THE NITROGEN CYCLE IN THE SEA

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(Text-figs. 1 and 2)

Brandt (1899) first suggested that phytoplankton organisms must, by removing from the illuminated surface layers of the sea the nutrients required for their further growth, place a limit on their continued multiplication, and he and Raben for long worked on the problem (see Brandt, 1927, for review of earlier work). The thesis has been proved true beyond all doubt by work which has followed improvements in analytical technique by Atkins and Harvey. In many waters reserves of nitrate are formed during the winter months and are used up, often completely, with the coming of longer days. Interest is now centred on the mechanism by which the nitrate reserve is built up and depleted and on the role played by other compounds of nitrogen. The time has come to review the nitrogen problem as a whole and to assess the importance of many possible reactions in which nitrogen may be involved. The plan of this paper is based on Fig. 1, in which associated reactions are

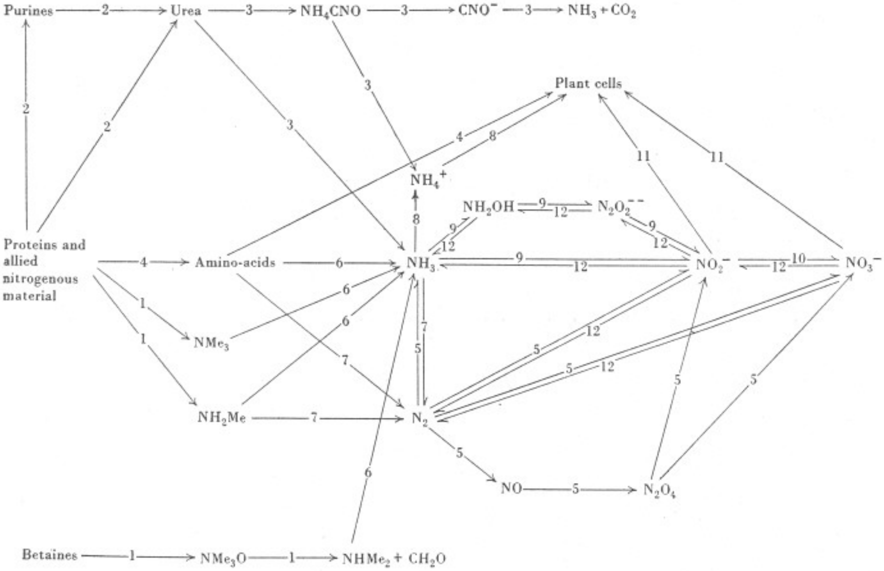


Fig. 1. The numbers indicate the reaction group in which the reaction is considered in the text.

numbered in reaction groups. Reactions bearing the same number and the products to which they lead are discussed together.

A complete historical survey would not best serve the purpose of the paper, which is to state the position as seen to-day. Reference to work in the last ten years does not necessarily imply that this was the first in the field in question, but rather that it states the present position and by its aid earlier work may be tracked down. Some reactions are examined from the thermodynamic standpoint, but it must be remembered that thermodynamics is concerned only with initial and final or equilibrium states and, without other help, can give no information as to the rate at which such equilibrium is attained.

Nitrogen in Particulate Matter (including Plankton)

In Atlantic water below 40 m. von Brand (1937) found about 1 mg.-atom nitrogen per cu. m. in particulate matter, including plankton, brought down by a co-precipitation method. In the upper layers where plankton was present in quantity there was two to three times as much. In the Sargasso Sea he found only 0·1 mg.-atom or less.

In 1934 at Station L 4, 8 miles S. 37° W. from this Laboratory, many plankton hauls were taken by Dr Harvey with the quantitative net which has 200 meshes to the linear inch. Analyses were made on some of these by the micro-Kjeldahl method (Cooper, 1934). Between 0·3 and 0·7 mg.-atom N per cu. m. was found (Cooper, 1937*b*, Table I). Since nannoplankton and detritus escaped the net, the concordance with von Brand's results in similar waters is good.

Dissolved Organic Nitrogen

Robinson & Wirth (1934 *a*, *b*) have determined albuminoid and organic nitrogen in the waters of the Pacific and of Puget Sound and have summarized earlier investigations. At four deep Pacific stations they found an average of 3 mg.-atom albuminoid-N and 6 mg.-atom organic-N per cu. m. (cf. Brandt, 1927).

Reaction Group 1. Formation of Methylamines

In animals considerable quantities of betaines, such as choline, exist and on hydrolysis give rise to trimethylamine oxide. This oxide occurs as such in the muscles of all marine fish yet examined and in many invertebrates, apparently as an end-product of their metabolism; trimethylamine also exists in these fish and in *Fucus vesiculosus* and *F. serratus* (Kapeller-Adler & Krael, 1930 *a*, *b*). The distribution of these compounds in animals has been reviewed by Kutscher & Ackermann (1933, 1936). After death the amine and its oxide are likely to escape into the sea where the oxide may split into dimethylamine and formaldehyde. Mono-, di- and trimethylamine have all been found amongst the excretory products of animals, are generally recognized as breakdown products of proteins, and must be produced in sea water in considerable quantities. When ammonia is determined in sea water by distillation, these methylamines will be included. They will have less effect on Nesslerization methods such as those of Wattenberg and of Buch and Witting (cf. Cooper, 1933*a*, p. 720, Wattenberg, 1937).

Reaction Group 2. Formation of Urea

Purines are commonly formed in the degradation of proteins, and in Lake Mendota in Wisconsin, Peterson, Fred & Domogalla (1925) found 0·7 mg.-atom purine-N per cu. m. By further degradation urea is formed. Large quantities must be liberated into sea water and play there an important although probably transient role. In Lake Mendota, Peterson, Fred & Domogalla (1925) found 0·9 mg.-atom amide-N per cu. m. which may well have been largely urea. Their method of concentration was, however, likely to lead to loss of urea.

Reaction Group 3. Decomposition of Urea

In aqueous solution urea is in equilibrium with ammonium cyanate but in the concentrations usually studied the amount of cyanate is small compared with that of urea. Calculation shows that the conditions in sea water are quite different. The increase in free energy, ΔF°_{298}, in the reaction

$$CO(NH_2)_2 \rightleftharpoons NH_4^+ + CNO^-$$

is +6160 cal. (Lewis & Randall, 1923, p. 587), and from this the equilibrium constant may be computed:

$$\Delta F^\circ_{298} = -RT \ln \frac{a_{NH_4^+}\, a_{CNO^-}}{a_{CO(NH_2)_2}} = 6160.$$

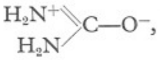
$$\therefore \frac{a_{NH_4^+}\, a_{CNO^-}}{a_{CO(NH_2)_2}} = 3{\cdot}07 \times 10^{-5} \text{ at } 25^\circ \text{ C.}$$

$\therefore$ in sea water containing 28 mg. NH_4^+-N per cu. m. ($=2 \times 10^{-6}\, M$), we have

$$a_{CNO^-} = 15{\cdot}35\, a_{CO(NH_2)_2}.$$

At equilibrium, therefore, the activity of the cyanate ion is fifteen times that of the unchanged urea, and with lesser amounts of ammonium ion the equilibrium will still further favour the cyanate ion.

A consideration of the literature on the rate of decomposition of urea (Armstrong & Horton, 1912; Burrows & Fawsitt, 1914; E. A. Werner, 1918; Price, 1919) shows divergence of opinion due to lack of understanding of the structure of urea. To-day urea in solution in water is considered to have the internal salt structure,



where the two amino groups are in resonance and are identical (see Taylor & Baker, 1937, pp. 280–6). The older work needs to be reconsidered with this structure in mind. In the meantime, that the decomposition of urea is unimolecular seems a fair reading. The percentage rate of decomposition at the very great dilutions existing in sea water will be much the same as at higher concentrations and will be assisted by the displacement of the equilibrium discussed above.

The cyanate ion is also hydrolysable by water to ammonia* and carbon dioxide, although little can be surmised as to the state of equilibrium and the reaction kinetics. Even so it is likely that a purely chemical mechanism will suffice to account for the hydrolysis of urea in the sea.

Bacterial hydrolysis with the help of urease is also probable but is considered as augmenting rather than as replacing the chemical hydrolysis above described. Werner (1918) suggests that the mechanism of enzymic hydrolysis by urease is most probably different from the chemical hydrolysis, and his comment that urease is markedly amphoteric fits with the present views on the structure of urea to give the basis of a "lock and key" mechanism. ZoBell & Feltham (1935) claim to have isolated from the sea three different types of urea-splitting bacteria, two of which liberate ammonia.

Reaction Group 4. Formation and Utilization of Amino-acids

Amino-acids have never been positively identified in sea water but their formation by the degradation of proteins seems more than likely. By concentrating fresh water from Lake Mendota more than 1000-fold Peterson, Fred & Domogalla (1925) determined arginine (3·1 mg.-atom N per cu. m.), tryptophane (0·85 mg.-atom), tyrosine (0·85 mg.-atom), histidine (0·6 mg.-atom) and cystine (0·3 mg.-atom). Similar concentrations are likely to arise in sea water during periods of degradation and to be available to any organisms that can use them.

Since amino-acids are the bricks from which proteins are built, diatoms and other unicellular plants may well use them, when available, as sources of nitrogen. Schreiber (1927) found that there was little to choose between glycine, ammonia, nitrite and nitrate as sources of nitrogen for bacteria-free cultures of *Carteria*. Braarud & Føyn (1930) found that similar cultures of *Chlamydomonas* could use glycine, alanine and asparagine but less efficiently than inorganic nitrogen. Amino-acids must be reckoned as important sources of nitrogen in the sea, but they differ in two respects from available inorganic nitrogen compounds.

Ammonia exists in sea water as the kation NH_4^+ (see p. 189) and nitrite and nitrate as anions, and this ionic character is, no doubt, of importance for their assimilation. By contrast the amino-acids are "zwitterions" or internal

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* Throughout the paper, "ammonia" is used as an inclusive term for NH_3, NH_4OH, NH_4^+ and ammonium salts in solution.

salts. Glycine is formulated as $H_3^+N.CH_2.COO^-$, and for the simple amino-acids the amount of zwitterion is 250,000–1,000,000 times that of the uncharged amino-acid (Edsall & Blanchard, 1933). Using the revised values for the dissociation constants of glycine (N. Bjerrum, cited by Taylor & Baker, 1937, p. 108), the ratios of concentrations of the positive ion, $H_3^+N.CH_2.COOH$, m_{A^+}, and of the negative ion, $H_2N.CH_2.COO^-$, m_{A^-}, to the zwitterion, $m_{A^{+-}}$, have been calculated at a number of pH values (Table I).

Table I

pH	$\frac{m_{A^+}}{m_{A^{+-}}}$	$\frac{m_{A^-}}{m_{A^{+-}}}$
2	2	$1{\cdot}5 \times 10^{-8}$
4	2×10^{-2}	$1{\cdot}5 \times 10^{-6}$
6	2×10^{-4}	$1{\cdot}5 \times 10^{-4}$
8	2×10^{-6}	$1{\cdot}5 \times 10^{-2}$
10	2×10^{-8}	$1{\cdot}5$

Thus glycine in sea water will consist of 98·5 % zwitterion and 1·5 % negative ion, with negligible amounts of the positive ion and of the uncharged molecule, $NH_2.CH_2.COOH$. Leucine, α-alanine and β-asparagine will behave similarly to glycine.

Plants require many amino-acids for protein synthesis, and any which are not taken in from the water will have to be freshly synthesized by the plants. Those amino-acids which are taken in by *Chlamydomonas* and *Carteria* in excess of requirements would therefore have to be deaminated within the plant and the resulting ammonia resynthesized into the missing amino-acids.

REACTION GROUP 5. FIXATION OF FREE NITROGEN

Bacterial fixation of nitrogen has been discussed by Waksman, Hotchkiss & Carey (1933, p. 161). The aerobic *Azotobacter* has been shown to exist in sea water, possibly in symbiosis with marine algae, and anaerobic *Clostridium* in the sea bottom, but their ability to add appreciably to the store of combined nitrogen has yet to be demonstrated.

According to data summarized by F. W. Clarke (1924) the annual deposition of nitric-nitrogen over the sea is likely to be about 2 mg.-atom and of ammonia-N 4–15 mg.-atom per sq. m. In areas of frequent thunderstorms such as the tropics, fixation of nitrogen by electric discharge is likely to be considerably greater. Deacon (1933, p. 219) found 10 mg. nitrate-N per cu. m. in tropical rain water over the sea, and considers this to be the source of the small amount of nitrate found in tropical surface water. Much of the fixed nitrogen found in the sea to-day has no doubt originated by atmospheric fixation, but during a limited period of years the amount so fixed can scarcely affect appreciably the nitrogen balance of the sea as a whole.

REACTION GROUP 6. DEGRADATION REACTIONS PRODUCING AMMONIA

Although deamination of urea is probably a purely chemical reaction, deamination of amino-acids and methylamines in sea water is likely to take place only with the aid of bacteria or enzymes produced by these. The production of ammonia from organic matter in the sea has been shown by Waksman & Carey (1935), von Brand, Rakestraw & Renn (1937) and others. Beesley (1914) studied the rate of nitrification of a number of amino compounds and found that the course of production of ammonia, nitrite and nitrate was essentially the same from urea, uric acid, asparagine, glycine, acetamide, methylamine sulphate, ammonium oxalate and ammonium sulphate. The cultures had been innoculated with bacteria from a sewage filter bed but the results are very similar to those of von Brand, Rakestraw & Renn (1937) who used untreated plankton as source of nitrogen. There was strong evidence of an intermediary between ammonia and nitrite in Beesley's work. There is evidence in favour of this at the end of four weeks in series I of the American team, although the small fall may perhaps not be significant. They consider further that at least part of the amino nitrogen in decaying plankton is liberated directly to the water without first appearing in the form of amino-acids. Beesley's work suggests, however, that the bacterial hydrolysis of amino compounds to ammonia is rapid compared with the prior liberation of amino-acids, so that direct liberation of ammonia is not essential for an explanation of the sea-water results.

In the sea ammonia has been determined at all depths down to 4000 m. but is commonly found in highest concentration at the surface (Böhnecke, Hentschel & Wattenberg, 1930; Cooper, 1933*a*; Robinson & Wirth, 1934*b*).

REACTION GROUP 7. INTERACTION OF METHYLAMINE, AMINO-ACIDS AND AMMONIA WITH NITRITE

Possible loss of nitrogen from methylamine, amino-acids and ammonia by the well-known reaction with nitrite deserves to be considered. The reactions have been examined by Taylor (1928) who found that free nitrous acid is necessary for them to proceed. In each case the underlying mechanism appears to be the same, but for amino-acids, owing to their zwitterion structure, somewhat different velocity equations must be used. Thus for methylamine and ammonia he found

$$-\frac{dm_{\mathrm{R.NH_3^+}}}{dt}=km_{\mathrm{R.NH_3^+}}\,m_{\mathrm{NO_2^-}}\,m_{\mathrm{HNO_2}},$$

where $\mathrm{R}=\mathrm{CH_3}$ or H, and for glycine and α- and β-alanine:

$$-\frac{dm_{\mathrm{A^{+-}}}}{dt}=km_{\mathrm{A^{+-}}}\,m_{\mathrm{HNO_2}}^{2},$$

where $\mathrm{A^{+-}}$ represents the zwitterion form of the amino-acid.

Schümann (1900) determined the ionization constant of nitrous acid, $\frac{m_{\mathrm{H}^+} m_{\mathrm{NO}_2^-}}{m_{\mathrm{HNO}_2}} = 0{\cdot}00045$, whence in sea water containing 0·5 mg.-atom (7 mg.) nitrite-N per cu. m., the concentration of free nitrous acid will be $1{\cdot}1 \times 10^{-5}$ millimol per cu. m. If the water also contains an equal amount of nitrogen as ammonia, methylamine, glycine or α- or β-alanine, the concentration would be reduced by half in a million million years or more. Loss of nitrogen by interaction of amines and nitrite in sea water may therefore be dismissed as of no importance.

REACTION GROUP 8. DIRECT UTILIZATION OF AMMONIA BY PLANTS

Of the ammonia (see footnote, p. 186) determined by distillation and titration a substantial part is likely to be methylamines (p. 185).

The ionization constant of ammonium hydroxide at 18° C.,

$$\frac{a_{\mathrm{NH}_4^+} a_{\mathrm{OH}^-}}{a_{\mathrm{NH}_4\mathrm{OH}}} = 17{\cdot}15 \times 10^{-6} \text{ (Noyes \& Kanolt, 1907).}$$

Since at 18° C. when $p\mathrm{H} = 8{\cdot}00$, $p\mathrm{OH} = 6{\cdot}24$,

$$\therefore\ a_{\mathrm{NH}_4^+} = 29{\cdot}8\ a_{\mathrm{NH}_4\mathrm{OH}}.$$

The term NH_4OH includes anhydrous NH_3, if any. Since the activity of the ammonium ion is only thirty times that of the undissociated base, assimilation of the latter might occur from sea water. However, in terrestrial investigations assimilation of ammonia has been most often observed under acid conditions where the activity of the kation would be many times that of the undissociated base so that in sea water also assimilation of ammonia is most likely in the ionic state.

The availability of ammonia for growth of bacteria-free cultures of *Carteria* and *Chlamydomonas* has been shown by Schreiber (1927) and by Braarud & Føyn (1930) respectively. Schreiber also showed that *Biddulphia mobiliensis* and *Melosira*, and Harvey (1933) that *Nitzschia closterium*, grew on ammonia as well as on nitrate, but these cultures were not bacteria-free. ZoBell (1935*a*) also has shown that *Nitzschia closterium*, *N. bilobata*, *Navicula* sp., *Chlorella* sp. and certain mixed cultures flourished on ammonia nitrogen and, indeed, at the start of his experiments ammonia was even more effective than nitrate. Pearsall & Loose (1937) report a similar result with *Chlorella vulgaris* in fresh water. ZoBell discusses the assimilation of ammonia and there remains no doubt that it provides an excellent source of nitrogen for many marine plants.

It has been claimed that records of ammonia in the sea have failed to show its utilization by diatoms. As against this must be set the small number of results available and the complexity of the changes in which it is continually taking part. On the one hand ammonia in the water is being enriched by degradation processes and on the other it is being removed by oxidation processes and by diatoms. It is not surprising that the picture is less clear than for nitrate. Results at E 1 in the English Channel in mid-April 1931 (Fig. 2) supply unequivocal evidence that, on that occasion at least, ammonia was used during an intensive diatom outburst (Cooper, 1933*a*). Wattenberg & Meyer's results (1936) in Kiel Bay in April 1935 are consonant with this, although other explanations may there be possible. Rakestraw's (1936) results for his station 1739 on the continental shelf off Cape Cod in July give similar evidence.

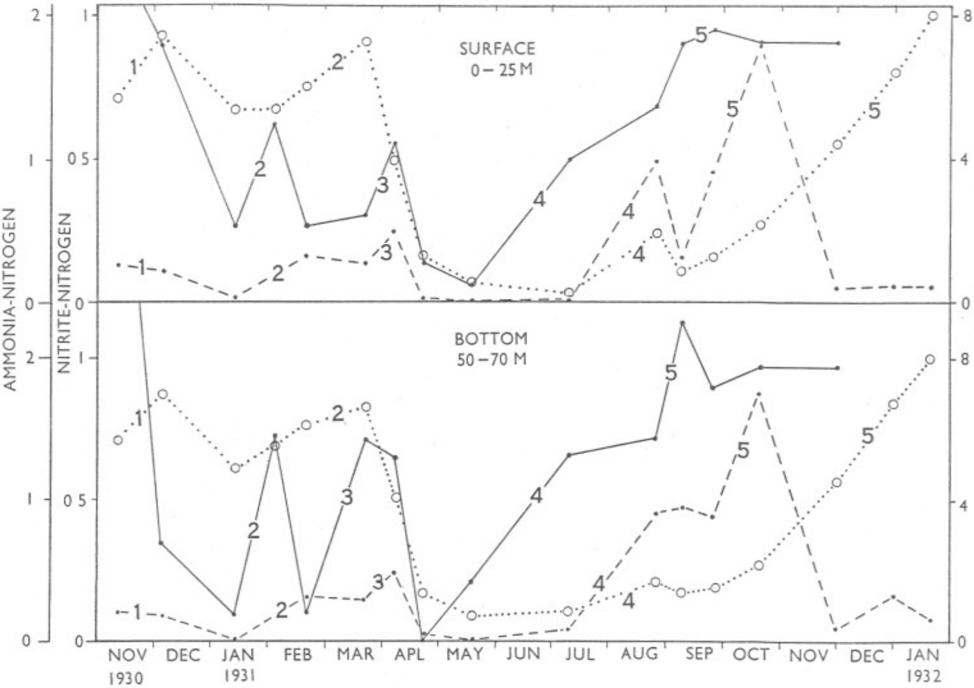

Fig. 2. Ammonia (full line), nitrite (pecked line) and nitrate (dotted line) at Station E 1 in 1930–1, surface and bottom. Stages in the conversion of ammonia to nitrite and thence to nitrate are shown by numbering. In April consumption of all three salts by plankton put a premature end to sequence 3. All as mg.-atom N per cu. m.

Reaction Group 9. Oxidation of Ammonia to Nitrite

The oxidation

$$NH_4^+ + OH^- + \tfrac{3}{2}O_2(\text{gas}) = H^+ + NO_2^- + 2H_2O$$

is accompanied by a decrease in thermodynamic potential or free energy of 59,400 cal. at 25° C. and so requires only to be suitably activated. This activation may be brought about by photochemical, chemical or bacterial agency, and more or less transient intermediates such as hydroxylamine or hyponitrous acid may play a part.

Photochemical Oxidation

Dhar strongly maintains that the oxidation of ammonia in tropical soils is mainly a photochemical process but that certain solid oxides are necessary as photosensitizers (Rao & Dhar, 1931). ZoBell (1933) demonstrated photochemical oxidation of ammonia to nitrite and nitrate by sunlight and by irradiation from a mercury vapour lamp. This result was also achieved by Corbet (1934) who further showed the intermediate formation of hyponitrite. ZoBell found that the reaction went better in natural sea water than in distilled water or artificial sea water. Whether silica exists in sea water in the colloidal or crystalloidal state is yet a matter for debate (Wattenberg, 1937, p. 17) but, since silica was amongst the sensitizers used by Dhar, presence of colloidal particles of silica is a likely explanation of ZoBell's results. He adds that autoclaving sea water at 120° destroyed its photochemical nitrifying power. Since at 120° the ionic dissociation of water is greatly increased, the increased concentration of hydroxyl ion would peptize the colloid, rendering it inactive. Rakestraw & Hollaender (1936) have further confirmed ZoBell's results and found an even greater difference between the behaviour of natural sea water and distilled water.

Photochemical oxidation of ammonia may occur in the surface layers of the sea but, due to the rapid absorption of ultra-violet light in sea water (Atkins & Poole, 1933), will be of no importance below about 1 m.

Chemical Oxidation

On a number of occasions under isothermal conditions we have observed more phosphate and ammonia at the surface than in the water beneath. This has been attributed to the decomposition of organic material floating up to the surface all over the area studied (Atkins, 1930 *b*, p. 829). The decomposition may be assisted by conditions which exist only in the surface layer. Many chemical reactions take place at surfaces and interfaces. Powell & Clarke's contention (1936), that increased light absorption immediately beneath the surface of the sea is due to minute bubbles of air, implies that the greatly increased area of the air-water interface should accelerate the rates of chemical oxidation and hydrolysis.

Bacterial Oxidation

The bacterial oxidation of ammonia to nitrite has been reviewed at length by Barritt (1933). That the oxidation may be brought about either by autotrophic bacteria obtaining their carbon from dissolved carbon dioxide or by heterotrophic bacteria living in symbiosis with diatoms appears in accord with the review and what we know about the sea. However, the work of Kingma Boltjes (1935) which is contrary to this view must be borne in mind. Corbet (1934) believes that in general the autotrophic *Nitrosomonas* is the most important factor in the biological production of nitrite from ammonia, a view which may need modification when applied to the sea and the sea bottom. Kingma Boltjes (1935) has shown that calcium ions, in which the sea is rich, are indispensable for *Nitrosomonas*.

Waksman, Hotchkiss & Carey (1933), Waksman, Reuszer, Carey, Hotchkiss & Renn (1933) and Carey & Waksman (1934) consider their own and earlier results of Thomsen, Issatchenko, Lipman, Harvey and others to be sufficient to show definitely that sea water, especially at the surface, has either no nitrifying bacteria at all or only very few of these organisms. On the other hand, the sea bottom, mud or sand, has an active population of nitrifying organisms to which the formation and accumulation of nitrate in the sea is largely due. From bottom deposits ZoBell (1935*b*) has isolated pure cultures of nitrifiers which oxidize ammonia to nitrite. The oxidation-reduction potentials of such cultures have been determined electrometrically using platinum electrodes. By adjusting the potential with poising substances, the optimum E^h for nitrification was found to be 0·30–0·55 V., multiplication without nitrite formation occurring at somewhat lower potentials. A detailed account of this work is not yet available, but the summarized record is highly suggestive. The optimum E^h is about that of sea water (*ca.* 0·45 V. due to the irreversible oxygen system; Cooper, 1937*a*) and far removed from the low values of E^h which ZoBell & Anderson (1936) have shown often to exist in bottom deposits. The nitrifiers are therefore ideally suited to oxidize ammonia in sea water but not in the deposits from which they were isolated.

There can be no doubt that nitrification immediately above the bottom is of great importance. For example, at the English Channel Station E 1 on August 16 1928, Atkins (1930*a*) found 2·22 mg.-atom nitrite-N per cu. m. immediately above the bottom, and this had increased to 2·67 mg.-atom by August 29. At this time the vertical stability was considerable. Similarly, Cooper (1933*c*) found 1·79 mg.-atom per cu. m. above the bottom at the same station on September 12 1932.

Von Brand, Rakestraw & Renn (1937) have followed the breakdown of plankton *in vitro* and have established clearly the conversion of ammonia to nitrite. Even so, attempts to follow the development of a specific bacterial flora were unsuccessful but opinion in their laboratory appears to consider organisms of the *Nitrosomonas* group to be responsible. Conditions were ideal in their experiments for heterotrophic bacteria perhaps in symbiosis with other organisms. Isolation of the nitrifying bacteria apart from their host would then be difficult.

Thus in the surface layers of the sea oxidation of ammonia to nitrite may be brought about by chemical or photochemical agency, and in and near the sea bottom by bacteria which have been isolated and grown. No one has yet demonstrated any means by which ammonia may be oxidized to nitrite in mid-water but, in spite of this, conversion to nitrite and thence to nitrate does appear to occur in mid-water, and the presence there of nitrite cannot always be accounted for by reduction of nitrate.

In the English Channel on August 16 1932 an intermediate layer of great stability existed (Cooper, 1933*c*), and intense regeneration of silica was there taking place. At the same time a kink in the nitrite vertical profile developed and, although not conclusive, suggests strongly that nitrification was taking place within this layer. A similar conclusion emerges from Atkins' (1930*a*) results in 1928 (Table II). Thus intense nitrification was taking place both at the bottom and in the intermediate layer. There was less activity in between, and the two regions of nitrification appear to have been independent. It is unreasonable to explain the results by oxidation of ammonia in the bottom water and by simultaneous reduction of nitrate in the intermediate layer, more particularly as this same layer of water had almost certainly suffered severe depletion of nitrate during the summer.

Table II. NITRITE AT STATION E 1, AUGUST 1928 (ATKINS). MG.-ATOM N PER CU. M.

	August 16		August 29	
Depth m.	Temp. °C.	NO_2-N	Temp. °C.	NO_2-N
10	15·78	—	15·67	0·00
15	15·67	0·015	15·08	0·31
20	14·92	0·015	13·60	2·78
25	12·70	2·64	13·57	—
40	—	—	12·90	2·06
50	12·65	1·68	12·76	—
65–70*	12·86	2·22	12·74	2·68

* 1–3 m. off bottom.

Similar evidence may be adduced from the survey of the English Channel in 1931 (Cooper, 1933*a* and also Fig. 2) and in Kiel Bight (Wattenberg & Meyer, 1936). At E 1 on a number of occasions formation of ammonia was followed about 3–4 weeks later by an increase in nitrite. In Fig. 2 each event is so numbered that the conversion of ammonia to nitrite and thence to nitrate may be the more readily followed. It may be argued that at a shallow-water station such as E 1, nitrification at the bottom followed by vertical mixing is sufficient to account for the observations. This is sometimes so, but it is very difficult to make the hypothesis fit all the facts. Wattenberg and Meyer's data also show the dependence of nitrite on ammonia but are compatible with the view that nitrification took place at the bottom in 20 m. Soot-Ryen's (1934) work in the neighbourhood of Tromsø gives poor support to the view that nitrification must take place in or near the bottom. Rakestraw (1936, p. 148) points out that at his stations in the Gulf of Maine, nitrite is produced near the surface and works down through the unstable layer during the winter until stratification again sets in.

At all stations investigated by *Discovery II* (1932) in the Southern Ocean, many deeper than 3000 m. (Stations 463, 492 and 646–671 between October and April 1930–1), between 0·3 and 0·7 mg.-atom nitrite-N per cu. m. was found above 150 m. and none below. Many of these stations were sampled in the neighbourhood of 60° S. in early spring or late autumn, so that photochemical oxidation was probably negligible. The results are explainable by reduction of nitrate but, if this is so, certain corollaries follow for which at present there is no other evidence.

In those waters living material is produced in great abundance and in due course dies or is voided to the water as animal faeces. Much of this admittedly sinks into deeper water but some must decompose *in situ* leading eventually to production of ammonia. A small part of this may be photochemically oxidized at the surface but inevitably, failing oxidation, ammonia must accumulate in considerable quantity between, say, 20 and 150 m. The point is capable of experimental test, but at present there is no evidence for such a "hold-up" in the nitrogen cycle in the sea.

Intermediate Formation of Hyponitrite

Beesley (1914) found some evidence for the formation of an intermediary in the bacterial oxidation of ammonia to nitrite, and this has now been identified by Corbet (1935). He pictures the micro-biological oxidation of ammonia as

$$NH_3 \rightarrow NH_2OH \rightarrow H_2N_2O_2 \rightarrow HNO_2 \rightarrow HNO_3,$$

a mechanism in accord with electrochemical views.

At *p*H 6 and over, hydroxylamine has only an ephemeral existence, being destroyed by purely chemical oxidation, and so is unlikely to accumulate in sea water. By contrast calcium hyponitrite is stable in cold aqueous solution and in presence of nitrite. When its aqueous solution was inoculated with soil micro-organisms Corbet found an accumulation of nitrite with no evolution of gas. Conditions in sea water are therefore favourable for the existence of hyponitrite as a stage in the bacterial oxidation of ammonia.

In the autumn of 1931 some experiments were made in this laboratory on the conversion of ammonia to nitrite and nitrate but were then difficult to interpret. They now suggest the intermediate formation of hyponitrite. Water was investigated from two stations, viz. E 1 (5, 50 and 70 m.), as typical of the open English Channel, and L 1 (Plymouth Sound) at high and low water, to give an idea of the behaviour of estuarine water, rich in organic matter and contaminated with sewage.

At Station E 1 the water collected on October 20 1931 was at once run into 300 ml. dark green milk bottles, sterilized at 110° C., in which the experiment was to be carried through. In each bottle an air space of 50 ml. was left. The samples from L 1 were collected in heat-sterilized Winchester bottles on October 23 at low and at high water. Exactly 250 ml. were transferred to sterilized flat-bottomed flasks (500–1000 ml. capacity) for each experiment. Measured quantities of heat-sterilized solutions of ammonia, of nitrite or of both together were added to certain samples in duplicate and some were preserved by mercuric chloride. Analyses for ammonia, nitrite and nitrate were made 8–13 days later (Table III); no nitrate analyses were made on L 1 samples owing to the known presence of much organic matter. The pipettes and measuring cylinders used were chemically clean (cleaning mixture followed by distilled water) but had not been specially sterilized.

Table III. CHANGES IN AMMONIA, NITRITE AND NITRATE + NITRITE IN SEA WATER AND ESTUARINE WATER ON STORAGE

		Initial concentration mg.-atom per cu. m.			Change in x days mg.-atom per cu. m.		
Station, depth and date	$HgCl_2$ present	NH_3-N	NO_2-N	NO_2-N + NO_3-N	NH_3-N per 13 days	NO_2-N per 10 days	NO_2-N + NO_3-N per 13 days
E 1, 5 m., 20. x. 31	−	0·4	0·92	1·9	−0·4	−0·07	−0·2
	−	5·0	0·92	1·9	−1·4	−0·06	Nil
	−	4·9	0·92	1·9	−0·8	−0·08	−0·2
	−	0·4	2·57	3·6	−0·2	−0·07	Nil
	−	0·4	2·76	3·8	−0·2	−0·24	Nil
	−	5·3	2·73	3·7	−1·6	−0·10	Nil
	+	5·2	2·71	3·7	−0·8	−0·05	−0·2
E. 1, 50 m., 20. x. 31	−	0·4	0·89	1·9	−0·1	−0·04	−0·4
	−	4·9	0·89	1·9	−1·3	−0·09	+0·5
	−	5·1	0·89	1·9	−0·6	−0·06	+0·3
	−	0·4	2·71	3·7	−0·4	−0·17	+0·1
	−	0·4	2·47	3·4	−0·4	−0·08	Nil
	−	5·4	2·76	3·7	−1·6	−0·17	−0·1
	+	4·9	2·60	3·6	−0·5	Nil	−0·1
E. 1, 70 m., 20. x. 31	−	0·8	0·86	2·4	−0·6	−0·05	−0·7
	−	5·5	0·86	2·4	−1·7	−0·09	−0·4
	−	5·6	0·86	2·4	−1·4	−0·06	Nil
	−	0·8	2·54	4·1	−0·4	−0·03	−0·8
	−	0·8	2·55	4·1	−0·3	+0·02	−1·0
	−	5·2	2·50	4·1	−1·3	−0·01	−1·1
	+	5·4	2·61	4·0	−0·6	−0·02	−1·0
					per 11 days	per 8 days	
L 1, 0 m., 25 min. before high water, 23. x. 31	−	3·0	0·22	.	+0·3	−0·02	.
	−	8·4	0·22	.	−0·2	−0·03	.
	−	8·4	0·22	.	−1·3	−0·01	.
	−	3·0	1·94	.	+0·1	−0·13	.
	−	3·0	1·94	.	Nil	−0·14	.
	−	8·4	1·94	.	−1·3	−0·14	.
	+	8·4	1·94	.	−1·6	−0·14	.
L 1, 0 m., low water, 23. x. 31	−	6·4	0·32	.	+2·1	+0·04	.
	−	11·0	0·32	.	+0·6	+0·02	.
	−	11·0	0·32	.	+1·4	+0·02	.
	−	6·4	2·04	.	+0·4	−0·15	.
	−	6·4	2·04	.	+0·5	Nil	.
	−	11·0	2·04	.	+0·9	−0·04	.
	+	11·0	2·04	.	−1·0	−0·28	.

In every sample from E 1, ammonia showed a fall. In those samples further enriched with ammonia this fall, amounting to much more than the experimental error, cannot be attributed to nitrite formation. It is highly probable that the ammonia had been oxidized to hyponitrite. Such change in nitrite as occurred was a small loss. In the samples containing mercuric chloride the loss of ammonia is smaller but is still greater than the experimental error. With this account the samples drawn from Plymouth Sound at high water agree. In the low-water samples, the ammonia, already large, increased further in every experiment except that in which mercuric chloride had been added. The bacterial liberation of ammonia in the water rich in organic matter was inhibited. We may conclude that ammonia may be set free from organic matter in sea water by bacterial action and may be converted into a substance such as hyponitrite by a mechanism which need not be bacterial.

Keys, Christensen & Krogh (1935) also found decreases in the ammonia content of sea water on storage but point out that their results are far from consistent. Kreps (1934) has suggested that enzymes, set free from dying bacteria, remain able to oxidize ammonia for a considerable time afterwards even in presence of a bactericide such as mercuric chloride. The stability of nitrite in the L 1 low-water sample subject to heavy contamination from river drainage and urban sewage is worthy of note.

REACTION GROUP 10. OXIDATION OF NITRITE TO NITRATE

The reaction $$NO_2^- + \tfrac{1}{2}O_2(\text{gas}) = NO_3^-$$

is accompanied by a decrease of free energy, ΔF°_{298}, of 18,000 cal., so that the reaction requires only activation, and like the oxidation of ammonia it may be used as a direct source of energy by suitably equipped bacteria. Since the equilibrium constant is related to this free energy,

$$\Delta F^\circ_{298} = -RT \ln \frac{a_{NO_3^-}}{a_{NO_2^-}\, a_{O_2}} = -18{,}000 \text{ cal.},$$

therefore with an oxygen activity of 0·2 atmosphere

$$a_{NO_3^-} = 3{\cdot}1 \times 10^{12}\, a_{NO_2^-},$$

and so, in aerated sea water containing 10 mg.-atom (= 140 mg.) nitrate N per cu. m., there will be an equilibrium activity of 3×10^{-12} mg.-atom nitrite N per cu. m., so that at equilibrium nitrite must always be undetectable.

In the building up of nitrate reserves in the sea nitrite is commonly considered an essential intermediary (cf. Atkins, 1930*a*). The investigation in the English Channel in 1931 showed clearly that increase in nitrate followed a few weeks behind the appearance of nitrite (Fig. 2). This result has since been confirmed by the laboratory experiments of von Brand, Rakestraw & Renn (1937) who allowed plankton to decompose in sea water whilst following the conversion of organic nitrogen to ammonia, nitrite and nitrate. Whilst the sea bottom, where nitrite-oxidizing bacteria have been shown to exist by Waksman, Hotchkiss & Carey (1933), is undoubtedly an important site of nitrification, the arguments advanced on pp. 192–4 in favour of oxidation of ammonia to nitrite in mid-water apply equally to the further oxidation of nitrite there.

REACTION GROUP 11. ASSIMILATION OF NITRITE AND NITRATE

The ability of phytoplankton to assimilate nitrate needs no discussion. Their power to assimilate nitrite is established on nearly as sound a basis (cf. Schreiber, 1927; Braarud & Føyn, 1930; ZoBell, 1935*a*), but nothing is known as to which would be preferred by a diatom presented with a mixture of salts of ammonium, nitrite and nitrate. At Station E 1 in the English Channel, between April 7 and 22 1931, all three salts decreased together (Fig. 2), strongly suggesting that they were being simultaneously utilized by diatoms. A close relationship between any one of them and diatom growth is hardly to be expected but, since in temperate and arctic latitudes nitrate is the one which collects in sea water during the winter months, it is available in quantity when the spring outburst of diatoms starts. Since Harvey's (1926, 1928) striking investigations in the English Channel, rapid falls in nitrate coincident with this spring outburst have been reported in many waters. At other seasons also, diatom outbursts may lead to depletion of nitrate, but the other nitrogenous compounds are likely then to be available in comparable amounts so that all need to be examined simultaneously if a clear picture of the nitrogen cycle is to be obtained.

REACTION GROUP 12. REDUCTION OF NITRATE AND NITRITE

Since Gran (1901) and Baur (1902) first showed the existence of denitrifying bacteria in the sea, reduction of nitrate and denitrification generally have been a fruitful source of controversy. The view long maintained by Brandt (1927), that true denitrification leading to loss of free nitrogen is of great importance in the sea, finds little support to-day (cf. Atkins, 1932). The literature has been reviewed by Lloyd (1931 *a*, *b*, *c*) and by Waksman, Hotchkiss & Carey (1933). The work of the Wood's Hole Oceanographical Laboratory has shown that the activities of most of the nitrate-reducing bacteria are limited to the reduction of nitrate to nitrite, a reaction leading to no loss of available nitrogen.

Gran (1901) considered that denitrifying organisms do not use nitrate if oxygen is available but observations by other workers suggest that this is not always so. Braarud & Klem (1931) found rapid depletion of nitrate in aerated sea water and Kreps (1934) has described a number of experiments to like effect. The fall in nitrate occurred whether or not mercuric chloride had been added. The writer in his 1931 experiments (Table III) also observed that depletion of nitrate occurred in bottom water from Station E 1 both in absence and presence of mercuric chloride. Kreps also found that the depletion of nitrate could go on in water which had been filtered through a Seitz filter and concluded that enzymes liberated by bacteria into the water were responsible for nitrate reduction and ammonia oxidation, and that these enzymes could pass a Seitz filter and withstand a bactericide such as mercuric chloride.

Waksman, Hotchkiss & Carey (1933) and Waksman, Reuszer, Carey, Hotchkiss & Renn (1933) say little of the effect of oxygen but aerated conditions are implicit in the description of their experiments demonstrating the existence in the sea of bacteria able to reduce nitrate to nitrite. Such bacteria may on occasion account for nitrite found between 25 and 150 m. (Rakestraw, 1933).

This reduction is accompanied by oxidation of organic matter, and the view has been expressed to the writer that it is a wasteful method for a bacterium to obtain energy from nitrate when oxygen is available. If dextrose be considered as source of carbon the changes in free energy or thermodynamic potential, ΔF°, are readily calculated, thus for 25° C.:

$$C_6H_{12}O_6(\text{aq.}) + 12NO_3^- = 12NO_2^- + 6CO_2(\text{aq.}) + 6H_2O(\text{liq.}); \quad \Delta F^\circ = -460 \text{ kg.-cal.}$$

$$C_6H_{12}O_6(\text{aq.}) + 8NO_2^- = 4N_2(\text{aq.}) + 4CO_3^= + 2CO_2(\text{aq.}) + 6H_2O(\text{liq.}); \quad \Delta F^\circ = -728 \text{ kg.-cal.}$$

$$C_6H_{12}O_6(\text{aq.}) + 6O_2(\text{aq.}) = 6CO_2(\text{aq.}) + 6H_2O(\text{liq.}); \quad \Delta F^\circ = -699 \text{ kg.-cal.}$$

The thermodynamic efficiencies of the oxidation of a sugar by oxygen and by nitrite are similar. With nitrate, itself simultaneously reduced to nitrite, the efficiency of the oxidation is reduced by one-third. Even so, bacterial reduction of nitrate in aerated water containing oxidizable organic matter cannot be dismissed as an excessively wasteful process. To assimilate the nitrate or nitrite, osmotic work must be done, but this amounts to not more than 1 or 2 kg.-cal. per g.-mol. of dextrose oxidized. If any other carbohydrate, fat or protein were taken a similar result would emerge. No account is taken of the mechanism of the oxidation. Both the straightforward oxidation of nitrite to nitrate and the respiratory reduction of nitrate to nitrite in presence of an organic substance are processes from which energy may be derived.

Korsakov (1929) has regarded the nitrate-nitrite system from the point of view of oxidation-reduction potential. The reduction can take place only if the compounds in the medium can be activated by the bacteria in such a way that one becomes the acceptor and one the donator of hydrogen. Aerobic bacteria are able to activate not only the system, donator of hydrogen (organic substance) + acceptor (oxygen), but also systems involving other acceptors (e.g. nitrate ion). Owing to the thermodynamic irreversibility of the nitrate-nitrite system arguments such as this are somewhat dangerous but, if the reduction of nitrate is regarded as dependent on oxidation-reduction potential, sea water provides a very uniform medium. There is evidence arising out of work on the iron system in this laboratory that diatoms possess a skin or shell where the potential is much lower than in the water. In this, extracellular reduction of nitrate might take place in the way visualized by ZoBell (1935*a*). Under some circumstances part of this nitrite may escape assimilation and be returned to the water. Production of nitrite in nitrate-enriched diatom cultures has often been observed (Orr, 1926; ZoBell, 1935*a*), whilst Warburg & Negelein (1920) found that nitrate was reduced to ammonia by *Chlorella* in pure culture with great rapidity, a fact repeatedly confirmed by Pearsall & Loose (1937).

Photochemical reduction of nitrate to nitrite has been suggested (Moore, 1919) but Villars (1927) showed that the quantum yield at *p*H 8·0–8·3 for wave-lengths greater than 2800 A. was very low. Since only light longer than 2900 A. reaches the earth (Pettit, 1932) and at 3030 A. is cut down to 0·01 % in the first 2·4 m. of sea water (Atkins & Poole, 1933), photochemical reduction of nitrate cannot occur in the sea.

Hyponitrites also occur as intermediates in the reduction of nitrite to nitrogen (Blom, 1928*b*; Lloyd & Cranston, 1930). Owing to the absence of data for hyponitrite the free-energy change of the reaction cannot be calculated. The heat set free by the reaction

$$C_6H_{12}O_6(\text{aq.}) + 12NO_2^- = 6N_2O_2^= + 6CO_2(\text{aq.}) + 6H_2O(\text{liq.})$$

is 376 kg.-cal., and the decrease in free energy is unlikely to differ greatly from this. Reduction of nitrate in the sea may well go as far as hyponitrite.

The evidence is therefore strong that reduction of nitrate in aerated sea water is readily brought about under laboratory conditions, and such reduction should sometimes occur. But as yet, in mid-water, we are not justified in attributing all nitrite formation to reduction of nitrate and dismissing oxidation of ammonia as of no account.

DISCUSSION

Our clear-cut picture of the annual cycle of nitrate in the sea has been obtained because, during the winter in temperate and arctic latitudes, nitrate is accumulated in quantity greater than other available forms of nitrogen. It is therefore ready to be consumed in quantity during the vernal diatom outburst when its variations may be followed by analysis. It is also clear why nitrate formation, requiring a number of successive stages, lags behind phosphate formation.

Since growing plants are well able to use other forms of nitrogen when these are present, analyses of nitrate alone are less likely to give an intelligible picture of events in summer and autumn. The analytical methods for nitrate determine nitrite as well, but when considerable quantities of nitrite are present, results will usually be somewhat too low but, even so, are usually a sufficient measure of nitrate + nitrite.

Our present knowledge of hyponitrite rests on soil and sewage cultures. Its probable existence in the sea requires study for an understanding of the transformations of inorganic nitrogen compounds. Ammonia is certainly of great value, but its determination still presents analytical difficulties (cf. Wattenberg, 1937). In addition, the results may be uncertain due to the presence of methylamines whose value as immediate sources of nitrogen for phytoplankton is unknown.

We can only speculate as to the existence and behaviour of amino-acids and urea in sea water. Amino-acids are known to be of value to certain marine plants, but their assimilation, due to their "zwitterion" structure, may follow a different mechanism from the inorganic ions. They are probably of value only as accessory nutrients. Analytical methods for amino-acids in sea water have still to be developed. The presence of urea in sea water is probably transient but important in the degradation of nitrogen.

It is clear that no one explanation will cover all the oxidation and reduction reactions in which ammonia, nitrite, nitrate and possibly hyponitrite take part. The oxidation of ammonia to nitrite and thence to nitrate may take place photochemically or chemically in a thin surface layer or bacterially in and near the bottom. No bacteria able to bring about the oxidation of ammonia or nitrite have yet been found in sea water well away from the bottom, and the view is maintained by some investigators that no such oxidation can take place there. Bacterial reduction of nitrate to nitrite undoubtedly can and does occur in the sea. In spite of this the writer feels that reduction of nitrate fails to explain all cases of nitrite occurrence in mid-water, that there is strong chemical evidence that oxidation of ammonia does take place away from the bottom and from the immediate surface layer, and that any theory which fails to take these into account cannot cover all the observations.

Summary

The nitrogen cycle in the sea is reviewed as a whole in accordance with the scheme set out in Fig. 1. This summary includes only original matter, since the survey of other work does not admit of further condensation.

The metabolism has been discussed of the following sources of nitrogen available to plants in sea water: mono-, di- and trimethylamine, trimethylamine oxide, urea, amino-acids, ammonia, hyponitrite, nitrite and nitrate. The methylamines will interfere in analyses of ammonia by distillation.

Thermodynamic methods have been extensively used. The equilibrium between urea and ammonium cyanate at sea-water concentrations favours the cyanate. In sea water containing 28 mg. ammonia N per cu. m., the equilibrium mixture will contain fifteen times as much cyanate as urea. Hydrolysis of urea is probably purely chemical.

The importance of the "zwitterion" or internal salt structure of amino-acids in studies on their assimilation is examined. In sea water, the common amino-acids will consist of about 98·5% zwitterion, $H_3{}^+N.R.COO^-$, and

1·5 % anion, $H_2N.R.COO^-$, with negligible amounts of kation and uncharged molecule, $H_2N.R.COOH$. If ever an amino-acid should form the sole source of nitrogen for diatoms, at least part must be deaminated within the plant before other necessary amino-acids can be synthesized.

It is shown from kinetic measurements that no loss of amino-nitrogen can occur by interaction with nitrite in sea water. The activity of NH_4^+ in sea water is about thirty times that of NH_4OH. The change in thermodynamic potential or free energy during the oxidation of 1 g.-mol. of ammonia to nitrite or of nitrite to nitrate is 59·4 or 18 kg.-cal. respectively.

The greater efficiency of photochemical oxidation of ammonia in sea water than in distilled water may be due to the presence of colloidal silica but such oxidation is of no importance below about 1 m. Purely chemical oxidation at the interface with minute air bubbles may be possible immediately beneath the surface of the sea.

Although bacteria able to oxidize ammonia and nitrite have been satisfactorily demonstrated only in bottom water, strong evidence is presented that similar oxidations must proceed away from the sea bottom, by mechanisms yet to be established.

Evidence is marshalled in favour of the intermediate formation of hyponitrite during the oxidation of ammonia to nitrite.

At thermodynamic equilibrium, the activity of nitrate will be 10^{12} times that of nitrite.

Oxidation of 1 g.-mol. of dextrose by nitrate ion, nitrite ion and dissolved oxygen is accompanied by a free energy decrease of 460, 730 and 700 kg.-cal. respectively. Denitrification in aerated water is therefore not excluded on thermodynamic grounds. Even so, reduction of nitrate cannot explain all cases of nitrite occurrence in mid-water. Photochemical reduction of nitrate cannot occur in the sea.

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