cardiac muscle and that the activation front runs perpendicularly to the endocardiac and epicardiac surfaces.

Riassunto. Gli autori hanno studiato l'e.c.g. intracardiaco di alcuni Teleostei, un Ganoide ed alcuni Selaci. Le caratteristiche del tracciato endocavitario e di quello epicardico sono risultate assai simili; ciò fà pensare che l'onda di eccitazione si irradia, nel cuore dei Pesci, nel medesimo tempo in tutto lo spessore della massa miocardica e che il fronte di attivazione si sposta nello stesso tempo rimanendo normale rispetto alle due superfici.

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Coagulation Defect Following Non-Toxic Doses of *Echis viper* Venom

In their previous papers, the authors have drawn attention to the fact that the *Echis viper* venom, when applied subcutaneously in small doses to experimental animals, prolongs the coagulation time of the plasma[1]. To analyse this phenomenon more thoroughly, the effects of thirteen other snake venoms (*Vipera russellii, Vipera berus, Vipera amodytes, Naja naja, Bitis gabonica, Vipera lebetina, Crot. viridis, Crot. atrox, Crot. terrificus, Bothrops alternat., Agkistr. piscivorus, Agkistr. contortrix, Agkistr. halys.*) have been tested in the same manner, i.e. 24 h after the administration of doses ranging from 10 to 200 γ/200 g of body weight of the rat. The prolongation of the coagulation time has been ascertained in the *Echis viper* venom only. The experiments have been carried out on groups of white rats, each batch containing 10 animals of 200 g body weight. The tests were carried out 24 h after the application of the venom. A significant prolongation of the coagulation time developed already after a dose of 2 γ/rat. If 10 γ or more were applied, no clotting of the plasma could be observed even after 5 min. The changes were more obvious when assessing the thrombin time (0.1 ml of plasma, 0.1 ml of thrombin) than when determining the prothrombin time according to Quick. If 20 γ were administered to one animal, the thrombin time was significantly prolonged already 4 h after the application. When the coagulation was examined in dogs 24 h after a dose of 50 γ/kg, it was found that changes of the cofactors of the plasmatic thromboplastin were not involved in the defect. Both the level of prothrombin and its consumption were normal. The assumption is made that the coagulation defect was caused by changes which were due to the conversion of fibrinogen or to the fibrinolysis. The disturbed dynamics of the third and fourth phase of the coagulant activity is evident from the thromboelastographic pattern taken 12 and 16 h following the administration of 50 γ of toxin per rat (Figure 1).

The inhibition of the fibrin formation could be caused by a change in the fibrinogen molecule due to the inhibition of its polymerization or to the activation of the fibrinolytic system. The method employed for the determination of the fibrinogen level after its conversion into fibrin cannot be used to assess the effect of higher doses of *Echis* toxin, because under these conditions no fibrin is formed at all.

Dog plasma has, therefore, been analysed with the aid of electrophoresis 24 h after the application of 50 γ/1 kg. In all six experimental animals, no qualitative changes have been observed in the zone, corresponding to fibrinogen, which would deviate from the standard methodic error or the physiological variability (Figure 2). There are two possible explanations for this fact. The toxin could influence the molecular structure of fibrinogen in such a way that, though it does not change its electrophoretic migratory properties, it inhibits the conversion of fibrinogen into fibrin. This hypothesis will have to be corroborated by a closer analysis of the fibrinogen molecule after the application of toxin to experimental animals.

The active components of the toxin could act as an antipolymerase. The quantitative relations would, however, have to be taken into consideration. The direct inhibitory bonding of the toxin to the molecules of fibrinogen or its intermediary polymeres would have to follow stoichiometric laws where the ratio of the molecules in the reaction

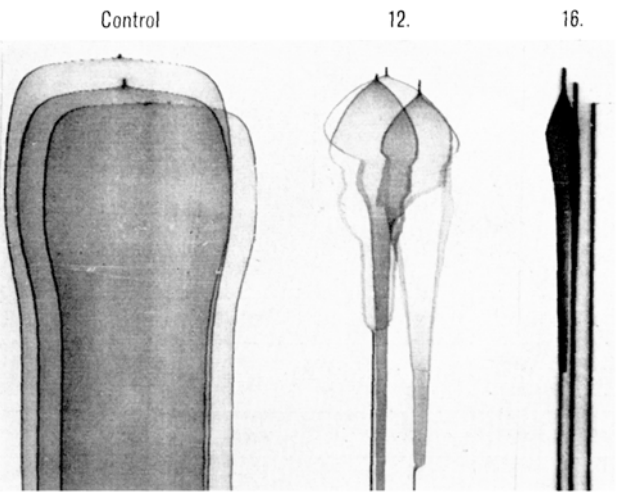


Fig. 1. Thromboelastographic pattern in control and experimental rats.

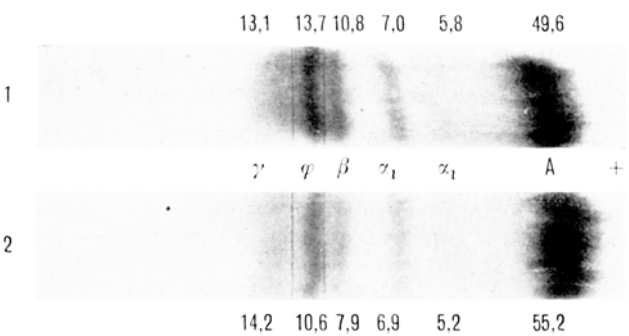
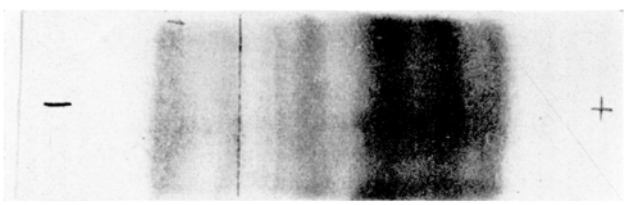


Fig. 2. Electrophoretic examination of dog plasma 24 h after application of *Echis* toxin. Number 1 control, number 2 24 h after application.

[1] F. Kornalík, Arch. exp. Path. Pharmak. *240*, 72 (1960).

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.