accumulation. These results suggest that chloroquine resistance in *P. falciparum* and multidrug resistance in mammalian cells may be due to the same (or to a similar) mechanism.

The inhibition of chloroquine release observed with calcium channel blockers (verapamil, diltiazem, and TMB-8) (Table 1) suggests that alterations in intracellular calcium may be necessary for the release of chloroquine from the resistant parasite. The mechanism of daunomycin action is not clear but may be related to its ability to act as a calcium antagonist in some systems (*16*). The effects of vinblastine (a known inhibitor of microtubular function) (*17*) suggest that cytoskeletal proteins may be involved in the release of chloroquine from the resistant parasite. Alternatively, these compounds may inhibit chloroquine efflux by competing with chloroquine for binding to a carrier analogous (or identical) to the P-170 glycoprotein of the multidrug-resistant cancer cell (*18*).

However, even in the presence of these drugs, the chloroquine release observed with the resistant parasite was greater than observed with the susceptible parasite (Table 1). This resulted in a greater chloroquine accumulation in the susceptible than in the resistant parasite (Fig. 2) (*6, 7, 12*).

Although there may be more than one mechanism of chloroquine resistance in *P. falciparum*, verapamil reduces the inhibitory concentrations of chloroquine for resistant isolates from West Africa (*19*) and we have observed the rapid efflux phenomenon in a resistant isolate from South America ($t_{1/2}$, 1.9 minutes). These results indicate that the rapid efflux resistance phenotype is present in each of the three continents with chloroquine resistance.

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Diel Periodicity of Photosynthesis in Polar Phytoplankton: Influence on Primary Production

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In the Southern Ocean, primary production estimated from seasonal chemical and geochemical changes is two to four times greater than the value calculated from carbon-14 uptake. Since carbon uptake had typically been measured only during midday incubations, the influence of diel periodicity of photosynthesis on daily productions was not considered. Phytoplankton from McMurdo Sound, Antarctica, exhibited distinct, but seasonally variable diel patterns of light-saturated and light-limited photosynthesis. Maximum photosynthetic capacity occurred about noon in early September, and its occurrence progressively shifted to about midnight by late October. This shift was accompanied by a concomitant phase shift in the occurrence of minimum photosynthetic capacity from midnight to midday. Daily production estimated from time-of-day corrected photosynthetic characteristics and from 24-hour incubations was 2.5 to 4 times greater than that predicted from 6-hour midday incubations. If similar diel periodicity in photosynthesis occurs in other polar oceans, primary production would be significantly higher than previously estimated from carbon-14 uptake measurements.

THE SOUTHERN OCEAN SUPPORTS large populations of zooplankton, marine mammals, and seabirds (*1*) and is the site of approximately 80% of global biogenic silica sedimentation (*2, 3*). Despite this indirect evidence suggesting high primary production (*4–6*), direct measurements of production are low and similar to those of tropical and subtropical oligotrophic oceans (*7–10*). Primary production in the Antarctic is generally measured only during the austral summer and is often based on single, midday measurements (*7, 10*); thus the influence of diel periodicity of photosynthesis on estimates of daily production has not been considered (*11–13*).

Diel periodicity in photosynthesis is a common characteristic of temperate and tropical phytoplankton (*12–14*), and temporal changes in photosynthetic characteristics can significantly influence estimates of areal production (*12, 15*). Polar phytoplankton are subject to a unique photic regime where 4-month periods of continual darkness or light are separated by 2-month transition periods when the photoperiod changes by about 20 minutes a day. Thus the diel patterns of photosynthesis of phytoplankton in polar regions could differ from those of phytoplankton in temperate and tropical regions that evolved under regularly alternating periods of light and dark (*16*). Departures of maximum photosynthetic capacity from the midday period could account for part of the discrepancy between direct [carbon-14 (^{14}C)] and indirect estimates of primary production.

We have found that phytoplankton from McMurdo Sound, Antarctica, show diel periodicity in rates of both light-limited and light-saturated photosynthesis. Maximum photosynthetic capacity, P_{max} (*17*), shifted from about noon in early and mid-September to midnight by late October. Throughout the austral summer, minimum photosynthetic capacity, P_{min} (*17*), occurred midday and the P_{max}:P_{min} ratio was about 15. Primary production estimated from time-of-

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day corrected photosynthetic characteristics was about three times greater than from extrapolating single midday incubations to 24 hours. If similar diel patterns of photosynthesis occur throughout other regions of the Southern Ocean, primary production could be significantly greater than the value estimated from ^{14}C measurements.

During early September, when daylight was <13 hours per day, P_{max} and P_{min} occurred around noon and midnight, respectively (*18, 19*) (Fig. 1, A and B). As the daylight portion of the photoperiod increased (*20*), the occurrence of P_{max} progressively shifted to later in the day until the period between late October and January (*21*) when it occurred at about midnight (Fig. 1, D and E). There was a concomitant shift in the occurrence of P_{min} from midnight to midday (Fig. 1). The low photosynthetic capacity during the midday period was not the result of photoinhibition of photosynthesis. The amplitude of the P_{max}:P_{min} ratio increased (Table 1) from between 2 and 3 in September to 15 in November through January (*21*). Both the photosynthetic capacity (P^B_m) and the slope (α) of the light-limited region of the photosynthesis-irradiance (*P-I*) relation (*18, 19*) had similar diel patterns and were highly correlated (Fig. 2) (*22*). A linear relation between P^B_m and α requires proportional changes in both parameters; thus, the light-saturation parameter, I_k (*22*), and the relative shapes of the *P-I* curves (*23*) remained relatively constant throughout the season. Superimposed upon these diel patterns of photosynthesis was a seasonal decrease in maximum photosynthetic capacity (*24*) from 8 to 1 μg of carbon per microgram of chlorophyll *a* per hour (Table 1). The periodicity in the *P-I* relationship was independent of changes in concentrations of chlorophyll *a* and inorganic nutrients. The concentration of chlorophyll *a* was measured coincidently with photosynthesis and showed only minor diel oscillations (Table 1). Dissolved inorganic nitrogen, phosphorus, and silicon in the upper 50-m depths were >20, >4, and >25 μ*M*, respectively. Throughout this period, the phytoplankton species composition was relatively constant (*25*); thus seasonal shifts in the diel patterns of photosynthesis reflected physiological responses to changes in the photoperiod or irradiance. Species-specific diel oscillations in photosynthesis were also measured for several of the more abundant diatoms in our samples by means of single-species radioisotope techniques (*19, 26*): these diel patterns were qualitatively similar to those of the natural assemblage.

In some temperate phytoplankton, persistent endogenous rhythms of photosynthesis can be entrained to alternating cycles of light and dark by a circadian oscillator (*27*). During September and early October, when incident irradiance was periodic, the diel patterns of photosynthesis of polar (Fig. 1, A through C) and temperate phytoplankton were qualitatively similar; daily maxima and minima occurred during the light and dark periods, respectively (*12–14*). During the remainder of the austral spring and summer, diel patterns differed from those of temperate phytoplankton: P_{max} occurred at midnight and P_{min} at midday (Fig. 1, D and E). Although circadian rhythms of photosynthesis can persist for several days to several weeks in algal cultures maintained in

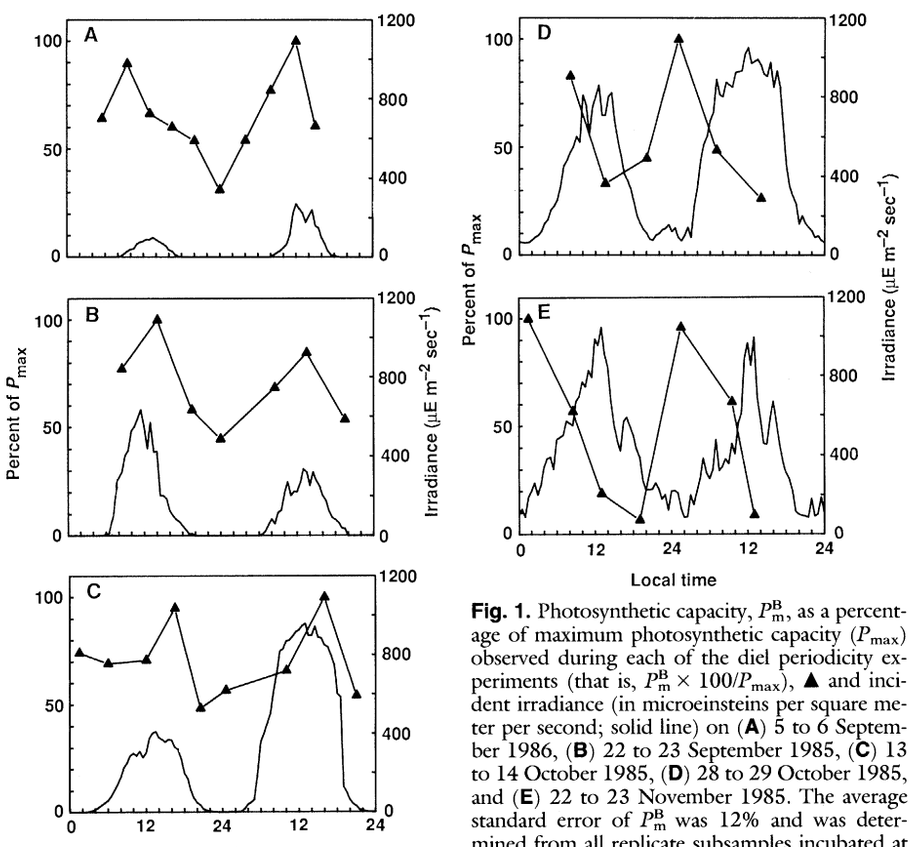

Fig. 1. Photosynthetic capacity, P^B_m, as a percentage of maximum photosynthetic capacity (P_{max}) observed during each of the diel periodicity experiments (that is, $P^B_m \times 100/P_{max}$), ▲ and incident irradiance (in microeinsteins per square meter per second; solid line) on (**A**) 5 to 6 September 1986, (**B**) 22 to 23 September 1985, (**C**) 13 to 14 October 1985, (**D**) 28 to 29 October 1985, and (**E**) 22 to 23 November 1985. The average standard error of P^B_m was 12% and was determined from all replicate subsamples incubated at photosynthesis-saturating irradiances (n = 6 to 12).

Table 1. Daily insolation and summary of photosynthetic and biomass characteristics during the diel periodicity experiments.

Date (1985)	Incident insolation*	P_{max}†	P_{max}:P_{min}‡	Chl *a*§	Max:min‖	Ratio of daily production¶
9/5 9/6	1.74 3.96	8.1	3.2	4.4 (7.6)	1.2	0.9
9/22 9/23	13.6 6.96	3.4	1.8	3.8 (11)	1.1	0.7
10/13 10/14	13.1 31.3	1.2	2.2	32 (13)	1.6	0.8
10/28 10/29	28.1 37.9	3.5	3.8	74 (13)	1.4	1.5
11/22 11/23	61.8 50.4	0.9	15	52 (16)	1.6	3.1

*Daily incident insolation (einsteins per square meter per day). †Maximum photosynthetic capacity (in micrograms of carbon per microgram of chlorophyll *a* per hour). ‡Ratio of maximum to minimum photosynthetic capacity. §Mean chlorophyll *a* concentration (nanograms per liter). Chlorophyll *a* was analyzed at 4- to 5-hour intervals during each periodicity experiment. The coefficients of variation (in parentheses) were calculated from all replicate samples during each experiment (n = 18 to 24). ‖Ratio of maximum to minimum concentration of chlorophyll *a* during each diel periodicity experiment. ¶Ratio of daily production calculated from (i) time-of-day corrected photosynthetic characteristics and (ii) a 6-hour (1200 to 1800) incubation extrapolated to a 24-hour incubation.

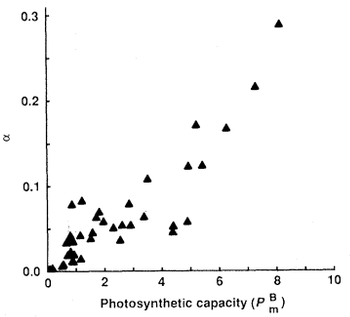

Fig. 2. Relation between 39 coordinate pairs of photosynthetic capacity P^B_m (in micrograms of carbon per microgram of chlorophyll *a* per hour) and α (in micrograms of carbon per microgram of chlorophyll *a* per hour/[microeinsteins per square meter per second]) during the five diel periodicity experiments. Each point represents a single photosynthesis-irradiance curve. The equation describing the linear relation is in (*22*).

constant dim light (*27, 28*), it is unlikely that the diel oscillation of photosynthesis in these polar phytoplankton was simply a persistent endogenous rhythm entrained during the early austral spring. From mid-October through mid-February (*20*) incident irradiance is continuous but not constant; diel variations in intensity of five- to tenfold are typical. These diel oscillations in incident irradiance may entrain and maintain the diel periodicities of photosynthesis.

The spatial and temporal distribution of phytoplankton biomass and primary production in the Southern Ocean is highly variable (*7, 10, 11*). Production estimated from seasonal chemical and geochemical changes (*3–6*) is two to four times greater than that measured by ^{14}C uptake (*7*). Since most studies of primary production were done during the austral summer and were based on single, midday incubations when photosynthetic capacity is at its daily minimum (Fig. 1, D and E) (*21*), daily primary production may have been significantly underestimated. Part of the apparent discrepancy between direct and indirect estimates of production may be resolved by considering the influences of the diel oscillations of photosynthesis on the measurement of primary production. During September and early October (when there was a dark period), daily production estimates from a 6-hour midday incubation, or from time-of-day corrected photosynthetic characteristics, were similar (Table 1). In contrast, during the austral summer, daily production calculated from time-of-day corrected photosynthetic characteristics (Table 1 and Fig. 1) (*21*) was about three times greater than predicted from a single 6-hour midday incubation (*29*). The direct measurement of ^{14}C uptake was 2.6 to 4.2 times greater during 24 hours than either 6-hour or 12-hour incubations (*30*). Our results, combined with those on the role of episodic high production in the marginal ice zone (*9, 10*), support the classic view (*31*) of a highly productive Southern Ocean.

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18. Samples were collected in a 10-liter Niskin bottle at a depth of 15 to 20 m at 4- to 5-hour intervals for 36 to 48 hours through a hole (1m in diameter) in the annual sea ice at our seasonal field station, 25 km north of Cape Armitage in McMurdo Sound (78°25′S, 166°30′W). Photosynthesis was measured within 30 minutes of collection by inoculating samples with $NaH^{14}CO_3$ (final activity 0.25 to 0.40 μCi/ml) and incubating replicate (n = 2 to 4) 1-liter subsamples, at −1.8°C for 1 to 2 hours at 6 to 7 irradiances (1 to 400 μE m^{-2} sec^{-1} provided by SHO fluorescent lights F48T12 CW/SHO, 1500 mA). The particulate material collected on GF/C filters was oxidized with 0.2*N* perchloric acid, and radioactivity was counted with Biofluor (New England Nuclear) as a scintillant. Chlorophyll *a* was analyzed fluorometrically (*19*). The photosynthesis-irradiance (*P-I*) relation parameters of photosynthetic capacity (P^B_m); in micrograms of carbon per microgram of chlorophyll *a* per hour and the slope of the light-limited portion of the *P-I* curve, α, in micrograms of carbon per microgram of chlorophyll *a* per hour/[microeinstein per square meter per second]) were derived from the hyperbolic tangent function [A. D. Jassby and T. Platt, *Limnol. Oceanogr.* **21**, 540 (1976)]. Photoinhibition of photosynthesis was typically not observed during these experiments (R. B. Rivkin and M. Putt, in preparation).
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22. The equation for the linear relation between P_{max} and α for the 39 *P-I* curves measured during the five diel experiments is:

$$\alpha = 0.0008(\pm\ 0.0003) + 0.0257(\pm\ 0.0023)P_{max} \qquad n = 39$$

The values in parentheses are the standard errors. The inverse of the regression coefficient is the light-saturation parameter I_k = 39 ± 4 μE m^{-2} sec^{-1} [C. S. Yentsch and R. W. Lee, *J. Mar. Res.* **24**, 319 (1966)].
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29. Daily production calculated from the time-of-day corrected photosynthetic characteristics was compared to the carbon incorporated during a 6-hour incubation (1200 to 1800) extrapolated to 24 hours. Although we have assumed that photosynthesis was light-saturated throughout the day, since α and P_m^B covaried (*22*), the diel patterns of photosynthesis would be similar at both photosynthesis-limiting and saturating irradiances. Incident irradiances during the austral summer are typically >200 μE m^{-2} $sec.^{-1}$ at midnight and 1200 to 1600 μE m^{-2} sec^{-1} at noon. If we assume a diffuse attenuation coefficient of 0.15 m^{-1} [(*5, 8*); R. B. Rivkin, unpublished data] and an I_k of 39 μE m^{-2} sec^{-1}, photosynthesis would be light-saturated in the upper 10-m depths at midnight and 15- to 25-m depths during the day. Similar irradiances saturated photosynthesis for phytoplankton assemblages collected both under the sea ice and in open water at the ice edge (*19*).
30. During time-course incubations under natural photoperiods and at photosynthesis-limiting and saturating irradiances in December and January, carbon uptake was 2.6 to 4.2 times greater for samples incubated for 24 hours than during either 6-hour (0600 to 1200) or 12-hour (0600 to 1800) incubations extrapolated to 24 hours. Daily production was 950 to 1200 mg of carbon per square meter per day and 225 to 356 mg of carbon per square meter per day for 24-hour and 6- or 12-hour incubations, respectively {a euphotic zone depth of 0.1% incident irradiance was assumed [(*5, 11*); R. B. Rivkin, unpublished data]}.
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A G Protein Directly Regulates Mammalian Cardiac Calcium Channels

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A possible direct effect of guanine nucleotide binding (G) proteins on calcium channels was examined in membrane patches excised from guinea pig cardiac myocytes and bovine cardiac sarcolemmal vesicles incorporated into planar lipid bilayers. The guanosine triphosphate analog, GTPγS, prolonged the survival of excised calcium channels independently of the presence of adenosine 3′,5′-monophosphate (cAMP), adenosine triphosphate, cAMP-activated protein kinase, and the protein kinase C activator tetradecanoyl phorbol acetate. A specific G protein, activated G_s, or its α subunit, purified from the plasma membranes of human erythrocytes, prolonged the survival of excised channels and stimulated the activity of incorporated channels. Thus, in addition to regulating calcium channels indirectly through activation of cytoplasmic kinases, G proteins can regulate calcium channels directly. Since they also directly regulate a subset of potassium channels, G proteins are now known to directly gate two classes of membrane ion channels.

GUANINE NUCLEOTIDE BINDING (G) proteins couple a variety of plasma membrane receptors to voltage-dependent calcium channels, and, for cardiac β-adrenoreceptors, the mechanism is indirect and involves cytoplasmic second messengers (*1*). G proteins can also directly couple membrane receptors to ion channels independently of cytoplasmic mediators. The G protein G_k directly activates subsets of potassium channels that normally are activated through muscarinic cholinergic (*2*) or somatostatin receptors (*3*), or both. In this report, we tested whether G proteins also modulate Ca^{2+} channels directly.

Single Ca^{2+} channel activity was recorded in guinea pig ventricular myocytes, first in the cell-attached configuration and then, after excision, in the inside-out configuration. We compared voltage-dependent activation of Ca^{2+} channels under different experimental conditions (Table 1), using a protocol in which the membrane patches were subjected to depolarizing test pulses of 200-msec duration at 0.5 Hz, before and after excision. Confirming a well-known finding, activatable responses survived only briefly after excision from nonstimulated cells (*4*). However, survival was enhanced by stimulation of channel activity during the cell-attached mode by the β adrenoreceptor agonist isoproterenol (ISO). For quantification, we both counted the number of test pulses during which activity was observable after excision (Table 1) and evaluated the current conducted by the membrane patches during the depolarizing pulses (Fig. 1). The results depend on the number (N) of channels in the patch and on their probability of opening during the test pulse. To provide a record of the entire experiment we measured, before and after patch excision, the proportion of open time (P) for the N channels in the patch (NP) for each pulse (Fig. 1, row 2). NP values were also summed to give cumulative activities in each patch (Fig. 1, row 3). To normalize for patch to patch variations in channel number, the cumulative activities summed over comparable number of traces (usually 30) before (cum NP_{CA}) and after (cum NP_{IO}) patch excision were compared (Table 1). All the results were from high-threshold Ca^{2+} channels; no low-threshold, dihydropyridine-insensitive Ca^{2+} channels (*5*) were observed, and polarizing the membrane to −90 mV from the usual values between −40 and −60 mV before applying test pulses failed to unmask any that might have been inactivated.

A small number of events was recorded in nonactivated control cells; the test potentials used to detect Ca^{2+} channel activity in cell-attached patches produced average opening probabilities (P_o's) of 0.01 to 0.05 at room temperature. The very brief survival after excision provided few data for comparison (Table 1). Consequently, we tried to increase NP with ISO. When ISO at $10^{-5}M$ was present only in the patch pipette ($n = 7$), P_o seemed greater, and prolonged openings, although still less than 1% of the events (*6*), seemed more frequent. Activity was not recorded without ISO in the pipette, so the significance of the changes was unclear (*7*). We then added ISO at $10^{-6}M$ to the bath; this was followed after several seconds by a large increase in NP ($n = 3$), confirming previous reports (*8*). Subsequently, we incubated the myocytes with ISO ($10^{-6}M$). The increase in NP was due to both increased frequency of opening and prolongation of events (*8*) and was associated with an increase in survival (Table 1). After excision, the mean open times were unchanged but the amplitudes could change, reflecting a change in driving force (*9*). When guanosine 5′-*O*-(3-thio)triphosphate (GTPγS) (100 μM) was present in the bath, survival was enhanced considerably (Fig. 1 and Table 1). In fact, activity was

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