Note added in proof: Subsequent to the completion of this study a published article on the protective effects of yeast extracts on irradiated organisms has been located in the Russian literature (*7*).

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Audiogenic Abnormality Spectra, Twenty-four Hour Periodicity, and Lighting

Under standardized conditions, certain stocks of mice show 24-hour periodicity in incidence of audiogenic convulsions and in mortality from them (*1*). Thus the proportion of mice with convulsions will differ significantly between comparable groups exposed to identical auditory stimulation at the times of daily high or low in eosinophil count (*1*). At either time of stimulation and in several stocks, the sequence of abnormal events following exposure to noise consists of dashing ("uncontrolled" running) "clonic" convulsion, "tonic" convulsion, and death, in this order, but this sequence is not necessarily started or completed in each animal. In response to noise, a given mouse may only crouch, or walk, or at best run, with relative "control" of its movements (*1, 2*). Herein we raised the questions whether dashing, an early audiogenic abnormality, also may be 24-hour periodic and whether the entire spectrum of periodic audiogenic abnormality can be influenced by the schedule of light and dark. If, as in the case of physiologic rhythms, the timing of abnormal responses to noise can be set by manipulation of lighting, a potentially useful model for the experimental pathologist will be more reliably defined (*3*).

D_8 mice, of both sexes, were weaned at 21 ± 2 days of age and immediately singly housed, with Purina Dog Chow and tap water available *ad libitum*. The cages were kept in rooms maintained at 24 ± 0.5°C and illuminated by artificial light only. One group of mice was in light from 06:00 to 18:00, another in light from 18:00 to 06:00, alternating with 12 hours of darkness in each case. Individual mice from these two groups were transferred from their cages to a stimulator (*4*), within less than 30 seconds. Each stimulation was of 60-second duration, one subgroup from each group being exposed to noise between 20:00 and 22:00, the other between 07:00 and 09:00.

Figure 1 shows "within-day" differences for the entire spectrum of abnormal responses to noise, which stand out clearly irrespective of the lighting regimen used. χ^2-tests were carried out on each difference in the proportion of mice exhibiting a given response at 08:00 and at 21:00, respectively, these differences being analyzed separately for mice on the two lighting schedules. Without exception, the P values were smaller than 0.05.

Figure 1 further reveals that mice in light from 18:00 to 06:00, as compared with those in light from 06:00 to 18:00, have shifted the time of day associated with a higher proportion of abnormal responses. This shift in timing of peak abnormality applies to dashing, to the two types of convulsion studied, as well as to the end-point death. Quite clearly, the lighting regimen on which the mice are kept determines the temporal placement within the 24-hour period of all of the abnormal rhythms studied herein, as long as other things remain comparable. Conceivably, the standardization of genetic background, past history, and age has substantially contributed to the significance of the results. The difference in over-all incidence of abnormality, irrespective of time, between the groups on the two regimens of lighting, however, cannot be accounted for with the data on hand. A possible increase in over-all susceptibility to convulsion immediately following a phase-shift of rhythm deserves study.

An earlier suggestion, that lighting is ordinarily the dominant synchronizer of various physiologic rhythms in the mouse (*5*), can now be extended to several physiopathologic periodicities in the same species. It seems noteworthy that (as yet ill-defined) changes underlying abnormal responses to acoustic sensory inflow in mice are among multitudinous 24-hour periodic changes governed in their timing by optic stimulation.

Finally, the present results on the experimental animal have an approximate clinical counterpart. For well over a century, epileptologists have discussed the unequal "within-day" distribution of seizures in some of their patients (for references, see *6*) and convulsive periodicity was studied in the clinic as a potential clue to seizure mechanisms (*6*). Yet progress in the field may have been hindered by the lack of suitable experimental animal models. From this point of view, periodicity analysis on D_8 mice, about 5 weeks of age, yielding the data of this report, may constitute a tool of the experimental pathologist. Most

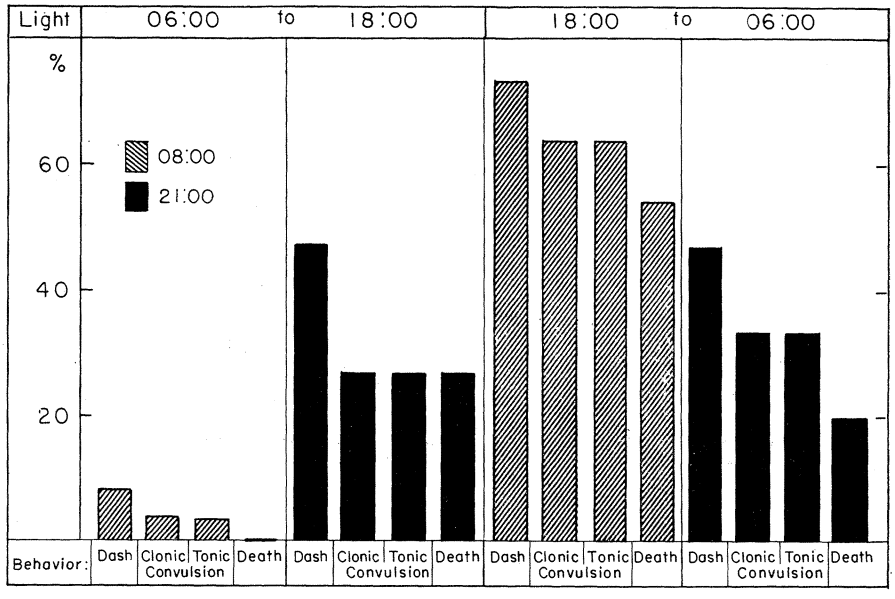

Fig. 1. Abnormal audiogenic responses in D_8 mice, on two schedules of light and darkness, alternating at 12-hour intervals. Note the difference in incidence of abnormality at 08:00 and at 21:00, on each lighting regimen. Note also the difference in time of high abnormality, in mice exposed to light from 18:00 to 06:00, as compared with that in mice exposed to light from 06:00 to 18:00. Total tested: 102 mice, about 5 weeks of age, of both sexes.

likely, nocturnally active D_8 mice with an evening high in audiogenic abnormality may be counterposed to certain types of "morning-fitters" among diurnally active human beings.

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Agar Substrates for Study of Microepidemiology and Physiology in Cells in vitro

Despite the general usefulness of monolayer cell and tissue cultures on glass, there are experimental situations in which multilayered systems are desirable. Certain shortcomings in monolayer cellular systems for the study of infectious disease are apparent, particularly if a succession of cells must be parasitized to obtain full development of infection or to demonstrate the resistance of cells. The relatively great expanse of cells and fluid nutriments permits exposure of sensitive agents to inhibitory components in the liquid phase. There is loss of viral and bacterial agents and of infected cells during renewal of the supernates (*1*). In the case of microorganisms which can proliferate independently, streptomycin (*2*) or other extracellular inhibitors must be added to ensure an intracellular type of infection.

Combinations of infectious agents with cells or cell colonies on agar provide a contiguity of cells which is closer than that in animal tissues; extracellular inhibitors are of less concern; neither the agent nor the cells can escape the experimental arena. Many microepidemiological problems, therefore, may be studied more advantageously than in the whole animal or in monolayer cell cultures.

The human tubercle bacillus, strain H-37Rv, grows in cell colonies of Earle's L strain of mouse fibrocytes and Gey's strain HeLa. The failure of *Mycobacterium lepraemurium* to achieve more than the usual 2.5- to 3-fold multiplication (*3*) reveals that the problems of this organism are more complicated than the simple need for continuous existence within host cells.

The reservoirs of nutriment provided in the agar systems to be described have also permitted the maintenance of cell cultures for extended periods with minimal care and have proved useful in the study of organized tissue fragments (see also *4*).

Agar substrates were prepared by combining at 50°C double-strength nutriments with equal volumes of 2 to 4 percent purified agar (*5*) in BSS just prior to the preparation of plates, impregnated filter paper strips (*6*), or agar slants (*7*). Plates and impregnated papers were inoculated with cells or tissue fragments without prior drying, while agar slants to carry circumscribed cell colonies were dehydrated in inverted cotton-stoppered tubes overnight at 37°C. Cell inocula were standardized by transferring 2-mm loops (0.0025 ml) from dense, enumerated suspensions of strain L and strain HeLa. Several assemblies of agar systems which provide a reservoir of renewable, slowly available liquid nutriments are shown in Fig. 1.

The development of large bacterial colonies after 24 to 72 hours on agar indicates that diffusion rates are more than adequate for tissue cells. On slants containing 3 ml of serum, 2 percent, Eagle's supplement, and 0.5 percent Bacto-peptone (*3*) without liquid phase, strain L produced relatively thick colonies within 2 weeks at 34°C. Cells at the margins of such colonies were round and clear. If 0.5 ml of liquid phase (1/7 of the system) was replaced each 2 weeks, very thick mucoid colonies developed within 2 months. In such media supplemented with pyruvate (50 mg/100 ml) and citrate (0.03 percent) the maintenance of uniformly high viability after 3 months was shown by exclusion of eosin (*8*) and by vigorous growths following transfers to new media.

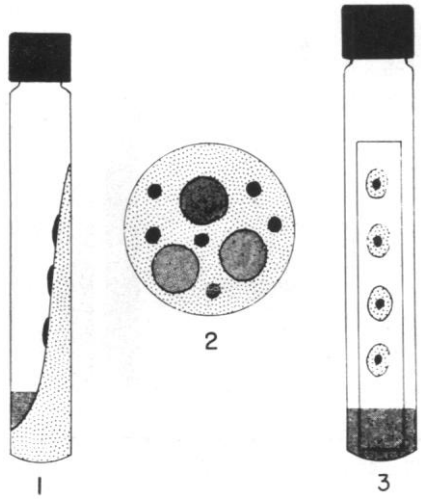

Fig. 1. Agar systems with diffusible nutriments: 1, agar slant; 2, agar plate with three "wells"; 3, agar-impregnated filter paper carrying four cell colonies.

Approximately 5000 L cells were required per site for reliable inoculation of the medium mentioned above. Although incubation in oxygen tensions of 1 to 4 percent, or in the presence of 20 percent serum, decreased the required inoculum, uniform growth was not obtained from less than 2000 cells. Growth of strain HeLa has been initiated with as few as two cells.

Quantitative samples are recoverable from plates or slants by the application of 0.25 percent pancreatin to each cell colony, and by several rinses with known volumes of diluent. Cells are recovered quantitatively from single colonies on plates, slants, or impregnated papers by first removing a disc of agar which includes the colony, and then by use of the foregoing procedure. A bacteriological wire transfers sufficient cells for quantitative determinations of cell types and ratios, the proportion of cells infected, the percentage of cells capable of excluding eosin, and for transfers of viable inoculum.

Fragments of guinea pig bone marrow were explanted on agar slants, plates, and impregnated papers containing 10 percent guinea pig serum and the aforementioned supplements. After 24 hours the migrating cells produced 5- to 10-mm halos in which neutrophils preponderated. The cells within the explants retained typical morphology and staining characteristics for 2 to 3 days. Following the death or modification of various cell types, or both, and after 4 weeks, some 20 to 30 percent of the cells in explants plus deteriorating halos were viable (*9*).

Continuous ciliary beating was observed on the surface of fragments of embryonic chick and embryonic and adult human bronchial tissues during at least two weeks on agar substrates containing 25 percent chicken or human serum and the supplements mentioned (*9*).

It seems, therefore, that cell colonies and tissue fragments on agar substrates are useful for the study of many problems in infectious disease and cellular physiology or differentiation, and also for the maintenance of cell lines with minimal effort (*10*).

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