Entrainment of the Body Temperature Rhythm in Rats: Effect of Color and Intensity of Environmental Light

Abstract. *The daily rhythm in body temperature in rats was continuously monitored during exposure to low-intensity environmental illumination of various colors in the visible and near-ultraviolet spectrum. The ability of phase shifts in the lighting schedule to induce concomitant changes in the rhythm was used to determine the spectral sensitivity of the retinal photoreceptor systems mediating rhythm entrainment. Green light ($\lambda = 530 \pm 45$ nanometers) was most potent, and red ($\lambda = 660 \pm 19$ nanometers) and ultraviolet ($\lambda = 360 \pm 34$ nanometers) were least potent in entraining the temperature rhythm.*

The body temperature of many mammalian species exhibits a circadian rhythmicity about a mean value (*1*). In the nocturnally active rat, the onset of darkness is accompanied by a 1° to 2°C rise in body temperature, which is reversed with the onset of light (*2*). Although this rhythm is closely entrained by the presence of other environmental cues besides light, including feeding activity and sound (*3, 4*), it persists with a period close to 24 hours in animals exposed to a regular photoperiod and isolated from other known external rhythmic stimuli (*5*). Consequently, it appears that environmental light constitutes the single most effective stimulus capable of entrainment (*6*). Little information is available concerning the nature of the retinal photoreceptor or photopigment that mediates the photic entrainment of the temperature rhythm, or of any circadian rhythm in mammals. Therefore we have studied the spectral dependence of the systems mediating this entrainment.

Table 1. Fraction of animals tested entraining to environmental light rhythm (12 hours light : 12 hours darkness) of given color and intensity within 14 days after 6-hour phase shift. Intensities tested are given in microwatts per square centimeter at the cage.

Color (peak wavelength ± peak half width)	Intensity	Response
Vita-Lite	11	All
	4	6/6
	0.3	2/3
	0.1	1/3, 3/6
Red	30	8/8
(660 ± 19)	11	2/6, 4/6
Gold	8	3/3
(590 ± 80)	4	1/5
Green	0.1	5/5
(530 ± 45)	0.07	2/3
	0.02	1/4
Blue	9	2/3
(435 ± 54)	3	0/3
Ultraviolet	13	3/4
(360 ± 34)	9	1/3

Body temperature was determined automatically in 100-g male Charles River CD rats by using a modification of the apparatus described by Holmquest (*7*). While rats were under ether anesthesia, we placed small temperature-sensitive radio transmitters loosely in the peritoneal cavity. After recovery from the surgery, temperatures were recorded every 10 minutes for the duration of the experiments, and the data were plotted and calculated automatically. Each experiment consisted of a 7- to 10-day control period, during which the animals were exposed to a known cyclic entraining signal (*8*) on a 12-hour light : 12-hour darkness schedule, followed by a 14-day experimental period. Each experimental period was introduced as a change in color and/or intensity of the light source concurrent with a 6-hour phase shift in the lighting schedule (produced by insertion of a single 6-hour dark period in the normal 12-hour light : 12-hour darkness schedule). Light intensities were measured at the cage by using an Eppley thermopile, connected to a Keithley nanovoltmeter.

Entrainment to the new source was scored as the fraction of the animals in each group whose temperature rhythms became entrained, within the 14-day test period, to the 12 : 12 light : dark cycle. Menaker and Eskin established two criteria for an entraining signal: first, the rhythm under study must assume the same frequency as the entraining signal; and second, the rhythm under study must have changed phase during entrainment (*4*). Since response to a phase shift involves at least a temporary alteration in the frequency of the rhythm, the assumption after a phase shift of the prior relationship with the entraining signal will satisfy both of these criteria. All animals which became entrained to a particular lighting environment did so within 7 days, and in no case did it appear likely that animals not entrained after 14 days would have done so after a longer exposure. However, this possibility cannot be excluded, and may occur especially in those groups containing both entrained and unentrained animals.

The results of these experiments, tabulated in Table 1, demonstrate a marked sensitivity of the temperature rhythm to the green portion of the visual spectrum. From these results a 50 percent response dose was estimated for each spectrum studied, and is plotted in Fig. 1, along with the relative spectral sensitivity of rat rhodopsin (*9*). Although the ordinates of the functions are not directly comparable, they demonstrate a similar sensitivity to green, and a low sensitivity to the red and blue ends of the visual spectrum. They are thus consistent with the hypothesis that retinal rod cells participate in the photic control of circadian rhythms that do not depend on perceived visual inputs.

In the only other study to our knowledge in which rhythms were entrained to other than broad spectrum sources, Winget *et al.* demonstrated a slower rate of response to phase shifts in chickens exposed to red light compared with a control white light group (*10*). In nonrhythmic systems the broad spectrum source used as a control in this study, which contains a significant ultraviolet output, has been shown to stimulate calcium absorption in humans (*11*) and to affect organ growth in rats (*12*). Narrow-band light sources have been used to define the action spectra of two nonrhythmic physiologic processes in mammals. Messing, using wavelengths above 480 nm, demonstrated a sensitivity similar to rhodopsin in the behavior of rats allowed to

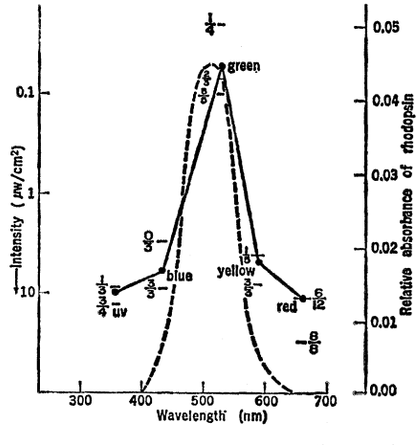

Fig. 1. Estimated 50 percent effective intensity for entraining sources used. Fractions represent numbers of rats in each group entrained by the particular intensity of the light source. The relative sensitivity of rat rhodopsin is also shown (*9, 14*) as a dashed line.

choose between differing light sources (*13*). Cardinali *et al.* measured the effectiveness of the sources used in our experiments in inhibiting pineal hydroxyindole-*O*-methyltransferase activity in the rat (*14*), and observed spectral sensitivity similar to that reported here. Whether the same receptor cells are involved in these functions and in vision has not been determined; the separation of photic information mediating these various responses could occur further along the visual pathways from the retina (*15*).

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Immunity to Colon Cancer Assessed by Antigen-Induced Inhibition of Mixed Mononuclear Cell Migration

Abstract. *A purified preparation of mixed human peripheral blood lymphocytes and monocytes was used in an inhibition-of-migration assay for cell-mediated immunity to cancer of the colon. This preparation was reproducibly antigen-responsive and migrated with greater reliability than did a more complex cell mixture. Of 27 patients with this disease, cells from 24 showed inhibited migration in response to colon carcinoma antigen. Uninhibited migration patterns were found in each of the 52 cancer-free controls, including eight patients with nonmalignant disease initially diagnosed as cancer of the colon, and in nine patients with surgically cured adenocarcinoma of the colon.*

The lymphocyte-mediated cytotoxicity (LC) test and related techniques have been widely used for the in vitro demonstration of immunity against tumors in animals and man (*1*). Assays of this type are also useful for studying serum "blocking" activity, an effect that is probably mediated by tumor antigen–antibody complexes and that may be the in vitro counterpart of in vivo "enhancement" of tumor growth (*2*). Several factors limit the clinical applicability of the LC test, however. Target tumor cells are required, incubation of cultures requires 48 to 72 hours, and cytotoxic effect must be assessed by time-consuming morphologic or isotope-release techniques. Tests of this type are not useful for assessing immunity to preparations of tumor-specific antigens. Finally, the LC test is of limited diagnostic and prognostic usefulness in that it is frequently negative in the presence of growing cancer, is often positive in tumor-free relatives and contacts of cancer patients, and, when positive in a cancer patient, tends to remain so after surgical cure (*3*).

Another in vitro correlate of cellular immunity, inhibition of leukocyte migration, was originally described by Soborg (*4*) and Bendixen (*5*) in bacterial infection and autoimmune disease, and attempts have also been made to demonstrate human tumor immunity by this means (*6*). Although the test is simple and rapid, results obtained with soluble antigens in delayed hypersensitivity are characterized by a lack of reproducibility, poor correlation with skin test reactivity, and the need to accurately distinguish the antigen-responsive mononuclear cell migration pattern from the antigen-unresponsive granulocyte margin (*7*). In studies of human tumor immunity with this assay, negative responses to tumor antigen were reported in a significant proportion of patients (*6*). We suspected that the incomplete correlation between clinical status and in vitro tumor immunity reflected as inhibition of leukocyte migration was not necessarily the result of absence of tumor immunity, but might be related to the complex and variable leukocyte preparations used. In this report, we describe the use of purified populations of antigen-responsive lymphocytes and migrating monocytes in a migration inhibition test, and we correlate the results of this assay with clinical status of colon carcinoma.

Tumor antigens were prepared as membrane-rich dilutions of homogenates of colon adenocarcinomas obtained at surgery. Homologous colon adenocarcinoma antigen was generally used, although in a few cases tumor antigen from the patient being studied gave identical results. One gram of tumor tissue was homogenized for 15 minutes in nine volumes of phosphate-buffered saline at 60,000 rev/min in a VirTis blade-type homogenizer. The homogenate was frozen, thawed, and rehomogenized for 15 minutes, and particulate matter was removed by filtration through several layers of sterile gauze. The filtrate was exhaustively dialyzed against Seligmann's buffered salt solution (a modified Hanks solution free of calcium and magnesium) and stored at -20°C in RPMI 1640 tissue culture medium at 1 : 100 dilutions (1 g of original tumor per 100 ml of medium). For use as antigen, the concentrated portions of tumor extract were further diluted to 1 : 3000 for final inclusion in migration chambers. Whole blood (40 ml) from patients with colon adenocarcinoma and controls was added to 1.4 ml of 5 percent ethylenediaminetetraacetic acid (EDTA), diluted with 100 ml of Seligmann's buffered salt solution containing 500 mg of EDTA, and divided into two parts. Under each portion was injected 20 ml of a mixture of 24 parts of 9 percent Ficoll (Pharmacia, Uppsala, Sweden) and 10 parts of 34 percent Hypaque (Winthrop Laboratories, New York), and samples were sedimented at 400*g* for 30 minutes (*8*). Platelets contaminating the monocyte-lymphocyte layer were removed by sedimentation through triple sucrose layers (6, 12, and 16 percent) at 200*g* for 15