The Roles of Sodium and Potassium Ions in the Generation of the Electro-Olfactogram

SADAYUKI F. TAKAGI, GORDON A. WYSE, HARUNOBU KITAMURA, and KATSUHIRO ITO

From the Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan 48103, and the Department of Physiology, School of Medicine, Gunma University, Maebashi, Japan. Dr. Takagi's present address is Department of Physiology, School of Medicine, Gunma University, Maebashi, Japan. Dr. Wyse's present address is Department of Zoology, University of Massachusetts, Amherst, Massachusetts 01002

ABSTRACT In order to clarify whether or not the electronegative olfactory mucosal potentials (EOG) are generator potentials, the effects of changed ionic enviroment were studied. The EOG decreased in amplitude and in some cases nearly or completely disappeared, when Na^+ in the bathing Ringer solution was replaced by sucrose, Li^+, choline$^+$, tetraethylammonium$^+$ (TEA), or hydrazine. In the K^+-free Ringer solution, the negative EOG's initially increased and then decreased in amplitude. In Ringer's solution with increased K^+, the negative EOG's increased in amplitude. When K^+ was increased in exchange for Na^+ in Ringer's solution, the negative EOG's decreased, disappeared, and then reversed their polarity (Fig. 6). Next, when the K^+ was replaced by equimolar sucrose, Li^+, choline$^+$, TEA^+, hydrazine, or Na^+, the reversed potentials recovered completely only in Na^+-Ringer's solution, but never in the other solutions. Thus, the essential role of Na^+ and K^+ in the negative EOG's was demonstrated. Ba^{++} was found to depress selectively the electropositive EOG, but it hardly decreased and never increased the negative EOG. Hence, it is concluded that Ba^{++} interferes only with Cl^- influx, and that the negative EOG's are elicited by an increase in permeability of the olfactory receptive membrane to Na^+ and K^+, but not to Cl^-. From the ionic mechanism it is inferred that the negative EOG's are in most cases composites of generator and positive potentials.

Since the pioneer work by Hosoya and Yoshida (1937) in the dog, and by Ottoson (1954, 1956) in the rabbit and frog, the electrical phenomena elicited in the olfactory epithelium by application of odors have been extensively studied and much new information has been obtained. Besides the electronegative slow potentials of the "on" type found by the above workers, and named "electro-olfactogram (EOG)" by Ottoson (1956), electronegative potentials of the "off" type (Takagi and Shibuya, 1959, 1960 *a*, *b*, *c*; Takagi,

Shibuya, Higashino, and Arai, 1960; Shibuya, 1960; Higashino, Takagi and Yajima, 1961; Ai and Takagi, 1963; Shibuya and Takagi, 1963 *a*, *b*; Gesteland, 1964; Gesteland, Lettvin, and Pitts, 1965; Higashino and Takagi, 1964; Takagi and Yajima, 1964, 1965; Takagi and Wyse, 1965) and electropositive ones of the on type (Takagi, Shibuya, Higashino, and Arai, 1960; Higashino and Takagi, 1964; Takagi, Wyse, and Yajima, 1966) and of the off type (Shibuya, 1960; Gesteland et al., 1965), and electropositive after potentials (Takagi et al., 1966) have been discovered.

There is no doubt that all these potentials are generated in the olfactory epithelium which is composed of the olfactory cells, the sustentacular cells, the basal cells, and Bowman's glands. However, the origins of these potentials in the epithelium have not been determined as yet. Ottoson (1956, 1963 *a*), on the basis of his own experiments, concluded that the electronegative EOG is a generator potential which elicits afferent discharges in the olfactory nerve. However, when the olfactory mucosal potentials were recorded together with the induced wave in the olfactory bulb, some questions were raised regarding his hypothesis (Takagi et al., 1960; Takagi, 1967 *a*). A more important finding against the hypothesis was recently obtained by Shibuya (1964), who showed that the mucosal potential can be dissociated from the olfactory nerve twig discharges when the olfactory mucus is absorbed with soft absorbent paper. On the other hand, a finding which seemed favorable for the hypothesis was obtained when the mucosal potentials disappeared in the olfactory epithelium whose olfactory nerve had been sectioned previously (Takagi and Yajima 1964, 1965). However, none of this research is considered to be conclusive at present (Takagi, 1967 *a*; Moulton and Beidler, 1967). Consequently, the origins and roles of the olfactory mucosal potentials are still topics for discussion.

The present research treats the problem by studying the roles of sodium, potassium, chloride, and calcium ions in the electronegative mucosal potentials of on and off types. The effects of barium and other ions are also examined. Thus, the ionic mechanisms underlying the electronegative EOG's are clarified. Finally, the ionic basis of these potentials is compared with that of other receptor potentials and a similarity of the negative EOG's with the end plate potential is suggested. A preliminary report has been published elsewhere (Takagi and Wyse, 1965), and the ionic mechanism of the electropositive EOG has been described in previous papers (Takagi and Wyse, 1965; Takagi, Wyse, and Yajima, 1966; Takagi, 1967 *b*).

METHODS

Preparation

Bullfrogs, *Rana catesbiana* and swamp frogs, *Rana grylio* were used. The olfactory epithelium of the roof of the olfactory cavity was excised and used for experiments.

Solutions

Normal Ringer's solutions used had the following compositions (mM): Na^+, 116.0; K^+, 2.5; Ca^{++}, 2.2; Cl^-, 116.9; HCO_3^+, 6.0. In later experiments, a solution containing Na^+, 117.6; K^+, 2.5; Ca^{++}, 2.0; Cl^-, 121.5; HPO_4^{++}, 1.1; $H_2PO_4^+$, 0.4 was used with no significant difference in results. In order to make sodium-deficient solutions, normal Ringer's solution was mixed with isotonic (6% Overton, 1902) sucrose solution containing the same concentrations of potassium and calcium as above. Sodium-free Ringer's solutions contained the same concentrations of other ions as normal Ringer's solution. In place of sodium chloride, equimolar choline chloride, tetraethylammonium chloride, or lithium chloride was used. Potassium was substituted for sodium in the bicarbonate or phosphate buffer. A sodium-free hydrazine Ringer solution was prepared after Koketsu, Cerf, and Nishi (1959). K^+-Ringer's solution was made by replacing Na^+ by K^+ and K^+ by Na^+ in Ringer's solution. This solution contained 117.6 mM K^+, 2.5 mM Na^+, 2.0 mM Ca^{++}, 121.5 mM Cl^-, 1.1 mM HPO_4^{++}, and 0.4 mM $H_2PO_4^+$.

Stimulants

Menthone and/or amyl acetate vapors were used to elicit the electronegative EOG of the on type. Saturated vapors of these odorants were mixed with purified air and were diluted to one-sixth or to one-thirty sixth. The negative-on EOG's became smaller when the concentrations of the vapors were lowered. Consequently, saturated vapors were applied to elicit bigger EOG's in most cases. Ethyl ether vapor was used to elicit the electronegative EOG of the "on-off" type. Nonanesthetic and anesthetic effects of ethyl ether have been studied and discussed previously (Takagi et al., 1960; Ai and Takagi, 1963; Higashino and Takagi, 1964). When the vapor was applied at the low concentrations (one-sixth or one-thirty-sixth), the negative-off EOG disappeared, leaving only the negative-on EOG. Consequently, the saturated vapor was applied to generate the on-off EOG. As long as the duration of application was short (4 sec in the present case) and the interval between applications was a minute or more, this on-off EOG could be elicited repeatedly and without decrease. In addition, chloroform vapor was applied to elicit the "electropositive EOG" and changes of the EOG in the above solutions were studied for comparison with the negative EOG's.

Recording Apparatus

Olfactory mucosal potentials were recorded by means of a pair of nonpolarizable (Ringer-gelatin-zinc sulfate-zinc metal) electrodes. The potential changes between these electrodes were amplified with a DC amplifier and recorded with a Sanborn 150 recorder and later with a Nihon-Kohden ink-writing recorder (Tokyo).

Experimental Procedure

The excised olfactory epithelium was spread flat, receptor side upwards, on filter paper overlying a perforated Lucite platform which was suspended across a groove made in paraffin wax inside a Petri dish. An exploring electrode was put on the center of the epithelium and an indifferent electrode on the peripheral part of the filter paper. After

a few immersions in Ringer's solution and a few stimulations, the receptor potentials attained fairly constant amplitudes (Fig. 2). Odorous vapors were applied at intervals of a minute or more so that potentials of the same order of magnitude might be obtained consistently.

At the beginning of each series of experiments the olfactory epithelium was immersed in Ringer's solution for 5 min. Then, the fluid level was brought down to the level of the filter paper, and the EOG's were recorded as controls for subsequent trials. Ringer's solution was next replaced with a test solution by means of a syringe and a pipette. The test solution was changed two or three times during 5 min immersion. After the fluid level had been lowered to the level of the filter paper, EOG's were

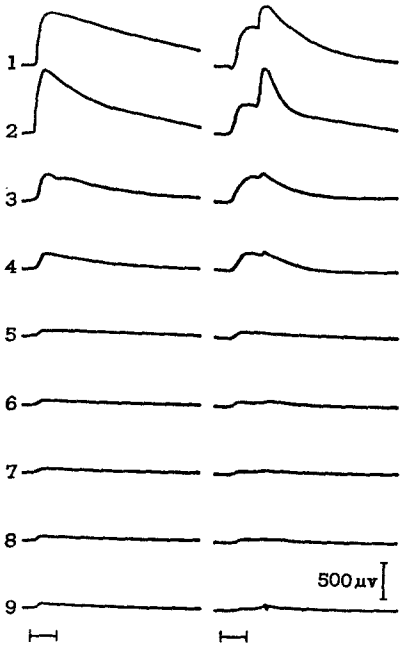

FIGURE 1. Effect of Na^+-free, sucrose-Ringer's solution. The negative-on potentials elicited by menthone vapor are shown in the left column, and the on-off potentials elicited by ethyl ether vapor in the right one. 1 indicates the negative EOG's in normal Ringer's solution. 2 to 9 indicate changes in magnitude of the negative on and off EOG's in Na^+-free Ringer's solution. The records were taken at intervals of 20 min. Note the temporary increase in magnitude of the on and off EOG's in 2, and the later decreases below 2. Short horizontal lines below columns of records indicate the time and duration (4 sec) of stimulation.

recorded to study the effect of the solution. Three recordings were made for each of the negative-on and -off and positive-on and-after potentials, with the positive potentials being used for comparison and for separate analysis. Then, the remaining test solution was removed and the epithelium was immersed in a fresh test solution for 5 min. By repeating these procedures, temporal changes of the EOG's were recorded. In order to study the recovery process of the potentials, the test solution was replaced by Ringer's solution, and the same procedure was repeated. Replacement of test or Ringer's solution and recording of nine EOG's took about 20 min, including 5 min for immersion. The average amplitude of the EOG's of the same kind was plotted at intervals of 20 min with or without standard deviation (Figs. 2, 4, 5, 7–10, 12, 13). Changes in amplitude of the negative-on and -off potentials have been described in a preliminary report (Takagi and Wyse, 1965). Since the similarity between the two negative EOG's has been shown (Higashino and Takagi, 1964), and since the changes

of the negative-off potentials in test solutions generally resemble those of the negative-on potentials, only the behavior of the latter is shown in many figures to save space. Experiments were not performed in midsummer or midwinter.

RESULTS

Effect of Sodium Ions

(a) REPLACEMENT BY SUCROSE

When the olfactory epithelium was immersed in sodium-free sucrose solution, the negative-on and -off EOG's initially increased in magnitude by as much as

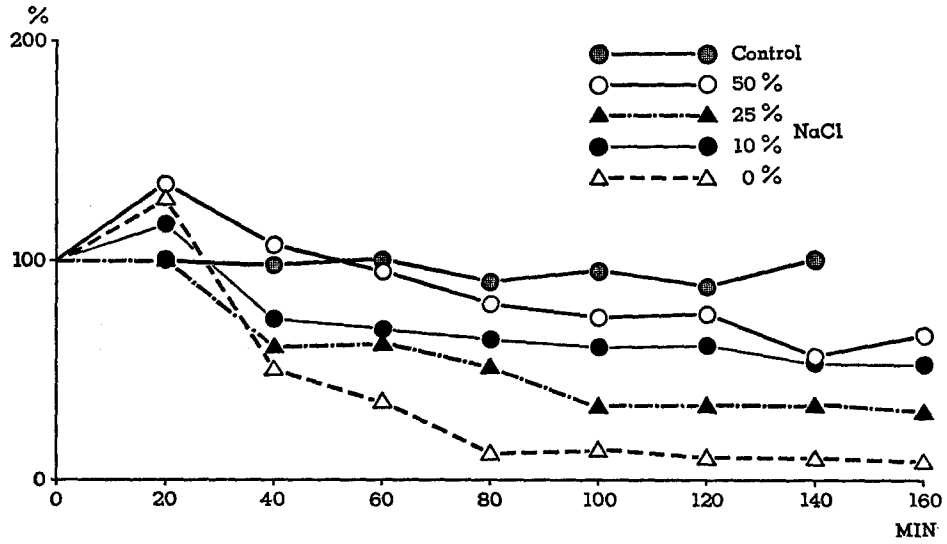

FIGURE 2. Temporal changes in amplitude of the negative-on EOG's in Na^+-free (shown in Fig. 1) and low Na^+ solutions. Ordinate, amplitude of EOG's is expressed as percentage of the initial EOG's. Abscissa, minutes after first immersion in the Na^+-free or low Na^+ solutions. Control means changes in amplitude of EOG's in normal (100% NaCl) Ringer's solution. Full explanation in the text.

188% (the average of eight experiments was 137%) and then a decrease in amplitude set in as indicated in Figs. 1 and 2. The decrease of the negative EOG's was initially very slow and became much slower in the later stages. The negative-on potentials decreased from 30 to 58% of the original values after three immersions (1 hr) (eight experiments, mean 45.2%), from 7 to 14% after six immersions (2 hr) (four experiments, mean 11.5%), and to about 2% or disappeared after seven and more immersions (over 150 min) (two experiments). The rates of decrease were similar even when the olfactory epithelium was immersed in the sucrose solution for 18 instead of 5 min (usual method) in each immersion. When the sodium-free solution was replaced by Ringer's

solution after several immersions, the negative-on and -off EOG's decreased further, and began to increase only after two or three more immersions in Ringer's solution. However, the recovery was slight and far from complete. The recovery was far less after longer immersion in the sucrose solution.

In 10% Na^+-Ringer's solution (one part of Ringer's solution mixed with nine parts of sucrose-Ringer's solution), the negative-on and -off EOG's showed similar changes in most cases, namely an initial increase followed by decrease (Figs. 2 and 10). The initial increase of the negative-on EOG reached 179% in one experiment. Then, the amplitudes of the potentials in this solution began to decrease and became 42–67% of the original one after three immersions and 40–60% after six immersions.

In the 25 and 50% Na^+-Ringer solutions, the decreases in amplitude of the potentials were much more moderate and were often not preceded by an initial increase (Fig. 2). The negative-on potentials were found to be 60-95% of the original ones after three immersions and 30–75% after six immersions. The rate of decrease of the negative-on EOG's in these latter solutions depended upon the olfactory epithelium employed rather than upon the concentrations of Na^+. Thus, it was difficult to relate the amplitudes exactly to the concentrations of Na^+ as was done in muscle (Nastuk and Hodgkin, 1950), in nerve (Huxley and Stämpfli, 1950, 1951), in the Pacinian corpuscle (Diamond, Gray, and Inman, 1958), and in the retina (Hamasaki, 1963).

Changes in the negative-off EOG were in general similar to those in the negative-on EOG (Fig. 1). This similarity was also found in the succeeding experiments (Fig. 4). Consequently, the two types of negative EOG's may have the same or similar ionic mechanisms. The recovery of the negative EOG's after immersion in these low Na^+ solutions was incomplete in all cases, even after 3 hr immersion in normal Ringer's solution.

In these Na^+-free and low Na^+ Ringer's solutions, the positive EOG's also increased initially and such increases often persisted for several immersions before they began to decrease in amplitude. In Na^+-free and 10% Na^+-solutions, the maximal amplitudes were found after one immersion, but in the other solutions only after further immersion. The greatest increase thus far obtained was 427% in Na^+-free solution.

(b) RELATION BETWEEN AMPLITUDE AND RATE OF RISE

The rate of rise and the amplitude of the negative EOG's were studied in 10% Na^+-Ringer's solution. The changes with time in rate and in amplitude were closely parallel (Fig. 3). Such a result is expected if Na^+ movement generates the potential: both amplitude and rate of rise (rate of Na^+ entry) would be proportional to external Na^+ concentration. If the potential were not due to the ion movement across the olfactory membrane, the parallel changes would be less likely. The gradual changes in rate and amplitude with time may be

due to the gradual decrease of Na^+ in the immediate vicinity of the olfactory receptive membrane (part 3 of the Discussion, see below).

(c) REPLACEMENT BY Li^+

It is well-known that Li^+ can substitute for Na^+ in maintaining the normal activity of the frog muscle and nerve (Overton, 1902; Huxley and Stämpfli, 1950, 1951). When Na^+ in Ringer's solution was replaced by Li^+, the amplitudes of the negative-on and -off EOG's decreased without initial increases (Fig. 4). The decrease was relatively rapid, the amplitude of the negative-on

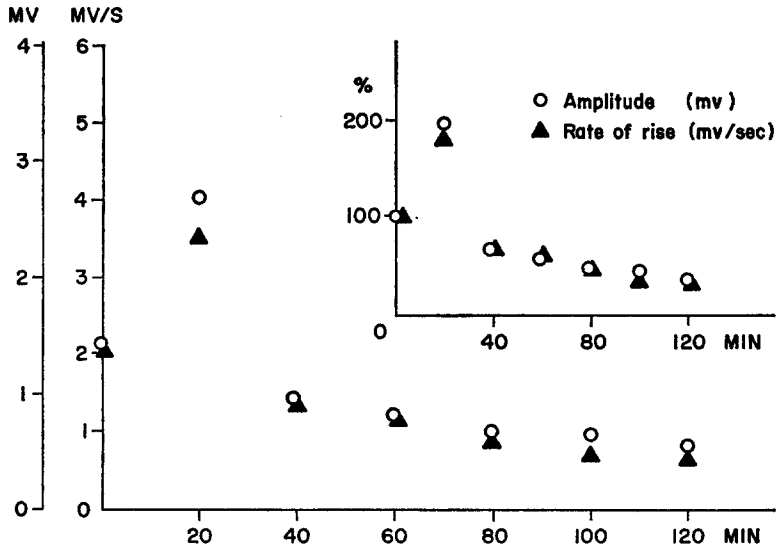

FIGURE 3. Rate of rise and amplitude of the negative-on EOG's. A, the ordinate at the extreme left shows the amplitude of the EOG in millivolts and the one next to it shows the rate of rise in millivolts per second. The abscissa indicates time in minutes after the first immersion in 10% Na^+ Ringer's solution. The inset at the upper right shows the same relation in percentage. The amplitude of the EOG decreased with time, but it coincided well with the rate of rise in these figures. Further explanation in the text.

EOG falling to 17–22% of the original amplitude after three immersions and nearly disappearing after six immersions. This rapid decrease may occur because Li^+ has a depolarizing action (Gallego and Lorente de Nó, 1947; Hamasaki, 1963). When the Li^+ solution was replaced by normal Ringer's solution, the negative EOG's further decreased for some time but then began to increase. It is worthy of note that within 2–3 hr the amplitudes of the negative EOG's always recovered completely or nearly completely (Fig. 4.)

The positive EOG also decreased in amplitude without an initial increase, and also nearly completely recovered after replacement of the solution with

normal Ringer's solution. This was the only solution with which complete recovery of the EOG's was obtained.

(d) REPLACEMENT BY CHOLINE

In choline-Ringer's solution the negative-on and -off EOG's decreased in amplitude (Fig. 5), but were often preceded by an initial increase. The rates of decrease were very gradual; in one case the on potential decreased only to

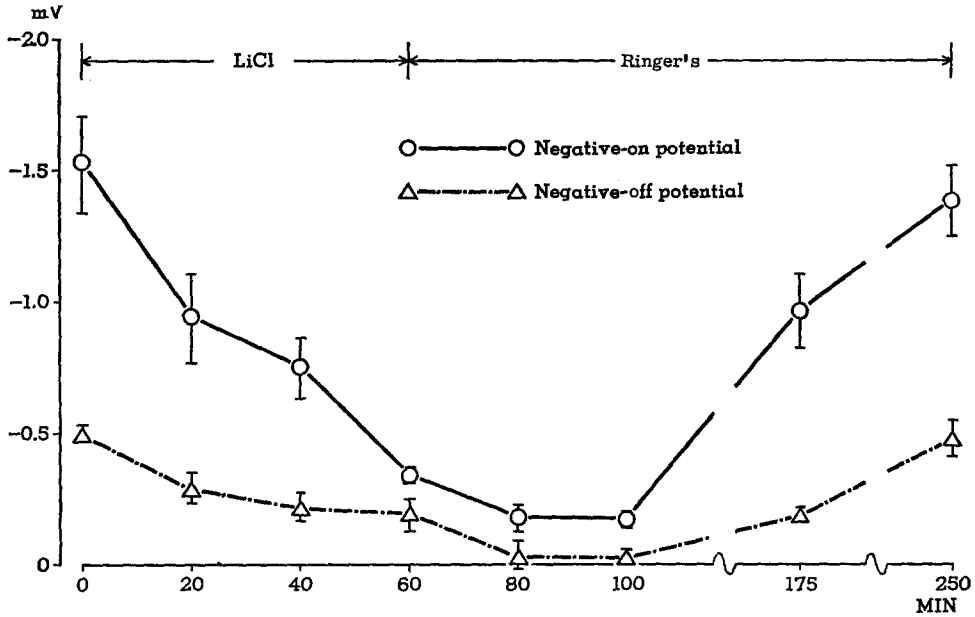

FIGURE 4. Negative EOG's in Li^+ solution. In Ringer's solution in which Na^+ was replaced by Li^+ the negative-on and -off EOG's decreased without initial increase. After three immersions (60 min) the solution was replaced by Ringer's solution. The potentials recovered completely or nearly completely after 3 hr. The vertical bars give standard deviations of the amplitudes of the EOG's.

70% of the original value after 2 hr immersion in this solution. The positive EOG always increased in amplitude (in one case up to 220%) and then began to decrease.

(e) REPLACEMENT BY TETRAETHYLAMMONIUM (TEA)

In the tetraethylammonium chloride–Ringer solution, the negative-on and -off EOG's decreased relatively rapidly in amplitude (24–40% of the original amplitude after the third immersion) without initial increases (Fig. 5). The positive EOG increased initially (one to three immersions) and then began to decrease. Recoveries of the negative-on and -off EOG's in normal Ringer's solution were prompt and striking. This was very different from the findings

with the other solutions. In most cases, however, recovery ended after several immersions in Ringer's solution and the potentials again began to decrease. The positive EOG showed little recovery in normal Ringer's solution.

(f) REPLACEMENT BY HYDRAZINE

When Ringer's solution was replaced by Na^+-free hydrazine-Ringer's solution, both the negative and positive EOG's decreased in amplitude and nearly

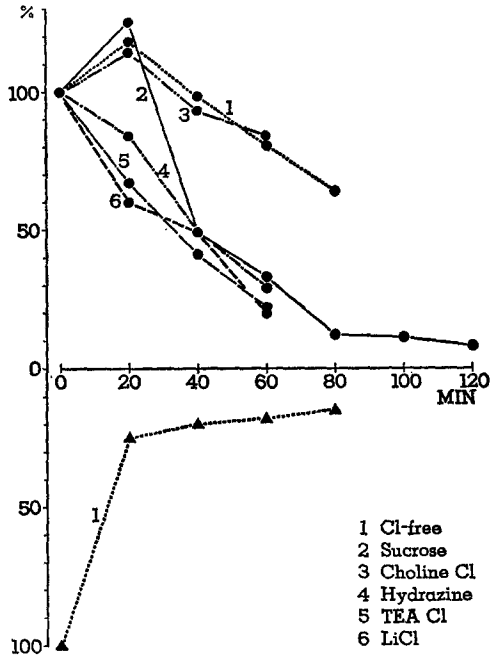

FIGURE 5. The gradual changes of negative EOG's in various sodium-free media in contrast to the abrupt decrease of positive EOG. Temporal decrease in amplitude of the negative EOG's in sucrose-, Li^+-, choline$^+$-, TEA^+-, and hydrazine-Ringer's solutions. In some cases, the decrease was preceded by an initial rise, but in other cases it was not. For comparison, the changes of the negative (above the abscissa) and positive (below the abscissa) EOG's in Cl^--free solution were added (indicated by 1). Note that the changes in the negative EOG's are very slow, as compared with the abrupt decrease of the positive EOG in Cl^--free solution (see also Fig. 13). Ordinate, EOG amplitude as per cent of control level. Abscissa, duration of immersion in various Na^+-free solutions.

disappeared after three or four immersions (Fig. 5). Recoveries of both potentials in normal Ringer's solution were always partial and incomplete.

Thus far, no substitute ion for sodium has been found among the above ions and chemicals which have been found to substitute for Na^+ in various excitable tissues. The search for such a substitute ion has been continued among many other mono- and divalent cations, but without success as will be

shown in a paper to be published (Takagi, Kitamura, Imai, and Takeuchi, unpublished data). Consequently, it appears that sodium is the only cation which can maintain the negative EOG's in the olfactory epithelium, although other ions may contribute to the potentials (see below).

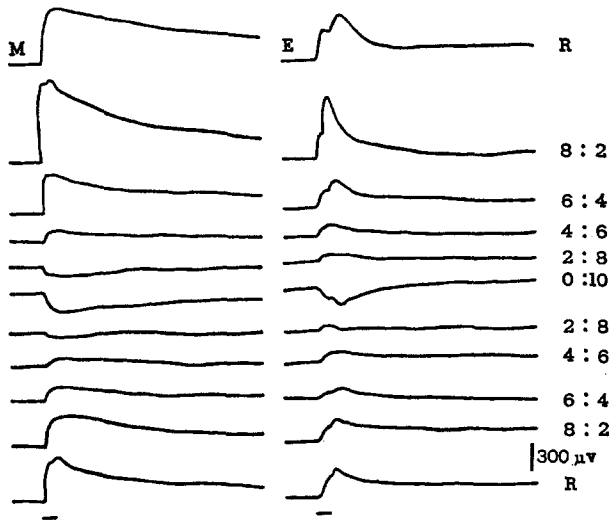

FIGURE 6. Reversal and recovery of EOG's. When Na^+ in Ringer's solution was replaced in steps by K^+ keeping the total quantity of both ions constant, the negative EOG's decreased in amplitude, disappeared, and then appeared with reversed polarity. With subsequent stepwise replacement of K^+ by Na^+, the negative EOG's recovered (shown from top to bottom). Negative-on EOG's were elicited by menthone (*M*), and negative on-off EOG's by ethyl ether (*E*) Composition of the bathing solution is given at the right as the ratios of normal Ringer's to K^+-Ringer's solution (in which Na^+ and K^+ concentrations are reversed). Thus, *R* is normal Ringer's solution and 8:2 is a mixture of eight parts Ringer's solution and two parts K^+-Ringer's solution. Short horizontal lines below columns of records indicate the time and duration (4 sec) of stimulation.

Effect of Potassium Ion

(a) REVERSAL OF EOG'S

When Na^+ in the normal Ringer solution was decreased and K^+ was increased in steps, by mixing normal Ringer's solution with the K^+-Ringer's solution (see Methods), gradual changes occurred in the amplitudes of the EOG's. The negative-on and -off EOG's decreased in amplitude, reversed their polarity, and then increased in amplitude with reversed polarity (Fig. 6). Conversely, when K^+ in K^+-Ringer's solution was replaced in steps by Na^+, the EOG's changed in the opposite direction and recovered to a considerable extent, although the recovery was usually not complete. With shorter immersion in K^+-Ringer's solution, recoveries were greater and after one immersion

the recoveries were complete or nearly complete in many cases (one such case is shown in Fig. 8). Thus, the negative EOG's change remarkably with changes in the concentration of external K^+.

In the above cases the effect of concurrent Na^+ variation is shown by the following experiment to be minor. In K^+-Ringer's solution, the concentration of Na^+ was increased from zero up to 20 mM, but the reversed (originally

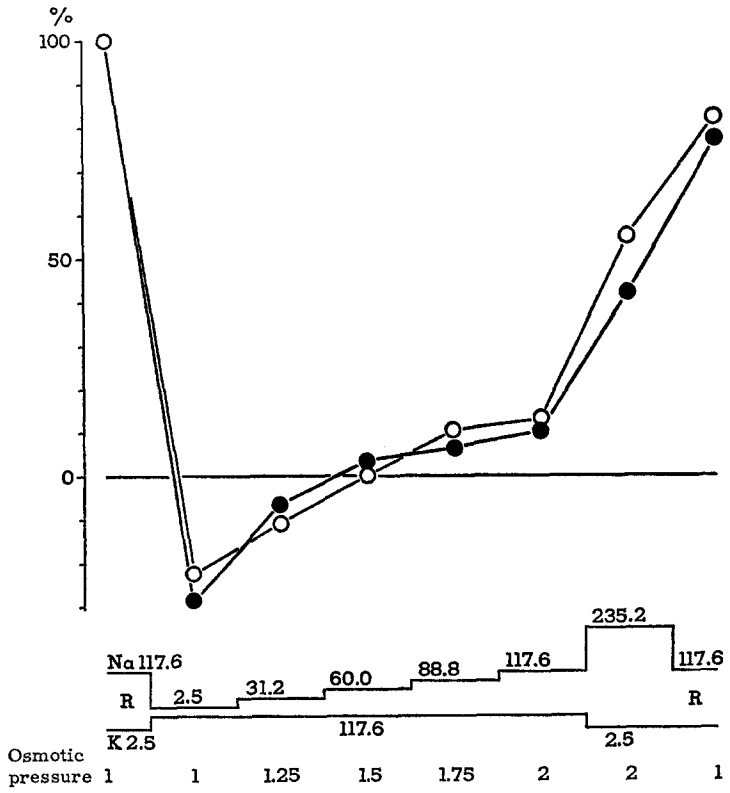

FIGURE 7. Effects of K^+ and Na^+. When the olfactory epithelium was immersed in K^+-Ringer's solution, the negative-on EOG's shown by a thick line with filled circles (amyl acetate) and by a thin line with open circles (menthone) became positive. Concentrations of Na^+ and K^+ and osmotic pressure are shown at the bottom of the figure. Then, with $[K^+]$ kept at 117.6 m*M* the concentration of Na^+ was increased from 2.5 to 117.6 mM (from left to right). Full explanation in text.

negative) potentials showed little change. Even when the concentration of Na^+ was increased up to 117.5 mM in the solution, the reversed potentials showed only a slight recovery (Fig. 7). However, when the concentration of K^+ was again decreased to 2.5 mM (i.e., returned to normal Ringer's solution), the reversed potential suddenly and remarkably recovered (Fig. 7). Since the effect of increasing the osmotic pressure to twice normal (produced by Na^+

or sucrose) does not have any significant influence upon the EOG's (unpublished data); and since the membrane potential of the receptor cell presumably depends mainly upon the concentration ratio of the external and internal K^+; it may be concluded from the above experiments (Figs. 6 and 7) that the amplitudes of the negative EOG's depend largely upon the magnitudes of the membrane potentials. Thus, a possible contribution of K^+ to the generation of the negative EOG's was indicated.

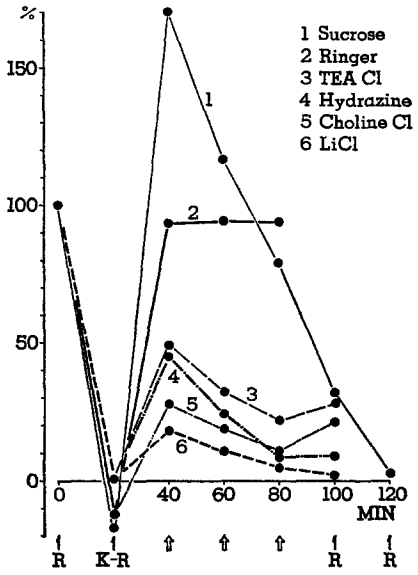

FIGURE 8. Comparison of recoveries of the reversed EOG's in various Na^+-free solutions After the negative EOG's were reversed in K^+-Ringer's solution recoveries were compared in various Na^+-free solutions in which Na^+ was replaced by Li^+, choline$^+$, TEA^+, sucrose, or hydrazine. Three hollow arrows at the bottom center indicate three immersions (during 60 min) in all these Na^+-free solutions except in sucrose solution. Vertical arrows at the bottom indicate from left to right one immersion in Ringer's solution (control), one immersion in K^+-Ringer's solution, and after the three hollow arrows two immersions in Ringer's solution. In the case of sucrose-Ringer's solution (indicated by 1), immersions in this solution were repeated five times. Further explanation in text.

(b) RECOVERIES OF THE REVERSED EOG'S

Recoveries of the reversed (originally negative) potentials were studied in various modified Ringer's solutions in which Na^+ was replaced by other ions. When K^+ in K^+-Ringer's solution was replaced by Li^+, the reversed potentials recovered to some extent and became negative. When, however, immersion in the same Li^+-Ringer solution was repeated, recovery of the potentials stopped and the potentials that had recovered again decreased in amplitude (Fig. 8). Similar phenomena were observed in other solutions in which Na^+ was re-

placed by sucrose, TEA^+, hydrazine, or $choline^+$ (indicated by 1, 3, 4, or 5 in Fig. 8). In these solutions, the negative EOG's recovered once and to various degrees, but then they began to decrease in amplitude. Thus, no further recovery was found in these solutions. In sucrose-Ringer's solution, the initial recovery was remarkable (up to 150%), but the negative EOG's soon decreased and disappeared after several immersions (Fig. 8).

In these experiments, only replacement of K^+ in K^+-Ringer's solution by Na^+ led to complete or nearly complete recovery of the reversed potential and to maintenance of the recovered potential (2 in Fig. 8). These experi-

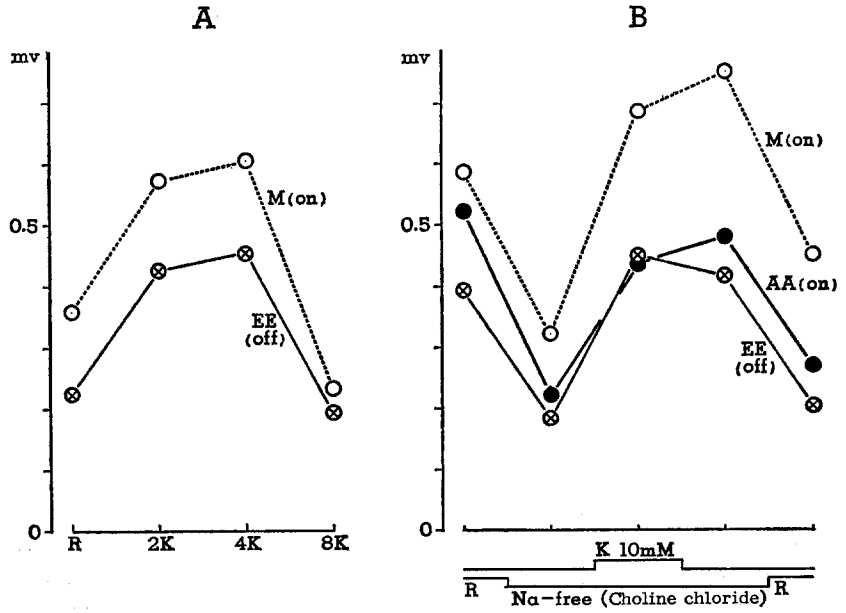

FIGURE 9. Effects of high K^+. A, the negative EOG's of the on type (indicated by *M*, menthone) and the off type (indicated by *EE*, ethyl ether) increased in amplitude when the concentration of K^+ in Ringer's solution was increased two or four times normal. At higher concentrations than four times normal (10 mM), the amplitude of the EOG decreased in this case. B, a similar phenomenon was observed in Na^+-free Ringer's solution (with substitution of choline chloride). The negative EOG's of the on type are indicated by *M* (menthone) and *AA* (amyl acetate) and the off type by *EE* (ethyl ether). Interval between trials, 20 min. Full explanation in text.

ments, therefore, provide further evidence for the essential and irreplaceable role of Na^+ in the negative EOG's.

(c) EOG's in K^+-free or excess solutions

When the olfactory epithelium was immersed in the K^+-free Ringer solution, both the positive and negative EOG's initially increased in amplitude and then began to decrease (Fig. 6 in Takagi et al., 1966; Fig. 13). The changes in

the positive EOG in this solution were discussed in this paper. Since dependence of the negative EOG's upon the membrane potential was shown in Figs. 6 and 7, the initial increase of the negative potentials could be explained by the initial increase of the membrane potential which occurs temporarily in the K^+-free Ringer solution. The subsequent decrease of the EOG's could be explained again by the subsequent decrease of the membrane potential in the

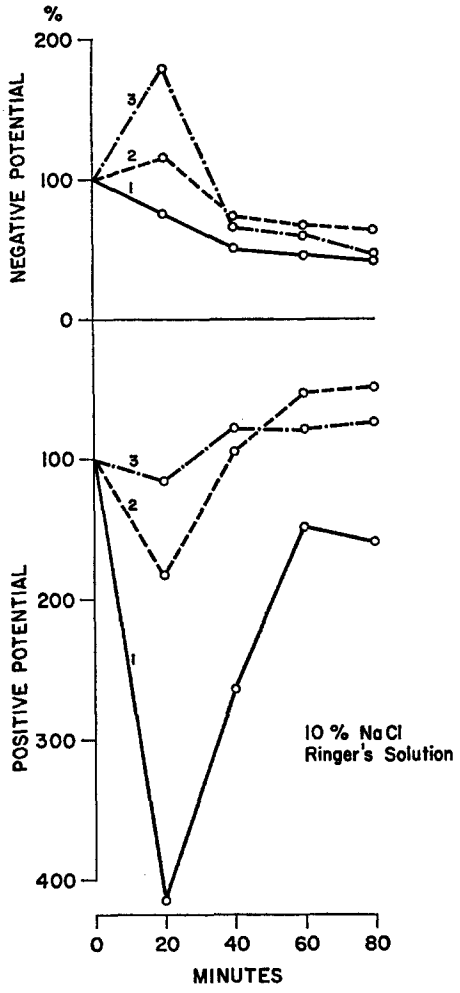

FIGURE 10. Comparison of the initial increases of negative and positive EOG's in 10% Na^+-Ringer's solution. Negative EOG's are shown above the horizontal line indicating 0% and positive EOG's are shown below the line. Increase of the negative EOG is indicated by an upward deflection of the lines (2 and 3), and that of the positive EOG by a downward deflection of the lines (1, 2, and 3). (Examples of increase or decrease in amplitude of the positive EOG are found in Fig. 11.) 1, 2, and 3 represent different preparations. 1, initial decrease in the negative EOG, with marked initial increase in positive EOG; 2, moderate initial increases in both negative and positive EOG's; 3, marked initial increase in negative EOG, with only slight initial increase in positive EOG. Full explanation in text.

K^+-free solution (Desmedt, 1953). Conversely, when K^+ was increased in Ringer's solution, the negative EOG's increased in amplitude but beyond a certain limit (10 mM in Fig. 9 A) they began to decrease (compare Fig. 6). A similar phenomenon was found when K^+ was increased in a Na^+-free choline$^+$-Ringer's solution. In Fig. 9 B, it is shown that the negative EOG's began to decrease in amplitude in the Na^+-free choline$^+$-Ringer solution but

recovered their amplitudes, when K^+ in the solution was increased to 10 mM. This experiment also indicates a contribution of K^+ to the generation of the negative EOG's.

(d) INTERACTION BETWEEN THE NEGATIVE AND THE POSITIVE EOG'S

It was very frequently observed that the amplitudes of the negative and the positive EOG's were inversely related: when the negative potential is relatively large, the positive potential is relatively small and vice versa. A similar relation was found in a 10% Na^+-Ringer solution: the increase in amplitude was very striking in the negative potential, but it was not so striking in the

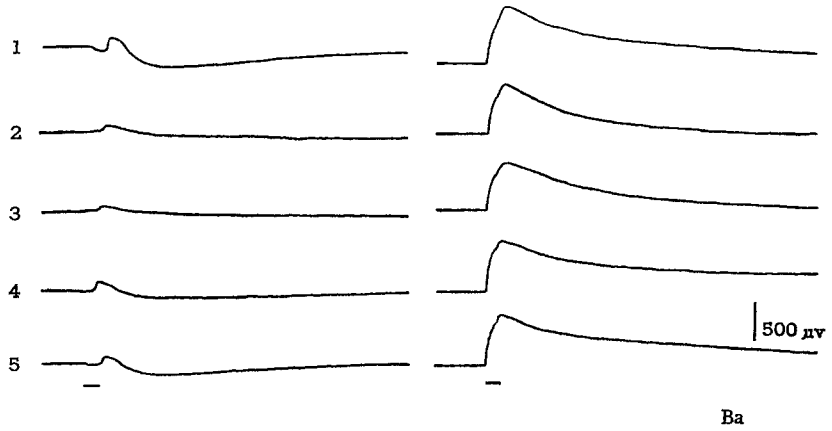

FIGURE 11. Effect of Ba^{++} on positive and negative EOG's. The positive EOG (1 in the left column) decreased in amplitude strikingly when 1 mM Ba^{++} was added to Ringer's solution (2 and 3), while the negative EOG's (right column) were hardly affected. Thus, Ba^{++} specifically affects the positive EOG. The positive EOG recovered to some extent with repeated immersion in normal Ringer's solution (4 and 5). Further explanation in text.

positive potential (3 in Fig. 10) and vice versa (1 in Fig. 10). But when both potentials increased simultaneously, the increases in both potentials were only moderate (2 in Fig. 10). These findings also indicate a contribution of K^+ to the negative EOG's that will be discussed later.

Effect of Barium Ion

Previous work has shown that the positive EOG depends mainly upon the entry of the external chloride and the exit of the internal potassium ions (Takagi et al., 1966). Similar ionic mechanisms have been demonstrated in various positive or hyperpolarizing potentials (Takagi et al., 1966). In the spinal motoneuron of the cat (Eccles, 1964) drugs were found to affect one such positive potential (inhibitory postsynaptic potential or IPSP). Strychnine,

tetanus toxin, and other antiinhibitory drugs are known to depress the IPSP. However, an ion which affects the positive potential has not yet been discovered.

Ba^{++} has such an action and depresses nearly selectively the positive EOG at a concentration of 1 mM or so (Figs. 11 and 12). Hence, it is probable that either Cl^- or K^+ movement or both are blocked by this ion. On the other hand, the negative EOG's were at most only slightly depressed (Figs. 11 and 12). This indicates that the entry of Na^+ may be affected by Ba^{++}, but the exit of K^+ is not. The effect of Ba^{++} on K^+ movement was further examined by

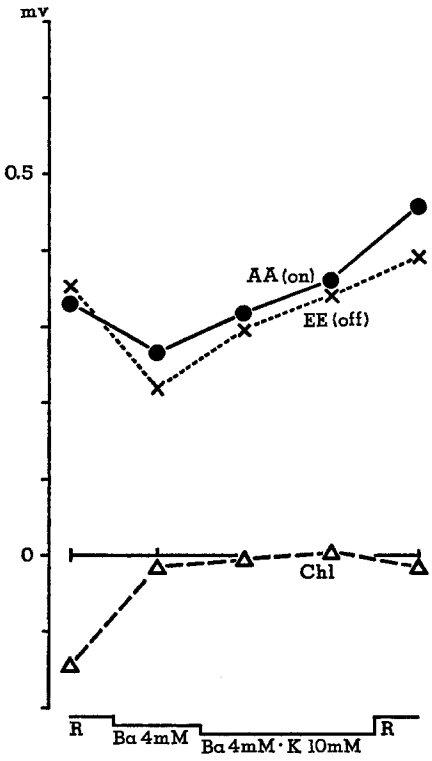

FIGURE 12. Effect of Ba^{++} and K^+. As in Fig. 11, Ba^{++} (in this case 4 mM) decreased the positive EOG elicited by chloroform vapor (*Chl*), while it slightly decreased the negative on and off EOG's elicited by amyl acetate (*AA*) and ethyl ether (*EE*) vapors respectively. When 10 mM K^+ was added to this Ringer's solution with Ba^{++}, the negative on and off EOG's increased in amplitude, although the decreased positive EOG did not show a striking change. Addition of Ba^{++} and K^+ to Ringer's solution is indicated at the bottom of the figure.

increasing K^+ up to 10 mM in the Ringer solution containing Ba^{++}. When K^+ was so increased, the negative EOG's increased in amplitude beyond the original level (Fig. 2). If K^+ movement were blocked by Ba^{++} and if the decrease of the concentration gradient of K^+ across the receptive membrane were not related to the generation of the negative EOG's, such increases in high K^+-Ringer's solution could not be expected. Thus, it is clear that K^+ movement is not blocked by Ba^{++} in the negative EOG's. Consequently, it appears that Ba^{++} selectively blocks the entry of the Cl^- but not the exit of K^+ in the positive EOG. Now, if the entry of Cl^- contributed to the generation of the negative EOG's, and if such entry of the ion were blocked by Ba^{++},

augmentation of the negative EOG's would be expected. As stated above, the negative EOG's were never augmented by this ion. Consequently, it is unlikely that Cl^- contributes to the generation of the negative EOG's.

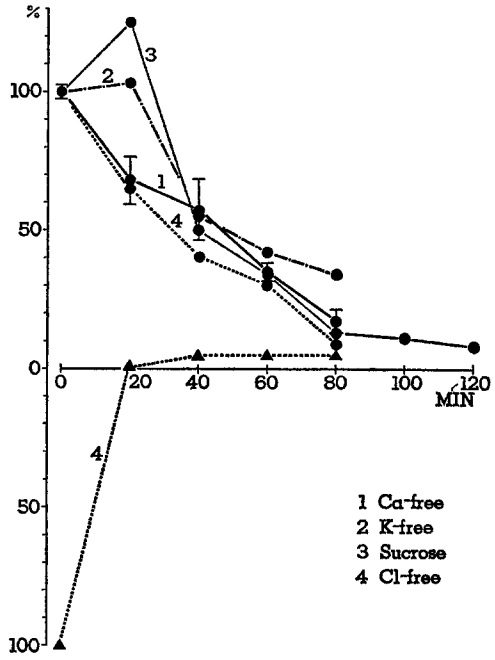

FIGURE 13. Effects of Ca^{++}-free Ringer's solution. When Ca^{++} was removed from the Ringer solution, the negative EOG's (shown above the abscissa) decreased in amplitude without initial increase. The rates of decrease are shown with standard deviation (indicated by 1). For comparison, the changes of the EOG's in Na^+-free (sucrose) (3), K^+-free (2), and Cl^--free (4) Ringer's solution are added. It is worthy of note that the negative EOG's in all these solutions decreased nearly uniformly after the first immersion, and that they decreased very slowly when compared with the positive EOG (shown below the abscissa) in Cl^--free solution (4). Further explanation in the text. Ordinate, amplitude of the EOG's shown in per cent. Abscissa, duration of immersion shown at 20 min intervals.

Effect of Calcium Ion

When the olfactory epithelium was immersed in Ca^{--}-free Ringer's solution, the negative (and positive) EOG's gradually decreased in amplitude without initial increase (Fig. 13).

Once the EOG's decreased in this solution, they never recovered even after immersion in Ringer's solution for 3 hr. Occasionally, a slight transient recovery was found, but it was always followed by a further decrease. In the light of this lack of recovery we feel that the Ca^{++}-free solution has an irreversible deteriorative action on the olfactory receptive membrane, as has been

found in the other excitable tissues and that Ca^{++} is essential to the normal activity of the olfactory receptive membrane.

DISCUSSION

1. *The Role of Na^+*

Ionic mechanisms of the receptive membranes which produce electronegative potentials have been studied in several receptors. In the Pacinian corpuscle, the receptor potential decreased in amplitude in Na^+-free media, and the decreases in amplitude and rate of rise of the receptor potential were found to be related to the Na^+ concentration in the bathing solution (Diamond et al., 1957, 1958). Although the decrease was slow and no direct result was shown, it was concluded that Na^+ plays an important role in the generation of the negative receptor potential. The basis for this conclusion was that either internal anions must leave or external cations must enter the receptive membrane, that Na^+ is the principal cation in the external fluid, and the inward electrochemical gradient of this ion is large, while there are no anions inside the cell which seem likely to effuse. The slow decrease was assumed to be due to the lamellae which surround the receptive membrane and slow down the change in concentration of Na^+ in the immediate vicinity of the receptive membrane.

Similar findings were obtained in the retinas of the toad, frog, and hermit crab (Furukawa and Hanawa, 1955; Hamasaki, 1963; Stieve, 1965), in the lateral eye of the horseshow crab (Kikuchi, Naito, and Tanaka, 1962), in the retinula cell of the crayfish (Eguchi, 1965), in the muscle spindle of the frog (Ottoson, 1963 *b*, 1964; Calma, 1965), in the stretch receptor cell of the crayfish (Edwards et al., 1963), and in the sugar receptor of the fleshfly (Morita, Hidaka, and Shiraishi, 1966; Morita, 1967). Although in some cases residual receptor potentials remained in sodium-free solutions (10% in the Pacinian corpuscle, about 30% in the lateral eye, and 20–30% in the muscle spindle), and the ionic mechanisms of these remaining potentials have not been the subject of research, it is concluded that the sodium ion does play an important role in these negative receptor potentials just as it does in the nerve action potential (Hodgkin, 1951). The above data derived from these experiments, however, are still rather indirect, and do not conclusively demonstrate the essential role of the ion. Furthermore, the role of the sodium ion in many nervous tissues has been questioned in recent years and action potentials in the absence of Na^+ have been demonstrated (Koketsu, 1961; Tasaki, Singer, and Takenaka, 1965).

When the olfactory epithelium was immersed in Na^+-free Ringer's solutions in which Na^+ was replaced by sucrose, Li^+, choline$^+$, TEA^+, or hydrazine, the EOG's slowly decreased in amplitude and in some cases nearly disappeared. In order to clarify the ionic mechanism of the negative EOG's, the arguments

used by Diamond et al. (1958) may be applied to our experiments. In addition to this logical approach, some positive evidence for a Na^+ hypothesis was obtained. First of all, none of the cations tested has been able to substitute for Na^+, which seems to be the only cation which can maintain the negative EOG's. Second, in the course of recovery after the negative EOG's were reversed in K^+-Ringer's solution, several cations, sucrose, and hydrazine were substituted for Na^+. Many other mono- and divalent cations were used in a similar manner (Takagi et al., unpublished data). None of them could restore the negative EOG's; only with Na^+ was there complete or nearly complete recovery and subsequent maintenance of the negative EOG. In the present stage of experiments, this may be the most powerful evidence for the essential role of Na^+ in the negative EOG's. A third line of evidence for the role of Na^+ is the finding that the amplitude and rate of rise of the negative EOG's may be related to the concentration of Na^+ around the receptive membrane. Similar experimental results and interpretations led Diamond et al. (1958) to conclude that the generator potential in the Pacinian corpuscle is principally a result of Na^+ entry. Although the result in the present experiments is indirect, it lends support to a supposition that the Na^+ plays an essential role in the negative EOG's. From the three results mentioned above, it is concluded that the sodium ion plays an important role in the generation of the negative EOG's.

Tucker and Shibuya (1965) studied the "underwater" negative EOG in the olfactory epithelium of the box turtle, *Terrapene carolina*. The odorous molecules were introduced into flowing isotonic saline solution containing 1.4 mM $CaCl_2$ and then the odorous solution was flowed over the olfactory area. When Na^+ was replaced by equimolar sucrose, the underwater EOG, which was usually less than 5 mv, became extraordinarily large (over 60 mv). Unfortunately, Tucker and Shibuya recorded EOG's in sucrose-Ringer's solution for only 15–20 min (personal communication). It is likely that the augmented potentials they recorded correspond to the initial increases recorded in sucrose-Ringer's in the frog electroretinogram (Hamasaki, 1963) and in the present study. We feel that with longer exposure to the Na^+-free solution the underwater EOG would decrease as the EOG's in our study did. The extreme augmentation of the underwater EOG (about 1200%, as opposed to 133% in the frog electroretinogram and a maximum of 188% for the negative and 427% for the positive EOG in our experiments) remains to be explained.

2. *Initial Increase in the Negative EOG's in Na^+-Free Solutions*

As was mentioned in part 1 of the Discussion, see above, the negative-on and -off EOG's initially increased in amplitude when Ringer's solution was replaced by Na^+-free or Na^+-deficient solutions. The resistance between a pair of

recording electrodes the usual distance apart was measured in normal and in Na^+-deficient Ringer's solutions. The resistance in 10% Na^+-solution was 1.7 times that in Ringer's solution, and the resistance in Na^+-free solution was 2.1 times that in Ringer's solution. These increases in resistance of the bathing solutions naturally decrease the electric current flowing through intercellular spaces from the inner resting part of the cell membrane (source) to the outer depolarized site (sink), and conversely increase the electric current passing through the cytoplasm of the neighboring inactive cells. The membranes and cytoplasm of these cells have greater electric resistances than the intercellular spaces, and the EOG's recorded by means of such large electrodes as in the present experiment are composed of the potentials produced by the electric current flowing through the neighboring cells. Consequently, most or at least a greater part of the initial increase of the negative EOG's can be explained by this increase of the current flowing through the neighboring inactive cells. The same explanation can be applied to explain why the rates of decrease in amplitude of the negative EOG's are so slight and so slow. Without the increase in the resistance of the solution, the negative EOG's should have decreased much faster.

It is well-known that the resting membrane potential of a neuron depends mainly upon the potassium equilibrium potential, but it is maintained at a lower level than the potassium potential, due to the presence of the sodium and chloride equilibrium potentials. When Na^+ in Ringer's solution is replaced by sucrose, and the influence of the sodium equilibrium potential is removed, it is possible that the resting membrane potential increases, although it did not in the case of the muscle experiments of Mullins and Noda (1964). If the sodium ion is retained in the immediate vicinity of the receptive membrane by a mechanism suggested in part 3 of the Discussion, see below and enters it in this hyperpolarized state, increase in amplitude of the negative EOG's results. In this unnatural condition, such hyperpolarization continues only temporarily, and then depolarization sets in and developes due to the movement of other ions. Although these changes in the membrane potential are conjectural they may be used to explain some of the initial increase followed by the decrease of the negative EOG's, if they do in fact occur.

Movement of Na^+ across the Receptive Membrane

The negative EOG's decrease in amplitude very slowly in various sodium-free solutions (Figs 2 to 5). The rates of decrease in five Na^+-free Ringer's solutions are all comparable and are considerably slower than the rate of decrease of the positive EOG in Cl^--free solution (Fig. 5). There are similar slow rates of decrease for the negative EOG in K^+-free, Ca^{++}-free, and Cl^--free Ringer's solutions (Fig. 13). Recovery of the negative EOG is also very slow after a decrease in Na^+-free solutions. Recovery often commences only after two or

three immersions in Ringer's solution (except following TEA^+ solutions), in contrast to the rapid recovery of the positive EOG after exposure to Cl^--free solutions (Fig. 3 in Takagi et al., 1966). Although the rates of decrease are not as slow as in the negative EOG's, similar slow decreases in amplitude in Na^+-free solutions have been found in all other receptor potentials previously mentioned. In the Pacinian corpuscle, the receptor potentials took 11 to 30 min to decrease 10% of the initial amplitude (Diamond et al., 1958). In the lateral eye, the ommatidial action potential required 47 min to decrease to about 30% (Kikuchi et al., 1962). Somewhat greater rates of decrease were found in the muscle spindle (10 min for the potential to decrease to 20–30%; Ottoson, 1964) and in the retina (disappearance of the electroretinogram in 9–15 min; Hamasaki, 1963). Thus, slow decreases in amplitude of the receptor potentials in Na^+-free media seem common in most receptive membranes.

The rate of decrease of the negative EOG in Na^+-free solutions is still slower than in the above cases, requiring 60 min to fall to 20–50% of the initial amplitude. It is also much slower than the rate of decrease of the positive EOG in Cl^--free media, recorded under the same conditions. These facts indicate either that the negative EOG does not result from increased Na^+ permeability, or that the Na^+ immediately overlying the receptive membrane is impeded by an ion barrier from exchanging with the surrounding fluid. The existence of such an extracellular ion barrier is indicated by the demonstration of EOG's in various animals that live in freshwater (Shibuya, 1960; Shibuya and Takagi, 1963 *a*, *b*). The olfactory epithelia of these animals are presumably exposed to freshwater and to the resultant leaching of ions from around the olfactory cells. Since apparently normal EOG's are generated, an ion barrier is likely.

The olfactory epithelium is covered by a layer of mucus about 100 μ thick, secreted by Bowman's glands and sustentacular cells. This mucus layer may be the postulated barrier to ion loss to the outside solutions. Imamura, Takeda, and Sasaki (1965) have found a layer of frog skin, apparently of mucoprotein, that accumulates Na^+ and Ca^{++}. Thus mucus apparently can serve as an ion trap or barrier. Shibuya's (1964) finding that the EOG disappears when the olfactory mucus layer is removed further demonstrates the close relation between the mucus and the potentials.

An impediment to free exchange of ions with the bathing solutions, presumably by the mucus layer, would explain the relatively slow decrease of the negative EOG in Na^+-free media. The relatively rapid decrease of the positive EOG in Cl^--free medium is not necessarily inconsistent with such a barrier. The hydrated sodium ion is 1.55 times the diameter of the hydrated chloride ion, and of course has the opposite polarity. This size and charge difference may radically affect the ability of the ions to penetrate the mucus layer. Other factors such as differential effects of Na^+-free and Cl^--free media on the resting potential may also affect the different rates of decrease.

Role of Cl^-

If the entry of Cl^- contributes to the generation of the negative EOG's, removal of Cl^- from the bathing Ringer solution should result in increase in amplitude of the negative EOG's, at least in the beginning. In Cl^--free Ringer's solution, the changes in amplitude of the negative EOG's were very irregular. In some cases, the EOG's simply decreased in amplitude (Fig. 13), while in other cases, they initially increased and then began to decrease (Fig. 5). Thus the results obtained did not provide conclusive evidence for the solution of the problem.

In the present experiments, it was shown that 1 mM Ba^{++} blocks only the Cl^- influx, but not the K^+ efflux through the membrane which produces the positive EOG. If the Cl^- should play a role in the generation of the negative EOG's, and if such an influx of the ion should be blocked by Ba^{++}, augmentation of the negative EOG's could be expected. In fact, the negative EOG's were never augmented by Ba^{++}. Consequently, the contribution of Cl^- to the generation of the negative EOG's could very probably be ruled out. The decrease in amplitude of the negative EOG's at higher concentrations of Ba^{++} (Fig. 12) indicates that the entry of Na^+ may be affected by this ion, but the efflux of K^+ is never affected.

Role of K^+

Although the role of Na^+ has been studied in a variety of receptor potentials (part 1 of the Discussion, see above) the role of K^+ has been studied only in the lateral eye of the horseshoe crab (Kikuchi et al., 1962) and in the stretch receptor cell of the crayfish (Edwards et al., 1963). Kikuchi et al. assumed that the initial increase in permeability of the receptive membrane to Na^+ is responsible for the rising phase of the ommatidial action potential and the subsequent increase in permeability to K^+ for the falling phase, although Edwards et al. could not determine the role of K^+ in the generator potential of the stretch receptor cell.

In the present experiments the contribution of K^+ to the negative EOG's was examined: (*a*) the negative EOG's reversibly decreased in amplitude in proportion to the increase of K^+ concentration in the bathing solution (Fig. 6). (*b*) Within a certain limit, however, they increased in amplitude (Fig. 9). The membrane resistance is reduced in high K^+ media, hence the generator potential decreases in other sensory neurons (Edwards et al., 1963); and the membrane potential is supposed to decrease in these high K^+ Ringer's solutions; but, nevertheless, such an increase in amplitude was observed in the present experiments. This may indicate an active role for K^+ in the negative EOG's. (*c*) The negative EOG's increased in amplitude in K^+-free Ringer's solution. (*d*) An interaction was observed between the amplitudes of the negative EOG's and that of the positive one (Fig. 10). It has been shown that the posi-

tive EOG is produced primarily by the entry of Cl^- and secondarily by the exit of K^+ (Takagi et al., 1966). The contribution of K^+ to the generation of both the positive and negative EOG's may then explain their apparent interaction. A decreased exit of the internal K^+ should increase the amplitude of the negative EOG's and simultaneously decrease that of the positive EOG, while an increased exit of the internal K^+ should elicit the opposite results. Thus the interaction of the two kinds of EOG's lends further support to the conclusion that K^+ movement contributes to generation of the negative EOG. From these four findings, it may well be concluded that K^+ contributes to the generation of the negative EOG's.

In the olfactory epithelium, K^+ as well as Na^+ appears to contribute to the negative EOG. Since there is no evidence of afterhyperpolarization following the EOG, it is likely that the Na^+ and K^+ movements are simultaneous, rather than the sequential movements inferred from afterhyperpolarization in the horseshoe crab eye (Kikuchi et al., 1962). From the experiments with Ba^{++} (Figs. 11 and 12) it seems likely that Cl^- movement does not contribute to the negative EOG. The ionic mechanism of the negative EOG thus resembles that of the muscle end plate potential, which results from simultaneous increase in permeability to Na^+ and K^+ but not to Cl^- (Takeuchi and Takeuchi, 1960).

Role of Ca^{++}

The importance of Ca^{++} in the excitable tissues is well-known (Brink, 1954; Koketsu, 1965). Nonmyelinated and myelinated axons lose excitability in Ca^{++}-free solutions (Frankenhaeuser and Hodgkin, 1957; Frankenhaeuser, 1957). The same phenomenon was found in the Purkinje fiber (Weidmann, 1955). The indispensability of this ion in the EOG's of the turtle has already been established (Tucker and Shibuya, 1965). In the present experiments, an apparently deteriorative effect of Ca^{++}-free Ringer's solution was shown in the olfactory receptor potential, just as in the excitable tissues.

Recently the role of Ca^{++} as a charge carrier was proven in the lower animals (Fatt and Ginsborg, 1958; Hagiwara and Naka, 1964) and in the heart muscle of the frog (Hagiwara and Nakajima, 1966). In the end plate potential during acetylcholine action and with low sodium media, a slight increase in the membrane permeability to Ca^{++} was suggested (Takeuchi, 1963). From the similarity in ionic mechanism of the EOG with the end plate potential, it is conceivable that Ca^{++} may play a similar role in the EOG's. However, even if the active role of Ca^{++} were proven in the negative EOG's, it would never contradict the above conclusion about the essential roles of Na^+ and K^+. The possibility that Ca^{++} acts as a charge carrier in the negative EOG will be studied in the future.

CONCLUSION

In the present experiments the ionic mechanisms of the negative EOG's were clarified, and were compared with those of various receptor potentials, electroretinograms, and muscle end plate potential. The negative EOG's resemble the receptor potentials in many respects, and they resemble most the muscle end plate potential. Although the final proof can come only from a direct comparison of the EOG with single cell activity by means of an intracellular electrode, it is highly probable that the negative EOG's are composed of the generator potentials.

However, it has been shown that an electronegative EOG is elicited by ethyl ether at low concentrations, but that it decreases in amplitude when the concentration of ether is increased, and that in extreme cases of saturated vapor, the EOG disappears, or even appears with a reversed polarity (Takagi et al., 1960). Even in the latter instances, remarkably clear induced waves appeared in the olfactory bulb as in the former cases. These paradoxical phenomena were explained by the dual action of ether: (*a*) its odor, which stimulates the olfactory receptive membrane, producing electronegative EOG's and (*b*) its anesthetic action which effects the membrane in the opposite way, producing an electropositive EOG. So far, several odorous substances have been found to have similar dual action. It is assumed, therefore, that in most cases the EOG'S are composed of negative and positive components. In the case of some odors, the electronegative component surpasses the electropositive one, thus eliciting an electronegative EOG; while with other odors, the electropositive component is predominant and elicits an electropositive EOG (Takagi et al., 1966). If such is the case, the paradoxical phenomena found in the studies on the EOG's can be explained. As for Shibuya's case against the generator potential hypothesis (1964), the dissociation of the EOG and the olfactory nerve twig discharge has already been discussed elsewhere (Takagi, 1967 *a*; Ottoson and Shepherd, 1967). One of the authors (S.F.T.) believes that the EOG decreased and disappeared because some physical conditions around the receptive membrane were altered when the olfactory mucus was removed and the consequent recording of the EOG's was not very successful. In fact, in olfactory epithelia deprived of mucus, Shibuya recorded a slow potential by means of a microelectrode, and he believes this to be a real generator potential (personal communication).

Two types of similar potentials have been recorded in single olfactory receptors of insects: one is the negative (depolarizing) potential, and the other positive (hyperpolarizing) potential. It was found that the role of the negative potential is excitation (generator potential) and that of the positive potential, inhibition (Boeckh, 1967; Boeckh, Kaissling, and Schneider, 1967). These

findings in insects also support the authors' view on the interaction between the two kinds of EOG's with opposite polarities.

In the light of the ionic mechanisms and the two opposing potentials elicited by many odorous substances, it may well be concluded that the negative EOG's are in most cases composites of the two potentials: true generator potentials, the ionic mechanism of which was studied in the present experiments; and the other potentials of opposite polarity, the origins and functions of which are now being investigated.

Addendum Hosoya and Yoshida (1937) found that the surface of the olfactory epithelium is always 1–6 mv negative with respect to the basal side of the epithelium. The origin of this standing potential is still entirely open to question. Consequently, our conclusion implying two potentials does not necessarily negate the possibility of other unknown potentials contributing to the EOG's.

The authors are indebted to Miss T. Yajima and Miss T. Tamura in the physiological laboratory of Gunma University for their collaboration during the preparation of this research paper.

The first half of this work was supported by a United States Public Health Service Grant for Information Processing in Living Systems (Grant NIH-MH08607). The latter half of the work was supported by a grant for scientific research from the Waksman Foundation of Japan and the Ministry of Education of Japan and by Air Force Office of Scientific Research Grant through DA-CRD-AG-S92-544-66-G34 of the United States Army Research and Development Group (Far East), Department of the Army.

Received for publication 12 June 1967.

REFERENCES

AI, N., and S. F. TAKAGI. 1963. The effect of ether and chloroform on the olfactory epithelium. *Japan. J. Physiol.* **13:** 454.

BOECKH, J. 1967. Inhibition and excitation of single insect olfactory receptors and their role as a primary sensory code. *Olfaction and Taste, Proc. Intern. Symp., 2nd, Tokyo.* **2:**721.

BOECKH, J., K. E. KAISSLING, and D. SCHNEIDER. 1967. Insect olfactory receptors. *Cold Spring Harbor Symp. Quant. Biol.* **30:** 263.

BRINK, F. 1954. The role of calcium ions in neural processes. *Pharmacol. Rev.* **6:** 243.

CALMA, I. 1965. Ions and the receptor potential in the muscle spindle of the frog. *J. Physiol., (London).* **177:** 31.

DESMEDT, J. E. 1953. Electrical activity and intracellular sodium concentration in frog muscle. *J. Physiol., (London).* **121:** 191.

DIAMOND, J., J. A. B. GRAY, and D. R. INMAN. 1957. Receptor potentials and Na^+ concentration. *J. Physiol., (London).* **138:** 31P.

DIAMOND, J., J. A. B. GRAY, and D. R. INMAN. 1958. The relation between receptor potential and the concentration of sodium ions. *J. Physiol., (London).* **142:** 382.

ECCLES, J. C. 1964. The Physiology of Synapses. Springer-Verlag, Berlin.

EDWARDS, C., C. A. TERZUOLO, and Y. WASHIZU. 1963. The effect of changes of the ionic environment upon an isolated crustacean sensory neuron. *J. Neurophysiol.* **26:** 948.

EGUCHI, E. 1965. Rhabdom structure and receptor potentials in single crayfish retinula cells. *J. Cellular Comp. Physiol.* **66:** 411.

FATT, P., and B. L. GINSBORG. 1958. The ionic requirements for the production of action potentials in crustacean muscle fibres. *J. Physiol., (London).* **142:** 516.

FRANKENHAEUSER, B. 1957. The effect of calcium on the myelinated nerve fiber. *J. Physiol., (London).* **137:** 245.

FRANKENHAEUSER, B., and A. L. HODGKIN. 1957. The action of calcium on the electrical properties of squid axons. *J. Physiol., (London).* **137:** 218.

FURUKAWA, T., and I. HANAWA. 1955. Effects of some common cations on electroretinogram of the toad. *Japan. J. Physiol.* **5:** 289.

GALLEGO, A., and R. LORENTE DE NÓ. 1947. On the effect of several monovalent ions upon frog nerve. *J. Cellular and Comp. Physiol.* **29:** 189.

GESTELAND, R. C. 1964. Initial events of the electro-olfactogram. *Ann. N. Y. Acad. Sci.* **116:** 440.

GESTELAND, R. C., J. Y. LETTVIN, and W. H. PITTS. 1965. Chemical transmission in the nose of the frog. *J. Physiol., (London).* **181:** 525.

HAGIWARA, S., and K. NAKA. 1964. The initiation of spike potential in barnacle muscle fibers under low intracellular Ca^{++}. *J. Gen. Physiol.* **48:** 141.

HAGIWARA, S., and S. NAKAJIMA. 1966. Effects of the intracellular ion concentration upon the excitability of the muscle fiber membrane of a barnacle. *J. Gen. Physiol.* **49:** 807.

HAMASAKI, D. I. 1963. The effect of sodium ion concentration on the electroretinogram of the isolated retina of the frog. *J. Physiol.*, **167:** 156.

HIGASHINO, S., and S. F. TAKAGI. 1964. The effect of electrotonus on the olfactory epithelium. *J. Gen. Physiol.* **48:** 323.

HIGASHINO, S., S. F. TAKAGI, and M. YAJIMA. 1961. The olfactory stimulating effectiveness of homologous series of substances studied in the frog. *Japan. J. Physiol.* **11:** 530.

HODGKIN, A. L. 1951. The ionic basis of electrical activity in nerve and muscle. *Biol. Rev.* **26:** 339.

HOSOYA, Y., and H. YOSHIDA. 1937. Ueber die bioelektrische Erscheinungen an der Riechschleimhaut. *Japan. J. Med. Sci. III. Biophysics.* **5:** 22.

HUXLEY, A. F., and R. STÄMPFLI. 1950. Direkte Bestimmung des Membranpotentials der markhaltigen Nervenfaser in Ruhe und Erregung. *Helv. Physiol. Pharmacol. Acta.* **8:** 107.

HUXLEY, A. F., and R. STÄMPFLI. 1951. Effect of potassium and sodium on resting and action potentials of single myelinated nerve fibres. *J. Physiol., (London).* **112:** 496.

IMAMURA, A., H. TAKEDA, and N. SASAKI. 1965. The accumulation of sodium and calcium in a specific layer of frog skin. *J. Cellular and Comp. Physiol.* **66:** 221.

KIKUCHI, R., K. NAITO, and I. TANAKA. 1962. Effect of sodium and potassium ions on the electrical activity of single cells in the lateral eye of the horseshoe crab. *J. Physiol., (London).* **161:** 319.

KOKETSU, K. 1961. Mechanism of active depolarization. Dispensability of sodium. *In* Biophysics of Physiological and Pharmacological Actions. (A. M. Shanes, editor). American Association for the Advancement of Science, Washington, D. C. 145.

KOKETSU, K. 1965. The role of calcium in membrane excitation. Proceedings 23rd International Congress Physiological Sciences, Tokyo. 521.

KOKETSU, K., J. CERF, and S. NISHI. 1959. Further observations on electrical activity of frog spinal ganglion cells in sodium-free solutions. *J. Neurophysiol.* **22:** 693.

MORITA, H. 1967. Effects of inorganic salts on the sugar receptor of the fleshfly. *Olfaction and Taste, Proc. Intern. Symp., 2nd, Tokyo.* **2:**787.

MORITA, H., T. HIDAKA, and A. SHIRAISHI. 1966. Excitatory and inhibitory effects of salts on the sugar receptor of the fleshfly. *Mem. Fac. Sci., Kyushu Univ., Ser. E.* Biology. **4:** 123.

MOULTON, D. G., and L. M. BEIDLER. 1967. Structure and function in the peripheral olfactory system. *Physiol. Rev.* **47:** 1.

MULLINS, L. J., and K. NODA. 1964. The influence of sodium-free solutions on the membrane potential of frog muscle fibers. *J. Gen. Physiol.* **47:** 117.

NASTUK, W. L., and A. L. HODGKIN. 1950. The electrical activity of single muscle fibres. *J. Cellular and Comp. Physiol.* **35:** 39.

OTTOSON, D. 1954. Sustained potentials evoked by olfactory stimulation. *Acta Physiol. Scand.* **32:** 384.

OTTOSON, D. 1956. Analysis of the electrical activity of the olfactory epithelium. *Acta Physiol. Scand.* **35** (Suppl. 122): 1.

OTTOSON, D. 1963 *a*. Generation and transmission of signals in the olfactory system. *Olfaction and Taste, Proc. Intern. Symp., 1st, Stockholm.* **1:**35.

OTTOSON, D. 1963 *b*. Activity of muscle spindle and sodium concentration. *Acta Physiol. Scand.* **59:** 118.

OTTOSON, D. 1964. The effect of sodium deficiency on the response of the isolated muscle spindle. *J. Physiol., (London).* **171:** 109.

OTTOSON, D., and G. M. SHEPHERD. 1967. Experiments and concepts in olfactory physiology. *In* Progress in Brain Research. Y. Zotterman, editor. Amsterdam, Elsevier Publishing Co. **23:**83.

OVERTON, E. 1902. Beiträge zur allgemeinen Muskel- und Nervenphysiologie. *Arch. Ges. Physiol.* **92:** 346.

SHIBUYA, T. 1960. The electrical responses of the olfactory epithelium of some fishes. *Japan. J. Physiol.* **10:** 317.

SHIBUYA, T. 1964. Dissociation of olfactory neural response and mucosal potential. *Science.* **143:** 1338.

SHIBUYA, T., and S. F. TAKAGI. 1963 *a*. A note on the on- and off-responses observed in the olfactory epithelium of a newt. *Gunma J. Med. Sci.* **11:** 63.

SHIBUYA, T., and S. F. TAKAGI. 1963 *b*. Electrical response and growth of olfactory cilia of the olfactory epithelium of the newt in water and on land. *J. Gen. Physiol.* **47:** 71.

STIEVE, H. 1965. Interpretation of the generator potential in terms of ionic processes. *Cold Spring Harbor Symp. Quant. Biol.* **30:** 451.

TAKAGI, S. F. 1967 *a*. Are EOG's generator potentials? *Olfaction and Taste, Proc. Intern. Symp., 2nd, Tokyo.* **2:** 167.

TAKAGI, S. F. 1967 *b*. Ionic basis of olfactory receptor activity. International Symposium on Theories of Odor and Odor Measurement Organized by NATO. Robert College, Istanbul, Turkey. in press.

TAKAGI, S. F., and T. SHIBUYA. 1959. 'On'- and 'off'-responses of the olfactory epithelium. *Nature.* **184:** 60.

TAKAGI, S. F., and T. SHIBUYA. 1960 *a*. The 'on' and 'off' responses observed in the lower olfactory pathway. *Japan. J. Physiol.* **10:** 99.

TAKAGI, S. F., and T. SHIBUYA. 1960 *b*. The electrical activity of the olfactory epithelium studied with micro- and macro-electrodes. *Japan. J. Physiol.* **10:** 385.

TAKAGI, S. F., and T. SHIBUYA. 1960 *c*. Electrical activity of lower olfactory nervous system of toad. *In* Electrical Activity of Single Cells. (Y. Katsuki, editor). Igaku-Shoin, Tokyo. 1.

TAKAGI, S. F., T. SHIBUYA, S. HIGASHINO, and T. ARAI. 1960. The stimulative and anaesthetic actions of ether on the olfactory epithelium of the frog and the toad. *Japan. J. Physiol.* **10:** 571.

TAKAGI, S. F., and G. A. WYSE. 1965. Ionic mechanisms of olfactory receptor potentials. Proceedings 23rd International Congress Physiological Sciences, Tokyo. 379.

TAKAGI, S. F., G. A. WYSE, and T. YAJIMA. 1966. Anion permeability of the olfactory receptive membrane. *J. Gen. Physiol.* **50:** 473.

TAKAGI, S. F., and T. YAJIMA. 1964. Electrical responses to odours of degenerating olfactory epithelium. *Nature.* **202:** 1220.

TAKAGI, S. F., and T. YAJIMA. 1965. Electrical activity and histological change of the degenerating olfactory epithelium. *J. Gen. Physiol.* **48:** 559.

TAKEUCHI, A., and TAKEUCHI, N. 1960. On the permeability of end-plate membrane during the action of transmitter. *J. Physiol., (London).* **154:** 52.

TAKEUCHI, N. 1963. Effects of calcium on the conductance change of the end-plate membrane during the action of transmitter. *J. Physiol., (London).* **167:** 141.

TASAKI, I., I. SINGER, and T. TAKENAKA. 1965. Effect of internal and ionic environment on excitability of squid giant axon. A macromolecular approach. *J. Gen. Physiol.* **48:** 1095

TUCKER, D., and T. SHIBUYA. 1965. A physiologic and pharmacologic study of olfactory receptors. *Cold Spring Harbor Symp. Quant. Biol.* **30:** 207.

WEIDMANN, S. 1955. Effects of calcium ions and local anaesthetics on electrical properties of Purkinje fibres. *J. Physiol., (London).* **129:** 568.