brane-bound, and their activities are affected by many of the same substances that affect circadian rhythms (*9*). In addition, recent measurements on mammalian cells in culture have demonstrated fluctuations in cyclic AMP levels associated with the cell cycle (*10*) and have suggested the possibility of an oscillatory feedback system in which the levels of cyclic AMP itself affect the synthesis of these two enzymes or their activities (*11*). In view of the possible relation between circadian clock cycles and cell cycles (*12*), oscillations in cyclic AMP levels offer an attractive possibility for a role in the control of circadian clocks. This report presents our initial attempts to determine whether cyclic AMP is involved in the circadian clock of *Neurospora* and shows a lengthening of the period of the circadian conidiation rhythm by three methyl xanthine inhibitors of cyclic AMP phosphodiesterase.

The following strains of *Neurospora crassa* were used in these experiments: *band*, which serves as the "wild type" (*13*), and three mutants derived from *band*, each of which has a period length different from *band*: *frq-1*, *frq-2*, and *frq-3* (*14*). All culture conditions, media, and procedures for measuring period length of the conidiation rhythm on "race" tubes were as previously described (*14*).

Theophylline, an inhibitor of *Neurospora* cyclic AMP phosphodiesterase (*15*), causes a small increase in the period lengths of the conidiation rhythm in wild-type and in each of the three mutant strains (see Fig. 1). Aminophylline, a derivative of theophylline that enters the cells more readily than theophylline and therefore inhibits phosphodiesterase in vivo at lower concentrations than theophylline (*16*), also causes a significant period lengthening and, as expected, at lower concentrations than theophylline. Finally, caffeine, another inhibitor of cyclic AMP phosphodiesterase (*15*), also causes significant increases in period length (Fig. 1).

Although at high concentrations all of these compounds are toxic to the organism, period lengthening occurred at concentrations that correspond to those necessary to inhibit phosphodiesterase (*15*) and that did not inhibit growth rate. In fact some low concentrations actually caused a small increase in linear growth rate (Table 1), a phenomenon similar to that previously reported in *Neurospora* (*16*).

These results indicate that inhibitors of cyclic AMP phosphodiesterase lengthen the period of the *Neurospora* clock. In addition, at least one previous report (*17*) demonstrated a period lengthening effect of theophylline and caffeine in higher plants, although their relation to cyclic AMP metabolism was not recognized at that time.

Table 1. Effect of aminophylline on linear growth rate of *Neurospora*. All standard deviations were 0.2 cm or less.

Strain	Linear growth rates (cm/day) at the following concentrations (m*M*) of aminophylline			
	0	1	2	5
frq$^+$	2.51	2.69	3.07	2.74
frq-1	2.48	2.49	3.04	2.70
frq-2	2.57	2.66	3.08	2.72
frq-3	2.46	2.71	2.91	2.74

It would be premature to conclude, however, that such results demonstrate that cyclic AMP is involved directly in the control of the circadian clock. First of all, it is not known definitely that the effect of the drugs is mediated through their inhibition of phosphodiesterase, since they are known to affect other cellular processes, such as ion transport and macromolecular synthesis. However, the effectiveness of all three inhibitors at concentrations known to inhibit *Neurospora* phosphodiesterase and at concentrations that do not inhibit growth tend to reduce, although do not eliminate, this possibility. Even if the inhibitors are acting through phosphodiesterase to change the cyclic AMP levels, it cannot yet be inferred that cyclic AMP metabolism is part of the clock, for this can be done only when changes in endogenous cyclic AMP levels can be correlated quantitatively with changes in the overt behavior of the clock.

Nevertheless, it is interesting that one important property of circadian clocks—namely, sensitivity to light—could be accounted for in terms of cyclic AMP metabolism. In both isolated frog retinal tissue (*18*) and the fungus *Phycomyces* (*19*) visible light causes a significant decrease in the endogenous cyclic AMP levels, and at least for frog retinal tissue, this effect is due to the light activation of phosphodiesterase. This type of phenomenon is exactly what has been predicted in several molecular models for the clock (*20*).

JERRY F. FELDMAN
Thimann Laboratories, University of California, Santa Cruz, Santa Cruz 95064

References and Notes

1. E. Bünning and J. Baltes, *Naturwissenschaften* **49**, 19 (1962); *ibid.* **50**, 622 (1963).
2. J. T. Enright, *Z. Vgl. Physiol.* **72**, 1 (1971); *ibid.* **75**, 332 (1971).
3. V. G. Bruce and C. S. Pittendrigh, *J. Cell Comp. Physiol.* **56**, 25 (1960); J. F. Feldman and V. G. Bruce, *J. Protozool.* **19**, 370 (1972).
4. A. Eskin, *J. Comp. Physiol.* **80**, 353 (1972).
5. E. Bünning and I. Moser, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2732 (1972).
6. B. M. Sweeney, *Plant Physiol.* **53**, 337 (1974).
7. D. Njus, F. M. Sulzman, J. W. Hastings, *Nature (Lond.)* **248**, 116 (1974).
8. G. A. Robison, R. W. Butcher, E. W. Sutherland, *Cyclic AMP* (Academic Press, New York, 1971).
9. G. S. Levey, *Ann. N.Y. Acad. Sci.* **185**, 449 (1971); R. A. Johnson and E. W. Sutherland, *J. Biol. Chem.* **248**, 5114 (1973).
10. M. M. Burger, B. M. Bombik, B. Breckenridge, J. R. Sheppard, *Nat. New Biol.* **239**, 161 (1972).
11. J. P. Schwartz, N. R. Norris, B. Breckenridge, *J. Biol. Chem.* **248**, 2699 (1973).
12. V. G. Bruce, in *Circadian Clocks*, J. Aschoff, Ed. (North-Holland, Amsterdam, 1965), p. 125; C. F. Ehret and J. J. Wille, Jr., in *Photobiology of Microorganisms*, P. Halldal, Ed. (Wiley, New York, 1970), p. 370.
13. M. L. Sargent, W. R. Briggs, D. O. Woodward, *Plant Physiol.* **41**, 1343 (1966).
14. J. F. Feldman and M. N. Hoyle, *Genetics* **75**, 604 (1973).
15. W. A. Scott and B. Solomon, *Biochem. Biophys. Res. Commun.* **53**, 1024 (1973).
16. W. A. Scott, N. C. Mishra, E. L. Tatum, *Brookhaven Symp. Biol.* **25**, 1 (1973).
17. S. Keller, *Z. Bot.* **48**, 32 (1960).
18. N. Miki, S. Keirns, F. Marcus, M. Bitensky, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3820 (1973).
19. R. Cohen, *Nature (Lond.)* **251**, 144 (1974).
20. T. Pavlidis and W. Kauzmann, *Arch. Biochem. Biophys.* **132**, 338 (1969); F. Cummings, *J. Theor. Biol.*, in press.
21. Supported by NIH grants GM-19850 and GM-22144. I thank Marian Hoyle for her excellent assistance.

9 May 1975; revised 30 July 1975

Immunosurveillance of Naturally Occurring Feline Leukemia

Abstract. *When compared to their housemates that subsequently developed leukemia, cats that remained healthy had five- to tenfold higher (geometric mean) humoral antibody titers to the feline oncornavirus-associated cell membrane antigen. This is compatible with the application of the immunosurveillance hypothesis to the natural development of leukemia in an outbred mammalian species.*

People with either the genetic immunologic deficiency diseases or drug-induced immunosuppression have an increased risk for developing lymphoid leukemia or lymphoma (*1*). This type of observation led Burnet to propose that an immunosurveillance mechanism may operate under natural conditions to eliminate malignant cells (*2*). Most studies with inbred mice and other laboratory rodents support this hypothesis (*3*). Some observations, however, such as the apparent lack of an increased risk for development of some types of tumors in genetically athymic (nude) mice have provided arguments against the immunosurveillance concept (*4*). It has been difficult to design experiments that test the application of the immunosurveillance hypothesis to spontaneous neoplasms of outbred mammals because the etiologic agents for naturally occurring tumors are generally unknown,

and antigens specific for tumor cell membranes have rarely been identified. However, in spontaneous leukemias and lymphomas of cats both the causative agent (*5*) and a tumor cell membrane antigen (*6–8*) have been described. We now describe studies with naturally occurring feline leukemia that strongly support the concept of immunosurveillance.

In our previous studies with cats' neoplasms that were induced by inoculation of feline sarcoma virus (FeSV) or feline leukemia virus (FeLV), we found a high correlation between resistance to tumor development or progression and the humoral antibody response to the feline oncornavirus-associated cell membrane antigen (FOCMA) (*6–8*). When cats inoculated with FeSV or FeLV developed progressively growing tumors they had little or no antibody to FOCMA. Inoculated cats that developed regressor tumors or no tumors had both an early response to FOCMA (*9*) and high antibody titers (*6–9*). Virus-neutralizing antibody titers, on the other hand, were not similarly correlated with regression of the tumors (*9*).

We then examined pet cats with spontaneous leukemia or lymphoma, and found that their FOCMA antibody titers were also low or negative (*7, 8, 10*). As a control group analogous to virus-inoculated laboratory cats that remained healthy, we checked healthy cats from leukemia cluster housholds. The healthy cats that were exposed to leukemic cats had both high FOCMA antibody titers and a high frequency of viremia with FeLV (*8, 11*). However, since the cats with spontaneous leukemia or lymphoma had only been tested after presentation with advanced disease to a veterinary clinic, whether or not the poor immune response to FOCMA preceded tumor development was unknown. Conceivably, the low antibody titers in spontaneous cases were due to (i) a nonspecific suppression of the immune response, which is often seen in advanced neoplasia, or (ii) a "soaking up" of FOCMA antibody by the enlarging tumor mass. Our results indicate that the poor response to FOCMA precedes the development of naturally occurring leukemia.

A prospective seroepidemiologic survey was conducted on 51 cats from a single private household that had previously been identified as a leukemia cluster. The status of each cat for both FOCMA antibody titer and FeLV antigen was determined at intervals of 3 to 5 months for a period of up to 2½ years. Three breeds of cats were present in the house, but all three had high incidences of FeLV viremia, antibody to FOCMA, and leukemia. Cats from similar genetic backgrounds that were kept isolated from FeLV and FeSV did not have detectable antibody to FOCMA (*6–9, 12*). Demographic, pathologic, and serologic details that established the house as a leukemia cluster have been described (*8, 11*). During our 2½-year study, 8 healthy cats spontaneously developed leukemia and 43 remained healthy (Table 1). All 51 were judged at the beginning of the study to be healthy on the basis of both clinical and hematologic examination (*8, 11*). No significant age differences were found between the cats that subsequently developed leukemia and those that remained healthy. All cats in the house that were available for testing for at least 1 year were included in the study except those that developed diseases other than leukemia (*8, 11*). Of the eight cats that developed leukemia, none had an antibody titer to FOCMA higher than 2 when checked 3 to 5 months before the first clinical or hematologic signs of leukemia, whereas 86 percent of the cats that remained healthy had titers of 4 or higher (Table 1). The geometric mean titer for the healthy cats was about tenfold higher than that for cats that subsequently developed leukemia; as examined by Student's *t*-test this difference is significant at $P < .0005$. Although the mean FOCMA antibody titer for healthy cats that are currently FeLV negative is substantially higher than the mean for FeLV-positive healthy cats, the latter group had a mean that was significantly higher than the mean for preleukemic cats ($P < .001$; Student's *t*-test).

Table 1. Antibody titers to FOCMA in cats that subsequently developed leukemia as compared to those living in the same household that remained healthy. For the cats that subsequently developed leukemia, the titers are based on the last sample that was taken while still healthy, at 3 to 5 months before clinical leukemia was detected. For the cats that remained healthy, the figures reported represent the last sample checked. FeLV positivity or negativity is based on the standard test for presence of viral antigen in circulating leukocytes.

Status	Number of cats with FOCMA antibody titers of:										Total	Titer (geometric mean)
	0	1	2	4	8	16	32	64	128	256		
Subsequently developed leukemia												
	1	5	2								8	1.36
Remained healthy												
Total	2	3	1	10	8	10	3	0	3	3	43	11.01
FeLV Positive	1	3	0	4	2	4	1				15	5.36
FeLV Negative	1	0	1	6	6	6	2	0	3	3	28	15.90

The FOCMA antibody titers for cats that subsequently developed leukemia were also compared to the titers for those that remained healthy on the basis of the geometric mean of the titers of the first sample checked on each cat. The mean antibody titers for cats that remained healthy were more than five times higher than the comparable values for cats that subsequently developed leukemia (Fig. 1). This difference was statistically significant ($P < .005$; Students' *t*-test).

These results clearly indicate that a low FOCMA antibody titer in the presence of FeLV positivity is a risk indicator for leukemia, while high antibody titers are associated with resistance to leukemia development. We interpret this as supporting the concept of immunosurveillance in an outbred species of mammal.

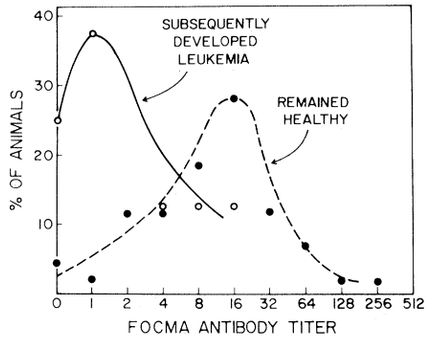

Fig. 1. FOCMA antibody titers upon first testing for cats that subsequently developed leukemia as compared to those that remained healthy.

M. Essex
A. Sliski, S. M. Cotter
Department of Microbiology, Harvard University School of Public Health, Boston, Massachusetts 02115
R. M. Jakowski
Department of Pathobiology, University of Connecticut, Storrs 06268
W. D. Hardy, Jr.
Memorial Sloan-Kettering Cancer Center, New York 10021

References and Notes

1. For review of this subject, see I. Penn, *Cancer* **34**, 858 (1974); R. A. Gatti and R. A. Good, *ibid.* **28**, 89 (1971).
2. M. Burnet, *Prog. Exp. Tumor Res.* **13**, 1 (1970).
3. K. Hellmann, R. I. Hawkins, S. Whitecross, *Br. Med. J.* **2**, 533 (1968); M. S. Hirsch and F. A. Murphy, *Nature (Lond.)* **218**, 478 (1968); A. C Allison and L. W. Law, *Proc. Soc. Exp. Biol. Med.* **127**, 207 (1968).
4. R. S. Schwartz, *Prog. Immunol.* **5**, 229 (1974); R. T. Prehn, *Am. J. Pathol.* **77**, 119 (1974).
5. W. F. H. Jarrett, E. Crawford, W. B. Martin, F. Davie, *Nature (Lond.)* **202**, 567 (1964); T. G. Kawakami, G. H. Theilen, D. L. Dungworth, R. J. Munn, S. G. Beall, *Science* **158**, 1049 (1967); C. G. Rickard, J. E. Post, F. Noronha, L. M. Barr, *J. Natl. Cancer Inst.* **42**, 987 (1969); G. H. Theilen,

D. L. Dungworth, T. G. Kawakami, R. J. Munn, J. M. Ward, J. B. Harrold, *Cancer Res.* **30**, 401 (1970).
6. M. Essex, G. Klein, S. P. Synder, J. B. Harrold, *Int. J. Cancer* **8**, 384 (1971); *Nature (Lond.)* **233**, 195, (1971); M. Essex. S. M. Cotter, J. L. Carpenter, *Am. J. Vet. Res.* **34**, 809 (1973); M. Essex and S. P. Synder, *J. Natl. Cancer Inst.* **51**, 1007 (1973); W. Jarrett, O. Jarrett, L. Mackey, H. Laird, W. D. Hardy, Jr., M. Essex, *ibid.*, p. 833; M. Essex, S. P. Snyder, G. Klein, in *Unifying Concepts of Leukemia*, R. M. Dutcher and L. Chicco-Bianchi, Eds. (Karger, Basel, 1973), pp. 771–777.
7. M. Essex, *Adv. Cancer Res.* **21**, 175 (1975).
8. ———, A. Sliski, S. M. Cotter, W. D. Hardy, Jr., *Cancer Res.*, in press.
9. J. Schaller, M. Essex, R. G. Olsen, D. S. Yohn, *J. Natl. Cancer Inst.*, in press.
10. S. M. Cotter, W. D. Hardy, Jr., M. Essex, *J. Am. Vet. Med. Assoc.* **166**, 449 (1975); M. Essex, S. M. Cotter, W. D. Hardy, Jr., P. Hess, W. Jarrett, O. Jarrett, L. Mackey, H. Laird, L. Perryman, R. G. Olsen, D. S. Yohn, *J. Natl. Cancer Inst.* **55**, 463 (1975); M. Essex, *Pathobiol. Annu.*, in press.
11. W. D. Hardy, Jr., L. J. Old, P. W. Hess, M. Essex, S. Cotter, *Nature (Lond.)* **244**, 266 (1973); W. D. Hardy, Jr., P. W. Hess, M. Essex, S. Cotter, A. J. McClelland, G. MacEwen, in *Comparative Leukemia Research 1973*, R. M. Dutcher and V. Ito, Eds. (Univ. of Tokyo Press, Tokyo, 1975), pp. 105–111; M. Essex, W. D. Hardy, Jr., S. M. Cotter, R. M. Jakowski, in *ibid.*, pp. 431–436; S. M. Cotter, M. Essex, W. D. Hardy, Jr., *Cancer Res.* **34**, 1061 (1974); W. D. Hardy, Jr., P. W. Hess, E. G. MacEwen, A. J. McClelland, E. E. Zuckerman, M. Essex, S. M. Cotter, *ibid.*, in press; M. Essex, W. D. Hardy, Jr., S. M. Cotter, R. M. Jakowski, A. Sliski, *Infect. Immun.* **11**, 470 (1975); M. Essex, R. M. Jakowski, W. D. Hardy, Jr., S. M. Cotter, P. Hess, A. Sliski, *J. Natl. Cancer Inst.* **54**, 637 (1975).
12. M. Essex, S. M. Cotter, J. L. Carpenter, W. D. Hardy, Jr., P. Hess, W. Jarrett, J. Schaller, D. S. Yohn, *J. Natl. Cancer Inst.* **54**, 631 (1975).
13. Supported by grants from the Anna Fuller Fund, the Jane Coffin Childs Fund for Medical Research, the Massachusetts and National Branches of the American Cancer Society (DT-32), the Cancer Research Institute, Inc., the Oliver S. and Jennie R. Donaldson charitable trust, the PHS (CA-13885 and CA-15579), and by scholarships to M.E. and W.D.H. from the Leukemia Society of America. We thank E. Jagher, K. DeSanti, E. Zuckerman, and W. Khan for technical assistance.

27 August 1975

Mefloquine (WR 142,490) in the Treatment of Human Malaria

Abstract. *Mefloquine hydrochloride, a new 4-quinolinemethanol, was administered as a single oral dose to 47 volunteers infected with malaria. Treatment resulted in rapid clearance of fever and parasitemia. No recrudescence of parasites was observed after treatment of chloroquine-sensitive infections of* Plasmodium falciparum. *More significantly, in nonimmune persons with chloroquine-resistant infections, 1 gram of mefloquine cured 10 of 12 patients and 1.5 grams cured all 8 patients who received this dose of the drug. The marked activity of a single dose of mefloquine against chloroquine-resistant strains of* Plasmodium falciparum *suggests that this agent may be more useful than currently available drugs are for the treatment of drug-resistant malaria.*

Malaria remains one of the most important health problems in the world today. The emergence of chloroquine resistance in *Plasmodium falciparum*, the most pernicious plasmodial species for man, has resulted in an intensive search for new drugs and the reappraisal of older ones. At present, no completely suitable regimen is available for treatment of patients infected with chloroquine-resistant strains of *P. falciparum* (*1*).

About three decades ago, a large number of 4-quinolinemethanols were investigated for their antimalarial activity in avian malaria models. The agent with the greatest activity, SN 10,275 [α-(2-piperidyl) - 6,8-dichloro-2-phenyl-4-quinolinemethanol], was tested in volunteers infected with the Chesson strain of *Plasmodium vivax* (*2*). Although phototoxic side effects prevented the general use of this compound, it was an effective blood schizonticidal agent with a remarkably long duration of activity. A derivative of this compound, WR 30,090 [α-(dibutylaminomethyl) - 6,8-dichloro-2-(3′,4′ - dichloro)phenyl-4-quinolinemethanol], has since been found to be highly effective in man against both chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum*. This 4-quinolinemethanol showed minimal evidence of phototoxicity but demonstrated a relatively short duration of action (*3*, *4*).

Mefloquine hydrochloride [WR 142,490; α - (2 - piperidyl) - 2,8 - bis(trifluormethyl)-4-quinolinemethanol] is an analog of SN 10,275 and WR 30,090 (Fig. 1). Preclinical studies revealed that this agent was more effective than WR 30,090, had the long duration of action characteristic of SN 10,275, and showed no evidence of phototoxicity (*1*, *5*). On the basis of these findings, studies were initiated to determine the safety and efficacy of a single oral dose of this drug in the treatment of malaria in man, particularly in individuals infected with chloroquine-resistant strains of *P. falciparum*.

During the first phase of these studies, the tolerance and safety of mefloquine were appraised in noninfected individuals (*6*) by a single-dose, double blind design with 19 dose levels rising from 5 to 2000 mg. At each dose level, two persons in the group received the drug and two received the placebo. To evaluate drug tolerance, the following procedures were performed twice before and several times during the 3 weeks after drug administration: interview, physical examination, electrocardiogram, hematocrit, total white blood cell count, white blood cell differential, platelet count, prothrombin time, total serum bilirubin, serum creatinine, serum glutamic oxalacetic transaminase, blood urea nitrogen,

Table 1. Response of 35 nonimmune volunteers infected with *Plasmodium falciparum* to treatment with mefloquine.

Strain of *Plasmodium falciparum*	Dose of mefloquine hydrochloride		No. treated	Asexual parasites mm³ (No.)*	Maximum rectal temperature (°C)	Clearance time (days)		No. cured
	mg	mg/kg				Fever†	Asexual parasites	
Ethiopian (Tamenie)	400	5.4‡ (4.6– 6.2)	2	1,610‡ (1,520– 1,700)	41.5‡ (41.5–41.5)	5.5‡ (5–6)	4.0‡ (3–5)	1
Vietnam (Marks)	400	5.5 (3.9– 7.5)	8	2,195 (240– 8,880)	40.4 (39.8–40.6)	4.1 (2–6)	5.1 (2–7)	1
Cambodian (Buchanan)	400	5.6 (5.2– 5.9)	2	2,835 (2,550– 3,120)	40.3 (40.1– 40.6)	5.0 (4–6)	5.0 (4–6)	0
Ethiopian (Tamenie)	1,000	10.4 (7.7–12.5)	3	810 (520– 1,130)	40.1 (39.4–40.9)	5.0 (4–6)	3.0 (2–4)	3
Vietnam (Marks)	1,000	14.6 (13.6–15.4)	8	3,437 (760– 8,480)	41.0 (40.6–41.6)	5.25 (4–7)	3.1 (3–4)	8
Cambodian (Buchanan)	1,000	13.9 (10.7–17.2)	4	10,090 (740–28,200)	41.2 (40.9–41.3)	4.5 (4–6)	4.75 (4–7)	2§
Cambodian (Buchanan)	1,500	21.2 (16.8–26.2)	8	8,437 (830–18,750)	40.1 (39.1–41.3)	3.5 (2–6)	4.0 (3–5)	8

*Maximum parasite count during the first 48 hours of treatment. †Interval between treatment and the first day of a 48-hour period during which the rectal temperature remained below 38°C. ‡Mean, with range in parentheses. §The individuals who were not cured received 10.7 and 13.2 mg of mefloquine per kilogram and those who were cured received 14.5 and 17.2 mg of the drug per kilogram.