daloid regions are directed toward some integrative level of the brain, probably the hypothalamus, since Bard and Mountcastle (*6*) have demonstrated that the hypothalamus is a critical center for the integration of emotional display. Furthermore, hypothalamic connections have been established for both the septal and amygdaloid areas. It appears that, in the rat, the septal area may normally act to "dampen" the hypothalamic activity associated with emotional states, while the amygdala may facilitate this diencephalic center.

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24 April 1958

Radiation-Protective Effects of Yeast Extract and Yeast Ribonucleic Acid

Most of the work with biological substances in the field of radiation protection has been with proteins, amino acids, animal cells, and their extracts. Studies involving the administration of embryonic cells, viable spleen and bone marrow cells, either in the pre- or postirradiation period, have been voluminous and unequivocally show varying degrees of protection.

On the other hand, experience with plant substances and yeasts in particular has been quite limited. It has been demonstrated by Jaraslow *et al.* (*1*) that the administration of an autolysed yeast extract to rabbits is capable of protecting the postirradiation response to certain immunologic stimuli. Hollaender and Doudney (*2*) have demonstrated that irradiated *Escherichia coli* grown aerobically in nutrient broth recover from x-ray effects to a considerable degree if they are plated after irradiation on agar containing yeast extract. In studies designed to evaluate the role of properdin in radiation protection, Ross *et al.* (*3*) studied postirradiation survival of rats and mice after injections of zymosan. A moderate protective effect was demonstrated. Because of the suggestion from these studies that yeast and yeast extracts might have radiation-protective properties, an evaluation study in rodents was performed.

Autolysed yeast extract was prepared according to the method described by Jaraslow *et al.* (*1*) in which dried brewer's yeast was incubated with isotonic phosphate buffer at *p*H 7.4 at 37°C for 12 hours and then centrifuged at 20,000 *g* for 30 minutes. The clear supernatant autolysate was used for injection. For each experiment fresh yeast autolysate was prepared. Within from 15 to 30 minutes prior to irradiation, 200-g Sprague-Dawley rats were injected either intravenously or intraperitoneally with 1.0 ml of the autolysate.

In the first experiment, a total of 27 injected rats were compared with a group of 50 uninjected controls. In the second experiment, 15 eleven-week-old C_3H mice were injected either intraperitoneally or intravenously with 0.5 ml of the yeast autolysate. The survival rate of these mice was compared with that of 105 uninjected controls. Thirty minutes after injection the rats were placed in a polyethylene box and irradiated in pairs so that each animal received a total body dose of 900 r of x-ray over a period of 17 minutes. In a similar fashion the mice were placed in a plastic container and given 700 r of total-body radiation of x-ray over a period of 20 minutes. The number of surviving animals then was checked at daily intervals during the 30-day postirradiation period. All but one of the uninjected control rats were dead by the 13th postirradiation day, and this animal succumbed on the 21st day. On the other hand, 11 of the 27 injected rats (41 percent) were alive on the 30th day after irradiation. Four of the 15 yeast autolysate-injected mice (27 percent) were alive on the 30th day after irradiation, whereas all of the uninjected control mice were dead by the 14th postirradiation day. In both groups of animals the results obtained with intravenous and intraperitoneal injections were comparable. It also was noted that even the nonsurviving rats and mice lived longer than did the irradiated controls.

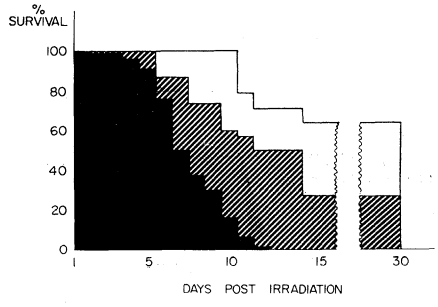

Fig. 1. Percent survival of three groups of C_3H mice following 700r total body irradiation. (a) Solid black area—105 controls (b) shaded area—15 yeast autolysate injected and (c) clear area—14 yeast RNA injected.

The results of these experiments indicated that the crude yeast autolysate provided a moderate degree of radiation protection to lethally irradiated animals. Yeast autolysate is extremely rich in ribonucleic acid (RNA), and there is good evidence that RNA is essential for protein synthesis. Studies with transplanted, irradiated nuclei of amoebae (*4*) suggest that irradiated cytoplasmic constituents of these cells are deleterious to normal nuclear function. Daniels (*5*) has suggested that non-nucleated cellular components of normal amoebae are capable of restoring nuclear function to irradiation-damaged cells. The high RNA content of cellular cytoplasm of many protective tissue extracts made it seem possible that the RNA of the yeast autolysate was its most important protective constituent and prompted investigation of the radiation-protective effects of this nucleic acid.

A group of 14 eleven-week-old, C_3H mice were injected intraperitoneally with either 10 or 20 mg of a commercial preparation of yeast RNA containing less than 1 percent protein (Schwarz Laboratories). The RNA concentration of the solution used for injection was 2.0 g/100 ml. Fifteen to 30 minutes later these animals were exposed to 700 r of total-body irradiation. Nine of the 14 injected mice (64 percent) were alive at the end of 30 days. These results are to be compared with no survival in the control group of 105 mice, and with 27 percent survival in the group injected with crude yeast autolysate (Fig. 1). There is some indication from these data that the dose of RNA may be of importance since only one of the five mice that died received the 20-mg dose.

In each of the three cases, a chi-square test was applied to determine whether the difference in survival rates between the two groups is significant. The resulting *P*-values are less than 0.001 in all three. These results indicate that the preirradiation injection of a crude yeast autolysate exerts a moderate radiation-protective effect in rats and mice. Postirradiation survival is considerably enhanced with a commercial yeast-RNA preparation. It is of interest that a plant extract devoid of viable cells and rich in RNA is capable of exerting protective effects to a degree comparable to that of many mammalian cells and tissue extracts. Preliminary results indicate that RNA, or a substance associated with it in the yeast autolysate, may be the principal radiation protective factor (*6*).

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

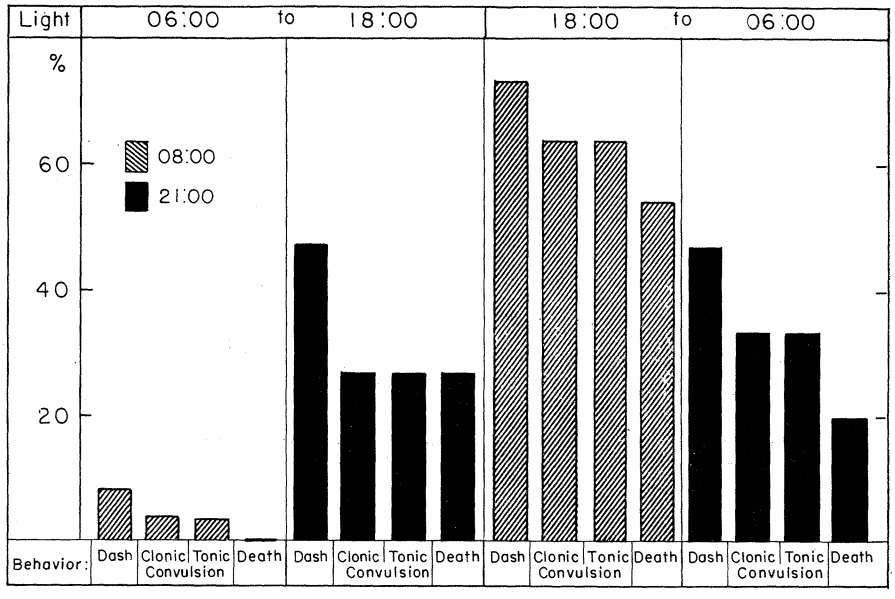

Fig. 1. Abnormal audiogenic responses in D_8 mice, on two schedules of light and darkness, alternating at 12-hour intervals. Note the difference in incidence of abnormality at 08:00 and at 21:00, on each lighting regimen. Note also the difference in time of high abnormality, in mice exposed to light 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.