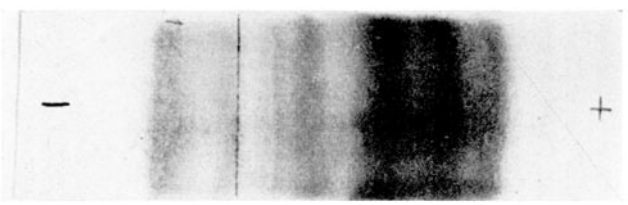
is 1:1. From the dose of the toxin, its molecular weight, the amount of fibrinogen and its molecular weight in the plasma of the experimental animal, one can, however, calculate that the ratio fibrinogen:toxin is at best 100:3. A reaction of an enzymatic type between the toxin and the intermediary polymeres cannot be excluded either. As will be shown elsewhere, *Echis* toxin *in vitro* is able to denature fibrinogen, to dissolve fibrin and to activate plasminogen. Thus it is possible to assume that the activation of the plasminogen, or the direct fibrinolysis, is the main cause of the functional deficiency of fibrinogen due to the administration of *Echis* toxin. The initial stage of the conversion is actually started by the action of thrombin; nevertheless, the fibrin strands formed are immediately hydrolyzed. The level of fibrinogen might remain unchanged, in analogy to various pathological conditions where the activity of the fibrinolytic system has been increased[2;3]. Thus one can compare the effect of the *Echis* venom with the activity of streptokinase, urokinase or other activators of plasminogen. When streptokinase was used, however, the effect occured much earlier and disappeared faster. The intensity of the effect of the toxin is evident from the fact that the experimental rats are resistant to 500 units of NIH thrombin applied intravenously and show no thromboembolic sequellae. This is a dose many times exceeding the tolerance of experimental animals where other activators of the fibrinolytic system have been employed.



Fraction No.	1	2	3	4	5	6	7	8	Control
Coag. *in vitro* time in sec	48	49	55	55	12,6	<2	5,3	37	39
Coag. *in vivo*					+	+	+		

Fig. 3. Electrophoretic division of *Echis* toxin.

When analysing the fibrinolytic properties of the other thirteen toxins, it was found that in seven out of them the fibrinolytic, plasminogen activating, and fibrinogenolytic component was more effective than in *Echis* toxin. It appeared that in the other venoms the active coagulant components were neutralized by the defense system of the organism to which the *Echis* venom is resistant. The specific properties of *Echis* toxin were evident also when influencing the coagulation *in vitro*. Contrary to all viper venoms whose coagulating effect *in vitro* is made possible by the presence of calcium and enhanced by the addition of kephalin, the effect of *Echis* toxin is not influenced by the presence of these two agents. In contrast to some venoms of the *Bothrops* and *Crotalus* kind, no fibrinogen converting effect is inherent to it either. We intended, therefore, to ascertain whether the influencing of the coagulation, which is dissimilar to all other toxins, is caused both *in vitro* and *in vivo*, by the identical fraction of *Echis* toxin. The venom can be divided by electrophoresis into eight fractions (Figure 3). The maximum effect in the experiment *in vitro* could be found in the fractions 5, 6, and 7. The single fractions were eluated and applied subcutaneously to dogs. Whereas *in vitro* they shortened the coagulation time by their thromboplastic activity, *in vivo* the same fractions prolonged or even inhibited the plasma coagulation in dogs. Both activities were destroyed by heating for 50 min to 72°C. It appears, therefore, that both effects are due to one identical component of *Echis* toxin.

Zusammenfassung. Von 14 untersuchten Schlangentoxinen ruft nur das Gift von *Echis carinata* in subtoxischen Gaben bei Versuchstieren Verlängerung bis Hemmung der Koagulation hervor. Es wird gefolgert, dass es sich um einen spezifischen Aktivator des fibrinolytischen Systems handelt. Der wirksame Faktor wurde papierelektrophoretisch teilweise isoliert.

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Institute for Experimental Pathology of the Charles University, Institute for Haematology and Blood Transfusion, Prague (Czechoslovakia), February 15, 1962.

[2] O. K. ALBRECHTSEN et al., Acta haematol. *14*, 309 (1955).
[3] V. LAKI and J. A. GLADNER, Nature (Lond.) *187*, 758 (1960).

Activity Patterns on Regimes Employing Artificial Twilight Transitions

If deer mice are kept under laboratory conditions in which twilight transitions and nocturnal illumination are simulated, their locomotor activity in running wheels can be synchronized to grossly unnatural periodicities and their phase of activity can be manipulated extensively[1]. Simulated dawn, particularly, is a potent modifier of locomotor activity of these nocturnal animals in circumstances in which the conventional technique of simply turning lights on is often ineffectual.

Recording of the speed and direction of wheel-running of deer mice in these studies has disclosed behaviour heretofore not noted, which can be summarized briefly as follows (see Figure 3). When the animals begin to run activity wheels at a time when ambient illumination is unchanging (whether bright, dim or dark), they customarily 'warm up' to maximum speed[2]. By contrast, when running begins during a simulated dusk, there is sometimes an initial spurt at high speed followed by deceleration to the subsequently sustained rate. If the animals happen not to be running at the beginning of simulated dawn, they frequently respond by running briefly at high speed; if already running at this time, they often increase the pace in a brief burst and then cease abruptly. When they run in long sustained sessions synchronized with the light cycle, they characteristically maintain the same direction of running[2] and, when possible, they run facing the source of nocturnal illumination. But when synchrony is poor or activity is sporadic, both speed and direction of running usually are haphazard.

[1] J. L. KAVANAU, Nature, in press.
[2] J. L. KAVANAU, Behaviour, in press.

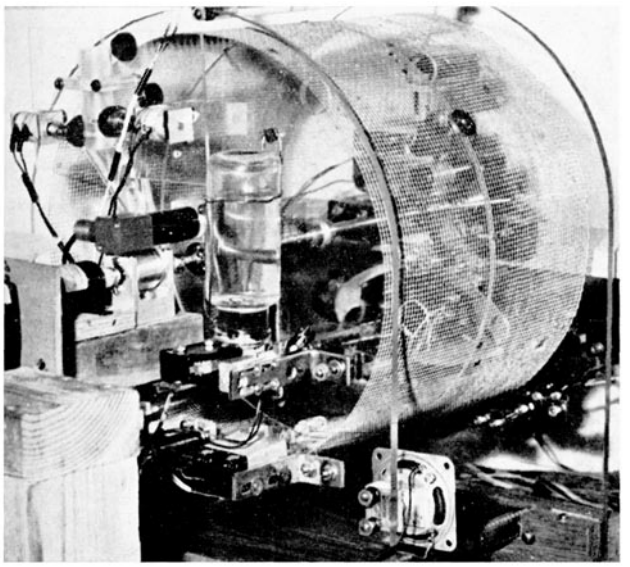

Fig. 1. Side view of the enclosure. Pertinent components are identified in Figure 2. A commercial Gerbrands pellet dispenser was used in place of that shown at the upper left.

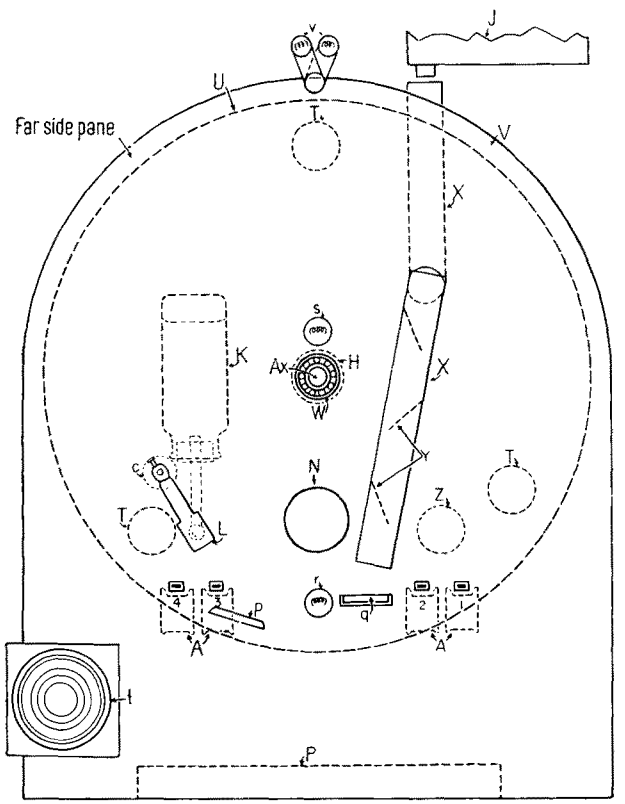

Fig. 2. Schematic scale diagram of the far sidepane of the enclosure as viewed from the wheel. Pertinent components are as follows: c, rotary solenoid of water shutter; p, platform for support while drinking; Q, food tray; r, s, wheel and axle infra-red electric-eye light sources; v, light bulbs; A, program microswitch levers; Ax, wheel axle; J', pellet dispenser; K, water bottle; L, water shutter; N, position of nest; T, closed passages in center-disc of wheel; U, wheel; V, far sidepane; X, pellet chute; Y, vanes slowing descent of pellets; Z, closed passage in far sidepane.

Using simulated twilight transitions, deer mice, *Peromyscus maniculatus*, can be synchronized to clear-cut phase-related 16 h periodicities of locomotor activity[1]. Since only the running of wheels or ambulatory activity are customarily employed as indices of activity in short-term animal rhythmicity studies, it is most pertinent to inquire as to whether animals synchronized to artificial periodicities of locomotor activity also display the same rhythmicity of food and water intake and sleep. It is possible that animals observing unnatural periodicities of locomotor activity in the laboratory, nonetheless tend to adhere to a circadian rhythmicity of other activities.

For a preliminary investigation, a female mouse showing the most clear-cut response on a 16 h regime was selected for further studies. This work employed an enclosure[3] (Figures 1 and 2) and techniques[1, 4] previously described that automatically detect and record up to 22 channels of information.

The passages (T) in the center-disc of the running wheel (U) were closed, thereby confining the animal to the side of the wheel containing facilities for food and water. The main nests, situated on the other side of the wheel, were inaccessible. An auxiliary nest (N) consisted of a 6.5 cm length of opaque Perspex (Plexiglas) tubing (4 cm I.D.), projecting into the wheel from the far sidepane (V), mounted 9 cm above the bottom of the running wheel (U).

Complete diet food pellets[3] (0.97 mg) were obtainable from a commercial dispenser (J') by pressing either of two microswitch levers (A, 1 or 2). The pellets were delivered into a small tray (q), no more than one being obtainable in any 10 sec interval. Water was obtainable[4] upon brushing aside a lightly spring-loaded shutter (L) exposing the spout of a water bottle (K).

Presence of the animal in the nest (N) was determined indirectly using two infra-red electric eyes (r, s). One of these (s) monitors presence upon, or crossing of, the axle (Ax); the other (r) monitors presence at, or crossing of, the bottom of the wheel (U). Whenever a mouse rests motionlessly in the loosely-turning wheel (frictional torque 56.1 g-cm), the beam of the latter eye is interrupted, since the wheel always comes to rest with the animal at the bottom. Presence in the nest can be inferred from failure of interruption of either infra-red beam.

Four General Electric No. 44 bulbs (v) were mounted to the sidepane directly overhead at a distance of 30 cm (only 2 bulbs are shown in Figure 2). Daytime illumination was at an intensity of roughly 3.96 ft-c; nocturnal illumination, of about 0.0008 ft-c, approximates that on a clear moonless night. Twilight transitions were effected by varying the filament voltage of the bulbs; these simulated the relative changes in intensity occurring in the hour before the end (and after the beginning) of local civil twilight[1].

The enclosure was housed in a light-proof, sound-deadening cabinet in an air-conditioned darkroom (20–22°C, 35–50% relative humidity) with an external background level of −4 db of thermal noise. Analog and digital automatic printing and strip-chart recording devices, and programing and systems controls techniques were used as previously described[1–4]. Direction and speed of running were determined using a miniature dc generator (not shown in Figures 1 and 2) and Speedomax G recorder. A microswitch actuated by an eccentric signaled the time and number of revolutions.

[3] J. L. Kavanau, Ecology *43*, 161 (1962).
[4] J. L. Kavanau, J. Mammal, in press.

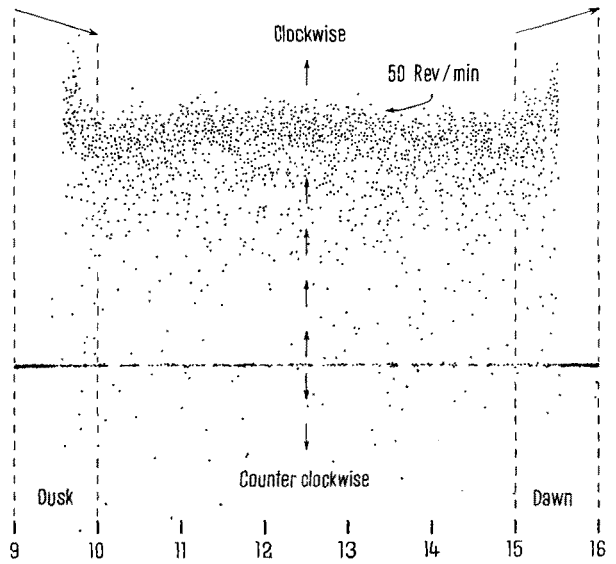

Fig. 3. Reconstruction of a Speedomax G record of instantaneous speed and direction of wheel-running during a nocturnal period. Values are recorded at 4 sec intervals at a chart speed of 1 inch per h.

In the first study of this animal[1], synchronization to a 16 h periodicity was accomplished by gradual phase shifts during a period of over 3 weeks. The sharpness of the 16 h rhythmicity achieved can be judged from the temporal plot of running for 11 16 h periods (LD 10:6) in Figure 4 (top). The ratio of wheel revolutions per unit time in the periods from the beginning of dusk to the end of dawn to those in the periods from the end of dawn to the beginning of dusk (D-L/L-D) was 2000/1. A record of speed and direction of running for this animal, which well illustrates responses to twilight transitions, is shown in Figure 3.

For the present study, the animal was placed directly upon a 16 h regime (LD 9:7), without preliminary phase shifting or gradual changes of periodicity. By the 4th day its running activity had adapted to and followed the unnatural regime closely. Results for running, food pellet procural, water consumption, number of drinks, time in nest and number of exits and entrances during 11 subsequent 16 h periods are given in Figure 4 (D-L/L-D = 410).

It will be noted that the animal displayed a clear-cut 16 h rhythmicity of all activities in phase with the light cycle. The few wheel revolutions registered during the light period (Figure 4, 2nd from top) were primarily coincidental with sporadic daytime excursions from the nest on which the animal ate, drank or excreted; they infrequently represent actual running of the wheel. It is typical of the behaviour of deer mice in the security of artificial enclosures under controlled environmental conditions that they eat, drink and eliminate during daytime excursions, even on a 24 h day[3-4]. They rarely spend more than 3 h at a stretch in the nest. In this connection, it is of interest that the animal never left the nest in the 2 h following dawn (Figure 4).

These findings suggest that, when the locomotor activity of small mammals is synchronized to and in phase with unnatural periodicities of illumination, their other activities follow the same periodicity. Relatively strenuous physical exertion, such as running a wheel, displays the sharpest synchrony with changes in ambient illumination of both natural and artificial periodicities, and would appear to be the index of choice for rhythmicity studies. A detailed report of these studies will appear elsewhere[5].

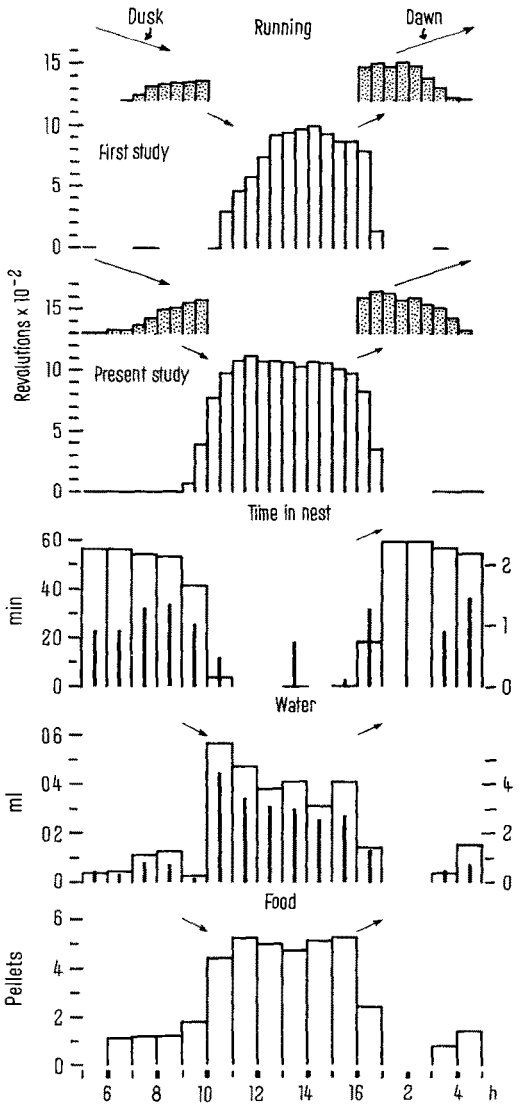

Fig. 4. Activity patterns during 11 16 h periods (LD 9:7). Left ordinate figures for food, water and time in nest are daily averages per h; those for running are per $^1/_2$ h, except for the stippled insets for running during dawn and dusk which are for 10 min intervals, including $^1/_2$ h preceding and following the twilight transitions. The central bars for water and time in nest give the number of drinks and the number of exits from and entrances of the nest, respectively (right ordinate scales). Periods of twilight transitions are indicated by inclined arrows.

Zusammenfassung. Eine Maus der Gattung *Peromyscus maniculatus* wurde mittels künstlicher Beleuchtung (Nacht sowohl als Tag) und künstlicher Dämmerung mit einem 16-h-Rhythmus synchronisiert. Das Tier zeigte eine eindeutige Periodizität in Laufaktivität, Nahrungs- und Wasseraufnahme sowie der Zeit, die es im Nest verbringt. Diese Tätigkeiten sind eindeutig vom Beleuchtungscyclus abhängig.

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Department of Zoology, University of California, Los Angeles (U.S.A.), April 9, 1962.

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