar A was left unaltered with a silica content of 0·92 mg. per litre and p_H 8·3. To jar B were added 68 mg. of Na_2SiO_3, $9H_2O$ equivalent to 4 mg. of silica per litre. A slight white turbidity was formed as the solid material was stirred into solution, but this later dissolved. Jar C received 176 mg. of the silicate (equivalent to 10 mg. SiO_2 per litre) dissolved in a little water. The resulting p_H was 8·6, and a small white precipitate formed, which dissolved on blowing carbon dioxide into the solution until the p_H had fallen to 8·4.

[1] For each litre of sea-water were added 2 cc. of solution A (20·2 g. KNO_3 in 100 cc. of water) and 1 cc. of solution B (4 g. Na_2HPO_4, $12H_2O$, 4 g. $CaCl_2$, $6H_2O$, 2 cc. of fused $FeCl_3$, 2 cc. concentrated HCl and water to 80 cc.).

The jars were covered with glass and placed on a table in front of a south window, where the mean temperature was about 21°.

During a week of cloudy weather there appeared to be no increase in the diatoms; indeed, many of the forms seemed to have died out. The concentration of silica, estimated on 100 cc. of solution withdrawn from the jars, was as follows: A, 0·65 mg. SiO_2 per litre; B, 9·9 mg., and C, 14·6 mg.

It was noticed, however, that while free forms were mostly absent, there was a growth of bottom forms, chiefly *Navicula*—at first *Navicula distans* and later, a minute form which was undetermined, which, with the advent of much sunshine, became very luxuriant during the next two days. This was especially marked in C, with B also showing a fair amount and A a little as well.

The soluble silica content of the jars fell rapidly with this burst of growth and after several days had fallen nearly to the vanishing point in all the jars. At this point the rapid increase in growth appeared to cease, clumping of the diatoms became noticeable and the cultures appeared to be becoming stagnant. After a further interval of a few days the cultures still appeared stagnant; the silica values had risen somewhat, probably due to re-solution of the skeleton of the diatoms. The jars were now placed in the dark in order that the diatoms might die off, disintegrate, and the mineral constituents, including the silica, possibly go back into solution.

The results of this experiment are summarised in Table I. It will be seen that there was a steady decrease of the silica in solution which ran parallel with

Table I. *Soluble silica in plankton cultures.*

Date 1931	Days	Soluble silica content in mg. per litre		
		Jar A	Jar B	Jar C
July 20	0	0·92	9·3	14·8
,, 24	4	0·74	8·6	12·8
,, 27	7	0·75	8·6	9·8
	(Bottom forms multiplying rapidly, especially in C)			
Aug. 1	12	0·22	3·5	2·0
	(Luxuriant growth in all jars)			
Aug. 3	14	0·15	0·28	0·15
	(Diatoms beginning to form clumps and float to top)			
Aug. 5	16	0·49	0·40	0·36
,, 10	23	0·52	0·80	1·50
,, 18	31	0·60	1·22	1·64
	(Jars placed in the dark)			
Sept. 14	58	0·88	1·44	—
Oct. 20	94	—	2·69	10·01
Nov. 20	125	—	5·1	11·28
Dec. 29	164	1·8	7·7	14·7

the growth of diatoms, and that after the cultures were dead and re-solution of the forms had commenced, there was a steady increase of soluble silica until the original values had almost been reached.

Since this experiment had demonstrated the possibility of increasing very markedly the growth of diatoms in Miquelised sea-water by the addition of silica in amounts 5 and 10 times that found in the sea-water in the Bay of Fundy region, it was thought worth while to investigate the influence of much larger concentrations of silica, namely, up to 200 times that found in sea-water. Since many more jars were required for this experiment, it was decided to use the

ordinary "Gem" pint sealers. It had been found impossible to coat these satisfactorily with any varnish, but since the quantities of silica to be used were very large compared with any amounts which might dissolve from the glass, it was decided to use the sealers uncoated.

An artificial sea-water prepared from the reagents available in the laboratory was found to have more silica than a natural sea-water. Several litres of the latter were accordingly Miquelised for the experiment. A dozen sealers were put up as follows:

1, 2. 250 cc. sea-water + 50 cc. distilled water + a few drops chloroform and no inoculation to determine the extent of dissolution of silica from the glass.
3, 4. 250 cc. sea-water + 50 cc. distilled water + inoculation*.
5. 250 cc. sea-water + 1·25 cc. of silicate solution† (1 mg. SiO_2 per cc.). +48·75 cc. distilled water.
6. ,, ,, + 2·5 cc. ,, +47·5 cc. ,,
7. ,, ,, + 5 cc. ,, +45 cc. ,,
8. ,, ,, +12·5 cc. ,, +37·5 cc. ,,
9. ,, ,, +18·75 cc. ,, +31·25 cc. ,,
10. ,, ,, +25·0 cc. ,, +25 cc. ,,
11. ,, ,, +37·5 cc. ,, +12·5 cc. ,,
12. ,, ,, +50 cc. ,,

* The inoculation was chiefly *Nitzschia closterium* (f.) *minutissima* which was in healthy condition but not in pure culture.

† The silicate solution consisted of 1·182 g. of $Na_2SiO_3, 9H_2O$ dissolved in 250 cc. of water, and contained 1 mg. of SiO_2 per cc.

The p_H of jars 1 to 6 was 8·4; 10 % HCl was added in small amounts to the others to bring them to a similar p_H.

Each jar was analysed for silica at the beginning of the experiment and at stated intervals, with the results illustrated in Table II.

Table II. *Utilisation of silica by phytoplankton.*

mg. of SiO_2 per 100 cc. solution.

Jar No.	1	2	3	4	5	6	7	8	9	10	11	12
Aug 16	0·40	0·44	—	—	0·92	1·29	2·42	5·56	8·43	10·33	16·00	20·75
,, 18	—	—	0·33	0·34	0·70	1·16	1·98	4·78	7·30	9·48	12·0	16·50
,, 24	0·40	0·44	0·20	0·19	0·29	0·92	2·01	4·43	6·60	7·80	9·60	16·00
Sept. 3	0·35	0·40	0·29	—	—	—	—	—	—	—	—	—
,, 8	—	—	0·29	0·14	0·52	0·84	1·64	3·50	5·1	4·9	8·2	16·0
,, 16	0·50	0·58	0·09	0·12	0·15	0·55	0·40	3·05	5·0	5·4	5·5	14·6
,, 25	0·47	0·61	0·04	0·04	0·05	0·03	0·04	1·37	3·3	4·1	4·3	14·2
	All cultures dead											
Oct. 5	0·67	0·82	0·84	1·12	1·60	1·37	1·47	2·78	4·8	4·9	5·9	14·1
Total SiO_2 in solution at conclusion of experiment			0·23	0·32	1·20	1·71	1·57	6·90	12·40	12·25	14·92	35·8
SiO_2 in precipitate (diatom valves, *etc.*)			3·5	4·1	5·9	3·6	7·7	12·6	10·4	17·0	23·7	15·0
Total SiO_2 recovered			3·73	4·42	7·10	5·31	9·27	19·50	22·80	29·25	38·62	50·8
Original SiO_2 present			1·00	1·10	2·30	3·22	6·05	13·90	21·75	25·82	40·00	51·9
Difference			+2·73	+3·32	+4·80	+2·09	+3·22	+5·60	+1·05	+3·43	−1·38	−1·1

The results (Table II A) indicated, as did those of the first experiment, that the addition of silicate to a plankton culture may cause a definite increase in the total amount of growth in a given period of time. It is interesting that this holds only for silicate concentrations up to about 100 mg. of silica per litre. When present in concentrations above this amount silicate seems to have an inhibitory and even definitely toxic effect on the plankton. This appears to confirm the finding of Coupin for high concentrations of sodium and potassium

Table II A. *Growth in cultures of different silica content.*

No. of cultures 1931	3	4	5	6	7	8	9	10	11	12
Aug. 20	[1] 66	60	85	118	55	69	121	114	71	4
,, 22	1216	2848	2960	3780	3000	6760 30 % *C. sociale*	4140 50 % *C. sociale*	812	628	0
,, 27	Active	Active	Active	Active	Active	Active	Max. culture	Active 30 % *C. sociale*	Weak	Dead
Sept. 4	[2] Stagnant	Stagnant	Stagnant	Stagnant	Stagnant	Stagnant	Stagnant	Max. culture	Stagnant	Dead
,, 8	Active	Active	Active	Active	Active	Dormant	Weak	Active	Active	Dead
,, 12	Active (weather very warm)	Active	Active	Active	Active	Dormant	Dormant	Weak	Dormant	Dead
,, 15	Weak (temperature in cultures rose to 30°)	Dormant	Dormant	Dormant	Dormant	Dormant	Dormant	Dormant	Active *C. sociale*	Dead
,, 18	[3] Dormant	Dormant	Active	Dormant	Active	Dormant	Dormant	Dormant	Active *C. sociale*	Dead
,, 22	Weak (weather very warm, temperature rising to 28°)	Weak	Weak	Weak	Weak	Active	Vigorous *C. sociale*	Weak	Weak	Dead
,, 25	[4] Weak	Weak	Active	Weak	Vigorous	Dormant	Dormant	Weak	Dormant	Dead
,, 30	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead	Dead

[1] Average number of diatoms in the counting cell, vol. 0·17 cc.
[2] *Stagnant*, refers to the colourless condition which developed in transit from St Andrews to Toronto.
[3] *Dormant*, no apparent new growth, some frustules alive but pale in colour; probably a period when many were dead and dissolving.
[4] *Weak*, healthy *N. closterium* specimens few.

Summary of changes observed in the cultures.

(1) *N. closterium* which was added as the inoculation in the whole series was predominant at all times except near the close of the experiment when bottom-living *Navicula* sp. became very abundant in the lower concentrations of silica and *C. sociale* had short periods of activity in the higher concentrations.

(2) The growth of *N. closterium* in dense patches after the first 3 or 4 days made even the counts of Aug. 22 indicative of a general condition only.

(3) *C. sociale* appeared in all the cultures. It was the dominant diatom in the unfiltered sea-water used for the cultures. Although a planktonic form accustomed to a temperature of 12° to 14°, it survived in culture a week of hot weather when the temperature reached 30°. None was found in cultures numbered 3 to 8 inclusive after the tenth day but it grew for short periods in the higher concentrations.

(4) After each period of growth empty frustules were found in the solution. *C. sociale*, which has a thin covering of silica, soon disappeared; the heavier coated *N. closterium* and *Navicula* lasted much longer. Many were apparent in the jars on Sept. 30.

silicates, and, since the p_H of all cultures in the present experiment was the same, to rule out the possibility that the toxic action was due to alkalinity, although this condition may have been a complicating factor in his experiments.

In those jars where growth was luxuriant the soluble silica values rapidly fell—in those of low and moderate initial concentration to very low values. At higher concentrations (jars 8, 9 and 10) the total drop was greater, although there was still much silica in solution at the time when maximum growth was reached. The two jars containing the highest concentrations of silicate likewise suffered a decrease of the silica in solution, but in this case the precipitated material appeared to consist mostly of gelatinous silicic acid rather than of diatom valves, as was the case in the other jars.

At the conclusion of the experiment the total amount of silica remaining in each culture was estimated by analysis of both the filtrate and the residue after filtering through a fine filter-paper. In all the jars except those two with the highest concentration of silicate, there was definite evidence of considerable dissolution of silica from the glass.

Dissolution of silica from boiled and unboiled diatoms.

Two litres of a "tow" of phytoplankton taken October 3rd and received October 7th, were centrifuged and the supernatant liquid discarded. The precipitate was shaken with distilled water and poured in equal amounts into two 200 cc. flasks. One flask was heated at 95° for 5 minutes and cooled. Both portions were now saturated with chloroform and transferred to 2 paraffined flasks. The p_H of the unboiled portion was 6·9 and that of the boiled portion 7·0.

Two 15 cc. portions were withdrawn from each flask (after shaking) and filtered clear through Whatman No. 42 papers. Silica was determined in 10 cc. of the filtrate. The results of the analyses (Table III) showed that a more ready dissolution of the silica was taking place in the unboiled than in the boiled sample.

Table III. *Silica in 10 cc. of filtered diatom solution.*

Days	Boiled	Increase	Unboiled	Increase
0	0·050	—	0·046	—
7	0·048	—	0·049	0·003
14	0·055	0·005	0·060	0·014
22	0·060	0·010	0·058	0·012
34	0·043	—	0·093	0·047
55	0·118	0·068	0·267	0·221

Addition of magnesia mixture gave no precipitate with these filtrates, indicating that there was very little phosphate present. The p_H did not change appreciably in either flask.

This experiment appears to indicate that there was possibly something of an enzymic nature in the unboiled portion which caused more ready dissolution of the silica. Since both solutions were originally part of the same, this agent (a silicatase?) must have been destroyed by the boiling. Alternative explanations to that of a specific agent acting directly on the silica or on some organic derivative thereof are that general autolysis of the organic parts of the diatoms left the skeleton more susceptible to dissolution, and that boiling in the one flask may have caused sufficient coagulation of the diatom bodies to render them less susceptible to dissolution. This experiment has been repeated several times. The unboiled portion of the diatom suspension has invariably dissolved more rapidly than that which was boiled.

Summary.

Addition of silicate to a culture solution already containing an abundance of nutrient salts caused increased diatom growth. In high concentration silicic acid appeared to have an unfavourable effect on the growth of diatoms.

Dissolution of silica appeared to be more rapid in an autolysing suspension of diatoms containing chloroform than in one which had been boiled.

The authors wish to record their thanks to Prof. A. G. Huntsman and his staff at the Atlantic Biological Station, St Andrews, New Brunswick, for much valuable assistance and for laboratory accommodation and facilities during part of this investigation.

REFERENCES.

Allen (1914). *J. Marine Biol. Assoc. U.K.* **10**, 417.
—— and Nelson (1910). *J. Marine Biol. Assoc. U.K.* **8**, 421.
Coupin (1922). *Compt. Rend. Acad. Sci.* **175**, 1226.
Fritz (1918). *Contrib. Can. Biol.* 63.
King and Lucas (1928). *J. Amer. Chem. Soc.* **50**, 2395.
Miquel (1892). *Le Diatomiste*, **1**, 73, 93, 121, 149, 165.
Peach and Drummond (1924). *Biochem. J.* **18**, 464.
Richter (1904). *Verhandl. Ges. deutsch. Naturforsch. Ärzte*, **2**, 249.
Van Heurck (1893). *Le Diatomiste*, **2**, 125.