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The Effect of the Venom of the European Water Shrew (*Neomys fodiens fodiens* Pennant) on Certain Experimental Animals

Działanie jadu *Neomys fodiens fodiens* Pennant na niektóre zwierzęta doświadczalne

I. INTRODUCTION

Results published in a preliminary report (M. Pucek, 1957) gave evidence of the toxicity of the saliva of *Neomys fodiens fodiens* Pennant. This species has proved to be the third mammal to have salivary glands, which produce a toxic secretion. Contrary to the belief held in ancient times, that the saliva of all *Soricidae* is poisonous, a view stated even in serious text-books (Grassé, 1955), no-one has yet demonstrated the toxicity of other European *Soricidae*.

Pearson's investigations (1956) carried out on *Neomys fodiens* from Switzerland gave negative results. It is possible that this was due to an error in identification. It is known that *Neomys anomalus* Cabrera a species difficult to distinguish from the European water shrew, is very common in Switzerland. This would support my observations, which, though they were carried out on only three specimens of *Neomys anomalus milleri* M o t t., disclosed no poisonous properties in the saliva of this species.

The aim of the present paper is to give further details and results, to examine certain pharmacological properties of the toxic substance of *Neomys fodiens* and to investigate the behaviour of this shrew towards other species.

The salivary glands of 39 *Neomys fodiens* P e n n a n t were used for these experiments, which were carried out on white mice (*Mus musculus* L.), field voles (*Microtus agrestis* L.) and on rabbits (*Oryctolagus cuniculus* L.). The mice and field voles were injected with the homogenized submaxillary glands, subcutaneously, intraperitoneally and intracerebrally, the rabbits — intravenously. For control purposes, parotid glands and 0,9% saline solution were injected the same way.

II. RESULTS AND DISCUSSION

The effect of the submaxillary glands was fairly characteristic and depended to a great extent on the amount administered and on the site of injection. The doses were calculated according to the weight of the body (per 20 g. body weight or per kg.).

Efforts were made to determine the smallest dose produce an effect on the white mice and on the field voles. The latter proved to be the more susceptible. The white mice showed no reaction to an intracerebral injection of 0.19 mg. per 20 g. whereas a strong toxic effects, including some deaths were observed in the field voles.

An intracerebral dose of 0.22 mg. / 20 g. body weight was the smallest to which the mice reacted while that for the field voles was about 0.01 mg. / 20 g.

These doses, injected into the body cavity, were not sufficient to induce any reaction. For example, the lowest dose to which the mice reacted was 21 mg. / per 20 g. body weight. It is interesting to note that the field voles reacted to a dose that was nearly twice as large, 40 mg., merely by a strong contraction of the abdominal muscles, so that a strong contraction of the animal affeared to take place (Plate VI, Fig. 5).

The same doses administered subcutaneously did not induce the changes observed when they were introduced into the brain or body cavity. Only slight reactions of the animals were noted.

Responses to the intraperitoneal injection were also less than those produced by intracerebral administration. No immediate symptoms were observed. The first appeared some minutes after the injection and were less violent than those seen after intracerebral injections.

It can be stated that, on the whole, the substance contained in the submaxillary glands of the water shrew has the most marked and the most rapid effect when given intracerebrally. The behaviour of the white mice and the field voles after such injections is shown in plates V—VII.

Disturbance of the locomotory system is characteristic of these animals. This is a result of an effect on the central nervous system, manifested by paraplegia of the hind limbs and the whole posterior region. The animals were seen sitting or lying with their muzzles resting on the floor, feet splayed as if to maintain balance (Fig. 1, 3, 7). Shaking and rocking of the animals was also observed. When they changed position, their movements were uncoordinated, floundering and helples (Fig. 2, 9). The characteristic rolling already described (M. Pucek, 1957) is partly shown in the photographs (Fig. 4, 8, 10, 11, 12).

After the injections the field voles turned over on their sides in the characteristic position — spine arched backwards, limbs extended and stiff, toos widely separated, head bent backwards or twisded in an unnatural manner (Fig. 6). In addition, spasms, convulsions and an abnormal increase in the tension of almost all visible muscles were observed in the white mice and field voles. The tail remained stiff,stretched and jutting upwards, almost in every case, practically at right angles to the longitudinal axis of the body. Marked respiratory disturbances were observed together with increased urination and inhibited reaction to external stimuli (sound, light). The animals retained, however, a very strong response to touch. After death anaemia of the limbs, ears and tail was noted.

The animals died after a variable period of time, according to the size of the dose. The smallest lethal dose for the mice was from 0.5 to 1.0 mg. / 20 g. body weight, that for the field voles, on the other hand was 0.2 to 0.4 mg.

The lenght of time in which death occurred varied for the two species. The field voles died earlier — about 5 minutes after the injection, whereas the mice died approximately 45 min. after the poison began to take effect.

These experiments carried out on white mice and field voles showed that the homogenized tissue from the submaxillary glands of *Neomys fodiens* Pennant caused symptoms similar to those observed in the experiments with *Blarina brevicauda* Say. (Pearson, 1942; 1950).

The strength of the toxic substance contained in the submaxillary glands of the *Blarina brevicauda* Say. is much more potent than that contained in these glands in *Neomys fodiens fodiens* Pennant. For example, 2.9 mg. intraperitoneal injections of fresh tissue from the submaxillary glands of the *Blarina brevicauda* Say., per 20 g. body weight caused the death of more than 50% of the mice used for the experiments and all the injections over 11.0 mg. / per 20 g. body weight brought about the death of 100%, whereas, almost double that dose — 21 mg. / 20 g. of the *Neomys* submaxillary glands administered intraperitoneally to white mice, only induced a slight reaction.

Pearson (1942, 1950) investigating the salivary glands of many species of *Soricidae*, found that the excretion of the *Sorex caecutiens* Laxmann (= *Sorex cinereus* Kerr.) submaxillary glands is also poisonous but that it is incomparably weaker than the *Blarina*. Other species of *Sorex* were slassified as comparatively non-toxic.

The changes brought about by the toxin in the circulatory and respiratory systems

The effect of the *Neomys* poison on functional changes in the blood vascular and respiratory systems was investigated in rabbits [1]).

For this purpose the rabbits were anaesthetized (amythal natrium — 75 mg. / kg. or methyl urethan — 1.4 mg. / kg.).

The experiments were designed to show graphical form any changes in depht of respiration and changes in blood pressure. The rabbits were injected intravenously (vena femoralis) with freshly homogenized submaxillary glands. For control purposes physiologi-

[1]) My sincere thanks are due to Professor Dr. J. Supniewski, Head of the P.A.Sc. Pharmacological Institute in Cracow, for enabling me to carry out these experiments there. I, also, particularly wish to thank Dr. T. Marczyński for his assistance.

cal saline solution and homogenized parotid gland were first injected into the same animals.

The parotid gland did not produce any marked changes. 30 mg./kg. of fresh parotid tissue introduced into the vena femoralis caused a slight, temporary fall in blood pressure. The blood pressure returned to normal level about 2 minutes after the injection (Fig. 13, A and B). No changes in the respiratory system were observed.

The rabbits were given the homogenized submaxillary gland denatured by heating almost to boiling. After introducing 20 mg. of the substance of these glands per 1 kg. of the body weight of the rabbits intravenously, it was found that the blood pressure was slightly raised, respiration became slightly shallower (Fig. 13, F).

Table 1.

Changes in the respiratory and vasculary systems of the rabbit after injections of the extract of water shrew's submaxillary gland.

Rabbit. Male, 1.62 Kg. dose: 20 mg./Kg.				Rabbit. Male, 1.70 Kg. dose: 30 mg./Kg.			
Time	Respiration: Amplitude in mm.	Respiration: No. per min.	Blood pressure	Time	Respiration: Amplitude im mm.	Respiration: No. per min.	Blood pressure
Before injection	43	21	78	Before injection	18	14	84
one min. after injection	47	25	48	one min. after injection	39	23	41
after five minutes	33	17	57	after fife minutes	9	18	26
after fifteen minutes	18	15	24	after fifteen minutes	—	—	—

On the other hand, after introducing fresh homogenized submaxillary gland in amounts of 30 mg./kg. body weight the following symptoms were observed:

Respiration. The moment it was given the toxin had a stimulating effect. This was manifested by a deepening of respiration. After about a minute extent of the respirations descreased and their frequency temporarily increased. Afterwards, a continually progressive depression of respiration was observed until death. The changes which took place are illustrated by the successive sections of the graphical record (Fig. 13, C, D, E; Plate VIII).

The blood pressure increased slightly at the moment of introduction of the extract, then suddenly decreased (Table 1).

In another experiment (Fig. 13. G, H, I) after one minute the blood pressure fell from 78 mm. of mercury to a level of 48 mm. then rose a little after which it again underwent depression.

Efforts to save the animals by administering adrenaline, coffeine, strophantine were not successful. Apparently irreversible process was taking place and 50 minutes after the injection the animals died.

It can therefore be concluded that death occurred as a result of paralysis of the nervous system manifested in disturbances of the respiratory and circulatory systems.

The results obtained are similar to those obtained by Ellis and Krayer (1955). These authors widened Pearson's field of investigation (1942) chierly from the point of view of the pharmacological properties of the *Blarina brevicauda* Say. toxin. The submaxillary gland extract from this animal caused at first an increase in depth and number of respirations after which in three minutes they were reduced to a quarter of the normal. In some cases even an immediate cessation of respiration was observed. Changes in circulation occurred simultaneosly. The blood pressure fell suddenly and within 40 minutes from the beginning of the experiments diminished to 18 mm. The heart beat became slower, progressively weaker and finally stopped.

As a result of the experiments performed on cats and rabbits Ellis and Krayer came to the conclusion that the *Blarina* toxin affects, above all, the respiratory system, having in the first phase a stimulatory effect and later an inhibitory one. Furthermore not only disturbances in the respiratory system but also changes in the heart rhythm and sudden fall in blood pressure bring about the death of the animals.

The properties of the toxin of *Neomys fodiens fodiens* Pennant

At the moment we cannot speak of a toxin in the true sense of the word as no toxic substance has been isolated in a pure form. The properties mentioned, therefore, pertain to the homogenized submaxillary tissue.

In 0.9 per cent sodium chloride solution the homogenized glands did not lose their toxic properties when kept for 24 hours at room temperature. After 48 hours the activity weakened and when kept for longer periods in these conditions the toxic properties were completely destroyed. It could be kept at cold temperatures (up to + 5° C) for 7 days with only a slight progressive weakening of its activity.

Heating to a temperature of 100° C destroyed the toxic properties a fact which would seem to indicate its protein nature. As regards its other properties, it can only be supposed that they are to a great extent similar to those of the *Blarina brevicauda* S a y.

Ethological observations

The behaviour of the *Blarina* in its natural state was the subject of investigations by S h u l l (1907) and H a m i l t o n (1930). One interesting observation was that *Blarina* seizes its victims, which are usually mice, by the ear then by the back of the head in the temporal area, piercing the skull. This type of attack, undoubtelly facilitates the introduction of the venom in to the brain, where, acting on the central nervous system, it can induce various types of paralysis. Thus, the venom of *Blarina* would have a neurotoxic character (La w r e n c e, 1954), acting fairly rapidly, if not as a lethal dose, at least sufficiently to immobilize the victim. (Probably this venom is effective in use on some invertebrates which comprise the primary element of the food of the *Blarina brevicauda* S a y.).

In view of this, it can be more readily understood, that a *Blarina* weighing 11.6 g. was able to kill a 17 gramme mouse in half an hour and forcibly attacked mice 10 g. heavier than itself (H a m i l t o n, 1930). It seems probable that the saliva excreted during battle is more toxic (P e a r s o n, 1942).

The observations made of the behaviour of *Neomys* towards other animals under laboratory conditions, showed that *Neomys* clearly attacks frogs, very often frogs bigger than itself. For example, a *Neomys* weighing 13.7 g. attacked a 30 g. frog after having killed smaller frogs easily. The manner in which *Neomys* attacked was similar to that observed in *Blarina*. It seized its victims from behind, usually by the posterior part of the head, or by the nearest part of the back. Frequently after a short struggle the shrew became separated from the frog. After being taken away from the shrew, the frogs seemed passive and immobilized. However, no obvious signs of paralysis were seem. The paralysis of the limbs and lack of reaction to touch, manifested in these animals were not satisfactory evidence of general paralysis. It is difficult to say whether the behaviour of the frogs was the results of the biting and injuries, e. g. nerve endings, or, if it was due to the action of the toxic substance contained in the saliva. In its attitude to the frogs, the *Neomys* is definitely the aggressor. This indicates that frogs may be part of their normal diet.

As regards its behaviour towards small rodents, *Neomys*, does not seem to exhibit the behaviour of a predator the mice, field voles and the shrew avoid one another. On encounter they do sometimes fight but then it is rather as a result of defensive action than a decided attack from one or the other.

The natural functions of this venom remain to be discussed.

Venomousness in the animal world is usefull in obtaining food, for defence etc. The appearance, in mammals, of glands producing toxic substances might be regarded as a phenomenon which is biologically superfluous, not being essential for the animal's existence — unless we regard them as a vestigial character. Nevertheless, careful observations show that as regards *Blarina*, venom plays an important role in the acquisition of food, this also appears to apply to *Neomys*. It is true that the food of this species differs from that of *Blarina* but the method of obtaining food, and the aggressiveness with which it falls on its prey, are similar. Hence it can be concluded that the toxic saliva of this animal is a contributory factor in the succesful aquisition of food. When they bite the *Neomys* excrete very large amounts of saliva as is easily seen when one takes the animal into one's hand. Possibly the saliva contains the toxic substance produced by the salivary glands, which, it can be supposed, serve to immobilize and, perhaps even to kill such victims as insects, small reptiles or fish.

Many authors endeavour to find links of an evolutionary character between venomous mammals and reptiles (L a w r e n c e, 1945). The present writer affirms that the *Blarina* toxin has many characteristic common to the venom of the *Elapinae (Ophidia)* as far as physicochemical properties are concerned. Both its neurotoxic action and the weak local reaction induced by the toxic substance contained in the saliva of the *Blarina brevicauda* S a y., emphasizes its similarity to the *Elapinae*.

According to L a w r e n c e (1945) certain ferment-like substances including some with proteolitic properties are present in the venoms of snakes. It is possible that the saliva of *Blarina* contains similar substances. As evidence Lawrence states, that the body walls of *Blarina* dissolve away very quickly after death and that the animal consumes large amounts of food daily.

The voracity of the *Soricidae* is well known, however (Tupikova, 1954) and is connected rather with their rapid metabolism than with proteolitic properties of their saliva. The experiments of Pearson (1950) on the effect of extract from the salivary glands of the *Blarina* on egg whites showed that the saliva of these animals do not contain any enzymes to decompose albumen. Thus the supposition of Lawrence based entirely on evidence from the literature does not withstand criticism. Reptiles and mammals are groups too highly specialised and too widely separated for us to seek such a great similarity between the two.

Nevertheless, it seems a significant fact that toxicity occurs among the *Insectivora* which are generally regarded as the most primitive of all the mammals of the *Eutheria*.

III. SUMMARY

The present paper extends investigations previously carried out on the toxicity of the European water shrew *(Neomys fodiens fodiens* Pennant), M. Pucek (1957).

When injected intracerebrally, intraperitoneally and intravenously, the extract from the submaxillary gland was found to have a toxic effect on white mice (*Mus musculus* L.), field voles (*Microtus agrestis* L.) and rabbits (*Oryctolagus cuniculus* L.). The strongest reaction was observed after intracerebrall injections (mice and field voles) and intravenous injections (rabbits).

The venom of the water shrew effects mainly, the nervous system, causing spasms, convulsions and paralysis of the limbs as well as a rise in the muscle tension and an inhibited reaction to some external stimuli.

There were also disturbances of the respiratory system (increase of the amplitude of breadth and its frequency) and of the vascular system (rapid decrease of the blood pressure).

The field voles were more sensitive to the shrew's venom than mice. The minimal dose to cause any reaction was 0.01 mg. of the substance from the submaxillary glands per 20 g. body weight and

0.22 mg. / 20 g. respectively. The smallest lethal dose for the *M. agrestis* L. was 0.2—0.4 mg. / 20 g. and for white mice, on the other hand, 0.5—1.0 mg. / 20 g. body weight.

The toxic substance contained in the saliva of the water shrew is undoubtelly excreted with the saliva, this being particularly copious during battle or when the animal is bitting its opponent, and it appears to be of assistance in killing or immobilising the prey (small reptiles and fish, insects and other invertebrates).

In the three specimens of *Neomys anomalus milleri* Mottaz, examined no toxic efect of the extract from their submaxillary glands was observed.

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EXPLANATION OF PLATES

Plate V.

Figs. 1 — 4. The effect of the extract from water shrew submaxillary glands on white mice. Intracerebral injection.

Plates VI & VII.

Fig. 5. Strong contraction of the abdomen in the *Microtus agrestis* L., caused by administration of submaxillary gland tissue into the body cavity.

Figs. 6 — 12. The action of the water shrew venom, its successive phase on being injected intra cerebrally into the *Microtus agrestis* L. Further details are given in the text.

Plate VIII.

Fig. 13. A, B — The effect of the parotid gland (30 mg. per kg.) on blood pressure and respiration in the rabbit. C, D, E — The effect of freshly homogenized water shrew submaxillary gland tissue (30 mg. per kg.) on blood pressure and respiration in the rabbit. F — The effect of denatured *Neomys fodiens* submaxillary gland tissue. G, H, I — As in C,D,E. Other experiments.

STRESZCZENIE

Publikacja niniejsza uzupełnia badania autorki (M. Pucek, 1957) nad jadowitością rzęsorka rzeczka (*Neomys fodiens fodiens* Pennant).

W wyniku injekcji domózgowych, dootrzewnowych i dożylnych ekstraktu gl. submaxillares stwierdza się jego toksyczne działanie na białe myszy, polniki (*M. agrestis* L.) i króliki. Najsilniejsza reakcja następowała po injekcjach domózgowych (myszy i polniki) oraz dożylnych (króliki).

Jad rzęsorka działa przede wszystkim na system nerwowy i objawia się drgawkami, konwulsjami, paraliżem kończyn a także wzrostem napięcia mięśni szkieletowych i zahamowaną reakcją na niektóre bodźce zewnętrzne (Tablice V do VII).

Obserwuje się również zaburzenia układu oddechowego (zmniejszenie amplitudy i wzrost częstotliwości oddechów) oraz systemu krążenia (gwałtowny spadek ciśnienia krwi) — Tabela 1, Tablica VIII.

Polniki są bardziej wrażliwe na działanie jadu rzęsorka niż myszy. Odpowiednie dawki minimalne dla wywołania reakcji wynoszą 0.01 mg masy gruczołu podszczękowego na każde 20 g wagi ciała i 0,22 mg/20 g. Najmniejsza dawka śmiertelna dla *M. agrestis* wynosi — 0.2—0.4 mg/20 g, dla białych myszy zaś — 0.5—1.0 mg/20 g wagi ciała.

Zawarta w ślinie rzęsorka substancja toksyczna jest niewątpliwie wraz z nią wydzielana, szczególnie obficie podczas walki i gryzienia i może pomagać przy uśmiercaniu lub unieruchamianiu jego ofiar (drobne płazy i rybki, owady i inne bezkręgowce).

U trzech badanych okazów *N. anomalus milleri* Mottaz nie stwierdzono toksycznego działania wyciągu z jego ślinianek podszczękowych.

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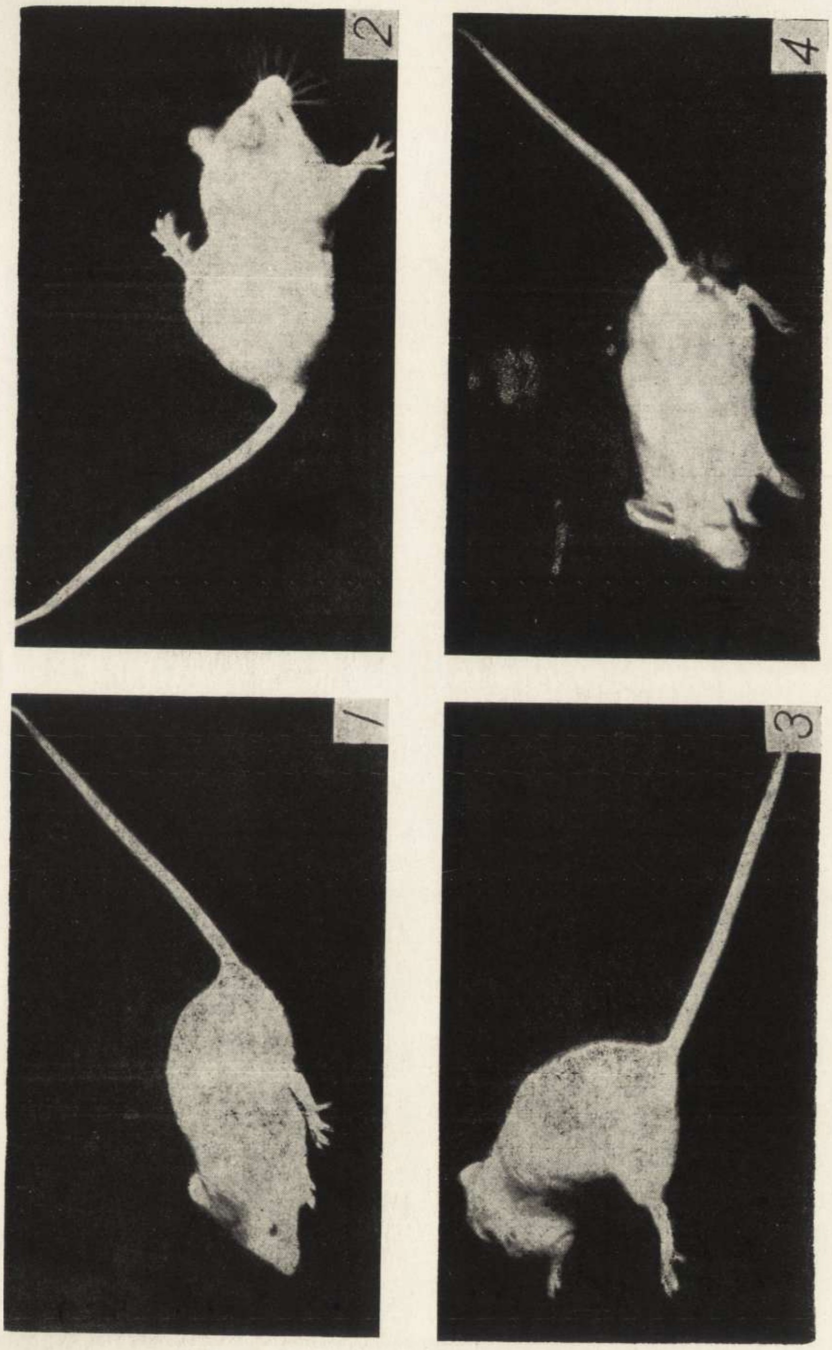

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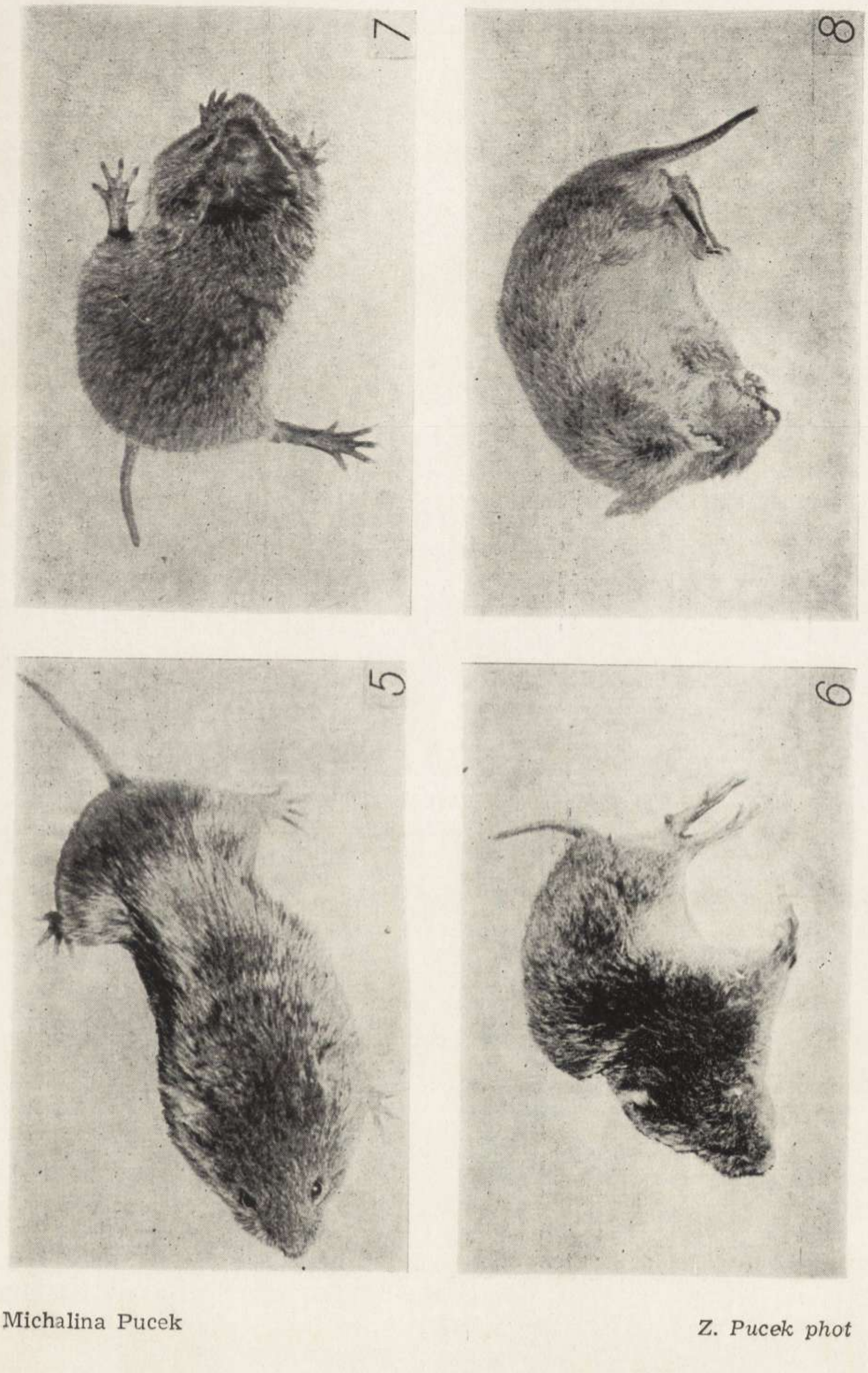

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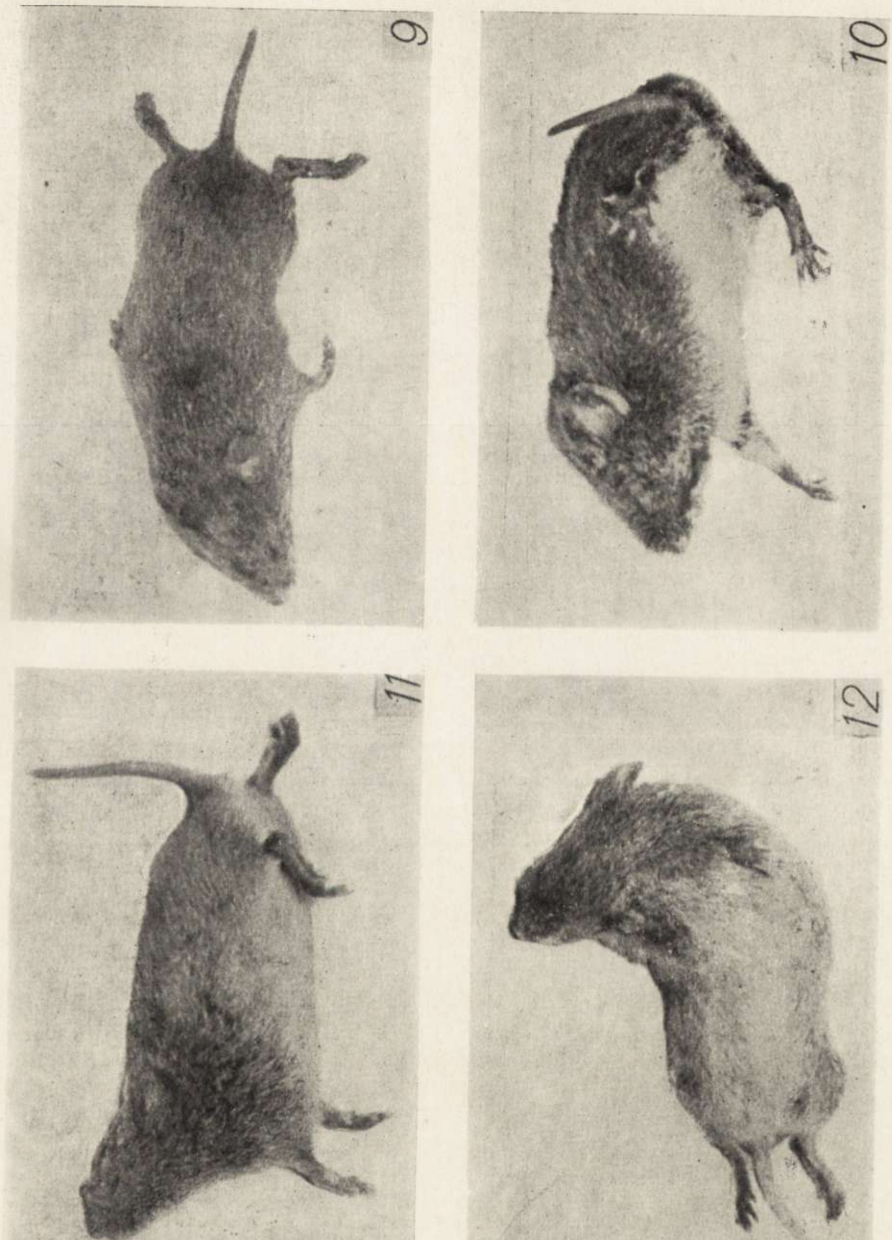

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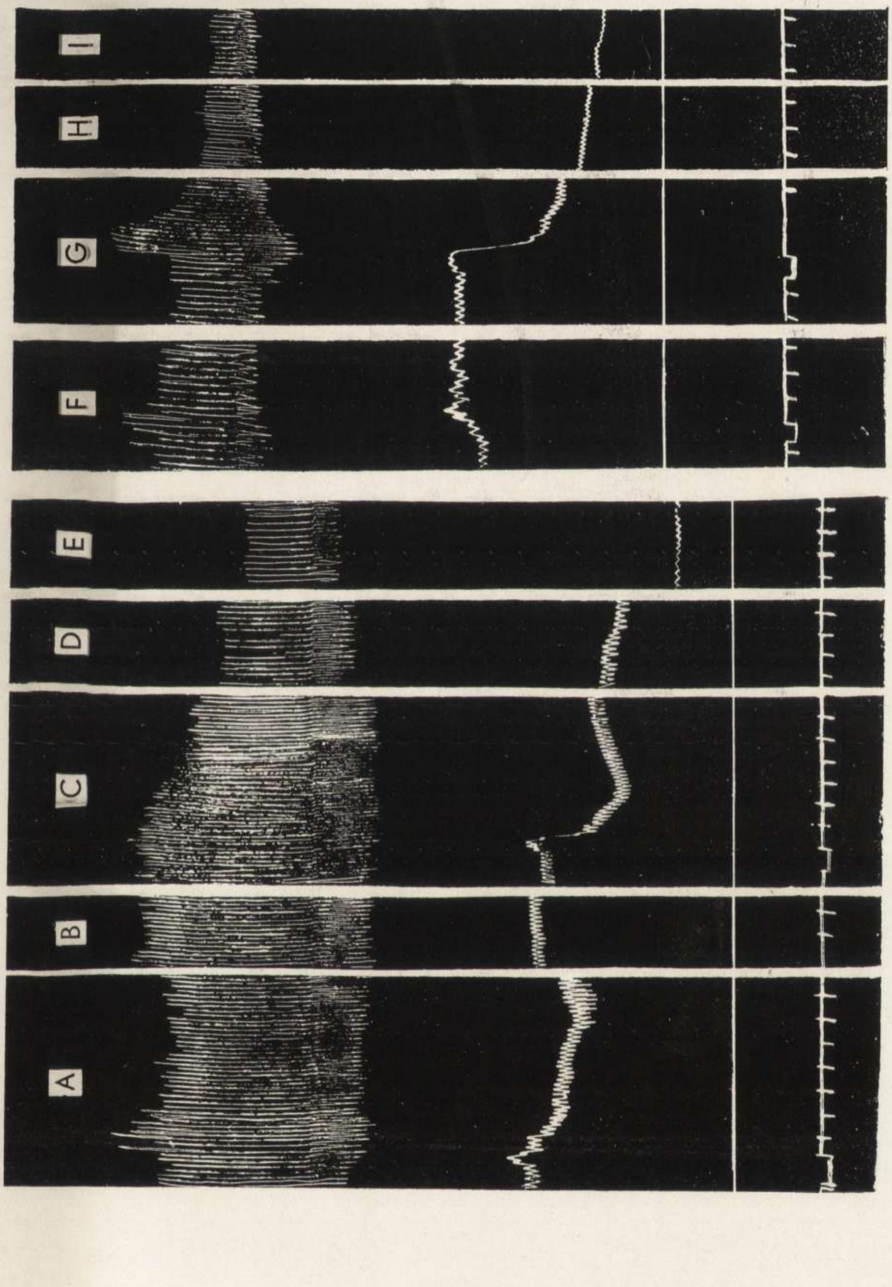
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