moved from the medium bathing the organ rudiments. Certain results of Urist and Ibsen (*8*) seem to suggest the latter alternative. Determinations of calcium in my culture medium have not, however, shown any diminution in soluble or ionic calcium during 12 days of cultivation in the presence of tetracycline at a concentration of 10 μg/ml (*9*).

Cultures of rudiments of embryonic organs seem to afford a valuable tool for the exploration and analysis of certain toxic and, perhaps, teratogenic effects of drugs. However, it should be stressed that at the present stage these conclusions are not applicable at the clinical level.

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3 May 1965

Productivity of Microalgae in Antarctic Sea Ice

Abstract. *Midsummer productivity of Antarctic microalgae, commonly occurring in brown sea ice along the west coast of the Palmer Peninsula, averaged more than 900 milligrams of carbon per cubic meter per hour, with an assimilation number of about 2.6. The rate of photosynthesis increased with light intensity to a maximum of about 18,000 lux, above which some inhibition was observed. The floral composition, genesis, and physiological properties of these ice communities are different from the epontic under-ice diatoms previously studied by other investigators in McMurdo Sound.*

At least two different kinds of microalgal communities exist in Antarctic sea ice which covers as much as 2 to 5 million square kilometers even in midsummer (*1*). One of these communities is the epontic diatom population that Bunt (*2*) found living on ice crystals and in the interstitial water of the ice matrix formed at the bottom surface of thick ice. This prolific flora of diatoms growing under several meters of sea ice, for example in McMurdo Sound, appears to be a shade-adapted community which can fix carbon dioxide slowly at low intensities of light (*3*). Another kind of ice population, studied by Fukushima (*4*) and Meguro (*5*), is commonly seen in yellow-brown bands of broken sea ice in the southern oceans south of the Antarctic Circle. As the weight of falling snow depresses the ice below the sea surface, sea water floods the interstices of the snow and provides a suitable place for development of a mixed diatom and flagellate community, in layers varying from 15 to 100 cm in thickness. The brown strata of ice plankton may exist as a slush during warm summer days or the layer may be frozen solid during very cold weather. We now present data concerning the amount of microalgae trapped in the ice and their photosynthetic capacity.

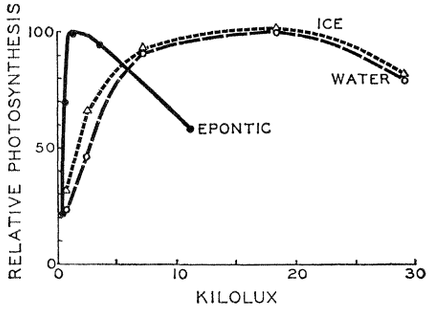

Fig. 1. Relative photosynthesis of ice and water organisms from Matha Strait, northeast of Adelaide Island, and the epontic under-ice community studied by Bunt (*3*) in McMurdo Sound.

On an expedition aboard the Argentine icebreaker *General San Martin* to Peter the First Island in February, 1965, we encountered much floating brown ice in Matha Strait and Marguerite Bay along the western coast of the Palmer Peninsula, in the vicinity of the Antarctic Circle. Samples of brown ice and sea water were obtained in plastic buckets for determination of carbon uptake by standard methods (*6*). The chlorophyll-bearing organisms were filtered onto Millipore membranes with pores 0.8 μ in diameter, then extracted in 90 percent acetone, and the pigment absorption was measured in a Beckman DU spectrophotometer aboard ship. Fixation of carbon was determined by incubation of samples in light with added $Na_2C^{14}O_3$, filtration of portions onto Millipore membranes, and counting in a Tracerlab counter (for beta particles). In order to obtain homogeneous suspensions of diatoms, portions of brown slush ice were melted in sea water and diluted appropriately to obtain practical working suspensions for experimental use. The temperature of incubation was maintained near zero with an ice-water bath.

Some typical results obtained in this study are shown in Table 1. Although the plankton was relatively abundant in these waters, the amount of chlorophyll *a* and C^{14} fixation per unit volume in the ice samples was enormous by comparison. In samples from Matha Strait, the chlorophyll *a* and C^{14} fixation of the ice organisms were approximately 30 times as great as for the phytoplankton in sea water in the same location. Chlorophyll *a* values were considerably higher than those reported in Arctic ice by Appolonio (*7*).

The assimilation numbers, expressing the milligrams of carbon fixed per hour per milligram of chlorophyll *a*,

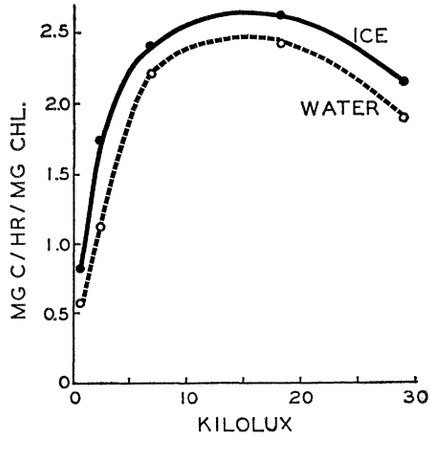

Fig. 2. Variation of assimilation numbers (milligrams of carbon fixed per hour per milligram of chlorophyll *a*) in ice and water organisms of Matha Strait, when exposed to different intensities of light (kilolux).

Table 1. Carbon fixation, chlorophyll *a*, and assimilation number of ice and water samples from the western coast of the Palmer Peninsula.

Locations	Carbon fixation (mg per m^3 per hr)	Chlorophyll *a* (mg per m^3)	Assimilation number*
Water samples			
Gerlache Strait	8.94	12.83	0.63
Bellingshausen Sea	0.62	0.24	2.58
Matha Strait	6.24	2.56	2.22
Ice samples			
Matha Strait	1080.0	407.22	2.65
Marguerite Bay	797.4	304.98	2.61

* Milligrams of carbon per hour per milligram of chlorophyll *a*.

when calculated at light saturation, gave values of about 2.6 for the samples of ice (Table 1). These values are a little higher than the calculated assimilation numbers for the plankton studied in samples of water collected at about the same time in Matha Strait and in the Bellingshausen Sea. The Bellingshausen phytoplankton consisted of *Corethron criophilum* and *Chaetoceros criophilum*. The low assimilation number of less than 1.0, found for the heavy blooms of plankton in the Gerlache Strait, may be characteristic of old cells of *Biddulphia striata*, which was dominant in the Gerlache community. In order to estimate the fixation of carbon by the ice organisms, it was necessary to subtract the calculated values contributed by the plankton of the water from the results obtained for the whole mixture (5 water : 1 ice) studied in Matha Strait, where the organisms in the ice portion occupied only one-sixth of the total experimental mixture. The calculated figure of 1080 mg of carbon per cubic meter per hour was found for brown ice. Similar reasoning was applied to obtain the portion of chlorophyll *a* contributed by the ice organisms.

When relative photosynthesis is plotted against the intensity of light (Fig. 1), the ice organisms and the plankton both increase in productivity up to about 18 kilolux and are somewhat inhibited by higher intensities at 29 kilolux. This situation is different from the response of the epontic community studied by Bunt (*3*), who observed a peak of productivity at 1 kilolux, accompanied by a low assimilation number, less than 0.15, and marked inhibition of photosynthesis in the range of light intensity between 1 and 11 kilolux.

In contrast, photosynthesis in the ice organisms increases with light intensity to a higher maximum rate than in the epontic population. The assimilation number of ice organisms at peak production under conditions of saturating light appears to be about 20 times as great as in the shade-adapted epontic community. The assimilation numbers of both plankton and ice organisms vary in a similar manner at different intensities of light (Fig. 2).

The microalgae present in the ice included large numbers of a very small diatom, moderate amounts of *Fragilaria curta* and *Nitzschia seriata*, and traces of many species, including *Chaetoceros dichaeta, Ch. pendulus, Corethron criophilum, Eucampia zoodiacus, Rhizosolenia truncata, Biddulphia striata, Navicula* sp., *Amphiprora* sp., *Gyrosigma* sp., *Tropidoneis* sp., and occasionally a few cells of dinoflagellates, small motile green flagellates, and *Phaeocystis pouchetii*.

Although some of the same species of diatoms were found in both the plankton and the ice organisms, the proportion of very small species was always higher in the ice. Diatoms commonly found in the waters near floating ice included the following: *Rhizosolenia alata, R. hebetata, Chaetoceros concavicornis, Ch. tortissimum, Thalassiosira hyalina, Biddulphia striata, Fragilaria antarctica, Corethron criophilum, Eucampia zoodiacus, Coscinodiscus radiatus*, and *Thalassiothrix antarctica*.

In view of the widespread occurrence of the ice organisms in Antarctic seas we have compared the organic productivity of ice-trapped microalgae with the oceanic and neritic phytoplankton. Calculation of daily productivity can be made from the data in Figs. 3 and 4. From the essential results obtained by incubating samples of melted and diluted sea ice at different intensities of light, the calculated data for carbon fixation were plotted as milligrams of carbon per cubic meter per hour in Fig. 3. A curve of diurnal incident sunlight was constructed from an average of the measurements made with a Weston illumination meter on 6 consecutive days in the vicinity where the ice samples were collected. Penetration of light into the sea ice probably varies considerably depending upon the amount of snow and thickness of the ice. Based upon the observations of Meguro (*5*), as well as our own, that 25 percent of the incident daylight

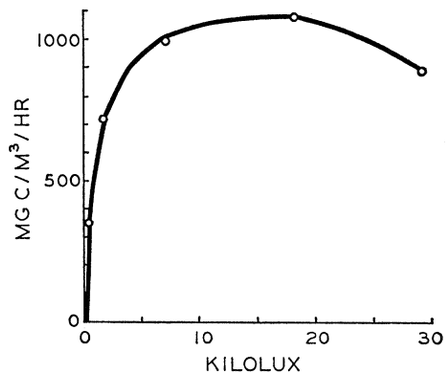

Fig. 3. Fixation of carbon (as milligrams of carbon per cubic meter per hour) in relation to the intensity of fluorescent light (kilolux) during incubation of ice organisms from Matha Strait.

penetrates to the depth of the ice organisms, another curve was drawn for one-fourth of the surface intensity, representing the values of diurnal light accessible to the microalgae in the brown ice. With this curve (Fig. 4*B*) and the photosynthesis graph (Fig. 3) as guides, the corresponding hourly values of productivity for each intensity of light throughout the day were calculated. The data for carbon fixation were then plotted for each hour of daylight in the usual way (not illustrated). By measuring the fractional area under the diurnal curve, the daily productivity was estimated to be at a rate of 1.27 g of carbon per cubic meter per day in the top portion of the ice community, where the light intensity is 25 percent of the surface illumination.

Our observations indicate that the thickness of the brown ice layers may

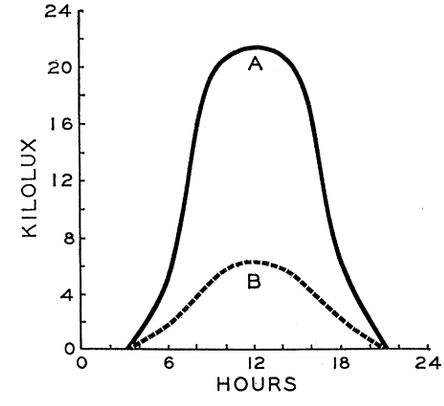

Fig. 4. Curves of diurnal incident radiation at the surface of the sea (*A*) and the radiation calculated at the top of the brown layer of ice organisms (*B*) in the vicinity of the Antarctic Circle along the western coast of the Palmer Peninsular, middle of February 1965.

vary considerably, with a conservative estimate for an average of 0.3 m. The attenuation coefficient of floating snow-ice was calculated from the data published by Meguro (*5*) and substituted in place of the value for sea water in the formula of Riley (*8*), to give an estimated attenuation coefficient of 12.8 for the ice and microalgae layer. The intensity of light reaching the bottom part of the ice community turned out to be about 1 percent of the incident radiation at the surface of the sea. Our calculations indicate that organic production is approximately zero at the bottom of the brown layer because of the low intensity of light. By integrating the production at the top and the bottom of the brown layer and assuming uniform distribution of the algae, we calculated that the algae under each square meter are able to fix about 0.19 g of carbon per day. It is of interest to compare this estimate with plankton productivity values which we have calculated as 0.09 g of carbon fixed per square meter per day in the Bellingshausen Sea and 0.66 g in the richer waters of the Gerlache Strait during the Antarctic summer.

It is not known how active the ice microalgae may be in the frozen condition; our experiments were conducted with slush communities transferred into sea water at about the same temperature. If the trapped ice algae have an activity *in situ* of the order calculated in our investigations, then the productivity of the algae, in a total of 2.6×10^6 km^2 of this kind of brown sea ice surrounding Antarctica in summer, would amount to about one half million tons of carbon fixed per day.

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Oxygen-Hemoglobin System: A Model for Facilitated Membranous Transport

Abstract. *Enhanced transport of oxygen in a Millipore filter containing a solution of hemoglobin can be accounted for by the diffusion of free oxygen as well as of hemoglobin-bound oxygen. A model shows that, at oxygen tensions at which the hemoglobin is fully saturated in a portion of the membrane, the enhanced transport is due to a steeper gradient for free oxygen, whereas in the rest of the membrane an "(oxy)hemoglobin shuttle" operates. A similar model may be useful for explaining facilitated diffusion in other systems.*

In 1960 Scholander (*1*) showed that the presence of hemoglobin solution in a Millipore filter membrane accelerated the transport of oxygen by an amount depending on the partial pressure of oxygen at the inlet side. Later it was shown (*2*) that the extra transport of oxygen due to the presence of hemoglobin was abolished by slight amounts of oxygen at the outlet side of the membrane. Although several models were proposed, no quantitative comparisons were made between these experimental results and the oxygen transport predicted by a model. Fick's diffusion law has been applied (*3*, *4*) to Scholander's system to derive a general expression for oxygen transport, but little or no effort has been made to test the equations against experimental data or to predict oxygen and oxyhemoglobin gradients within the membrane. Enns (*5*) measured the oxyhemoglobin gradient within the Millipore membrane and found that at high pressures of oxygen at the inlet nearly all the hemoglobin in the membrane existed in the oxyhemoglobin form. This finding led him to postulate that oxygen transport by hemoglobin results from oxygen exchange between binding sites of colliding hemoglobin molecules. Although Enns's calculations showed reasonable correspondence between theoretical and experimental values for oxygen transport, it was not immediately apparent by what force enhanced oxygen transport takes place in that portion of the membrane in which no gradient of oxyhemoglobin exists.

I now present a model based on the simultaneous diffusion of free oxygen and oxyhemoglobin. The results illustrate how transport can be enhanced even in that portion of the membrane that does not exhibit a gradient in oxyhemoglobin concentration.

A model for a steady-state transport of oxygen in a Millipore filter containing hemoglobin was constructed on the following assumptions: (i) one fraction of the oxygen is transported as free O_2 by diffusion, the rest by diffusion of hemoglobin-bound O_2(HbO_2); (ii) O_2 and HbO_2 are in instantaneous equilibrium at each point (*6*); and (iii) diffusion rates are determined by the local gradients in the membrane

$$\left(\frac{d[O_2]}{dx}, \frac{d[HbO_2]}{dx}\right)$$

The basic equations for the steady state are:

$$D_1 \frac{d[O_2]}{dx} + D_2 \frac{d[HbO_2]}{dx} = K \quad (1)$$

$$[HbO_2] = f[O_2] \quad (2)$$

where x is a variable distance through the membrane, D_1 is the diffusion coefficient of free O_2, $[O_2]$ is the concentration of free O_2 as a function of x, D_2 is the diffusion coefficient of HbO_2, $[HbO_2]$ is the concentration of HbO_2 as a function of x, and K is oxygen flux through the membrane. Equation 1 expresses Fick's law; Eq. 2, the oxyhemoglobin dissociation curve. Integration of Eq. 1 gives

$$D_1 \int_0^x d[O_2] + D_2 \int_0^x d[HbO_2] = K \int_0^x dx$$

or

$$D_1 ([O_2]_x - [O_2]_0) + D_2 ([HbO_2]_x - [HbO_2]_0) = Kx \quad (3)$$

where the subscripts 0 and x refer to the location in the membrane, so that $[HbO_2]_0$, for example, is the concentration of HbO_2 when $x = 0$. It is interesting to note that the steady-state transport of oxygen (K) through a given membrane depends only on the boundary concentrations of O_2 and HbO_2.

To calculate the steady-state oxygen transport (K) for a 0.015-cm thick Millipore filter (*1*) which is positioned between a given pressure of oxygen on one side and vacuum on the other, Eq. 3 can be simplified by setting $[O_2]_x$ and $[HbO_2]_x$ to zero and making x equal