Influence of the viscous substance at the tip of the labellar hairs of *Phormia regina* M. on the effectiveness of stimulation by cations and anions[1]

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Summary. The stimulating effectiveness of salt solutions on the labellar taste hairs of *Phormia regina* M. is discussed on the basis of ion diffusion properties across the mucopolysaccharidic layer at the hair tip.

A layer of viscous substance is located on the tips of the labellar hairs of *Phormia*[2]. This substance, which is secreted by the trichogen and tormogen cells, is of a mucopolysaccharidic nature[3]. The purpose of the present paper is to investigate the influence of this substance on the stimulating effectiveness of some salts, by evaluating the possible interaction between it and the stimulating agents. This interaction may influence the diffusion of the stimulating agents from the external environment to the chemosensory dendrites, thus influencing their stimulating effectiveness. In order to study the importance of the interaction on ion diffusion, we measured the resistivity of the hairs in contact with various groups of equiconductive salt solutions. The experiments were performed both on intact hairs, and on hairs the tips of which had been removed (amputated hairs), in order better to evaluate the role of the structures in the hair tips' resistivity.

Resistivity measurements were performed on labellar taste hairs of the blowfly (*Phormia regina* M., 6-8 days old) according to the method reported by Stürckow[4] for the chemosensory hairs of *Calliphora*. Only the first 2-3 long rostral labellar aboral hairs, following the classification by Den Otter[5], were used. Each hair was tested with a given group of equiconductive salt solutions, which were applied in a random sequence. 3 groups of salt solutions were used. The first comprised a 0.5 M NaF solution and a NaI one made equiconductive with it, the 2nd a 0.5 M KCl solution and an equiconductive KI one, the 3rd a 0.4 M NaCl solution and equiconductive KCl and LiCl ones. Equiconductivity was experimentally reached by adding NaI, KI, KCl or LiCl to water, so as to achieve a conductivity of the same order as the 0.5 M NaF, 0.5 M KCl or 0.4 M NaCl solutions respectively. Conductivity was measured by means of a Kohlrausch bridge. In the experiments on amputated hairs the tips of the hairs were cut off with microscissors.

As shown in table 1, the resistivity of the intact labellar hairs is greater in the NaF solution than in the NaI one. In the corresponding experiments on amputated hairs, no significant difference was detected between hairs treated either with NaF or NaI solutions. Intact hairs tested with a KCl solution exhibited greater resistivity than those in contact with the equiconductive KI solution; here again, the corresponding experiments on amputated hairs did not show any significant difference (table 2). As regards the hairs tested with the equiconductive NaCl, KCl or LiCl solutions (table 3), no significant difference was detectable either among intact hairs or among the amputated ones.

The results reported in tables 1 and 2 show that the changes in hair resistivity dependent on the kind of anion do not occur if the hair tip is cut off. On the contrary, no relationship was noted between the kind of cation and the resistivity of either the intact or amputated hairs when the cation of a given halide was changed (chloride, in our experiments) (table 3).

In our opinion, the variations in the resistivity of intact hairs obtained by changing the anion are likely to be related to the viscous mucopolysaccharidic layer on the hair tip. This layer separates the external environment from the surface of the chemosensory dendrites[2,3]. This layer may act as a chemical filter, bearing in mind that a lower resistivity is shown by the halides of a given cation with a smaller hydrated anion diameter[6].

In other words, the mucopolysaccharidic layer may hinder the diffusion of anions in proportion to their hydrated diameter. It is conceivable that the lower the resistivity, the greater the actual concentration, and consequently the stimulating effectiveness, of the salt at the dendrites. In effect, our resistivity data are in agreement with the electrophysiological results of Gillary[7] on the stimulating effectiveness of the halides of a given alkaline cation. As regards the cations, our data show no relationship between hair

Table 1. Intact and amputated labellar taste hair resistivity (MΩ) tested with a 0.5 M NaF solution or with an NaI solution made equiconductive to it. Mean values ± SE. Intact hairs tested with NaF differ significantly from the corresponding NaI treated (Student's t-test, $p<0.001$); there was no significant difference between the amputated hairs ($0.5>p>0.4$)

	Test solution NaF	NaI	Number of experiments
Intact hairs	31.94 ± 1.53	28.87 ± 1.50	38
Amputated hairs	8.83 ± 0.69	8.22 ± 0.59	50

Table 2. Intact and amputated labellar taste hair resistivity (MΩ) tested with a 0.5 M KCl solution or with a KI solution made equiconductive to it. Mean values ± SE. Intact hairs tested with KCl differ significantly from the corresponding KI treated (Student's t-test, $p<0.001$); there was no significant difference between the amputated hairs ($0.5>p>0.4$)

	Test solution KCl	KI	Number of experiments
Intact hairs	32.31 ± 0.89	28.80 ± 0.79	49
Amputated hairs	7.69 ± 0.66	8.69 ± 0.65	48

Table 3. Intact and amputated labellar taste hair resistivity (MΩ) when tested with a 0.4 M NaCl solution and with KCl or LiCl solutions made equiconductive with the first. Mean values ± SE. There were no significant differences either among intact hairs (Student's t-test; $0.2>p>0.1$ between NaCl and KCl; $0.5>p>0.4$ between KCl and LiCl and between NaCl and LiCl) or among the amputated ones ($0.6>p>0.5$ between NaCl and KCl and between NaCl and LiCl; $0.9>p>0.8$ between KCl and LiCl)

	Test solution NaCl	KCl	LiCl	Number of experiments
Intact hairs	37.56 ± 0.60	36.20 ± 0.73	36.88 ± 0.70	96
Amputated hairs	7.92 ± 0.54	7.44 ± 0.49	7.47 ± 0.59	93

resistivity and the cation stimulating effectiveness, even though some kind of interaction between cations and the mucopolysaccharide anionic groups is likely to take place, this is irrespective of the kind of cation. This may be true for the cations used in our experiments; otherwise our method would not be adequate to detect very small differences in ion diffusion. Anyway, our results indicate that the viscous mucopolysaccharidic substance at the tip of the labellar taste hairs of *Phormia* may act as a barrier that modulates the sensory response to the external environment, and therefore the reflex behaviour of the insect.

1 This work is partly supported by a grant from the Consiglio Nazionale delle Ricerche (CNR), Roma, Italy.

2 B. Stürckow, in: Olfaction and Taste, vol. 2, p. 707. Ed. T. Hayashi. Pergamon Press, Oxford 1967.
3 M. Moulins and Ch. Noirot, in: Olfaction and Taste, vol. 4, p. 49. Ed. D. Schneider. Wissenschaftl. Verlagsgesellschaft, München 1972.
4 B. Stürckow, Z. vergl. Physiol. *72,* 131 (1971).
5 C.J. Den Otter, Neth. J. Zool. *21,* 464 (1971).
6 B.E. Conway, in: Electrochemical data, p. 374, Elsevier Publ. Company, Amsterdam 1952.
7 H.I. Gillary, J. gen. Physiol. *50,* 359 (1966).

Importance of the viscous substance at the tip of the labellar taste hairs of *Phormia regina* M. on the salt-sugar interaction[1]

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Summary. The role of the mucopolysaccharide layer at the tip of labellar taste hairs of *Phormia regina* M. on the inhibitory mechanism of sugar on the stimulating effect of salt and vice versa has been investigated.

The addition of salt to sugar stimulating solutions reduces the responses of the sugar sensory units in the taste hairs of Phormia. On the other hand, salt sensory unit responses are lowered by adding sugar to the salt stimulating solutions[2-4]. In the present study, we have tried to investigate the importance of the viscous mucopolysaccharide layer at the tip of taste hairs of Phormia[5,6] in salt-sugar interaction, bearing in mind that the physical-chemical properties of the layer could determine changes in the concentration of the stimulating agents at the dendrites as compared to the external environment. The functional importance of the mucopolysaccharide layer has been evaluated by measuring hair electrical resistivity and considering this parameter as an index of ion diffusion and therefore of the probability of reaching the dendrites. We accordingly tested resistivity of the taste hairs by bathing them in NaCl or NaCl+sucrose equiconductive solutions. Control experiments were performed by repeating these measurements on hairs, the tips of which had been cut off (amputated hairs).

Material and methods. 1-2-day-old blowflies, *Phormia regina* M., were used. Resistivity measurements were performed, according to the technique described by Stürckow[7], on the first 2-3 long rostral labellar aboral hairs. Each hair was bathed in 2 solutions, containing either 0.5 M NaCl or 0.5 M sucrose in a random sequence. An adequate amount of NaCl was added to the latter to reach the same conductivity as the 0.5 M NaCl solution. The conductivity was evaluated by means of a Kohlrausch bridge. The tips of the hairs were cut off, in control experiments on amputated hairs, with microscissors.

Results and discussion. NaCl was added at a concentration of about 0.7 M to the 0.5 M sucrose solution to reach the same conductivity as the 0.5 M NaCl solution. As shown in the table, the resistivity of the intact hairs was lower when tested with the NaCl solution than with the equiconductive NaCl+sucrose solution. The difference was statistically significant. On the contrary, no statistically significant difference in resistivity was detectable when the same experiments were performed on amputated hairs. If one considers that it is necessary to enhance the NaCl concentration in the NaCl+sucrose solution in order to reach the same conductivity of the pure NaCl solution, it may be concluded that the presence of sugar can diminish NaCl stimulating effectiveness by lowering its activity and diffusion coefficients. This possibility has already been suggested by Wolbarsht[8] and reported by Dethier[4]. It is also conceivable that a similar influence may be exerted by NaCl on sucrose. Otherwise our results suggest that the salt-sugar mutual inhibitory effect cannot be related solely to the physical-chemical properties of the mixed sugar-salt solutions. In fact, the resistivity of the intact hairs was significantly lower with the NaCl solution than with NaCl+sucrose solution, even though both solutions were equally conductive. This difference did not occur when the same experiment was performed on amputated hairs, thus indicating its dependance on the structure of the hair tip. Although not providing direct evidence, these results may be explained if one considers the specific features of the mucopolysaccharide molecular structure, since the higher concentration of the solutes in the NaCl+sucrose solution than in the equiconductive NaCl one may cause the mucopolysaccharide molecular net to contract osmotically, resulting in a reduction of the diffusion coefficient of the solutes. Sucrose can also partly obstruct the 'channels' along which the solutes diffuse[9,10]. Both factors hinder diffusion of the solutes across the viscous mucopolysaccharide layer at the tips of the hairs, as shown by the higher resistivity of the hairs when bathed in the NaCl+sucrose solution, and thus lessen the probability of contact between the stimulating substances and the dendrites. In conclusion, in terms of molecular structure of the mucopolysaccharide,

Intact and amputated labellar taste hair resistivity (MΩ) tested with equiconductive 0.5 M NaCl or 0.5 M sucrose solutions. NaCl at an adequate concentration was added to the latter to reach the same conductivity of the 0.5 M NaCl solution.

	Test solution NaCl	NaCl+sucrose	Number of experiments
Intact hairs	27.47 ± 0.81	41.57 ± 0.97	42
Amputated hairs	10.80 ± 0.67	11.55 ± 0.77	44

Mean values ± SE. Intact hairs tested with NaCl differ significantly from the corresponding NaCl+sucrose treated (Student's t-test, $p < 0.001$); there is no significant difference between the amputated hairs ($0.5 > p > 0.4$).