THE SUPPLY OF IRON TO DIATOMS

By H. W. Harvey, Sc.D., M.A.
Hydrographer at the Plymouth Laboratory

(Text-figs. 1-2)

INTRODUCTION

Several factors which influence, and from time to time control, the production of phytoplankton can now be enumerated with some degree of certainty—the concentration of phosphate and of nitrate, the illumination and temperature, the rate at which the organisms are being eaten by zooplankton, and the extent to which vertical currents carry them down beyond the level of sufficient light.Instances are indeed known where one or other of these factors has played a preponderating part in regulating the plant life in the sea.

Based on growth in culture solution, Gran (1931, p. 41) concluded that lack or low concentration of iron probably limited plant growth at times and in areas of the sea where it was not replenished by land drainage. Previous data, and those obtained by Braarud & Klem (1931) off the Norwegian coast at his instigation, showed the iron content of sea water to be very small, ranging from 3 to 21 mg. Fe per m.³ Seiwell (1935), by a method which would detect 1-2 mg. per m.³, found the upper forty metre layer at one position in the Atlantic devoid of iron. Cooper (1935) obtained similar values to those of Braarud & Klem for the water of the English Channel. He also found the ratio by weight of iron to phosphorus in the diatoms, obtaining values of 4:2 during the spring outburst and 4:4 later during an outburst of Rhizosolenia. Brandt & Raben (1920) had found a high ratio for diatoms in the North Sea, and analyses by T. G. Thompson quoted by Wailes (1929) showed an even higher ratio for diatoms in Puget Sound. Cooper saw the implication of this; it indicated that all the iron in the English Channel water was taken up by diatoms several times during the course of the year.* Thompson & Bremner (1935) actually found a reduction in the quantity of the iron in the water of Puget Sound during the outbursts of diatoms which are exceptionally intense in that area.

* During the course of some 6 weeks in the spring of 1934 in the English Channel, 8 mg. phosphate P were used by the growing diatoms from each cubic metre of water. It follows from Cooper's ratio that some 33 mg. Fe are taken up by diatoms per cubic metre of water. Diatoms containing 8 mg. phosphate P and 33 mg. Fe are not at any time found per cubic metre of water, because more are eaten than are left to form the population. Since the total iron content of the water remained between 25 and 10 mg. Fe per m.³ during this period, it is concluded that iron taken up from the water by diatoms is given back when they are eaten and taken up again when other diatoms develop.
From this knowledge it appeared, and indeed it still seems probable, that
the growth of phytoplankton in the sea is at times delayed owing to lack of
available iron. Moreover, the growth rate of diatoms in culture was found to
be so dependent upon the supply of available iron that reproducible results
from experiment to experiment, as distinct from duplicate results in the same
experiment, could not be obtained unless iron could be supplied in equally
available quantities. It is thought that such experiments on the factors
affecting their growth rate will lead to a better understanding of changes
taking place in the sea. With these ends in view it was determined to seek
information regarding the forms of iron in sea water and the availability of such
forms for the growth of phytoplankton.

With regard to the nature of iron occurring in sea water Cooper calculated
that less than $10^{-12}$ mg. per m.$^3$ can exist in solution as ferric ions, owing to the
great insolubility of ferric hydroxide, once equilibrium had been attained. It
was observed early in this investigation that ferric hydroxide slowly gave off
ferrous ions into solution even on the alkaline side of neutrality. This led
Cooper (1937) to investigate further the “saturation value” of iron ions in sea
water. He showed this to be some $10^{-7}$ mg. per m.$^3$ for water at a pH slightly
over 8, and to consist for the most part of ferrous and Fe(OH)$^{++}$ ions. He con-
cluded (1935) that the remainder of the iron found in sea water, when equili-
brium had been attained, was in the form of hydroxide, mostly as colloidal
micelles. Some observations suggest that this view may be subject to a slight,
but rather important, modification. It was found that if a precipitate of ferric
hydroxide was formed by adding an iron salt to sea water rich in phosphate,
the quantity of phosphate in solution was reduced. Further, when sea water
containing 87 mg. phosphate P in solution per cubic metre was shaken with
freshly prepared ferric hydroxide, the phosphate in solution was reduced to
3 mg. P per m.$^3$ in the course of 10 days. Similar observations have been made
by Professor S. A. Waksman (private communication). This suggests that
part of the iron in natural sea water may be in the form of colloidal ferric
phosphate. It was found that various preparations of ferric phosphate sols
were more rapidly soluble in dilute acid than hydroxide sols. From this
it was inferred that iron phosphate would provide a more rapidly available
source of iron for diatoms than hydroxide. Direct experiment has con-
firmed this.

A considerable body of information exists concerning the formation and
properties of ferric hydroxide and similar sols, but this relates to concentrations
many hundred times greater than occurs in natural sea water, in which there is
only some 3–20 mg. Fe per m.$^3$. Direct observations, working down to con-
centration of 300 mg. Fe per m.$^3$, suggest that aggregation of molecules to
micelles and subsequent aggregation of micelles to flocs which sediment, is so
delayed with increasing dilution, that some very small aggregates may conceivably persist for a long time in natural sea water where the concentration of
total iron is far below the limits investigated.
Thus, at the outset, we are provided with a picture of sea water containing iron in the following forms:

(i) Iron in true solution as an equilibrium mixture of ferric, ferrous and \( \text{Fe(OH)}^{++} \) ions amounting in all to some \( 10^{-7} \) mg. per m.\(^3\). If these are present above this equilibrium or saturation value, not only hydrolysis to insoluble ferric hydroxide but intake by diatoms tend to bring their concentration down to saturation value.

(ii) Ferric hydroxide and phosphate as colloidal micelles and larger aggregates.

(iii) Stable organic compounds of iron, of which there is, as yet, no direct evidence.

As the investigation proceeded, information was obtained bearing upon the following questions: (i) the diffusion of iron ions from the surrounding water as a direct source of supply to diatoms; (ii) adsorption of ferric hydroxide and phosphate on the surface of diatoms; (iii) utilization of particles of ferric hydroxide and phosphate by diatoms; (iv) the quantity of iron needed for growth; and (v) the mechanism by which insoluble particles are used.

Information concerning iron in organic combination in sea water, and the part it plays in regulating the growth of phytoplankton, is left for a further communication.

**Calculation of the Maximum Daily Supply of Iron to Diatoms by Diffusion of Iron Ions from the Surrounding Sea Water**

It seemed questionable whether diatoms could obtain their requirement for rapid growth from iron ions, even if their “saturation value” of \( 10^{-7} \) mg. Fe per m.\(^3\) was kept up in the diatoms’ immediate vicinity by solution from ferric hydroxide particles. There is little more ionic iron in a cubic metre of water than found in a diatom of moderate size. Colloidal particles of ferric hydroxide dissolve only very slowly when the saturation value of ionic iron is raised several thousandfold by making a hydroxide sol slightly acid and the concentration of ionic iron is kept low by adding dipyridyl, acetylacetone or citrate which combine with, and remove from the sphere of action, ferrous or ferric ions.

The question can be investigated in the following manner:

(i) Assuming the most favourable conditions for diffusion of iron ions into the growing cell, the concentration gradient of these ions outside the cell can be calculated. This gives the depth and volume of the zone of water around the cell which is undersaturated with respect to ionic iron.

(ii) The renewal of ions from colloidal micelles of ferric hydroxide takes place in this undersaturated water. By taking a maximum rate at which the particles dissolve, we can arrive at a maximum value of the rate iron ions could diffuse into the cell.

(iii) The quantity of iron taken up daily by a growing diatom can be found
and compared with this calculated maximum rate at which it could be supplied from ions in the sea under the most favourable circumstances.

Then, if the quantity taken up daily is greatly in excess of the (possible) maximum daily supply, the calculation provides evidence that the cell obtains iron by some other mechanism than diffusion of ions from the surrounding water.

Proceeding along these lines, it is assumed that iron ions diffuse freely to and through the cell-water interface of the diatom and then at once combine with the cell contents, their concentration on the interior surface of the interface becoming zero.

Consider a spherical living cell of radius \( r \) cm. absorbing a solute from a solution at a rate of \( Q_1 \) g.-mol. per cm.\(^2\) per day. In course of time equilibrium will be attained, with a concentration \( C_x \) of the solute at an (infinite) distance from the cell, becoming zero at the surface.

At a distance \( x \) cm. from the centre of the cell the concentric spherical surface \( A \) in Fig. 1 is cut by the same cone as the spherical surface \( S \) on the cell. Through \( A \) will pass, in unit time, the same quantity of solute as passes through \( S \). Since the area of \( S \) to \( A \) is as \( r^2 \) to \( x^2 \), \( Q_1 \cdot \frac{r^2}{x^2} \) g.-mol. will pass \( S \) per cm.\(^2\) per day.

Fick's law for diffusion of a solute states \( \frac{dC}{dx} = \frac{Q}{Da} \), where \( Q \) = quantity of solute in g.-mol. per day diffusing through a boundary of area \( a \) cm.\(^2\); \( D \) = diffusion coefficient (cm.\(^2\) per day), and \( dC/dx \) = the concentration gradient (g.-mol. per litre per cm.).

For a concentric spherical surface at a distance \( x \) cm. from the centre of the cell, \( \frac{Q}{a} = Q_1 \cdot \frac{r^2}{x^2} \), and hence \( \frac{dC}{dx} = \frac{Q_1 \cdot r^2}{D \cdot x^2} \),

integrating \( C_a = k - \frac{Q_1 \cdot r^2}{Dx} \),

where \( C_a \) is the concentration at that boundary.
When \( x \) is infinity, \( C_\infty = k \).

Hence

\[
C_x = C_\infty - \frac{Q_1 r^2}{Dx}.
\]  

(i)

Consider the case where \( x = r \) at the surface of the cell, immediately within the cell-water interface where \( C \) is postulated as zero

\[
C_r = 0 = C_\infty - \frac{Q_1 r}{D},
\]

\[
Q_1 = \frac{C_\infty D}{r},
\]  

(ii)

If a spherical living cell of radius \( r \) is suspended in water containing a concentration \( C_\infty \) of a permeable ion which combines at once on reaching the inner surface of the membrane, then, after a time a state of equilibrium will be attained. When this has taken place equation (iii) gives the concentration in the water at varying distances from the cell and Fig. 2 shows the relation.

Undersaturation to an average extent of about \( 65\% \) occurs in a zone of water extending \( 4r \) cm. from the surface of the cell, that is \( 5r \) from the centre of the cell. For simplicity in calculation it is assumed that at this boundary the concentration \( (C_{5r}) \) of iron ions is their saturation value \( (1.78 \times 10^{-15} \text{ g.-mol. per litre}) \).
Applying these formulae to a concrete instance of a spherical cell having a surface area \(3.3 \times 10^{-5} \text{ cm}^2\) \((r = 1.62 \times 10^{-3} \text{ cm.})\), the quantity of iron ions entering the surface daily

\[= 3.3 \times 10^{-5} \cdot Q_1 \text{ g.-mol.}\]

\[= 3.3 \times 10^{-5} \cdot \frac{5r \cdot D \cdot C_{3r}}{5r - r} \text{ g.-mol. from (iv)}\]

\[= 2.8 \times 10^{-17} \text{ g.-mol.}\]

or \(1.6 \times 10^{-12} \text{ mg. Fe.}\)

The volume of water in the undersaturated zone extending \(4r\) from the surface of the cell = \(5.25 \times 10^{-7} \text{ c.c.}\). If this contained 20 mg. Fe as colloidal ferric hydroxide per cubic metre, the total quantity in the undersaturated zone amounts to \(10.5 \times 10^{-12} \text{ mg. Fe.}\).

If as much as one-tenth of this dissolved daily in the undersaturated zone, and was able to pass into the cell, in addition to that calculated above, the total daily supply would be

\[2.6 \times 10^{-12} \text{ mg. Fe.}\]

The diatom *Lauderia borealis* is a relatively quick-growing species, and in nature a growth rate of one division in 36 hr. would certainly not be excessive (Harvey, Cooper, Lebour & Russell, 1935). A culture of this species, having an average cell-water interface equal to that of the spherical cell considered above, was grown and found to take up from the culture medium \(3.5 \times 10^{-8} \text{ mg. phosphate P}\) for each new cell produced. Thus, when growing at the rate of one division in 36 hr., the daily supply per cell would normally be in the order of

\[2.2 \times 10^{-8} \text{ mg. phosphate P.}\]

The high ratio of iron to phosphorus found in diatoms indicates that this cell would in nature obtain daily some

\[8 \times 10^{-8} \text{ mg. Fe.}\]

That is over ten thousand times more than the \(2.6 \times 10^{-12} \text{ mg.}\) calculation showed it could possibly obtain from iron ions.

A second example provides a check on the magnitude of this result. The diatom *Biddulphia mobiliensis* has been grown in culture at a rate exceeding one division in 36 hr. under a rather wide range of light and temperature conditions. Its surface area approximated to that of a sphere having a radius of \(0.0035 \text{ cm.}\).

Applying similar calculations to such a sphere, its possible supply from sea water containing 20 mg. Fe per m.\(^3\) would be \(4 \times 10^{-11}\) daily.

In the mixed diatom community occurring during spring in the English Channel a relation was found (i) between the pigment content of the cells and their phosphorus content (Harvey, Cooper, Lebour & Russell, 1935); (ii) between the phosphorus and iron in the diatoms (Cooper, 1935).

The pigment content of *Biddulphia* cells in culture was found, and from it the iron content of a cell was assessed by applying these two relations. This amounted to \(1.12 \times 10^{-7} \text{ mg.}\) or a daily intake of \(7.5 \times 10^{-7} \text{ mg. Fe.}\).
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The big discrepancies found suggest that diatoms obtain iron by some other mechanism than diffusion of ions from the surrounding water.

It is thought that calculations of this nature do not allow any further conclusions to be drawn for the following reason. Iron is present in diatoms and most living organisms in the ferrous state; hence it is returned to the sea water as ferrous iron, which oxidizes to ferric iron; this hydrolyses to the insoluble hydroxide, the molecules of which aggregate to form particles. There is produced, finally, an equilibrium mixture of ferrous, ferric and Fe(OH)\(^{++}\) ions and ferric hydroxide particles. Both processes, oxidation and hydrolysis, take place at very great dilution and so may require a considerable time to reach completion. Hence it is likely that the sea is at times "supersaturated" with ferrous ions. However, such a condition could not persist indefinitely; it could only delay the final formation of hydroxide.

**Adsorption of Ferric Hydroxide, and Phosphate, on the Surface of Diatoms**

Adsorption compounds are very readily formed by ferric salts with a wide variety of other molecules. The iron atom is ready to accept electrons from other molecules or groups such as hydroxyl which can "donate" electrons. Ferric hydroxide molecules even tend to share electrons with other molecules of ferric hydroxide; thus, on ageing, the hydroxide forms a series of polymers containing finally many atoms of iron (Kolthoff, 1937). It is therefore, *a priori*, to be expected that micelles of ferric hydroxide, and phosphate, will be readily adsorbed upon many and diverse substances.

This was found to be so. The properties of a ferric hydroxide sol were found to be altered by the addition, before, or in some cases even after formation, of a minute quantity of albumen, agar, gum arabic, starch or casein. Such sols, containing sometimes no more emulsoid than iron, when added to sea water did not flocculate for a considerable time, whereas a sol without such emulsoid, when added in similar quantity, flocculates rapidly.

That adsorption of hydroxide takes place on diatoms was suggested in the first place by their high ratio of iron to phosphorus, and by the variable and often high ratio found in published analyses of sea weeds and freshwater algae. In order to obtain evidence concerning this possibility three series of observations were made.

The boundary, or interface, which regulates the diffusion of solutes into the cell and acts as a barrier to colloids is the plasma membrane, of whose lipoid nature there is much evidence. Therefore evidence of adsorption of ferric hydroxide on lipoids was sought.

A sol of ferric hydroxide, containing twice as much gum arabic by weight as iron, was prepared. This was very stable and remained in suspension, when added to sea water, for many weeks. It was added to sea water made faintly opalescent with an emulsion of lecithin, prepared by adding an ether solution
of ovolecithin to boiling water. In 2 days the water had cleared and become colourless, the lecithin having adsorbed the ferric hydroxide and sedimented.

The same hydroxide sol was next added to sea water and shaken with a fine emulsion of olive oil. On passing through a filter paper (Whatman No. 42) the filtrate was almost colourless, the hydroxide having been adsorbed on the oil globules which were retained. On filtering the sol in sea water, without addition of oil emulsion, it passed through with little if any adsorption on the filter paper.

These two experiments are interpreted as showing that ferric hydroxide micelles are readily adsorbed on a lipoid surface. Not only were the micelles adsorbed on gum arabcic previous to adsorption on the lipoid, but they and the lipoid carried an electronegative charge. The electrostatic repulsion between micelles and droplets was overcome by the forces bringing about adsorption.

The next step was to obtain some direct evidence of adsorption on the living cell. Unfortunately ferric hydroxide micelles, not already adsorbed on an emulsoid, when added to sea water in such quantity that observations can be made, soon flocculate and sediment. Flocculation certainly takes place by preference on diatoms living suspended in the water, indeed to such an extent that spiny and rather transparent species, such as Chaetoceros, may appear coated with the brown hydroxide. However, this shows no more than that they act as nuclei on which precipitation takes place by preference.

A sol of ferric hydroxide with gum arabic, as used in the previous experiments, was added to sea water, giving a concentration of 1120 mg. Fe per m.³. In one portion of this a bunch of Fucus, previously washed in sea water, was placed for a short time. In another portion a similar bunch of Fucus was kept for 20 hr., when the iron content of the two waters was compared. A reduction of 12 % was found where the Fucus had remained overnight.

A sol of ferric hydroxide with starch in place of gum arabic was prepared, containing ten times more starch by weight than iron. This was boiled, to complete hydrolysis of the ferric salt, and was found to remain in suspension when added to sea water for 2 months without flocculation taking place. A quantity amounting to 280 mg. Fe per m.³ was added to sterilized sea water, enriched with phosphate, nitrate and silicate. A portion of this was inseminated with Chaetoceros pseudocurvisetus. After 6 days part of this, and also of the water which had not been inseminated, was centrifuged, and the following analyses made, using dipyridyl (Cooper, 1935):

<table>
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<tr>
<th>Analysis</th>
<th>N 69</th>
<th>Fe (mg per m.³)</th>
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<tbody>
<tr>
<td>Diatom suspension</td>
<td>ca. 280</td>
<td>152</td>
</tr>
<tr>
<td>Centrifugate from diatom suspension</td>
<td>129</td>
<td></td>
</tr>
<tr>
<td>Water with no diatoms added</td>
<td>280</td>
<td></td>
</tr>
</tbody>
</table>

Hence roughly one-half of the iron hydroxide had been adsorbed on or utilized by the diatoms. Since preliminary oxidation of the suspension with
bromine gave no perceptibly greater quantity of iron than solution in hot
dilute hydrochloric acid, no large proportion had passed into stable organic
combination.

Finally observations were made on diatoms which had been grown in iron
rich media and on diatoms from the sea. Ferric citrate to the extent of
560 mg Fe per m.\(^3\) was added to sterilized sea water enriched with nitrate
and phosphate, and *Ditylum brightwelli* was grown in this medium, in which
ferric hydroxide was in the process of formation. The cells were then stained with
acidified ferrocyanide. Small flocs of ferric hydroxide, stained blue, were seen
adhering to the diatoms, as would be expected. Further, where the contents
had retracted allowing the colour to be seen, the whole cell surface appeared to
have taken on a faint blue tint. The same cells were also treated with a saturated
solution of dipyridyl in \(0.2N\) HCl. Within a minute a red tinge appeared
around the periphery of each cell, and the liquid slowly reddened as it does
when in contact with ferric hydroxide. In a similar experiment, with *Biddul-
phia mobiliensis* grown in an iron-rich medium, the whole contents appeared
red, and the red compound could be seen streaming from the cells into the
surrounding liquid.

Diatom plankton from the sea composed mainly of *Lauderia borealis*,
*Biddulphia sinensis* and *Thalassiosira gravida* was concentrated by centrifuging,
washed with acetone to dissolve out colouring matter, centrifuged again and
suspended in distilled water. A part of this was stained with acid ferrocyanide,
and the other part, to which a trace of alizarine yellow was added so that both
fluids were the same colour, was used as a control. The walls of the cells
stained with ferrocyanide were darker than those in the control, particularly in
the case of *Biddulphia* and *Thalassiosira*. The differences were distinct, but not
very distinct.

These various observations, no one of which is conclusive, taken together,
provide evidence that ferric hydroxide is adsorbed on the surface of diatoms.

**Evidence that Particles of Ferric Hydroxide and Phosphate are Used by Diatoms**

Allen-Miquel culture medium has been used successfully to support the
growth of many species of marine diatoms. It is made by adding ferric
chloride to sea water enriched with phosphate and nitrate, then adding sodium
bicarbonate which brings the hydrogen-ion concentration to ca. \(\text{pH } 8\). A
copious precipitate consisting of ferric phosphate with some hydroxide is
formed, and it is recorded (Allen & Nelson, 1910) that unless some of this
precipitate is retained in the liquid when it is decanted, the fluid will not
support the continued growth of diatoms. This observation has since been con-
formed by several workers, and is demonstrated in the following experiment.

The diatom *Biddulphia mobiliensis* was added in equal quantity to (i) Allen-
Miquel medium freed from precipitate by filtering, (ii) this filtrate with the
addition of residue left on the filter paper, and (iii) this filtrate with added ferric citrate to the extent of 400 mg. Fe/m.3 The three cultures were kept at 17° C. in artificial light for 48 hr., when the number of diatoms in samples from each culture were counted with the following result:

<table>
<thead>
<tr>
<th>Percentage increase in 48 hr.</th>
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<tbody>
<tr>
<td>In filtrate from Allen-Miquel medium</td>
</tr>
<tr>
<td>&quot; &quot; &quot; &quot; + residue</td>
</tr>
<tr>
<td>&quot; &quot; &quot; &quot; + 400 mg. Fe/m.3 as citrate</td>
</tr>
</tbody>
</table>

Two experiments were made in which sterilized sea water enriched with phosphate nitrate and silicate was inseminated with Chaetoceros pseudocurvisetus growing actively in culture. A ferric hydroxide sol was prepared by adding ferric chloride to a slight excess of sodium hydroxide, the colloidal solution having a pH ca. 9. The effect of adding this (final concentration 280 mg. Fe per m.3) was to discolour the suspended diatoms with ferric hydroxide and bring about in 10 days a considerable growth, as compared with the control to which no iron had been added. At the same time a similar sol had been prepared containing ten times more starch than iron. This was boiled, a treatment calculated to complete hydrolysis of the iron salt as it both increases the rate of reaction and temporarily raises the hydroxyl ion concentration to a high level. The addition of the same quantity of iron in this form to the diatom suspension did not give any perceptible deposition of ferric hydroxide on the diatoms, but it did bring about a considerable growth. This was judged to be as great as that due to the addition of the same quantity of iron in the form of citrate, and slightly less than that due to the same quantity of iron in the form of the sol without starch, which settled out on the diatoms.

For the second experiment a similar alkaline sol was prepared, but it was boiled before use; also a similar alkaline sol, containing starch, which was twice brought to boiling-point before use. Each was added to the diatom suspension to the extent of 560 mg. Fe per m.3 A rich growth compared with that in the controls was obtained in both cases; the growth in both was slightly greater than that where the same quantity of iron had been added in the form of citrate.

These two experiments indicate that ferric hydroxide particles of colloidal or of larger size were utilized by the diatom.

The diatom Nitzschia closterium var. minutissima is particularly suitable for experiments on iron intake, since its growth may be brought almost to a standstill through lack of iron without apparent injury. The cells are then pale in colour but grow rapidly on adding iron. A series of experiments has been made by transferring such cells in equal quantity to sterilized sea water, enriched with phosphate, nitrate and silicate, with and without added iron in various forms:

Exp. 62. A culture of Nitzschia, in which growth had ceased and the cells were yellow green in colour, was used for insemination. Additions of 280 mg. Fe per m.3
were made with ferric citrate; hydroxide sol freshly prepared; hydroxide-gum arabic sol (Fe: gum :: 1 : 2), pH ca. 9; the same after boiling; hydroxide-starch sol (Fe: starch :: 1 : 10) boiled.

After 7 days the greatest growth was in the flasks enriched with citrate and freshly prepared hydroxide sol, the least in flasks enriched with boiled alkaline hydroxide-gum arabic sol. In all the flasks with added iron there was more growth than in the control.

After 20 days the increased growth due to the various forms of added iron was indistinguishable.

Exp. 64. The same subculture of Nitzschia was used and additions of 280 mg. Fe per m.³ made with ferric citrate, with a hydroxide-starch sol (Fe: starch :: 1 : 10) at pH 8.5, and with the same after it had been boiled, also at pH 8.5.

During the first week, the most rapid growth occurred with citrate and the least rapid increase in growth with boiled sol.

After 18 days there was little or no difference between the growth in the flasks with added iron.

Exp. 67. The yellow-green Nitzschia had meanwhile been transferred to sterilized sea water and was actively growing when used for insemination. Additions of 280 mg. Fe per m.³ were made with citrate, hydroxide sol, the same boiled, hydroxide-agar sol. All the sols were at pH ca. 9.

No difference in growth rate could be distinguished, and after 13 days a considerable and similar production of diatoms had taken place in all the flasks to which iron had been added.

A further addition of iron citrate at this stage brought about no increase in diatoms, but a further addition of nitrate and phosphate caused a 50% increase in the crop after a further 10 days.

These experiments again indicate that colloidal and larger particles of ferric hydroxide, if in considerable quantity, can be utilized by diatoms. The amount added—280 mg. Fe per m.³—is over ten times more than ordinarily occurs in the sea, but the diatom population in these cultures is itself many times more than ordinarily occurs in the sea.

The effect of adding sols in very small quantity was then investigated. In the first series of experiments sols were made in alkaline solution at a concentration of 0.001 M with respect to iron. These were boiled, a process which probably both completes hydrolysis and gives rise to rather large colloidal aggregates. They were then diluted to 0.000005 M and added to the Nitzschia suspensions.

Concentration of iron in the culture due to addition:

<table>
<thead>
<tr>
<th>Amount (mg. per m.³)</th>
<th>Type of Sol</th>
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<tbody>
<tr>
<td>2.8</td>
<td>ferric hydroxide sol</td>
</tr>
<tr>
<td>5.6</td>
<td>ferric hydroxide-starch sol</td>
</tr>
<tr>
<td>1.3</td>
<td>ferric phosphate sol</td>
</tr>
</tbody>
</table>

In every case a perceptibly greater growth occurred than in the control with no added iron; and always the increase in diatoms was considerably less than that due to the addition of the same quantity of iron in the form of citrate. This considerable difference was thought to be due to the size of the micelles in these sols, made in a relatively concentrated form and then
boiled, compared with the size of micelles in process of formation at very
great dilution from citrate.* This view gains confirmation from the following
experiment.

Exp. 106. Additions of iron to flasks of *Nitzschia* suspension, in each case amounting
to 5.6 mg. Fe per m.³, were made with the following freshly prepared sols:
Ferric hydroxide sol (C) made by adding *M/250* ferric nitrate to an equal volume of
4N/250 sodium hydroxide and then diluting to *M/200,000* with respect to iron.
Ferric hydroxide sol (D) made by adding *M/100,000* ferric nitrate to an equal volume of
4N/100,000 sodium hydroxide.
Ferric phosphate sol (C) made by adding *M/250* ferric nitrate to an equal volume of
*M/250 Na₂HPO₄* and diluting to *M/200,000*.
Ferric phosphate sol (D) made by adding *M/100,000* ferric nitrate to an equal volume of
*M/100,000 Na₂HPO₄*.

At the same time, the same quantity of iron was added to flasks of the *Nitzschia*
suspension in the form of citrate, ferrous dipyridyl, and iron-ascorbic acid complex.
The two latter compounds hydrolyse in sea water less rapidly than the citrate.

Growth was most rapid in the flasks with iron added as phosphate sols, ferrous
dipyridyl and the ascorbic complex, less rapid in the flasks with ferric hydroxide sol
(D) and citrate, least rapid with ferric hydroxide sol (C). Very little growth took place
in the control.

After 26 days, a similar increase had taken place in all flasks with added iron
(ca. 3.68 x 10¹² cells per m.³) with the exception of the hydroxide sol (C), where there
was, by inspection, rather less and where many of the cells were misshapen and matted
together.

### THE QUANTITY OF IRON NEEDED FOR GROWTH BY DIATOMS

Four experiments have been made to determine the increased number of
*Nitzschia* cells which develop in culture due to the addition of small quantities
of iron.

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<thead>
<tr>
<th>Addition</th>
<th>Increase in number of cells compared with control, due to addition of 1 mg. Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>N 82</td>
<td>2.8 mg. per m.³ as citrate</td>
</tr>
<tr>
<td>5.6</td>
<td>4.3 x 10¹²</td>
</tr>
<tr>
<td>1933</td>
<td>2.0</td>
</tr>
<tr>
<td>5.0</td>
<td>3.0 x 10¹²</td>
</tr>
<tr>
<td>N 109</td>
<td>2.8</td>
</tr>
<tr>
<td>N 106</td>
<td>5.6</td>
</tr>
<tr>
<td>phosphate</td>
<td>3.2 x 10¹²</td>
</tr>
<tr>
<td>Mean value</td>
<td>3.2 x 10¹²</td>
</tr>
</tbody>
</table>

Since it is improbable that all the iron added was actually used and passed
into the cells of the diatoms, these experiments indicate that 1 mg. of iron is
contained in more than 3 x 10¹² cells.

Similar experiments were also made to determine the increase in numbers

* Iron citrate hydrolyses in sea water. The citrate stabilizes ferric hydroxide particles of
colloidal size, delaying their flocculation and precipitation. Thus a mixture of ferric chloride
and citrate does not give a precipitate on adding to sea water for some hours, whereas ferric
chloride alone does so.
due to the addition of phosphate to cultures rich in nitrate but poor in phosphate, with the following results:

<table>
<thead>
<tr>
<th>Exp. N 83</th>
<th>Increase in number of cells, compared with control, due to addition of 1 mg. P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14 \times 10^9</td>
</tr>
<tr>
<td></td>
<td>10.5 \times 10^9</td>
</tr>
</tbody>
</table>

| Exp. N 95 | 28 \times 10^9 |
|           | 21 \times 10^9 |

| Mean value | 18.4 \times 10^9 |

Hence, on the average, 1 mg. Fe and 175 mg. P caused similar increases in diatom population.

This leads to the inference that less iron than 1/7th of the phosphorus content was actually needed within the cell, although several times more iron than phosphorus is usually found in and on diatoms in nature.

The diatom *Nitzschia* is not only a pennate species but behaves differently in culture from centric species. Since it would be dangerous to generalize from experiments on *Nitzschia* alone, the following experiment was made.

Sea water enriched with phosphate nitrate and soil extract was inseminated with *Lauderia borealis* and kept until the growth of diatoms had utilized 360 mg. phosphate P per m.³. The total quantity of iron in the culture, fluid + diatoms, was then determined. This amounted to 25 mg. Fe per m.³. Hence the ratio of iron to phosphorus used by the diatoms was at most 25/360, probably much less, since much of the iron was, doubtless, not within the diatom cells.

**SUGGESTED MECHANISM BY WHICH DIATOMS UTILIZE FERRIC HYDROXIDE AND PHOSPHATE ADSORBED ON THEIR SURFACE**

In the pennate diatoms protoplasm streams out through holes from the interior of the cell, moves over the skeleton and passes into the cell again through other holes; a fresh surface of protoplasm is more or less continuously being exposed to the water. The centric diatoms have many small pores in the skeleton exposing the protoplasm, and there is evidence (Schütt, 1899) that it extrudes to form a film on the exterior of the skeleton. The cell sap is acid in reaction; Dr. F. Gross (unpublished data) found a pH of about 4.5 by crushing diatoms in an unbuffered fluid with indicators. The cell contents, in common with other living cells, probably have a low oxidation-reduction potential.

The free diffusion of colloids into the cell is barred by the “plasma membrane” situated at or close to the exterior surface of the protoplasm.* It is the

* This statement may conceivably require qualification. East & White (1933) obtained some evidence that colloid particles of small size may pass a plasma membrane. Marklund (1936) observed that large molecules penetrate into the cell of the diatom *Melosira* more readily than into most vegetable cells.
effective diatom-water interface with regard to the free diffusion of solutes and ions into and out of the cell. Since growing cells can build up a concentration of some particular ion many times greater than in the surrounding water, work is done in concentrating the ion. From impedance measurements Cole (1928) suggests that the thickness of the membrane may be even less than monomolecular, and that the transfer of ions into the cell may be largely due to electrostatic forces at this surface.

From these considerations it seems clear that ferric hydroxide particles, adsorbed on the surface of a growing diatom, are just where considerable changes in energy are normally taking place.

It is almost unanimously conceded that lipoids occur at this surface, where they would be so orientated that their carboxyl groups are exposed to the water. At such an interface the hydrogen-ion concentration can be different from that in the main body of water.

Danielli (1937) has calculated that this difference may amount to as much as two units of $\text{pH}$.

Hence there is reason to suppose that adsorbed particles of ferric hydroxide are upon a seat where their solution and passage into the diatom is possible, indeed imminent.

**Summary**

The nature of iron occurring in sea water, and its utilization by diatoms, is discussed.

Diatoms in the sea obtain many thousand times more iron than calculation shows they can obtain by diffusion of iron ions from the surrounding water. Evidence is presented that ferric hydroxide is readily adsorbed on the surface of diatoms.

It is shown that colloidal and larger particles of ferric hydroxide or phosphate can be utilized by, and support the growth of diatoms.

Experiments show that the diatoms *Nitzschia closterium* and *Lauderia borealis* require, for continued growth, a very small quantity of iron compared with that found on, and in, diatoms taken from the open sea.

It is contended that iron hydroxide adsorbed on diatoms is in contact with an interface where its solution, and subsequent passage into the cell, is probable.

The co-operation of Dr L. H. N. Cooper, who investigated the equilibrium between iron ions and oxidation-reduction potential in sea water, has been invaluable. I am also indebted to Dr Fabius Gross for cultures of diatoms and much information, to Mr G. M. Spooner for statistical analyses of counts of diatoms made in connexion with this enquiry and to Dr W. R. G. Atkins for reading the manuscript.
THE SUPPLY OF IRON TO DIATOMS

REFERENCES


