A leech-avoidance reaction of Physa fontinalis (L.) and Physa acuta Drap.

by
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1. Introduction.

DEGNER (1921) described the reaction of *Physa fontinalis* (L.) when its mantle border or its fringes [1]) are touched by a leech of the genus *Glossiphonia* (*Gl. complanata* (L.); *Gl. heteroclita* (L.); *Gl. papillosa* Braun). On contact with the leech the snail makes vigorous shaking movements with its shell and at the same time detaches its foot from the substratum, so that it moves jerkily away from the leech. The reaction occurs only on contact and not if the snail is some little distance away from the leech. Small amounts of mucus from one of these leeches can also produce this reaction. On contact between two specimens of *Physa* a reaction also occurs, but this is much less intense.

WREDE (1927) studied the reactions of *Physa fontinalis* (L.) as well as *Physa acuta* Drap. She found that there was no difference between the two *Physa* species in respect of their reactions to *Glossiphonia complanata* (L.). Contact of individuals of *Physa* with each other or with other fresh-water snails sometimes gave rise to a slow shaking movement of the shell.

Mechanical stimulation and contact with the leeches *Herpobdella octoculata* (L.) and *Haemopis sanguisuga* (L.) gave no reaction or an occasional weak reaction. WREDE succeeded in evoking the characteristic avoiding reaction by chemical stimulation: solutions of various salts, including NaCl, NH_4Cl, $Na_2Cr_2O_7$ and $(NH_4)_2Cr_2O_7$ in relatively high concentrations (1-10%) produced only the avoidance reaction, while various other salts produced not only this reaction but also other responses such as the contraction of the snail into its shell.

WREDE did not succeed in identifying a given ion as responsible for the reaction. She concluded that the mucus of certain leech species might contain substances which can produce the reaction, as can NaCl in high concentrations. It would appear that these substances are rather insoluble, as the reaction occurs only on direct contact between the animals.

[1]) The digitations of the mantle edge are termed f r i n g e s here in order to avoid the repetition of a long phrase.

2. Material and methods.

The specimens of *Physa fontinalis* (L.) and *Physa acuta* Drap. used in this investigation were collected during the autumn of 1954 and the winter of 1955 from the southern shore of a lake near Amsterdam (Nieuwe Meer, Amsterdamse Bos).

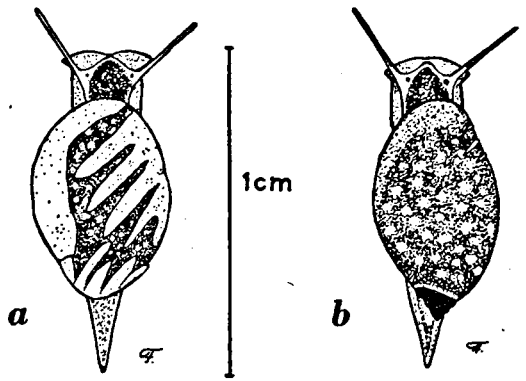
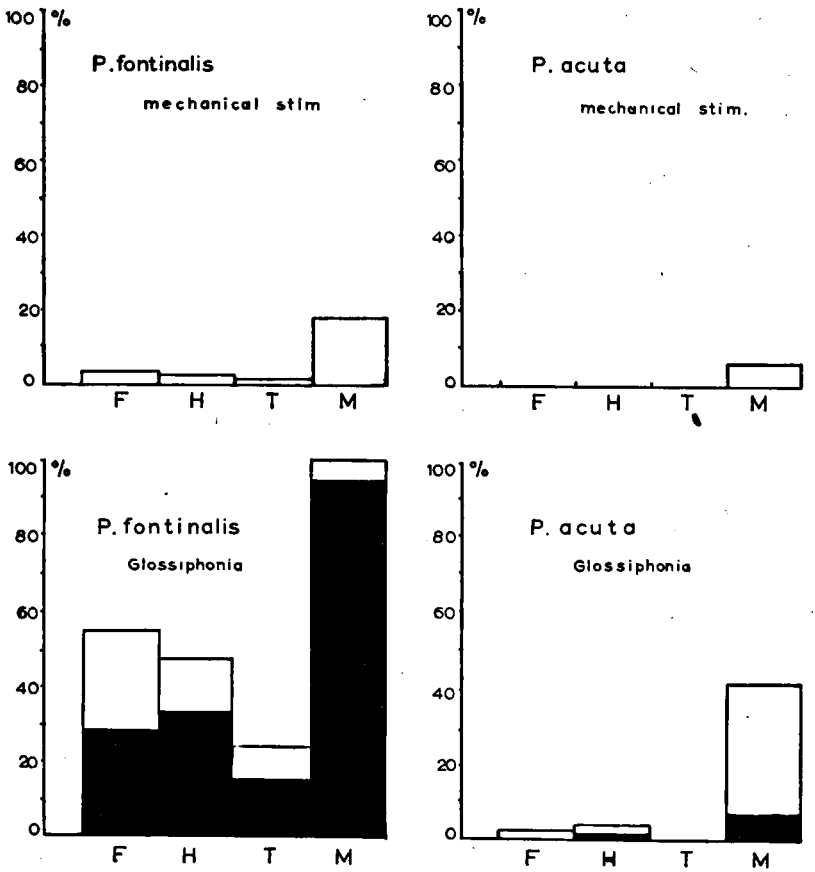


Fig. 1, a: *Physa fontinalis* (L.); b: *Physa acuta* Drap.

The leeches used, with the exception of *Hirudo medicinalis* (L.), were also collected there. In the laboratory, the snails and the leeches were kept in separate glass tanks, in which some water plants were placed. The death-rate among the snails was always high, and new specimens had to be added from time to time during the investigation, so that a large number of snails was used. For the actual experiments, the snails were taken out of the tank and placed in groups of about 10, in large Petri dishes half-filled with water. After transfer to the Petri dishes, the snails were left undisturbed until they began to crawl around slowly, after which the experiment was started.

3. Differences between *Physa fontinalis* (L.) and *Physa acuta* Drap.

(a) *Morphological differences.* — As regards external characters there is little difference in the form of the head, tentacles, shell and foot, but there is a considerable difference in the shape of the mantle-fringes. In *Physa fontinalis* (L.) these are broad flaps which cover most of the shell when the snail moves slowly along (Fig. 1a). In *Physa acuta* Drap. the appendices are narrow strips which cover only a very small part of the sides of the shell (Fig. 1b).



Graph I. Reactions of *Physa fontinalis* (L.) and *Physa acuta* Drap. to mechanical stimulation and stimulation by touching with *Glossiphonia complanata* (L.) at various parts of the body. (F = at the upper surface of the foot behind the shell; H = at the head; T = at the tentacles; M = at the mantle-fringes).

Defence reaction and leech-avoidance reaction (black) as a percentage of the total number of stimuli applied.

(b) *Differences in response to stimulation.* — A large number of specimens of both species were subjected to mechanical stimulation with pincers, and to stimulation, by touching with a piece of skin of *Glossiphonia complanata* (L.), at various parts of the body (head, tentacles, mantle-fringes and upper surface of the foot behind the

shell). Care was taken to ensure that the size of the area of contact was the same in all cases. The reactions of the snail were classified as leech-avoidance reaction (shaking of the shell together with detachment of the foot from the substratum); defence reaction (shaking without detachment) and no defence reaction (at the most a withdrawal of the stimulated portion of the body). The results are summarized in Table I. The number of times that a given reaction was observed is expressed as a percentage of the total number of stimuli applied. The data are plotted in Graph I.

		Leech avoidance reaction	Defense reaction	No defence reaction
Physa fontinalis (L.)	Foot	—	3	97
mechanical	Head	—	2	98
stimulation	Tentacles	—	1	99
	Mantle-fringes	—	17	83
Physa fontinalis (L.)	Foot	28	26	46
Glossiphonia	Head	33	14	53
complanata (L.)	Tentacles	15	9	76
	Mantle-fringes	95	5	—
Physa acuta Drap.	Foot	—	—	100
mechanical	Head	—	—	100
stimulation	Tentacles	—	—	100
	Mantle-fringes	—	6	94
Physa acuta Drap.	Foot	—	2	98
Glossiphonia	Head	1	2	97
complanata (L.)	Tentacles	—	—	100
	Mantle-fringes	7	34	59

Table I. Reactions of *Physa fontinalis* (L.) and *Physa acuta* Drap. to mechanical stimulation and stimulation by touching with *Glossiphonia complanata* (L.) at various parts of the body. In each case a total number of a hundred stimuli was applied.

From these results it appears that on mechanical stimulation both species display only the defence reaction. In both species the mantle-fringes are the most sensitive part of the body. Both species showed a higher percentage of defence reactions and leech-avoidance reactions to contact with a fragment of skin of *Glossiphonia complanata* (L.) than to mechanical stimulation. With *Physa fontinalis* (L.) the percentage of leech-avoidance reactions was much higher than with

Physa acuta Drap. In contrary to WREDE (1927), we thus found a marked difference between the two *Physa* species in respect of the reactions to stimulation by *Glossiphonia complanata* (L.).

	Leech avoidance reaction in %	Defence reaction in %	No defence reaction in %	Total number of stimuli applied 100 %
Mechanical stimulation	—	17	83	100
Snails:				
Physa fontinalis (L.)	—	90	10	40
Lymnaea ovata (Drap.)	—	50	50	40
Leeches:				
Glossiphonia complanata (L.)	95	5	—	100
Helobdella stagnalis (L.)	86	14	—	50
Hemiclepsis marginata (O.F.M.)	80	18	2	50
Hirudo medicinalis (L.)	75	25	—	20
Herpobdella octoculata (L.)	16	76	8	50
Piscicola geometra (L.)	—	70	30	20

Table II. Reactions of *Physa fontinalis* (L.) to contact of its mantle-fringes with various fresh water organisms.

4. Reactions of *Physa fontinalis* (L.) to contact of its mantle-fringes with various fresh-water organisms.

Experiments were conducted in which the mantle-fringes of these snails were subjected to contact with other specimens of the same species, with other species of snail and with 6 species of leech. The results are shown in Table II. The data for *Gl. complanata* (L.) are taken from table I, while the number of reactions to chemical stimulation is also included for comparison. In contact between two specimens of *Physa fontinalis* (L.) the defence reaction was observed in 90% of cases; in contact with *Lymnaea ovata* Drap. it was observed in 50%. Both these percentages are considerably higher than those for mechanical stimulation. The defence reaction is thus not a specific reaction to contact with leeches. Of the leeches, *Gl. complanata* (L.), *Hirudo medicinalis* (L.), *Helobdella stagnalis* (L.) and *Hemiclepsis marginalis* (O.F.M.) were notable for the high percentage of leech-avoidance reactions evoked by them. *Herpobdella octoculata* (L.) and *Piscicola geometra* (L.) are not enemies of the snails, whereas of the species to which *Physa fontinalis* (L.) reacted with a leech-avoidance reaction in 75% or more of the experiments, only *Glossiphonia complanata* (L.) and *Helobdella stagnalis* (L.) are known to feed chiefly on snails.

	Leech avoidance reaction in %	Defence reaction in %	Defence reaction followed by withdrawal of the snail into its shell in %	withdrawal of the snail into its shell in %	no reaction in %	total number of stimuli applied 100 %
Aqua dest.	—	7	—	—	93	60
NaCl	86	4	—	10	—	70
NaBr	95	—	—	5	—	60
Na_4SO_2	—	—	10	90	—	30
KCl	—	—	100	—	—	30
K_2SO_4	—	—	17	83	—	30
$MgCl_2$	—	47.5	—	—	52.5	40
$CaCl_2$	—	5	—	—	95	40
NH_4Cl	92.5	2.5	—	2.5	2.5	40

Table III. Reactions of *Physa fontinalis* (L.) to solutions of various salts, concentration 300 milliequivalent per litre.

5. Reactions of *Physa fontinalis* (L.) to solutions of various salts.

WREDE (1927) reported that 1-10% NaCl solutions evoked a "strong defence reaction" in *Physa*. Our investigations showed that 2% NaCl practically always elicited the typical leech-avoidance reaction in *Ph. fontinalis* (L.). The other salts investigated were therefore used in concentrations equivalent to this, i.e. 300 milliequivalents per litre. WREDE compared equal percentages, which are not comparable with respect to the number of ions present. The solutions were brought into contact by means of a capillary pipette on the mantle-fringes of *Ph. fontinalis* (L.), a blank test with distilled water also being done with the same pipette. To avoid changes in the surrounding medium as a consequence of these experiments, the water in the Petri dishes in which the snails were placed was regularly renewed.

Table III shows the reactions to solutions of various salts. In addition to the leech-avoidance reactions and the defence reaction, a distinction is made here also between defence reaction followed by withdrawal of the snail into its shell and contraction without defence reaction.

Distilled water did not produce the leech-avoidance reaction at all and only in a few cases did it give rise to the defence reaction, from which it can be concluded that the stimulus of the stream of water from the pipette is not responsible for the occurrence of the leech-avoidance reaction. In agreement with Wrede we found that NaCl and NH_4Cl produced the leech-avoidance reaction in a very high percentage of experiments. The common component of these

solutions is the chloride ion, but this cannot be the cause of the leech-avoidance reaction as neither $MgCl_2$ nor $CaCl_2$ produced this reaction, the former producing the defence reaction in rather less than half the experiments and the latter in only 5% (comparable with distilled water). WREDE concluded that the sodium ion, could not be the responsible agent either, as she found that Na_2SO_4 was inactive, we also found that this salt did not produce the leech-avoidance reaction. It appears probable, however, that the Na-ion in NaCl and NaBr is responsible for the leech-avoidance reaction, but that in Na_2SO_4 the action of the SO_4 ion predominates, so that the snail withdraws into its shell. K_2SO_4 produces contractions into the shell, so does KCl, although that is preceded by a brief defence reaction. The potassium ion, too, promotes contraction. Summing up these results, it can be stated that of the ions investigated, only Na and NH_4 are capable of producing the characteristic leech-avoidance reactions.

6. Substances in leeches that may evoke the leech-avoidance reaction.

As it proved impossible to collect sufficient mucus from *Gl. complanata,* aqueous extracts of triturated leeches were prepared. After centrifugation the supernatant was turbid, probably owing to the presence of proteins. On boiling it gave a precipitate which, when applied to the mantle-fringes of *Ph. fontinalis* (L.) gave rise to the leech-avoidance reaction. After removal of lipids by extraction with acetone the precipitate still produced the leech-avoidance reaction. It remained to be ascertained whether the reaction was caused by certain ions. A cloudy solution obtained as described above was dialysed through collodion to remove free ions. Upon heating it gave a precipitate which still elicited the leech-avoidance reaction. Thus free ions or other dialysable substances are not necessary for the production of the reaction.

In order to remove ions possibly bound to "protein", the above-mentioned solutions were now brought to low or high pH by the addition of acids or bases. Addition of KOH or NaOH gave a pH of approx. 11, a value at which negative ions are removed from the protein. A blank was also run with addition of distilled water (pH approx. 7). The "protein" precipitated from these solutions was washed repeatedly with distilled water and applied to the mantle-fringes of *Ph. fontinalis* (L.), which then gave the leech-avoidance reaction in 100% of experiments. This shows that after treatment with alkali or acid, which will remove the greater part of the ions bound to the protein, the latter still produces the leech-avoidance

reaction, so that it may be concluded that in all probability bound ions are not necessary for the production of the reaction.

The body of *Glossiphonia complanata* (L.) thus contains substances, presumably of protein nature, which are capable of producing the characteristic leech-avoidance reaction of *Ph. fontinalis* (L.).

SUMMARY

It has been shown that in both *Physa fontinalis* (L.) and *Physa acuta* Drap. there is a difference in sensitivity of different parts of the body to contact with *Glossiphonia complanata* (L.). Contact with the mantle-fringes was found to give the highest percentage of leech-avoidance reactions. These fringes are also the parts most sensitive to mechanical stimulation. *Ph. fontinalis* (L.) and *Ph. acuta* Drap. show considerable difference in their reactions to contact with *Gl. complanata* (L.), *Ph. fontinalis* (L.) being by far the more sensitive. In addition to *Gl. complanata* (L.), leech avoidance reactions of *Ph. fontinalis* (L.) were also produced by *Hirudo medicinalis* (L.), *Hemiclepsis marginata* (O.F.M.) and *Helobdella stagnalis* (L.) The characteristic leech-avoidance reaction of *Ph. fontinalis* (L.) could also be produced by solutions of NaCl, NaBr and NH_4Cl and it was shown that it is the sodium or ammonium ion and not the anion that is responsible for the reaction. Boiling of extracts of *Gl. complanata* (L.) gave a precipitate, presumable a protein, to which *Physa fontinalis* (L.) responded with the leech-avoidance reaction. The pH of the extracts did not influence the activity of the precipitate, so that it may be concluded that ions bound to the protein by salt-formation are not concerned in the reaction.

REFERENCES

DEGNER, E., 1921. Ueber einen Abwehrreflex bei *Physa fontinalis* (L.). Arch. Molluskenk., vol. 53, pp. 117—120.

WREDE, W. L., 1927. Ueber die Abwehrreaktion von *Physa*. Zool. Jahrb. (Zool.), vol. 44, pp. 315—322.