While it would be desirable to prepare A material completely free of B-chain, either by more drastic reduction, or by repeating the reduction of the isolated material, technical difficulties (the poor solubility properties of A-chain) now prevent this last step.

If Ab4 determinants reside only with the B-chain, one is nearly forced to accept the earlier stated assumption that the allotypic specificities (at least of the *b* locus) are due to primary structure changes, since B-chains have less than one carbohydrate molecule (hexose or hexosamine) per protein chain (*14*).

G. W. STEMKE
Service d'Immunochimie Analytique, Institut Pasteur, Paris, France

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Daily Sensitivity Rhythm of the Two-Spotted Spider Mite, Tetranychus urticae, to DDVP

Abstract. *Adult females of the two-spotted spider mite* (Tetranychus urticae *Koch), showed a daily rhythm of sensitivity to DDVP (dimethyl 2,2-dichlorovinyl phosphate). When the mites were maintained in alternating light-dark conditions, maximum susceptibility to the chemical occurred 2 hours after dawn and the mites were least sensitive 2 hours after nightfall.*

Although the information on daily rhythms of sensitivity to biologically active chemicals is still meager, the finding of such rhythms has emphasized the basic importance of circadian organization and provides an additional technique for studying it in organisms too small for the usual assays of locomotory activity. Halberg and his coworkers (*1–4*) have found that mice show daily rhythms in their response to a wide variety of chemicals, such as ethanol (*1*), ouabaine (*2*), *Escherichia coli* endotoxin (*3*), and Su-4885, an adrenocortical inhibitor (*4*). Insects and other arthropods are being used extensively in the study of circadian rhythms, and a great deal of attention has been devoted to sensitivity to chemicals, especially insecticides, in this group of animals. Beck (*5*) found that the German cockroach showed a marked rhythm of sensitivity to cyanide, which was phase-related to the daily respiratory rhythm, and also noted daily fluctuations of sensitivity to DDT and Dimetelon. The first finding of a circadian phenomenon in the two-spotted spider mite was of daily rhythms of sensitivity to ether, chloroform, and carbon tetrachloride (*6*). The experiments described herein show that sensitivity in the two-spotted spider mite to DDVP, a commercially used acaricide, also manifests a pronounced daily rhythm.

The mites used were of the Blauvelt strain, whose resistance to acaricides is known to be stable (*7*). They were reared on Fordhook lima bean plants and maintained in a modified refrigerator (*8*) at 25°C (± 2°C) and a relative humidity of about 70 percent. Fresh plants were introduced into the culture every other day between 7 p.m. and 9 p.m. Light was provided by two 20-watt white fluorescent bulbs set about 20 cm above the tops of the plants and connected to a time switch that maintained a light-dark cycle of 14 : 10 (light from 6 p.m. to 8 a.m.; dark from 8 a.m. to 6 p.m.).

Preliminary experiments on the fumigant action of DDVP indicated a daily fluctuation in sensitivity of the mites to the toxicant. We found that more toxicant was required to kill 50 percent of the mites (LD_{50}) during the first hours of the subjective day than around nightfall. We have not, however, found it practical to utilize its fumigant action in a detailed study of the effectiveness of DDVP at different times of day because (i) there are difficulties in determining the exact concentration of the vapor, and (ii) the number of tests necessary to establish an LD_{50} cannot easily be performed simultaneously. Staggering the tests would defeat the point of the study, which was to measure sensitivity at given points in the light cycle. Therefore, we utilized a method in which a single dose of DDVP could be used and equality of treatment in successive tests could be guaranteed.

The method consists of placing adult females on microscope slides (*9*) which are then dipped in a 0.005 percent emulsion of DDVP for 5 seconds. At each treatment time four groups of 25 mites each were tested. The operations of placing the mites on the slides and dipping the preparation in the acaricide for 5 seconds were carried out in dim white light at 25°C. Immediately after treatment the slides were returned to the refrigerator. The resultant mortality was determined 24 hours later. In all, at least 16 groups (each of 25 mites) were tested at each of 12 different points in the cycle, though not more than four of the treatment times could be tested during any one 24-hour cycle.

The results of these experiments are summarized in Fig. 1. The plotted points are the mean mortality figures expressed as percentages of the treated samples. The 95 percent confidence limits are shown. A marked daily rhythm of sensitivity can be seen, with a distinct peak of maximum susceptibility 2 hours after dawn, which falls gradually during the day to reach a

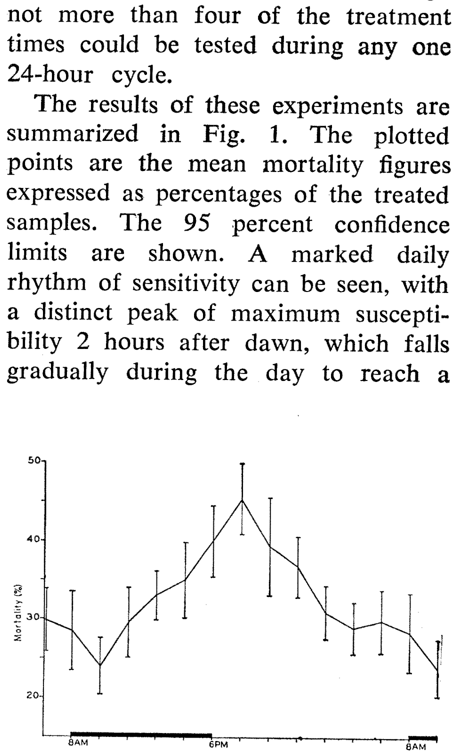

Fig. 1. Percent mortality at different times of day. (The 95 percent confidence limits for each of the test hours are indicated.)

minimum 2 hours after nightfall. The difference in the percentage mortality between the highest and the lowest points of the curve is significant to more than 99.9 percent. The 14-hour period of falling sensitivity and the 10-hour duration of rise in the pattern in these light-dark 14 : 10 conditions could indicate some relationship of the internal organization of the organism to the duration of the light and dark phases—that is, to the photoperiod. Thus not only is the phase of the rhythmic pattern set by the alternating light-dark conditions, but the duration of different parts of the cycle may be regulated by the photoperiod. We attempted to examine this suggestion by testing mites from a colony maintained in a light-dark cycle of 12 : 12, but under such short-day lighting conditions at 25°C the appearance of diapausing forms prevented the continuation of the experiment.

Many of the standard methods of investigation of circadian organization cannot be utilized with the two-spotted spider mite. The method used here can provide information on the temporal organization of organisms which are too small to study with the normally used actograph techniques.

The findings of a marked sensitivity pattern to the acaricide should emphasize the importance of considering circadian organization in the evaluation and interpretation of toxicological experiments with insecticides and all biologically active chemicals.

BRONISLAW POLCIK
JOZEF W. NOWOSIELSKI
JOHN A. NAEGELE
Waltham Field Station, University of Massachusetts, Waltham

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Gastric Erosions in the Rat: Effects of Immobilization at Different Points in the Activity Cycle

Abstract. *Predictable, cyclic patterns of activity were obtained from 30 male rats. Seventeen of these were subjected to physical immobilization just as they were approaching their period of peak activity and thirteen animals were restrained during the inactive phase of their cycle. Eight animals were found to have gastric erosions following the immobilization. All of these came from the group immobilized during the time they would have been in the active phase of their particular cycle.*

When rats are subjected to physical immobilization a certain proportion will develop gastric erosions in the body of the stomach. This is the lower, glandular portion of the stomach which contains acid-secreting cells. Previous research has demonstrated that animals found to have such erosions also have higher concentrations of pepsinogen in the plasma than animals without these lesions (*1*). Moreover, plasma pepsinogen concentrations, taken as a measure of gastric secretory potential, are predictive of erosion susceptibility (*2*). It was concluded (*2*) that a high pepsinogen concentration was neither necessary nor sufficient for the development of gastric erosions since all animals with high concentrations of pepsinogen did not develop erosions upon subsequent restraint, nor did a low pepsinogen concentration uniformly protect against such lesions. Apparently, then, different animals possessed of the same biological predisposition to gastric lesions responded differently to a constant stimulus situation. This is not unlike results which sometimes obtain in "stress" experiments with human subjects where it is hypothesized that an individual may not respond to what the experimenter defines as a "stress" because of the manner in which that person perceives or interprets the situation. The stimulus must be meaningful to the individual—and what may be meaningful is assumed to be a function of the individual's past history. There is no reason to assume that such an argument would not apply to animals lower than man, especially since there is a considerable amount of research in animals which indicates that past experiences influence responses to "stress," even though it is not always possible to predict the direction in which differentially stimulated animals will differ from controls. It was therefore of interest to determine whether differing perceptions of a particular nonspecific "stressful" stimulus on the part of animal subjects could be brought under experimental control.

One of the methods commonly used to induce gastric erosions in the rat is a period of physical immobilization accompanied by food and water deprivation. The greater the duration of immobilization, the greater is the percentage of animals that develop erosions (*2, 3*). Because of the nature of this stimulus, its opposite, "activity," suggested itself as a behavior which might predispose animals to lesions on a psychological or behavioral as well as a physiological level. It was hypothesized that an animal that was psychologically (and physiologically) prepared to be active would "perceive" stimulation in the form of bodily restraint as being more "stressful" than an animal that was psychologically (and physiologically) prepared to be inactive, and that, other things being equal (for example, the concentration of pepsinogen in the plasma), the former would be the more likely to develop gastric erosions.

Experimentally naive, male, Sprague-Dawley rats, each weighing approximately 400 g, were individually housed in cages adjoining activity wheels (Wahmann No. LC-34). Animals remained in these cages with free access to the wheels and with food and water always available for a period of 6 weeks. During the final 2 weeks the number of wheel revolutions was recorded by automatically photographing a bank of electrical counters at 3-hour intervals. To increase the variability in the time at which the peak activity periods would occur, the lights in the room housing these animals were kept on at all times.

A predictable pattern of high and low periods of activity was apparent in 30 of the 44 animals observed. Twenty-one of the 30 animals showed peak activity beginning somewhere between 6 p.m. and 3 a.m.; the maximum activity of the remaining animals occurred during the daylight hours. Some animals displayed a pattern in which the periods of maximal activity occurred within the same 3-hour period each day; other animals showed a cycle that was slightly greater than 24 hours in its periodicity. This latter type of rhythm is illustrated in the lower portion of Fig. 1. The upper graph is an example of a regular activity pattern.

The 30 animals were then subjected