of intracellular potential changes in taste cells[5,6] and in non-taste cells[7,8]. During the experiment, near zero potential was determined by punching a hole into the tissue, both solutions being identical. No potential was elicited when any stimuli were applied to the serosa. Relations between stimuli concentrations and the maximum magnitude of the responses to each stimulus are shown in figure 2. These relations, excluding the sucrose-response, are in approximate agreement with those of the receptor potentials in taste cells, and with those of neural activity in frogs[5]. The responses to each stimulus were reversibly depressed in the presence of 0.05 to 0.5% tetracaine or procaine on the mucosa, as was observed in frog taste cells[9]. The results indicate that the response to chemicals is not simply a physicochemical potential occurring at a fluid interface.

The magnitude of the response to each stimulus was not influenced when the air supply to the Ringer's solution was turned off, or 1 mM ouabain or 1 mM 2,4-dinitrophenol (DNP) was applied to the serosa and/or the mucosa for over 1 h. On the other hand, in the frog skin and bladder, NaCl-responses obtained using the same apparatus rapidly decreased to zero in the presence of ouabain or DNP, being associated with a marked reduction of the resting potential difference. These observations further indicate that the dorsal epithelium, including taste disks, can receive taste chemicals and produce the responses caused by a change of passive transport of some ions through the tissue rather than through active transport. Different results have been reported for the dog tongue[1,2]. This could be due to the fact that an amphibian isolated tissue is generally insensitive to oxygen and temperature and is therefore easier to maintain alive than a mammalian tissue.

The tissue resistance, which was measured by an application of 100-msec pulse currents (10^{-6} to 10^{-5} A, 0.5 Hz) through a Wheatstone bridge, was 710 ± 180 Ω/cm^2 in resting state. This value is similar to that obtained for the dog tongue[1,2]. During NaCl- and acetic acid-responses a marked decrease in the tissue resistance was observed. On the average, it was changed to 22.6% by 0.2 M NaCl and 56.3% by 5 mM acetic acid from 100% in resting state. Also, in the majority of cases, a small decrease in resistance was produced by 5 mM quinine and a small increase was produced by 0.5 M sucrose. This change in resistance gives further support to the explanation of the production of transepithelial potential changes. Thus, lingual epithelial responses to taste chemicals may have some influence on taste reception, as has been suggested for the dog tongue[1,2].

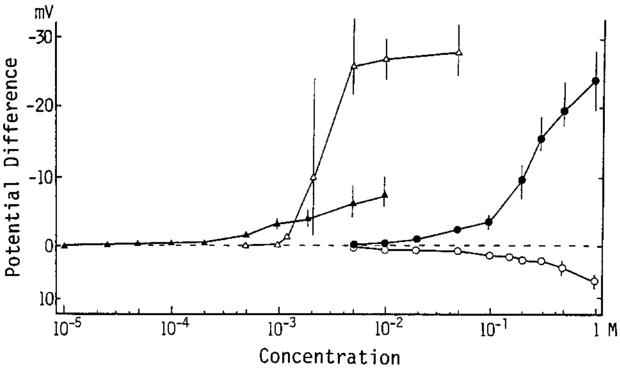

Figure 2. Relations between stimulus concentration and response magnitude, obtained from the dorsal epithelium. Stimuli indicated by solid circles, open circles, open triangles and solid triangles are NaCl, sucrose, acetic acid and quinine, respectively. Each point and its vertical bar in the figure represent the mean values of maximum magnitude of responses and their standard deviations obtained from 7–16 experiments.

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The effect of stimulus frequency on transmission in ganglia of Remak's nerve in the chicken

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Summary. Electrical stimulation of side branches of Remak's nerve evoked a synaptically-mediated spike discharge at the oral end of the nerve which increased in amplitude in response to successive stimuli. The maximum amplitude of the synaptic component was attained at a frequency of about 3 Hz but was almost completely absent at 31 Hz.

Key words. Chicken; ganglionic transmission; Remak's nerve; stimulus frequency.

The organization of Remak's nerve in the chicken is still only poorly understood. The information that is available has come from histochemical[1], pharmacological and electrophysiological studies[2–4]. In a recent study[5,6] evidence was presented suggesting that enteric cholinergic neurones formed part of a pathway which projected orally along Remak's nerve (the intestinal nerve) in the chicken. The function of these cholinergic neurones is not known nor is the identity and projection of the post-ganglionic neurones in Remak's nerve with which they synapse. During the course of the study it was noted that the response recorded from the oral end of the nerve trunk, evoked by stimulation of a side branch between the nerve and the gut, increased in amplitude in response to successive stimuli in a train, although the stimulus intensity remained unchanged. In the present study an attempt has been made to characterize the effect of stimulus frequency on the growth of the post-ganglionic response.

Methods. Nine chickens between 6 and 12 weeks of age were killed by cervical dislocation. About 2–3 cm of Remak's nerve trunk with side branches intact were removed and pinned onto silicone resin (Sylgard, Dow Corning) which lined the base of the organ bath. The bath contained Krebs solution of the following composition (mM): NaCl 118, KCl 4.75, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 11. The Krebs solution, which was vigorously gassed with 5% CO_2 in O_2, was kept within 0.5 °C of 37 °C and changed by overflow every 15 min. The main nerve trunk, and one or more side branches, were cleared of fat and connective tissue, and drawn into tightly-fitting suction electrodes. The electrode on the side branch was connected to a stimulator via an isolation unit. The activity evoked by side branch stimulation was recorded from the oral end of Remak's nerve, amplified with an AC preamplifier (band width 10 Hz to 3 kHz) and either displayed on the screen of a storage oscilloscope or transferred to magnetic tape for later analysis. The stimulus intensity used to excite fibres in the side branch was supramaximal for the steady state evoked oral response at 1 Hz. A train of 20 stimuli at various frequencies between 0.1 Hz and 31 Hz was applied to the side branch every 5 min. Mean values are given ± SEM.

Results and discussion. The records in figure 1 illustrate the increase in the amplitude of the oral response evoked by the second, third, tenth and twentieth stimulus in the train compared with that evoked by the first stimulus at four different frequencies. The growth in the amplitude and the maximum amplitude attained in this experiment was smallest at 0.1 Hz, greatest at 1 Hz and declined a little at 5 Hz. There was very little change in the amplitude of the first response from trial to trial. Two experiments were unlike the one illustrated in figure 1; the evoked oral response consisted of more than one component and the increase in the amplitude of separate components during the train of stimuli was not always identical. In these two cases only the component exhibiting the greatest increase in amplitude was considered. In the nine preparations the steady state amplitude of the evoked oral response was reached almost invariably by the tenth stimulus. The maximum steady state amplitude was attained at a frequency which varied with each preparation but the value was within the range 1 Hz to 3 Hz. At higher frequencies the steady state amplitude declined until almost no response was detected at 31 Hz.

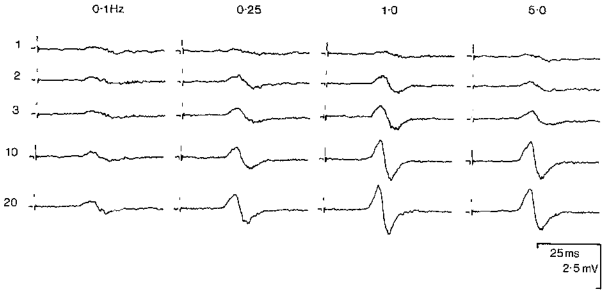

Figure 1. The effect of stimulus frequency on the increase in amplitude of the evoked oral response. The first, second, third, tenth and twentieth responses recorded at four different frequencies, 0.1, 0.25, 1.0 and 5.0 Hz, are shown. The maximum amplitude was attained at 1 Hz.

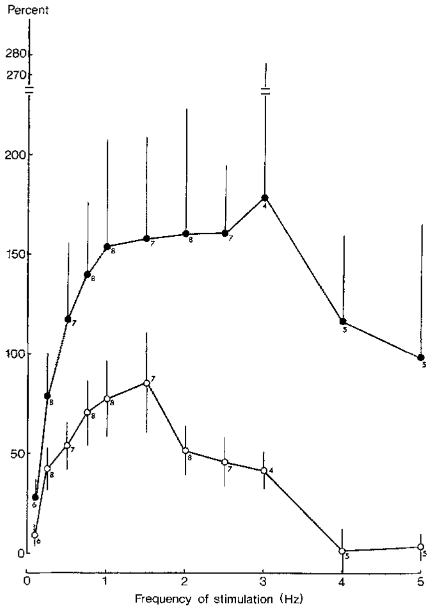

Figure 2. The relationship between the percentage increase of the second (P_2, open circles) and the tenth (P_{10}, closed circles) evoked oral responses over that of the first (ordinate), and stimulus frequency (abscissa). The numbers refer to the number of observations used to determine the mean values for both P_2 and P_{10}. Mean values are given ± SE.

The pooled data from eight experiments are presented in figure 2 in the form of the percentage increase (P_2) in amplitude of the second response (A_2) over that of the first (A_1), $P_2 = \frac{A2-A1}{A1} \times 100$; and the percentage increase (P_{10}) in the amplitude of the tenth response (A_{10}) over that of the first (A_1), $P_{10} = \frac{A10-A1}{A1} \times 100$. Although there was great variation from preparation to preparation in P_2 and P_{10} at the frequencies studied, a definite trend was discernible. Thus P_2 increased from about 9% at 0.1 Hz to a maximum of 85% at 1.5 Hz and then dropped below 4% at frequencies greater than 4 Hz. The value of P_{10} increased from 28% at 0.1 Hz to a maximum of 179% at 3 Hz then fell to 99% at 5 Hz. There was no significant difference between any of the mean values from 0.25 Hz to 3 Hz for both graphs suggesting a plateau to the relationship over this frequency range. The mean values at 0.1 Hz were, however, significantly different from all those over this range ($p < 0.02$, t-test).

Since about 93% of the amplitude of the evoked oral response results from cholinergic excitation of post-ganglionic neurones in Remak's nerve[6] then variations in the amplitude of the evoked oral response are presumably a reflection of the number of synapses at which transmission results in a suprathreshold post-synaptic potential. (This is supported by the finding that hexamethonium reduced or abolished those components that exhibited potentiated responses to successive stimuli.) It seems that this occurs at relatively few synapses in response to the first preganglionic spike in a train but that following subsequent spikes more post-ganglionic neurones are brought to threshold. This occurred in the greatest number of neurones at a frequency of about 3 Hz (fig. 2). At higher frequencies it seems that the number of post-ganglionic neurones reaching threshold is reduced, which may be the result of depression at some ganglionic synapses. The significance of the frequency plateau is unknown although it is interesting to note that spontaneous contractions of the circular muscle of the chicken ileum do occur at about these frequencies (Hodgkiss, unpublished observations).

The growth in the amplitude of the evoked oral response is a feature which is useful for identifying the synaptic components in a multicomponent evoked oral response and has been used in the determination of the conduction velocities of the pre- and post-ganglionic nerve fibres.

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