

ers were tagged at anthesis and the age of the fruit was determined from that date. Harvested fruits were sectioned with a sharp knife and the seeds were immediately separated from the locular material by gentle pressure with the fingers. Seeds were rapidly blotted free of surface moisture with filter paper and weighed on a Roller-Smith balance. They were then dried to a constant weight at 100°C. In all instances, ten seeds were pooled for weight determinations, and the mean weight per seed was calculated.

Experiments were performed to correlate the water content of various tissues with fruits older than 15 days.

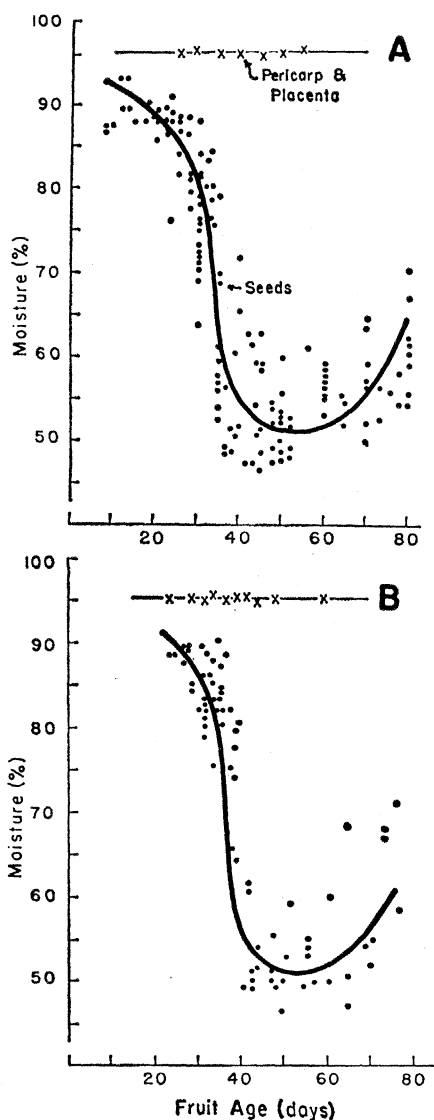


Fig. 1. Percentage moisture (on the basis of fresh weight) of seeds and fruit pericarp and placenta of Marglobe (A) and Bonny Best (B) tomato fruits of various chronological ages, from plants grown in greenhouses. For a given age, points represent samplings from different fruits.

Results indicated that whereas the percentage moisture of the placenta and pericarp showed little variation with the stage of maturity of the fruit, that of the seeds dropped sharply as the fruits matured (Fig. 1). For tomatoes grown in the greenhouse, this sharp decline in moisture content usually occurred when the fruits were between 25 and 50 days old and varied only slightly with variety or season. The dehydration period of seeds in tomatoes produced late in the growing season in the garden, however, tended to extend over a longer time. The moisture content of the seeds in the fruits always dropped to approximately 50 percent, stabilized at this level for a relatively short period and then the seeds began to reabsorb moisture (Fig. 1).

Since it was conceivable that the observed changes in the percentages of moisture content in the developing seeds merely reflected an accumulation of dry matter, without any change in water content, the total quantities of water in the seeds were also determined (Table 1). These data clearly indicated that although there was an accumulation of dry matter during development, the major factor contributing to the decrease in percentage moisture was an actual loss of water from the seeds.

It was also observed that it was not necessary that the tomato fruits remain attached to the shoot for the seeds to dehydrate. Analyses of the water content of seeds at various intervals subsequent to harvesting and storage of the fruits at 21°C showed that they continued to lose moisture. If the fruits were at the appropriate stage at the time of harvest, a dehydration pattern similar to that observed for fruits developing on the vine was observed. It was also demonstrated that the reduction in percentage moisture of such seeds was not merely a consequence of the accumulation of dry matter. The seeds in detached fruits also began to rehydrate after reaching a moisture content of about 50 percent.

Experiments indicated that mechanisms within the seeds, as well as within the whole fruit, participated in the dehydration system. It was found, for example, that excised seeds with moisture contents in excess of about 80 percent would dehydrate when placed in distilled water. Since in the intact fruit the seeds dry till they have a moisture content of about 50 percent, it

would appear that the tissues outside the seed are responsible for a portion of the dehydration. The mechanisms controlling the dehydration of seeds in the highly aqueous environment of the fruit have not been found. The movement of water from seed to fruit against a gradient of moisture concentration shows that an osmotic differential between seed and fruit cannot explain seed dehydration (2).

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2. This work was undertaken in cooperation with U.S. Army Natick Laboratories, Army Materiel Command under government contract No. DA19-129-qm-915. Also supported by the Clara A. and Wallace C. Abbott Memorial Fund of the University of Chicago.

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Activity Rhythm in *Peromyscus*: Its Influence on Rates of Recovery from Nembutal

Abstract. Injection of sublethal doses of Nembutal (sodium pentobarbital) into deer mice, *Peromyscus maniculatus rufinus* (Merriam), at different times in their daily activity cycle revealed large differences in rates of recovery. By taking the phase of the circadian rhythm into account, the accuracy obtained in pharmaceutical and physiological bioassays could be increased.

In recent studies of various circadian rhythms, particular attention has been given to overt locomotor or activity rhythms. These are well exemplified by nocturnal rodents such as *Peromyscus*, which generally become active at a fairly definite time in the evening and remain in this state throughout most of the night. Near dawn these mice cease their activity and do not renew it until the normal time of onset the following evening. Two distinct phases are thus discernible in this type of rhythm, one characterized by more or less continuous activity and the other by a state of rest.

Physiological rhythms of approxi-

mately 24-hour duration have also been noted, and such phenomena as heart rate, body temperature and overall metabolic rate have been shown to have daily maxima which coincide with the active phase of the locomotor rhythm (1). These physiological oscillations led us to assume that the time required to detoxify certain drugs might also vary with respect to the phase of the animal's activity rhythm.

To explore this possibility, we administered intraperitoneal injections of Nembutal (0.1 mg per gram body weight) to seven male deer mice (*Peromyscus maniculatus rufinus*) at each of six predetermined times in their activity rhythms. The mice had previously been kept in cages where their activity was recorded, and from these records we chose the six times to represent different levels of activity in the daily cycle (2). (Three of the six times occurred during the active phase and three during the inactive phase of each animal's locomotor rhythm.) During the 4-week experimental period, each mouse received a total of 18 injections—that is, three injections at each of the six times being studied. Care was taken to keep injections at least 12 hours apart to insure that consecutive readings for any one mouse would be independent of one another. Precautions were also taken to prevent drifting of the activity rhythms. At the conclusion of the study, the mice were returned to the cages where their activity was recorded, and we found that no significant phase shifting occurred.

Several measures of the duration of the anesthetic effects of Nembutal were used. Of these, the time elapsed from the moment the animal first lost the ability to right itself until this ability was regained proved to be the most reliable as well as the least subjective and consequently this measure was used in the data analysis.

The results of the study demonstrate that the quantitative response of the deer mice to Nembutal was not constant throughout the daily locomotor cycle. The rate of recovery from anesthesia was 10 to 20 percent more rapid during the active phase than during the inactive part of the cycle.

When a two-way analysis of variance was carried out on the data (Table 1) a highly significant difference ($p < .005$) was found to exist between the rates of recovery at the different injection times. Upon subdividing this

Table 1. Analysis of variance for rates of recovery of *Peromyscus* from injections of Nembutal. (The raw data used in the analyses were the reciprocals of the recovery times, in minutes, $\times 100$.)

Source	Degrees of freedom	Sums of squares	Mean square	F ratio	p
Times	5	301.37	60.27	8.70	$< .005$
Between active and inactive phase	1	239.22	239.22	34.51	$< .005$
Within active phase	2	30.02	15.01	2.17	$> .10$
Within inactive phase	2	32.13	16.07	2.32	$> .10$
Mice	6	749.13	124.86	18.01	$< .005$
Interaction	30	292.81	9.76	1.41	$> .10$
Error	80	554.54	6.93		
Total	121	1897.84			

"time" factor into its component parts, it can be observed that the recovery rate during the active phase differed significantly from that during the inactive phase of the rhythm, but that within either phase no such differences occurred. This indicates that variance can be greatly reduced when comparisons of recovery rate are limited to tests on animals in similar circadian states.

Whether a more detailed segregation of data would be profitable in further decreasing the amount of variance in the results would depend upon the variability of the circadian locomotor rhythms in the species in question.

The conclusion that the circadian rhythm has considerable influence on the rate of recovery from certain drugs is in accordance with the results of Halberg (3) and more recently of Davis (4) who showed a day-night periodicity in the response of *Mus musculus* to pentobarbital.

The implications of these types of studies should have far reaching importance in the fields of physiological and pharmaceutical research where various drugs are being tested for their effects on experimental animals. The role of circadian rhythms in studies of drugs has only recently received widespread attention and consequently, precautions are often not taken to control this source of variance. Animals kept in rooms which are not exposed to regular light-dark cycles can easily have their activity rhythms disrupted. The same is true of animals kept under conditions of constant light. If several animals, whose rhythms were greatly out of phase with each other, were tested as a group to note the effects of a drug or chemical, errors could ensue; proper results could be obscured by the high degree of variance due simply to differences in the times of locomotor phases of the experimental subjects.

We suggest, therefore, that experimental animals be kept under conditions of normal light-dark cycles (without interruptions), and that the phase of the activity rhythm during which a drug is administered be noted (5).

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5. We thank K. S. Rawson for his assistance and for the use of his equipment and E. T. Hooper and F. C. Evans for their critical examination of the manuscript.

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Retention in Rats: The Effect of Proactive Interference

Abstract. Four groups of rats were trained to make a simple spatial discrimination. Two of the groups had previously been trained to make the reverse discriminations, the other two had no prior training. Two groups were tested 1 day after training, while two others were tested 44 days after training. There was no retention loss unless prior interference had been provided.

It is generally believed that one of the major causes of forgetting is interference. The most recent version of interference theory was proposed by Underwood (1), who suggested that much of forgetting is due to the interfering effect of competing responses