sions during periods of strong stratification.

Note added in proof: A survey of oyster bars in the Choptank River on 6 to 7 September 1984 along a transect with bathymetries from 3 to 10 m revealed the mortality of all shellfish as well as their fouling organisms on bars below 6 m. Seed-bed areas and bars above 5 m were unaffected. This has since been verified by two independent surveys, involving personnel of the University of Maryland Sea Grant program and the Maryland Department of Natural Resources. During 4 to 12 September a precipitous drop in daily air temperatures by 12°C to below surface water temperatures produced sufficient instability to erode the pycnocline downward. This terminated the shallow pycnocline-anoxic water conditions in the tributaries, saving the remaining benthic stocks above 6 m. This finding raises two questions. Have earlier anoxic excursions occurred unnoticed and contributed to the decline of shellfish populations in the bay? Is the Chesapeake Bay ecosystem now less stable to extremes of climatic events than it was in the past?

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References and Notes

1. C. L. Newcombe and W. A. Horne, *Science* **88**, 80 (1938); C. L. Newcombe *et al.*, *J. Mar. Res.* **2**, 87 (1939).
2. J. L. Taft, W. R. Taylor, E. O. Hartwig, R. Loftus, *Estuaries* **3**, 242 (1980).
3. C. B. Officer, R. B. Biggs, J. L. Taft, L. E. Cronin, M. A. Tyler, W. R. Boynton, *Science* **223**, 22 (1984).
4. P. G. Falkowski, T. S. Hopkins, J. J. Walsh, *J. Mar. Res.* **38**, 479 (1980).
5. R. L. Swanson and C. J. Sindermann, *Natl. Oceanic Atmos. Adm. Prof. Pap. 11* (1979).
6. "Estimated Streamflow Entering Chesapeake Bay" (monthly summary available from the U.S. Geological Survey, Washington, D.C.).
7. K. F. Bowden, in *Estuarine Transport Processes*, B. Kjerfve, Ed. (Univ. of South Carolina Press, Columbia, 1978), pp. 11–36.
8. $<Q_f>$ is the monthly mean river discharge rate in cubic meters per second; A is the vertical cross-sectional area at the Bay Bridge in square meters; $\Delta\rho_t$ is the mean monthly density difference between the bottom and the surface of the water column; g is the acceleration of gravity, 9.8 m sec^{-2}; $<|U_t|^3>$ is the mean of the cube of the absolute value of the tidal velocity over a tidal cycle; C_D is the frictional coefficient at the water-sediment interface; and $<h>$ is the mean height of the water column in meters. In the Chesapeake Bay at the Bay Bridge area, $A = 7.63 \times 10^4$ m^2 [W. B. Cronin, *Chesapeake Bay Inst. Spec. Rep. 20* (1970), reference 71-2]; $C_D = 0.0025$ [R. D. Pingree and D. K. Griffiths, *J. Geophys. Res.* **83**, 4615 (1978)], $<h> = 10.5$ m; and U_t(max) is assumed to be 0.41 m sec^{-1}. We estimated the value of $<|U_t|^3>$ by assuming a sine function for the tidal velocity so that $<|U_t|^3> = 2/3\ |U_t(\max)|^3$.
9. Fish larvae (menhaden, spot, croaker) from spawning areas in coastal waters during winter-early spring [A. J. Lipson and R. L. Moran, *State Md. Power Plant Siting Program PPSP-MP-13* (December 1974); J. Nelson, *Rep. N.J. Agric. Exp. Stn. 183* (1911)]; diatoms during winter-spring (M. A. Tyler and H. H. Seliger, in preparation); *Prorocentrum minimum* during spring [M. A. Tyler and H. H. Seliger, *Limnol. Oceanogr.* **23**, 227 (1978); *ibid.* **26**, 310 (1981); H. H. Seliger, M. A. Tyler, K. R. McKinley, in *Toxic Dinoflagellate Blooms*, D. L. Taylor and H. H. Seliger, Eds. (Elsevier, New York, 1979), p. 239]; *Ceratium furca* in the southern bay in summer; oyster larvae within the tributaries in summer [H. H. Seliger, J. A. Boggs, R. B. Rivkin, W. H. Biggley, K. R. H. Aspden, *Mar. Biol.* **71**, 57 (1982)]; and phytoplankton recirculation and patchiness [H. H. Seliger *et al.*, *Mar. Biol.* **61**, 119 (1981)].
10. J. Namias and D. R. Cayon, *Oceanus* **27**, 41 (1984).

11. Major support for this research was furnished by NSF grant OCE-8208745. Additional funding beginning in July 1984 was provided by University of Maryland Sea Grant (National Oceanic and Atmospheric Administration). We gratefully acknowledge the participation of A. Brandt, C. Sarabun, and we thank M. A. Tyler for discussions and collaborative efforts in documenting this anoxia during May 1984. We thank Captain J. Wimsatt and the crew of the R.V. *Ridgeley Warfield* (Chesapeake Bay Institute) and Captain W. Keefe of the R.V. *Aquarius* (University of Maryland) for their cooperation.

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Circadian Timing of Cancer Chemotherapy

Abstract. *Animal studies have indicated that the time of administration of adriamycin and cisplatin, two widely used anticancer drugs, has a profound effect on their toxicity. This effect in cancer chemotherapy was studied in 31 patients with advanced ovarian cancer. Patients received at least eight monthly courses of adriamycin that were followed 12 hours later by cisplatin, with adriamycin randomly administered at either 6 a.m. or 6 p.m. The results show that in the group receiving adriamycin in the evening and cisplatin in the morning (i) twice as many patients required reductions in dosage and delays in treatment, (ii) four times as many treatments had to be delayed, (iii) drug dosages had to be modified downward three times as often, and (iv) even with more dose attenuation and treatment delays, treatment complications were still about two times more common as in the group receiving adriamycin in the morning and cisplatin in the evening. These findings show that the circadian stage at which anticancer drugs are given to patients should be carefully considered.*

Chronobiologic investigations have shown that the therapeutic indices of drugs can be affected by varying circadian drug timing. This phenomenon may be particularly important in anticancer chemotherapy. These powerful drugs can kill cancer cells but also kill or severely injure cells of normal tissues. Since the susceptibility of the normal tissues is rhythmically variable during the circadian cycle, whereas that of malignant tissue may be less so, the timing of chemotherapy may be an important element allowing greater therapeutic specificity.

The toxicity of at least 11 commonly used anticancer drugs has been shown in animal studies to depend on the time of administration (*1–5*). The therapeutic index of these agents and curability of several transplantable cancers in mice or rats are governed by the circadian time of drug treatment (*1, 2, 6*). Indeed, animal experiments had clearly indicated that the greatest toxicity of adriamycin is late in the activity cycle of the rodent and that of cisplatin near awakening (*7–11*). A test of whether circadian treatment time would affect human beings was designed.

A preliminary crossover study of 21 patients showed that post-treatment blood count fall and recovery, extent of decrement in creatinine clearance, and drug pharmacokinetics all differ substantially, depending on when patients are treated (*12–14*). Adriamycin given shortly before usual awakening (6 a.m.) and followed 12 hours later by cisplatin was better tolerated during the month after treatment than was this therapy when begun in the evening (usually 6 p.m.) (*15*). Because of these interesting acute toxicity results, a simple two-arm randomization of treatment timing was initiated.

Thirty-one women suffering from histologically verified ovarian cancer were each treated with an average of eight courses of adriamycin and cisplatin chemotherapy. None had received prior chemotherapy or irradiation therapy. All had widespread disease, which was limited to the abdomen in 22 (Figo stage 3), and which was also extra-abdominal in 9 patients (Figo stage 4). After patients were stratified by stage, the circadian treatment schedule was randomly allocated to each patient. The order of the two drugs and the span between them was kept constant. The planned doses were 60 mg per square meter of body surface for each drug. Circadian schedule A consisted of adriamycin infused between 6 a.m. and 6:30 a.m. (at or just before usual wakening) and followed 12 hours later by cisplatin from 6 p.m. to 6:30 p.m. Schedule B was administration of adriamycin at 6 p.m. and cisplatin at 6 a.m. This regimen was repeated approximately monthly to a planned total of nine treatments giving a total of 247 courses

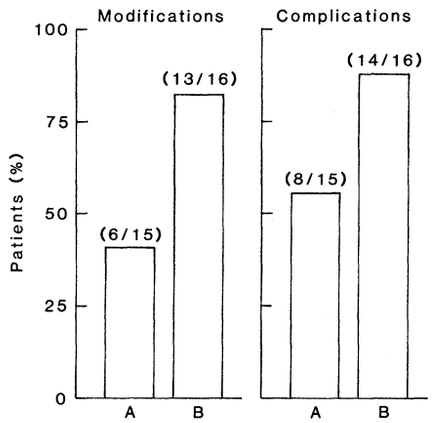

Fig. 1. Even though patients on schedule B required more reductions in dosage and more delays in treatment than those on schedule A, therapy-associated complications that included infections, transfusions, and bleeding still occurred more commonly in schedule B patients.

of therapy for evaluation. The cisplatin hydration regimen is begun immediately following the 30-minute adriamycin infusion. Normal saline with additional potassium chloride (20 meq per liter) was infused at a rate of 200 cm^3 per hour for 7 hours. This rate was increased to 350 cm^3 per hour 5 hours before the planned cisplatin infusion and was maintained for 4 hours after cisplatin administration. The average hourly urine output during the 9-hour peri-cisplatin infusion span for these patients approached 200 cm^3/hour. No diuretics were used.

Evaluation of toxicity after each of the 247 treatment courses included 8 a.m. weekly hemoglobin, total and differential white blood cell count, platelet count, and creatinine clearance. These weekly laboratory values combined with a monthly interim history and physical examination served to guide dose and schedule modifications.

Modifications of adriamycin dosage or schedule delays were forced by (i) a recovery (day 28) absolute granulocyte count below 1500 cells per cubic millimeter, (ii) a recovery platelet count under 100,000 cells per cubic millimeter, or (iii) interim infection or bleeding. Infection is defined as fever with temperature greater than 102°F during leukopenia that responds to the administration of antibiotics or culture-proven symptomatic infection requiring antibiotic administration. If any of these conditions obtained, a 25 percent reduction in adriamycin dosage or a 1-week treatment delay with subsequent reevaluation was instituted. Adriamycin dosages were more often reduced if an infection or bleeding complication supravened, and treatment delays were more common with a poor recovery of blood cell counts. Cisplatin was discontinued if creatinine clearance fell below 30 cm^3/mm.

Treatment complications were defined as interim clinical infections as described above, interim bleeding episodes of any kind whether or not platelet transfusions were administered, and anemia requiring a transfusion episode in order to maintain the hemoglobin level above 10 g per deciliter. Each transfusion episode usually required administration of three units of packed red blood cells.

The toxicity of chemotherapy was assessed in two ways: first, the number of patients from each timed (with regard to circadian rhythms) treatment group who suffered treatment complications or who had to have treatment modifications was compared; second, the number of individual monthly courses that were followed by treatment complications or that were modified was contrasted.

More patients treated on schedule B had to have both dose reductions and treatment delays (6 of 15 compared to 13 of 16 patients; $\chi^2 = 4.0$, $P < 0.05$). Individuals receiving diminished drug dosages and modified schedules would be expected to have fewer complications. However, even though schedule B patients had more dose reductions and more frequent treatment delays, episodes of infection, bleeding, and transfusion each occurred more commonly in these patients than in those treated more frequently and with higher doses of chemotherapy on schedule A (8 of 15 compared to 14 of 16; $\chi^2 = 2.9$, $0.1 > P > 0.05$) (Fig. 1).

A total of 247 courses of adriamycin-cisplatin therapy were administered; 115 of schedule A and 132 of schedule B. Dose reductions were three times more frequent when the drugs were on schedule B (31 percent compared to 9 percent; $\chi^2 = 17.4$, $P < 0.01$). Treatment delays were four times more frequent on schedule B (17 versus 4 percent, $\chi^2 = 8.4$, $P < 0.01$). Despite dose reductions and treatment delays for patients on schedule B, 44 percent of schedule B treatment regimes were associated with bleeding, infection, or transfusion requirement, whereas only 23 percent of schedule A treatments were associated with these complications ($\chi^2 = 10.5$, $P < 0.01$) (Fig. 2).

In the last 40 years, reports of the dependence of toxicity on the circadian stage of drug administration for the mouse and rat have been plentiful. The toxicity, therapeutic efficacy, or both, of alcohol, theophylline, steroids, adrenocorticotropic hormone, and other drugs are clearly time-dependent in human beings. There is every reason to expect this to be the case for most, if not all, toxins and medications. These circadian susceptibility and efficacy rhythms may be especially pertinent for drugs whose toxic-therapeutic ratios are narrow such as anticancer drugs.

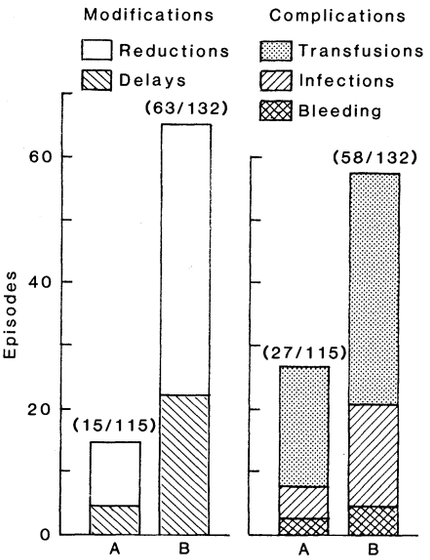

Fig. 2. For every reduction in dosage on schedule A there were three on schedule B. Four times as many treatment delays were required for schedule B as for schedule A patients. Despite more treatment modifications, nearly twice as many complications resulted from schedule B as from schedule A treatments.

This investigation indicates that circadian timing of administration of adriamycin and cisplatin, which are the two most commonly used anticancer drugs in the United States, influences toxicity substantially. Administration of adriamycin in the morning and cisplatin in the evening caused fewer complications, dose reductions, and treatment delays than administration of adriamycin in the evening and cisplatin in the morning. Too few patients have been studied to determine whether or not the timing of treatment affects tumor response or patient survival. There is, however, increasing evidence that any compromise of dosage or delays in treatment schedule diminish the likelihood of cancer control or cure (*16*).

The practical problem of administering chemotherapy at a specific circadian stage also deserves comment. The current practice of medicine largely disregards timing of drug administration. Both inpatient and outpatient medical practice make the precise stipulation of drug timing impossible. Even in medical

oncology wards where good communication among staff is the norm, precise drug timing cannot be achieved. However, implantable and extracorporeal programmable drug delivery systems will make clinical "chronotherapy" both possible and economical. Devices now available time therapy with an external clock, but a third generation of "closed loop" devices will eventually time each dose to the individual patient's internal circadian clock.

The most crucial element in the successful application of cancer chemotherapy is optimization of the antineoplastic effects and minimization of secondary toxicity to normal tissues, that is, selectivity (*17*). One approach to increasing this selectivity is administration of these highly toxic drugs at times associated with their best tolerance.

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References and Notes

1. F. Halberg, *Am. J. Anat.* **168**, 545 (1983).
2. L. E. Scheving, in *Cell Cycle Clocks*, L. N. Edmunds, Ed. (Dekker, New York, 1984), pp. 455–499.
3. W. J. M. Hrushesky, *Am. J. Anat.* **168**, 519 (1983).
4. W. J. M. Hrushesky *et al.*, in *Chronobiology: Principles and Applications to Shifts in Schedules*, L. E. Scheving and F. Halberg, Eds. (Sijthoff & Noordhoff, Alphen aan der Rijn, Netherlands, 1980), pp. 513–533.
5. F. Levi, W. Hrushesky, E. Haus, F. Halberg, L. E. Scheving, B. J. Kennedy, in *Chronobiology: Principles and Applications to Shifts in Schedules*, L. E. Scheving and F. Halberg, Eds. (Sijthoff and Noordhoff, Alphen aan der Rijn, Netherlands, 1980), pp. 481–511.
6. E. Haus *et al.*, *Science* **177**, 80 (1972).
7. R. B. Sothern, F. Halberg, H. H. Zinneman, B. J. Kennedy, in *Chronopharmacology and Chronotherapeutics*, C. A. Walker, C. M. Winget, K. F. A. Soliman, Eds. (Florida A&M University Foundation, Tallahassee, 1981), pp. 269–273.
8. W. J. Hrushesky, F. A. Levi, F. Halberg, B. J. Kennedy, *Cancer Res.* **42**, 945 (1982).
9. F. A. Levi *et al.*, *ibid.*, p. 950.
10. F. Levi, W. Hrushesky, F. Halberg, E. Haus, T. Langevin, B. J. Kennedy, *Eur. J. Cancer Clin. Oncol.* **18**, 471 (1982).
11. F. Levi *et al.*, *Cancer Treat. Rep.* **66**, 1933 (1982).
12. W. J. M. Hrushesky, *Ann. N.Y. Acad. Sci.* **397**, 293 (1982).
13. ———, R. Borch, F. Levi, *Clin. Pharmacol. Ther.* **32**, 330 (1982).
14. W. J. M. Hrushesky, in *Platinum Coordination Complexes in Cancer Chemotherapy*, M. Hacker, E. B. Douple, I. H. Krakoff, Eds. (Nijhoff, The Hague, Netherlands, 1984), pp. 165–186.
15. W. J. M. Hrushesky, in *Toxicity of Chemotherapy*, M. Perry and J. Yarbro, Eds. (Grune & Stratton, New York, 1984), pp. 449–477.
16. V. DeVita, *Cancer Lett.* **10**, 1 (1984); W. Hryniuk and H. Bush, *J. Clin. Oncol.* **2**, 1281 (1984).
17. G. Elion, address at the meeting of the American Association of Cancer Research, Toronto, Canada, 9 May 1984.
18. I thank V. DeVita, S. Carter, and M. Slavik for bringing the work of F. Halberg to my attention; F. Halberg for guidance and advice; and B. J. Kennedy for constant support. Supported by the NCI grant R01 CA 31635 and the Clinical Research Center grant 5M-01RR00400.

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Evidence for a Malarial Parasite Interaction Site on the Major Transmembrane Protein of the Human Erythrocyte

Abstract. *Soluble oligosaccharides derived from the surface of human erythrocytes were tested for their ability to competitively inhibit invasion of erythrocytes by* Plasmodium falciparum, *a malarial parasite. Invasion was most effectively inhibited by erythroglycan, a carbohydrate component of the band 3 transmembrane protein. The lactosamine chains of erythroglycan contributed much of the inhibitory activity. This indication of a primary parasite interaction site on band 3 supports a role for this protein in mediating the radical alterations of the erythrocyte cytoskeleton that accompany invasion.*

The molecular interactions at the cell surface which determine and control the infection of a human erythrocyte by the falciparum malaria parasite are critical to the life cycle of the parasite and the course of disease (*1*). During invasion the parasite binds to and deforms the erythrocyte membrane and enters the cell by a process resembling endocytosis. In so doing, it subverts the elements of the erythrocyte membrane responsible for maintaining cell shape and integrity. Because the mechanism of this subversion is unknown, we undertook to identity the components of the erythrocyte membrane with which the parasite interacts. We report that the oligosaccharide portion of the band 3 protein, erythroglycan (*2, 3*), is a primary interaction site for the *Plasmodium falciparum* merozoite (*4*).

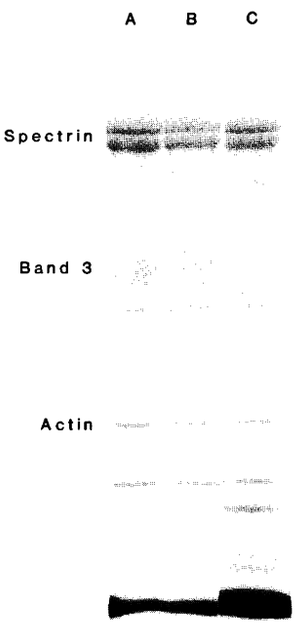

Fig. 1. (A to C) Effect of trypsin on erythrocyte membrane proteins. Washed human erythrocytes (10 percent by volume) were incubated for 60 minutes at 37°C with shaking in 25 m*M* Hepes, 135 m*M* NaCl, 5 m*M* $CaCl_2$ (*p*H 7.4), and tosylamide phenylethyl chloromethyl ketone–trypsin (Worthington). After two washes in 100 volumes of 25 m*M* Hepes and 135 m*M* NaCl (*p*H 7.4) (HBS), the cells were treated with 1 m*M* phenylmethylsulfonyl fluoride (Sigma) in HBS for 20 minutes at 22°C and washed two more times. Membranes from 10^8 cells were prepared by hypotonic lysis in 50 volumes of 10 m*M* tris-HCl (*p*H 7.8), washed two times in the same buffer, and analyzed by SDS-PAGE (*22*). The effects of trypsin at 0, 0.075, and 1 mg/ml are shown in (A), (B), and (C), respectively.

Ultrastructural (*5*) and biochemical studies of encounters between malarial parasite merozoites and erythrocytes reveal two distinct interactions: (i) initial attachment, mediated by erythrocyte surface sialic acid (*6*), and (ii) subsequent formation of a tight junction between the anterior end of the merozoite and the erythrocyte membrane. Freeze-fracture studies of the junction zone have shown that junction formation is associated with a closely packed assembly of intramembranous particles in the erythrocyte membrane. During invasion the junction zone changes from a cap to a constricting ring through which the merozoite enters an endocytotic vacuole of the host cell.

The cytoskeleton of the erythrocyte membrane is a network of structural proteins with unique dynamic interactions that allow the cell to first resist but then accommodate external forces (*7*). A spectrin-actin mesh underlies the lipid bilayer (*8*) and is in part responsible for preventing internalization of membrane by endocytosis. Thus antibody to spectrin within the cell can prevent drug-induced endocytosis by strengthening the spectrin network and preventing formation of the spectrin-free domain associated with endocytotic events (*9*). The spectrin-actin network is anchored to the membrane by the transmembrane proteins of the erythrocyte. Band 3, the major transmembrane protein, is exposed to the outside, crosses the bilayer several times, and is firmly bound to the cytoskeleton (*10*). Glycophorin A is normally attached weakly or not at all, but if the externally exposed domain of glycophorin A is constrained by antibody or lectin binding, this glycoprotein also becomes firmly bound to the skeleton (*11*).

It is reasonable to guess that the tight