tions of the enzyme ranging from 1 midgut per ml to 8 midguts per ml were used in the reaction mixture. To see the effect of end-products, corresponding sugars were added to the reaction mixtures; in controls they were added after stopping the reaction. The effect of dialysis on the melezitase activity was observed after dialyzing the enzyme extract against distilled water at 4 °C for 24 h. To see the effect of ions, different concentrations of KCl, $LiCl_2$ and *Tris* salt solutions were added to the reaction mixtures so as to bring their strength in different mixtures from 0.001 *M* to 1.025 *M*; and then relative melezitase activity was determined.

Results and discussion. The optimum activity of melezitase and of maltase from midguts of *S. inferens* was at pH 6.2 (Figure 1). KRISHNA[4] found 2 pH optima, 4.8 and 6.0 in case of melezitase from the gut of the larva of *Trogoderma*, while maltase from different insects showed optimal activity between pH 5.2–6.8[5–8]. The pH range of the midgut of *S. inferens* is 7.9–8.2. At this pH the activity of melezitase and maltase will be very low, that is only 20–26% and 13–18% of the optimum respectively.

Melezitase and maltase showed optimum activity at temperature of 35 and 40 °C respectively (Figure 2). As the larvae were reared at 32 °C, at this temperature the activity of melezitase and maltase will be 87.5% and 64% of the optimum (at pH 6.2) respectively.

The increase in the incubation period (Figure 3), the enzyme concentration (Figure 4) and the substrate concentration (Figure 5) enhanced the rate of hydrolysis of the substrates and the concentration of the hydrolytic end-products. The latter inhibited the activity of the enzymes.

Dialysis reduced the activity of melezitase by 4.8%; K^+ and Li^{++} ions increased its activity upto 0.001 *M* final concentration and thereafter an increase in their concentraion inhibited its activity; while *Tris* increased the activity at 0.025 *M* final concentration by 85.62%.

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Latency of Taste Nerve Signals in Frog (*Rana catesbeiana*)

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Summary. The latency of frog gustatory neural impulses to 1.0 *M* NaCl was a mean of 86 msec. Electrical stimulation of taste cell membranes produced gustatory neural impulses with the mean 5 msec latency. It is concluded that most of the 86 msec latency of taste nerve responses to 1.0 *M* NaCl is due to the latency of taste receptor potential following the onset of gustatory stimulation.

Vertebrate taste cells make functional contact with gustatory nerve fibres. According to present knowledge, transduction of taste stimuli into neural signals can be described by the following scheme: a) adsorption of taste stimuli onto the taste receptor membrane[2]; b) receptor potential of taste cells[3–7]; c) postsynaptic potential at the subsynaptic nerve fibre membrane[8]; and d) generation of gustatory impulses at the nerve terminal[8]. Because of technical difficulty, there is no full physiological understanding of the time course between the successive events mentioned above. However, electrophysiological investigations have revealed that the time required for the whole process, from the onset of strong gustatory stimulation of tongue to the initiation of the first gustatory nerve impulse, is about 35–50 msec[9,10]. When such a latency of gustatory nerve impulses produced by taste stimulation is compared with that produced by electrical stimulation of gustatory cells and nerve terminals, analysis of factors associated with the latency would be feasible. With this approach, I attempted to examine the properties of latency of gustatory impulses and to analyze the factors determining the latency.

Materials and methods. Tongues of bullfrogs (*Rana catesbeiana*) anesthetized with urethane were used in the experiments. A fungiform papilla was fully drawn into a recording suction electrode filled with Ringer saline[11], and the action potentials which were conducted antidromically from other papillae to the gustatory nerve fibres of the suctioned papilla were recorded (Figure 1a). The presentation of taste solutions was done with a taste-stimulus-delivering device, composed of electric interval timers and solenoid valves[12]. A nozzle of the gustatory stimulator was put in the centre of the fungiform papillae population functionally connected with the papilla studied. The solution from the nozzle flowed over the tongue sur-

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face at the rate of 0.13 ml/sec. The taste stimuli used were 0.1–1.0 *M* NaCl solutions. As shown in S of Figure 1b, the taste stimulus arrival on the tongue surface was signalled with the artifact of an electrical transient surge produced by initial contact of the first drop of solution.

Results and discussion. Figure 1b illustrate a train of gustatory neural impulses recorded from the suctioned fungiform papilla after the application of 0.5 *M* NaCl to the surrounding papillae. In this figure, there are neural impulses of 3 different gustatory units, which are distinguishable by spike potential amplitudes. 3 arrows indicate the first impulses appearing in each unit, the latency of which was 93, 102 and 118 msec, respectively. Figure 1c shows the frequency distribution of latencies of the first impulses in response to 0.5 *M* NaCl. The average latency was 158 msec (96 gustatory units) with a range of 19 to 437 msec. Figure 1d shows the relationship between NaCl concentrations of 0.1–1.0 *M* and latencies of the first gustatory neural impulses obtained from 13 different units. With the Ringer-adapted tongues, the threshold concentration for NaCl stimuli was 0.1 *M* and mean latency to 0.1 *M* NaCl was 2.3 sec. The latency of gustatory responses shortened as the stimulus NaCl concentration was raised. The mean latency in response to 1.0 *M* NaCl became 86 msec with a range of 16–145 msec.

A conduction time of impulses was included in the latency of the antidromically recorded spike potentials from the suctioned papilla. To measure this conduction time, another papilla drawn into a suction electrode was electrically stimulated with a square pulse of 0.01 msec duration, antidromic impulses being recorded from the neurally connected papilla (Figure 2a). Figure 2b–d illustrates an example of positive-negative going biphasic action potentials elicited by electrical stimulation with gradually increasing intensities. When the papilla surface was made anodal or cathodal, the latency of the first spike potentials (I in the figure) was a mean of 1.8 or 2.1 msec (40 units) and was extremely constant even through repetitive stimulation at more than 100 Hz was employed. Therefore the first spikes are considered to have originated from gustatory nerve fibre terminals, and to have conducted antidromically to the recorded papilla with the conduction time of about 2 msec. This value is negligibly small in comparison with the overall latent period of 86 msec in response to 1.0 *M* NaCl stimuli.

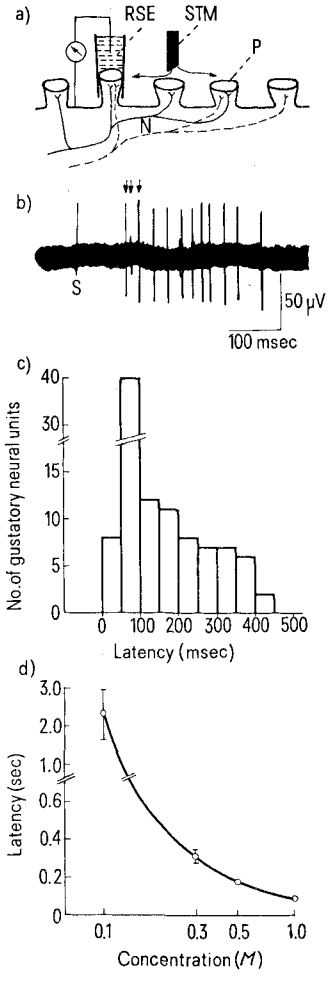

Fig. 1. a) Schematic experimental arrangement for taste stimulation and recording of antidromic impulses. P, fungiform papillae; RSE, recording suction electrode filled with Ringer solution; STM, output nozzle of gustatory stimulator; N, gustatory nerve fibres. b) Antidromically conducted gustatory nerve impulses in response to 0.5 *M* NaCl recorded with the suction electrode. 3 arrows denote the first action potentials in 3 different neural gustatory units. S, artifact produced by contact of taste solution with the tongue surface. c) Frequency distribution of latency of antidromic impulses to 0.5 *M* NaCl. d) Relation between latencies and 0.1–1.0 *M* NaCl concentrations. Each point is mean ± SE from 13 different units, except for the point to 0.1 *M*. 7 out of 13 units observed did not respond to 0.1 *M* NaCl, so that these units were omitted from calculation of the mean.

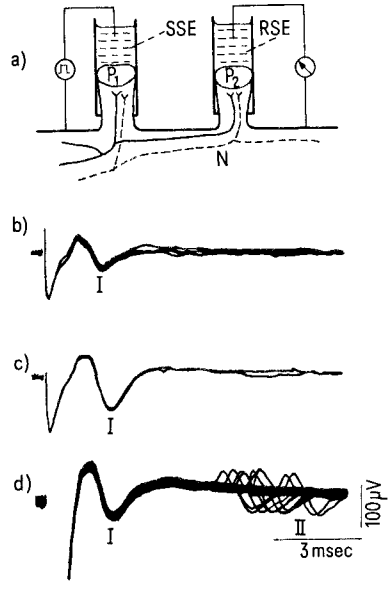

Fig. 2. a) Schematic arrangement for electrical stimulation of a fungiform papilla (P_1) and recording from another papilla (P_2) with 2 different suction electrodes. N. gustatory nerve fibres. b)–d) Action potentials evoked by electrical stimulation of the papilla with gradually increasing strength. The surface of papilla (P_1) in a) was made anodal. The record b) was obtained with threshold intensity, showing a biphasic action potential of one gustatory unit in the first group (I). In record c) an action potential of the other neural unit was superimposed on that of record b), showing simultaneous firing of 2 units. In record d), the first group of impulses (I) was followed by the second group of impulses (II) with long and fluctuating latencies. This record of 20 superimposed sweeps indicates that 20 electrical stimulations at 1 Hz produced intermittently 10 impulses of the second group (II), which were from the same unit as those in record b). Impulses of the same shape were also produced by 0.5 *M* NaCl applied to the surrounding papillae.

As shown in Figure 2d, electrical stimulation with 2–4 times threshold intensity produced the second group of action potentials (II), which exhibited long and fluctuating latency. The minimum latency of the second action potentials, corrected for conduction time, was a mean of 5.1 msec (26 units) with a mean fluctuation range of 4.9 msec. Similar values have been obtained in the frog water-sensitive fibres by NOMURA and KATSUHATA[13]. Since the 5 msec latency of the second impulses in response to electrical stimulation is much shorter than the 86 msec latency to a 1.0 *M* NaCl stimulus, it seems unlikely that the second impulses were induced by gustatory stimulation of the taste receptor sites with electrophoretically carried Ringer ions at the tip of Ringer-filled suction electrode. The second fluctuating impulses completely disappeared with repetitive electrical stimulation at 10 Hz. Even when the repetion rate was 1 Hz, the responses occurred intermittently, as shown in Figure 2d. Because of instability at low frequences of stimulation, it is not considered that the second group of action potentials was generated by direct electrical stimulation of gustatory nerve terminals. When the taste bud located at the summit of the fungiform papilla was mechanically destroyed, no second impulse after the first impulse was observed. Therefore, it is concluced that the second impulses with irregular latencies originated synaptically from the result of direct depolarization of taste cell membranes by electrical current. With neuromuscular junctions, it is well known that repetitive electrical stimulation of the presynaptic axon with low frequency produces endplate potentials with fluctuating latency and intermittent occurrence[14]. These properties are very similar to those of the second group of gustatory impulses under the present investigation.

Although the whole time course of transduction steps of a gustatory stimulus into nerve signals is as yet unknown, the present experiment indicates that the total time interval between the onset of taste cell depolarization and the initiation of gustatory neural impulse is about 5 msec. This indicates that about 94% of an 86 msec latency produced by 1.0 *M* NaCl is the time between the onset of gustatory stimulation and the generation of the taste receptor potential, i.e., the latency of receptor potential. Thus, most of the portion of the latency of NaCl-induced taste nerve responses may be due to the time required for effective adsorption of taste stimuli on the taste receptor membrane[2], because the latency is greatly dependent on taste stimulus concentrations as shown in Figure 1d.

SATO[5] has already shown that the time between the presentation of 0.5 *M* NaCl and the onset of intracellular receptor potential of frog taste cells was 100–300 msec. These values are similar to those seen in Figure 1c and d. Therefore, it is concluded that the most important factor determining the latency of gustatory neural impulses is the latency of taste cell depolarization following initiation of taste stimulation.

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Spontaneous Thermoregulatory Oscillations in Cutaneous Efferent Sympathetic Activity

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Summary. Patterns of changes in cutaneous efferent sympathetic activity which have previously been shown to occur during experimental manipulation of the temperature of various thermosensitive body sites, have now been shown to accompany spontaneous thermoregulatory activity. That is, under thermoneural conditions, concurrent spontaneous oscillations in skin blood flow and efferent sympathetic activity were observed.

Patterns of efferent sympathetic activity appropriate to regional circulatory responses elicited by changes in temperature of the hypothalamus, spinal cord or skin have recently been clearly demonstrated[2–4]. Such work has involved specific experimental manipulation of the temperature of an area of skin, the hypothalamus or spinal cord, and although normal thermoregulatory effector mechanisms accompany such changes (see reviews by HALES[5] and SIMON[6]), the accompanyment of spontaneous thermoregulatory activity by appropriate efferent sympathetic activity has not been reported; this is, of course, essential if proposed mechanisms are to be accepted as entirely valid, and the present study has examined this.

Methods. Observations have been made on 3 albino rabbits of either sex, weighing 2–3 kg; they were artificially ventilated while anaesthetized with sodium pentobarbital (initial dose of 30 mg kg^{-1}, followed by an infusion of 4 mg kg^{-1} h^{-1}) and immobilized with succinylcholine (20 mg initially, followed by an infusion of 100 µg kg^{-1} h^{-1}). Polyethylene thermodes were surgically placed in the vertebral canal, extending from the lower lumbar to the mid-cervical level.

Continuous monitoring was made of the skin temperature of both ears, rectum, spinal canal and ambient air, and of the electrical activity of a postganglionic nerve twig accompanying one of the retroauricular arteries (cutaneous 'ear' sympathetic). The nerve potentials were recorded directly and also after integration over 4 sec intervals. Full details of these techniques have been given previously[2–4].

At the beginning of an experimental period, the rabbit was placed on its side on a water perfused plate, and water and ambient air temperatures were adjusted until ear skin temperatures lay approximately mid-way between

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