J. Physiol. (1952) 116, 137–157

THE MICROPHONIC ACTIVITY OF THE LATERAL LINE

By RENSKE JIELOF, A. SPOOR and Hl. de VRIES

From the Natuurkundig Laboratorium der Rijks Universiteit,
Westersingel 34, Groningen, Netherlands

(*Received* 27 *June* 1951)

One of the present authors (de Vries, 1948*a*) found three years ago that electrical voltages could be recorded from the lateral lines of fishes when these organs were excited by water currents. This electrical activity is not a nerve response but is identical with the well-known microphonic activity of the cochlea ('Wever and Bray effect') and the other labyrinth organs.

The study of the response of the lateral lines is not merely an extension of the important work that has been done on the cochlea. For obvious mechanical and anatomical reasons the lateral line organs are more suited for investigations of the microphonic activity than the labyrinth organs are; the ontogenetical relation between all these organs implies that the fundamental properties found from the study of one special organ will also be true for the other organs. With respect to this a fundamental problem is the way in which the mechanical stimulus finally gives rise to the excitation of the sense cells.

In some previous papers de Vries (1948*a*, *b*, *c*) suggested that the microphonic activity of the labyrinth organs had a functional meaning. The mechanical energy was supposed to be transformed into electrical energy which could be concentrated on the sense cells in an efficient way. As an extension of the theory it was suggested that this transformation could take place in the tectorial membrane (or in the cupula). Anticipating the results of the present study of the lateral line we might say here that we have disproved this extension. The voltages are not generated by piezo-electric properties of the cupula, but they are related in some way to the stretching of the hairs on the sense cells. The present work also gave information about the functional significance of the microphonic activity.

Anatomical and histological description

The first descriptions of the lateral-line organs were given by Leydig (1850, 1851) and by Schulze (1861, 1870), according to a survey by Dijkgraaf (1933). One lateral-line organ consists of a group of sense cells, generally called hair cells, which are covered together by a jelly-like cap, the cupula (see Fig. 1). Sometimes the cupula projects free into the water, sometimes a proportion of the organs are in a canal covered by the skin. In both cases water movements give rise to forces acting on the cupula and these cause excitation of the sense cells in some way or other.

The lateral lines on the head of the ruff (*Acerina cernua*) are relatively large and therefore are most suited for our work.* One finds about forty organs on the head and gill covers, but only four on the top of the head could be used. It is shown in Fig. 1 that the organs are of the type that are placed in canals covered by the skin. Moreover, they are protected by bony 'bridges' which have been removed in the left canal of Fig. 1*c*. Fig. 1*b* shows that the cupula fits rather closely in the canal.

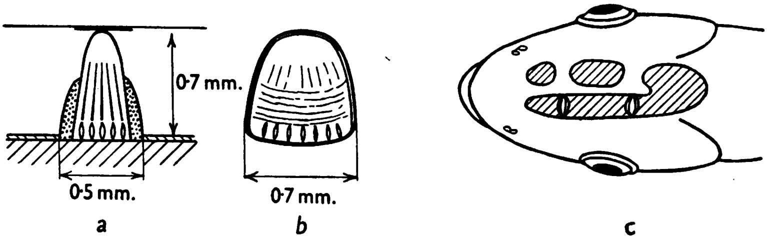

Fig. 1. Schematic drawing of the head of a ruff and one sense organ. *a*, vertical section, direction head-tail. The hairs of the sense cells seem to project into the striations. *b*, vertical section perpendicular to the plane of *a*, showing horizontal striations in the wall of the cupula. *c*, head of the ruff. The skin has been removed from the canals. From the left canal the bony bridges above the cupula have also been removed. The canals communicate just before they go downward along the edges of the gill covers.

Histological description. The cupula is a transparent, nearly invisible, jelly-like structure. In the literature (see Dijkgraaf, 1933) we did not find much information about its histology. We therefore tried to apply the methods that were also used by one of the present authors (de Vries, 1949) on the tectorial membrane of the cochlea.

Frozen sections in several directions were made from organs embedded in gelatine. Some of the material was fresh and some was fixed in formalin or Bouin's solution. The striations which are visible in the fresh cupula were not found in the sections. On the contrary, this technique, which gave very good results for the tectorial membrane, gave only a micellar structure in the cupula which was hardly different from that of the surrounding gelatine. The projections of the sense cells which are generally called 'hairs' were little lumps of material. We have not observed them in the living state, where Schulze described them as short hairs about 14 μ. long.

Double refraction. We found that the cupula showed *negative* double refraction with respect to the striations. This means that if light propagates itself

---

* We have to thank Prof. Dr S. Dijkgraaf for drawing our attention to this fish.

perpendicular to the cupula, the component which is polarized in the direction of the striations is faster than the other component. Generally the second component is faster in biological structures (positive double refraction).

The wall which surrounds the cupula of the ruff (see Fig. 1) shows a less pronounced fibrous structure. It can be removed from the cupula and was found to show positive double refraction. Obviously this wall is different from the cupula proper and we believe it to be only a supporting tissue. Indeed, removal of this wall had no influence on the electrical effects (see p. 145 below).

Specific gravity. The specific gravity of a cupula was determined from the velocity at which it settles in water. We found $\rho = 1{\cdot}01$ with a possible error of 0·002. We tried to obtain a more accurate result from its settling velocity in a salt solution with a density of 1·01, but after some seconds the cupula began to fall in this solution with the same velocity as in pure water. Evidently the diffusion of salt into the cupula, at least when it has been removed from the fish, is nearly complete in a few seconds.

APPARATUS AND METHODS

Stimulating devices. We have studied only periodic motions of the cupulae, which were brought about by water-currents. The final measurements were made with the apparatus shown schematically in Fig. 2. Since it proved to be essential to have very pure sinusoidal movements the piston and the cylinder were attached to a large tuning fork. The amplitude was determined from the voltage of the system M_2 (see Fig. 2). The relation between amplitude and voltage was calibrated by measuring the electrical output for one frequency and one amplitude of the piston. This amplitude could be determined by a microscope directly. It never exceeded about 0·1 mm.

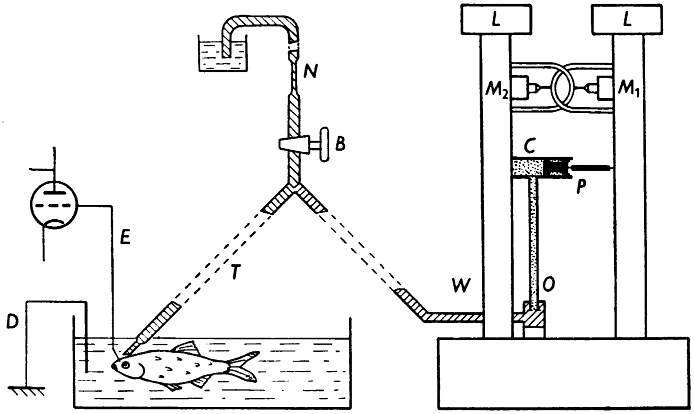

Fig. 2. Stimulating arrangements. L: lead blocks on the tuning fork, by which the frequency can be varied in steps from 12·5 up to 48 cyc./sec. M_1 and M_2: magnets with moving coils from an electrodynamic loudspeaker. M_1, driving coil; M_2, pick up coil for measurement of amplitude and phase. C and P: cylinder and piston (width 6 mm.). O and W: oil and water, separated by a thin membrane. B: stopcock. When B is opened, a constant current of water flows over the cupula. The velocity of the current depends on the width of the narrow tube N. T: glass tube; width 10 mm., narrow end 4 mm. D and E: ground electrode and pick up electrode.

The voltage of M_2 was also used for the phase measurements, since the voltage induced in the coil is in phase with the *velocity* of the tuning fork. This means that the voltage goes through zero when the tuning fork is in one of its extreme positions, etc. We have studied possible sources of phase-shifts between the motion of the tuning fork and the water close to the fish (air bubbles, clearance of the piston in the cylinder, etc.). Finally it was checked by stroboscopic illumination (see below) that the water at the outlet of tube T moved in phase with the tuning fork.

Stroboscopic illumination. For inspection of the motion of the water and especially of the cupula, stroboscopic illumination was used. A block diagram of this apparatus is given in Fig. 3. It is shown there that *one* a.c. generator feeds all the devices used, so that all parts have the same frequency and a constant phase-relation. The neon flash-lamp gives one short flash of light in each period of the vibration so that the observer sees the moving system always in the same position (and apparently at rest). He can adjust the moment of the flash, however, by turning the knob of the phase-rotator. For each revolution the system observed seems to perform one vibration. The peak on the sine-wave generated by the coil M_2 (see Fig. 3) gives the moment at which the light-flash appears, relative to the motion of the tuning fork. Rotation of the phase control makes this peak 'ride' over the hills of the sine-wave. The position given in Fig. 3 corresponds to zero voltage in M_2 and this means maximum deviation of the tuning fork. In fact, this is observed when the tuning fork is illuminated by the neon lamp, for this adjustment of the phase of the flash. The neon tube is ring-shaped in order to fit around the objective of the microscope. In that position the field is illuminated from all sides.

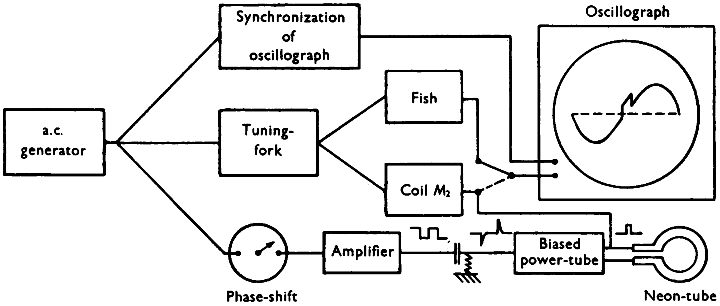

Fig. 3. Block diagram of the circuit providing for stroboscopic illumination.

Recording devices. The amplifier was a simple R–C coupled amplifier for low frequencies. It was checked by square-wave signals that the amplifier gave a constant amplification without phase-shifts at the frequencies studied. This is indicated by the fact that the curve on the oscillograph screen is a true reproduction of the input voltage.

The electrode was an enamelled copper wire (about 0·1 mm.) which was insulated except for a small point of about 0·1 mm. Since the electrodes also tend to give a phase shift at low frequencies the grid leak of the amplifier was omitted; moreover, the absence of phase shift was checked by connecting the electrode D in the tank which normally earthed the fish (Fig. 2) to the calibrating voltage. A square-wave was still reproduced without distortion.

Operative technique. The majority of the measurements were made on *free* organs, i.e. organs in canals from which the skin and bony bridges had been removed. This operation is partly illustrated by Fig. 1*c*. First the skin is cut with a knife and removed with a pair of forceps. Then the bony bridge is cut at both sides of the canal and removed. It is nearly impossible to avoid causing some damage to the organ. Sometimes the damage is invisible whereas the electrical output has nearly gone to zero. Very often the organ recovers in the course of some hours. After about 24 hr. there are only two possibilities: the cupula has disappeared or the organ gives a measurable electrical effect. Unless the fish dies, the same organ can be studied for weeks. The operations are easier when the cupula is made visible with powder of ZnO. This powder is suspended in water which is brought into the canal with a small pipette. Some of the powder settles on the cupula and adheres very well.

The fish-holder. The fish (length between 10 and 15 cm.) was tied on a glass strip with a conical tube in its mouth to prevent it from slipping out in a forward direction. Through this tube also water was supplied for the artificial respiration of the fish (about 10 ml./min.). In this way we were able to stop the respiratory movements of the fish. This was essential for the measurement of small displacements of the cupula, since these amounted only to a few microns.

The microscope. In order to avoid the influence of small ripples on the water, the amplitude of the vibrating cupula was determined with the objective immersed in the water. The objective should not interfere with the water-currents, and at least the interference should be the same when the objective is focused on the top of the cupula or on its base. This was checked by the fact that the amplitude and the phase of the output of the organ remained the same when the microscope was focused on different points. The magnification was about 250.

RESULTS

Electrical measurements

For the present work more than 300 fishes have been used but, of course, most of the early measurements only indicated that the apparatus had to be better adapted to the problem.

In the first measurements spurious electrical effects originated from various diffusion potentials, especially at the outlet of tube T, at the electrode E and at wounded regions of the fish, e.g. when the cupula or even the whole organ had been removed. These spurious voltages have the same frequency as the water-current; therefore they are easily distinguished from the microphonic potentials which have, for the lateral line, the double frequency. Moreover, they are well below 1 μV. when suitable precautions have been taken.

Unless stated otherwise, all measurements were made on free organs, i.e. organs from which the skin and the bony bridge had been removed. The output from organs under normal conditions is generally larger, on the average about 3 times. This is partly caused by the organ being damaged by the operation (see p. 140 above). The electrical situation after the operation, however, is also less favourable than before. The skin shields the canal from the surrounding water which is grounded. The shielding action is demonstrated by the fact that no electrical changes can be detected on the skin. The electrode has to be brought *into* the canal through a small hole. Furthermore, the normal situation is more effective from a purely mechanical point of view.

Amplitude of electrical effects. The output depends on the distance of the electrode from the centre of the epithelium; it decreases with increasing distance. At about 1 mm. from this centre (and in all directions) the output is still at least one half of its value very close to the organ at the base of the cupula. This fairly large spread of the output of one organ makes it necessary to destroy the adjacent organs if the organ studied has a small output. In the normal measurements the electrode was always close to the cupula.

We have measured the output for a large number of organs for various frequencies and amplitudes of the water-currents. Finally it turned out, however, that the results were not very consistent and that the relations became more simple when the amplitude of the cupula was used as the variable and not the amplitude of the water-currents. There is a relation between these two, but this relation proved to be more complicated than we expected at first (see p. 149 below). The sets of results represented in Fig. 4 were obtained from fishes where we also measured the amplitudes of the cupula.

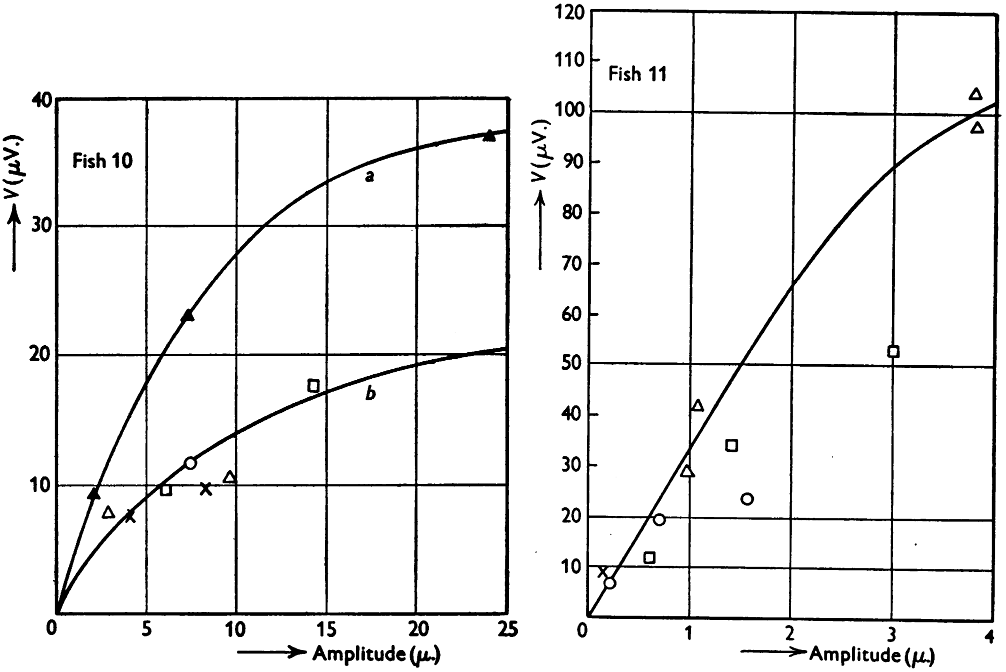

Fig. 4. Output of some organs for various frequencies and amplitudes. Note the different scales for fishes 10 and 11. V = effective (r.m.s.) voltage. △, frequency 48 cyc./sec.; □, 37 cyc./sec.; ○, 21 cyc./sec.; ×, 14 cyc./sec. Curves *a* and *b* correspond to the first and the second day respectively (see text).

Effect of conductivity of surrounding water. The majority of the measurements were made in water from the mains (specific resistance 1400Ω. cm.). Filling the tank with distilled water or saline solutions proved to have a reversible effect on the output measured. The effect did not depend on frequency or amplitude. Moreover, wave-form and phase were independent of the conductivity of the surrounding fluid. In distilled water the voltages were found to increase by a factor of about 3; in a 0·5% NaCl solution (specific resistance 95Ω. cm.) the voltages decreased by a factor of about 10.

Phase and wave-form. The most remarkable feature of the electrical response is its wave-form. It is not exactly sinusoidal, and its frequency is twice the frequency of the water-current at all amplitudes and all frequencies (14 cyc./sec. up to 600 cyc./sec.). The high frequencies were obtained from tuning forks which were pressed against the tank. The same wave-form was also obtained from four cupulae on the head behind the four which were generally studied, and from two smaller organs in front of these ones.

The phase relations and the wave-forms do not depend on the position of the electrode. For the phase, this fact is most obvious when the water movements are not exactly sinusoidal, so that the two peaks of the double wave are different. This makes it possible to recognize each peak, and even then the same wave-form is found on all sides of the cupula.

Phase relations between the electrical output and the velocity of the water-current (or tuning fork) can be found from Fig. 5 (curves 1–4). More important, however, is the relation between the microphonic activity and the motion of the cupula itself. In order to determine this we have to make two measurements: the phase of the microphonic activity relative to the tuning fork, and (afterwards) the phase of the cupula relative to the tuning fork. This last is always found from the output of coil M_2 in Fig. 2. We always checked that the phase relations did not change during the experiment. In a few cases they did. As a typical example we have given here fish 10. On the second day the cupula was very soft. Its mechanical phase was about 60° behind the phase on the day before. It was interesting, however, that the phase of the electrical output had changed in the same way. This meant that the relation between the microphonic activity and the motion of the cupula had not altered. In the course of the second day even this relation changed a little.

Some results are summarized in Table 1. A phase difference zero means that the negative peaks coincide with the maximal deviations of the cupula. At the lower frequencies the negative peaks come earlier. The possible error is between 5 and 10°. It mainly arises from the error in the measurement of the mechanical vibration.

TABLE 1. Phase difference in degrees between the mechanical motion and the electrical output (see text). A phase angle α means that the negative peak of the microphonic is α° ahead of the corresponding maximal deviation of the cupula.

	Frequency (cyc./sec.)			
Fish	48	37	21	14
4	0	22	76	—
5	20	16	66	—
10	0	41	—	40
11	0	5	76	60
18	0	40	50	70

Recording from below organ. It is possible to bring the electrode under the organ by making a small hole in the tissue which covers the bottom of the canal. One finds that the sign of the voltage reverses when the electrode is pushed through the hole (Fig. 5, curves 5 and 6). Apart from the reversal of sign, the shape of the curve remains the same. The voltages measured here are about one-half of the voltages at the other side, maximum output being found just under the organ.

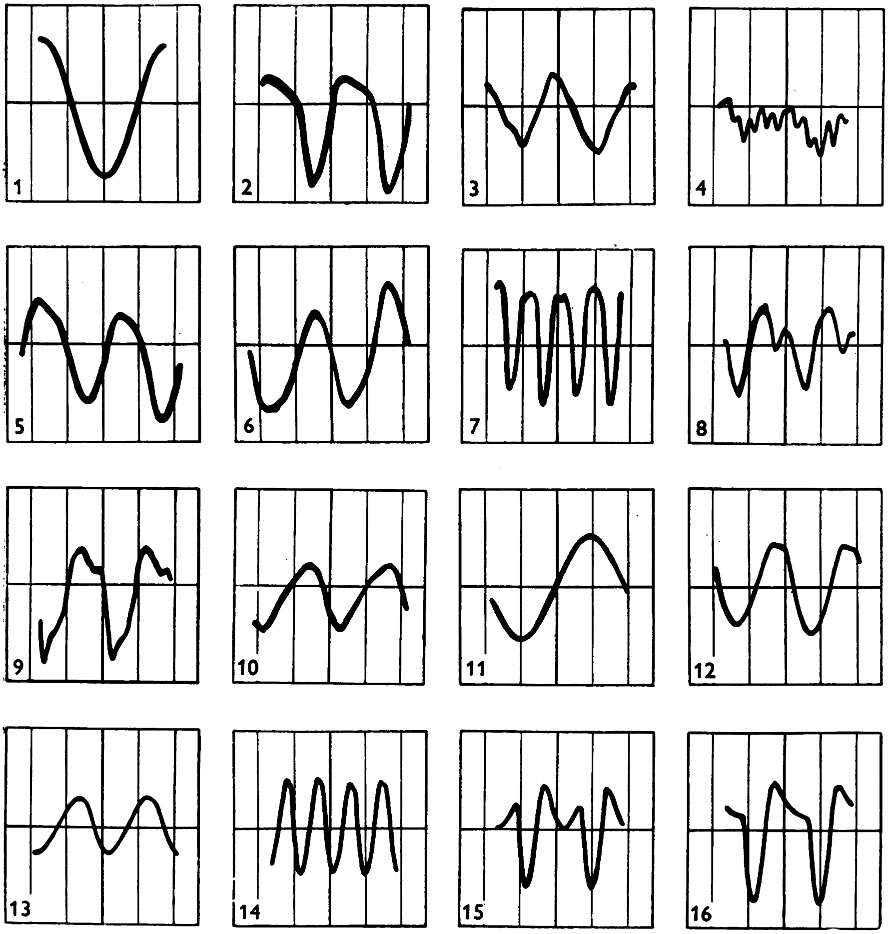

Fig. 5. Oscillograms. Negative voltage corresponds to downward deviation. The ruff studied here was not used in other experiments. Curves 1–6 and 10–13: frequency of time base equal to the frequency of the input (in general the tuning fork). Curve 1 represents the velocity of the water-current; curves 2–4 output of the organ at frequencies of 48, 21 and 14 cyc./sec. Curves 5 and 6: 48 cyc./sec. In curve 6 the electrode under the organ, in curve 5 in the normal position. Curves 7–9: 48 cyc./sec. Time base at 24 cyc./sec. In curve 7, normal output. In curve 8, the cupula had been deformed a little and in curve 9 the displacement was still somewhat larger; one of the peaks disappears. Curve 10: microphonic activity of the sacculus of a perch. Tuning fork 128 cyc./sec. Output 256 cyc./sec. Curves 11–16: output from the equivalent circuit (see text, p. 152).

Measurements on deformed and damaged cupulae. The wave-form can be altered in a reversible way by deforming the cupula. If it is bent *forward* by a stream of water, by a fine glass wire or by the electrode itself the peak which corresponds to the *backward* displacement of the cupula disappears (see Fig. 5). The other peak disappears when the cupula is bent backward. A very large displacement suppresses the whole microphonic activity. The constant displacement which is necessary to suppress one peak depends on the amplitude of the vibration. Thus we found for fish 6 at 48 cyc./sec. that displacements of 16, 9 and 6 μ. were necessary for amplitudes of 3, 2 and 1 μ. respectively. For all organs investigated in this way, the constant deviation had to be larger than the amplitude of the vibration. The wave-form remains the same for all positions of the electrode.

We have also tried to bring the electrode into the cupula close to the epithelium. Even this had no influence on the wave-form, at least when the electrode did not displace the cupula.

Removal of the 'wall' around the cupula (see Fig. 1) did not affect the electrical output.

More important were some measurements in which part of the cupula was removed. This was done with a red-hot platinum wire or by cutting it with a small knife. Especially the latter procedure generally damaged the whole organ so that we had to wait some days until it had recovered. We could remove the upper part until at most one-third of the cupula was left. Even then the same wave-form and phase were obtained. Generally the amplitude came back to the original value after some days.

Effect of temperature. Measurements were made at temperatures varying from 1° up to 26° C. (temperature of the water close to the cupula). No change of magnitude or wave-form of the electrical output was found. This is true for nearly maximal stimulation as well as at lower intensities.

Mechanical measurements

Elastic properties of the cupula. It was found that the force which had to be applied close to the top of the cupula in order to give a displacement of 100 μ. varied from about 2·5 to 10 dynes for different cupulae. The force was determined from the bending of a copper wire (0·13 mm. thickness, about 10 cm. length) which was pressed against the cupula. These figures refer to organs which were in a good condition. A cupula which does not give much electrical output is generally very soft. This is generally the beginning of complete destruction of the organ.

Displacement of cupula by constant water-currents. In several series of experiments the displacement of the cupula by a constant water-current was determined (Fig. 6). The position of the glass tube T (Fig. 2) was the same as for alternating currents.

Especially for the larger velocities the cupula 'creeps' still somewhat farther in the course of some seconds after the immediate initial displacement. Generally this creeping is more pronounced for soft cupulae (see fish 5, Fig. 6). The displacement of the base of the cupula is between about 0·25 and 0·40 times the displacement of the top. Evidently there is a bending as well as a displacement of the cupula as a whole.

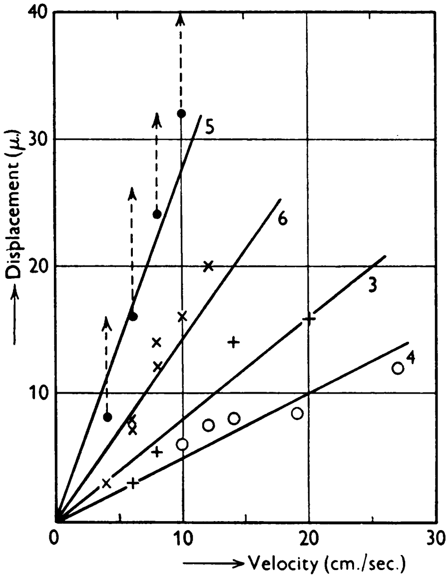

Fig. 6. Displacement of the top of the cupula for different (constant) water-currents. For fish 5 the heads of the arrows give the displacements after a few seconds.

The mobility of the base of the cupula is very obvious when the cupula is moved by a thin wire which is pressed against its base. It is very interesting to see how the cupula moves and even rotates a little (about a vertical axis) over the bottom of the canal as if it were free, while in fact it is fixed to the bottom at least by the wall.

Displacement of cupula by alternating water-currents. The amplitude was determined by making use of stroboscopic illumination, as described under Methods. Within the limits of error the amplitude of the cupula proved to be proportional to the velocity of the water. Therefore we have given in Table 2 the amplitude for a velocity of 1 cm./sec. This is the mean velocity of the water at the outlet of the tube *T*, at the moment that the sinusoidal current has its maximum velocity. The actual displacements of the cupula can be found from Fig. 4. Amplitudes below 1 μ. in Fig. 4 and Table 2 were not measured directly, but were calculated from the displacement in faster currents, assuming proportionality between the amplitude of the cupula and of the water-current (see above).

These data refer to the amplitude of the top of the cupula. The amplitude of the base is between 0·3 and 0·5 times the amplitude of the top. The distance between points on the cupula which moved and points on the bottom of the canal which were at rest was very small.

TABLE 2. Amplitude of the top of the cupula for a velocity amplitude of the periodic water-current of 1 cm./sec. Fish 10*a*: first day. Fish 10*b*: second day. See also Fig. 4. Amplitudes expressed in microns (0·001 mm.)

	Frequency (cyc./sec.)			
Fish	48	37	21	14
4	1·4	1·2	0·5	—
5	2·5	1·4	0·5	—
10*a*	2·0	—	—	—
10*b*	3·5	3·0	1·2	0·8
11	0·32	0·30	0·1	0·075
18	1·3	—	0·5	0·32

Phase measurements. The phase relation between the motion of the cupula and of the tuning fork is not of direct importance. Therefore we give here only the mean result of our measurements: the phase of the displacement of the cupula is 45° in front of the velocity of the water-current. This means that the cupula is already moving backward when the water has not yet reached its maximum velocity in the direction tail-head. At 14 cyc./sec. the phase difference was somewhat smaller. The phase relation did not depend on the amplitude, and the phase at the base of the cupula was the same as at its top within the limits of error, which were between 5 and 10°.

DISCUSSION

Mechanical measurements

The present discussion is mainly meant to show the general consistency of the data obtained in the foregoing section. Various details of the calculations have been omitted since they would require too much space. Moreover, they are merely an application of the common theory of vibrating systems and of hydrodynamics. The main difficulty arises from the fact that the possible error in some of the data is fairly large.

Theory. Considering the cupula as a vibrating system, three constants are important: its mass the stiffness and the friction. The mass m is easily found from the dimensions. For the vibrations, however, we have to add the mass of part of the surrounding water. This makes the total effective mass about 0·5 mg. In general the force K which is necessary to give a displacement u is proportional to u, or $K = fu$. The factor f is called the stiffness of the cupula; its value will be calculated below from the experimental data. Finally, the *friction* is proportional to the velocity of the system. The force for a velocity of 1 cm./sec. is denoted by b.

From m, f and b the behaviour of the system can be predicted. The resonant frequency n_0 equals $(f/m)^{\frac{1}{2}}/2\pi$. For low frequencies the amplitude equals P/f, where P = amplitude of the periodic force. For frequencies close to the resonant frequency the amplitude depends only on b. One value of

b is of special interest, namely the value which gives the so-called critical damping. A critically damped oscillator returns to its equilibrium position as fast as possible without overshooting it. In order to get this optimal damping b should equal $2\sqrt{(fm)}$. The critically damped oscillator has a flat response curve to periodic forces, i.e. there is no sharp and high resonance peak but the amplitude at the resonance point is $P/2f$. At low frequencies the displacement is in phase with the force; close to the resonant frequency the displacement is 90° later than the force.

Stiffness of the cupula. From the definition of f, combined with the measurements given on p. 145, we find that the stiffness of the cupulae varied from 250 to 1000 dyne/cm.

Displacement by constant water-currents. The force which acts on a cupula in a water-current can be calculated approximately by considering it as one-half of a sphere. From the well-known law of Stokes one finds that the force K equals $7 \times 10^{-3}\, v$ dynes, where v is the velocity of the water in cm./sec. For large velocities Stokes's law no longer holds, but a further analysis shows that it is still a good approximation at the velocities used in our experiments.

The displacement caused by the force K is K/f. Assuming $f = 250$ dynes/cm. (see above) we would expect that a water-current of 1 cm./sec. would give a displacement of $0{\cdot}28\,\mu$. and, furthermore, that the displacement would be proportional to the velocity v of the water-current. Indeed we believe that the deviations from linearity in Fig. 6 are not large enough to suggest a deviation from the theory. But the mean displacement for $v = 1$ cm./sec. is $1\,\mu$. instead of $0{\cdot}28\,\mu$. We do not believe that the difference can be completely due to the possible errors in our calculation. At least part of the discrepancy must be due to the fact that K is not exactly proportional to the displacement. Now f was calculated from measurements in which the displacement was $100\,\mu$. The displacement by the water-currents was much smaller and it is quite possible that the stiffness is smaller for these deviations. For the rest of the discussion we shall assume f to be 125 dyne/cm.

Displacement by periodic water-currents. Before we can start with this discussion we have to explain one apparent discrepancy. As stated on p. 147 above, the phase of the displacement of the cupula is 45° ahead of the velocity of the water-current. Since the force is in phase with the water-current this would mean that the cupula is ahead of the force. This is impossible, however, for any vibrating system; it can only lag behind in phase. This occurs at higher frequencies (see the theory). We have been able to remove this difficulty by considering the water-current in more detail. For the motion of fluid between parallel plates we could solve the problem rigorously; here we found a phase shift of 45° between the current at the plates and farther in the fluid. These calculations will be published in more detail elsewhere, since they are also of interest from the hydrodynamical point of view. The motion of the tuning fork gives us the phase of the main part of the water-current. The cupula, however, is driven by the thin layer close to the surface and the phase of this layer is about 45° ahead of the phase of the rest of the water. As the frequency is reduced this phase shift approaches zero at a few cycles per second, depending on the dimensions, etc.

Inserting the values of f and m found above in the formula for the resonance frequency we find 80 cyc./sec. as an average value. Evidently this frequency is well above the highest frequency studied (48 cyc./sec.). Indeed the majority of the cupulae were still 45° ahead of the water-current even at 48 cyc./sec. Only the soft cupula of fish 10 (see p. 143 and Table 2) was later; this indicates that its resonance frequency was close to 48 cyc./sec.

Finally, we consider the amplitude of the cupula. Comparing Fig. 6 and Table 2 we find that at 14 cyc./sec. the amplitude is nearly equal to the displacement in the same constant current. Perhaps the latter is somewhat larger, as a result of the creeping of the cupula (p. 146). With increasing frequency the amplitude increases appreciably. Even if the damping of the cupula were zero the increase would be only a factor 1·6 (from 14 to 48 cyc./sec.) for a natural frequency of 80 cyc./sec. In Table 2, however, one finds an increase by a factor of about 4. This discrepancy can be removed as follows. The calculations of the distribution of the water-current mentioned above predict that between parallel plates the velocity amplitudes close to the boundaries increase proportionally to the square root of the frequency for a constant velocity amplitude of the tuning fork. The real situation is more complicated and therefore we measured directly the amplitude of the water-current in the canal by observing small suspended particles. The velocity amplitude increased by a factor of 2·6, whereas the theory mentioned above predicted a factor of 1·85. Since the force acting on the cupula will be proportional to the velocity of the water close to it, of the factor 4 mentioned above only $4/2{\cdot}6=1{\cdot}5$ is really due to the cupula itself.

The number of steps involved in the calculation of this factor 1·5 is too large to base a calculation of the damping constant b upon it. We only wish to show that the cupula behaves as a simple (physical) system. The damping of the cupula cannot have been much less than critical, since even fish 10 (Table 2), which had a natural frequency close to 48 cyc./sec., gave the same increase of amplitude as the other fishes. From the formula given in the theory one finds for critical damping $b=0{\cdot}5$ c.g.s. unit. So we shall assume that $b=0{\cdot}3$ for the cupula. An indication that it will not be very much lower can be obtained from the fact that for the sacculus otoliths of the ruff (see de Vries, 1950) we found $b=10$ c.g.s. units. This otolith was also nearly critically damped.

Electrical measurements

The most interesting property of the electrical output is its double frequency. We have seen that the frequency of the vibration of the cupula was equal to the frequency of the water-current. Therefore, the double frequency of the electrical response is a property of the organ itself. We recorded similar double curves from the sacculus of a perch (*Perca fluviatilis*) (see Fig. 5). Measurements of this microphonic effect of the sacculus have already been made by Zotterman (1943). Though he does not discuss the frequency of the recorded potentials it is evident from the curves given in his article that the second harmonic was very strong. Indeed, in our measurements, the wave-form depended on whether the otolith was displaced already by the electrode or not; we could suppress more or less completely one of the two waves by the orientation of the electrode.

The measurements on the deformed cupula suggest that the double wave should be interpreted as two negative (downward) peaks. One of them corresponds to a forward and the other to a backward displacement. This interpretation was also checked by some preliminary observations on the response to clicks, produced by tapping at the walls of the tank. Whatever the direction of the click, the response always began with a negative peak. Since the microphonic activity did not depend on the temperature it is very improbable that these negative peaks are due to a nerve response. We will discuss these problems in more detail on pp. 152–5, together with the phase relations.

The relation between the *amplitude* of the vibration and the *electrical output* is demonstrated in Fig. 4. One finds that for a certain amplitude nearly the same voltage is generated whatever the frequency. Since a complete set of measurements takes a long time, the output sometimes decreased in the course of one experiment. Generally, 37 cyc./sec. was the last frequency studied, so that the corresponding points were often too low. A complete discussion would therefore require the knowledge of all details of the experiment. Our final conclusion is that there exists a small but unimportant decrease of the output with decreasing frequency. The amplitude of the vibration decreases appreciably with decreasing frequency and this is the reason why the output at low frequencies is easily masked by irregularities of the water-current (see Fig. 5, curve 4).

For weak vibrations the output is proportional to the amplitude; for larger deviations the output finally shows a 'saturation'—it approaches a maximum value. This maximum did not depend on the frequency between 14 and 400 cyc./sec. A similar saturation is also found for the cochlea and for the other organs of the labyrinth.

Finally, it is interesting to note that this maximal output is reached for an amplitude of about 10 μ. This may seem to be a very small deformation, but probably the deviations of the cupulae in the semicircular canals are of the same order of magnitude under *normal* conditions. It follows from an analysis of subjective measurements (see Groen & Jongkees, 1948) that the cristae of the semicircular canals act normally in all respects only up to an angular velocity of the head of about 60°/sec. It can be calculated that the displacement of the cupula is then about 10 μ.

Comparison of mechanical and electrical energies

From a simple calculation one finds that the mechanical energy dissipated by the organ equals $\frac{1}{2}b\,(2\pi nu)^2$ erg/sec. Here n stands for the frequency and u for the amplitude, while b has already been defined as the frictional force for unit velocity.

The electrical energy is more difficult to estimate, since we do not know what happens in the organ itself. As stated on p. 143, the electrical effect has opposite sign above and below the organ. Therefore we can represent the organ schematically as in Fig. 7. The upper side of the internal source of e.m.f. (voltage V_i) is connected to ground by the resistance r_e. The other side is grounded by an unknown resistance r_2. The voltage at Q is somewhat smaller than what is found above the cupula (V_1) (p. 144), but it has the opposite sign. Furthermore, the 'cell' has an internal resistance r_i. Since r_2 represents mainly the resistance of the bony bottom of the canal, both r_i and r_2 will be independent of the conductivity of the water in the tank. One easily calculates that

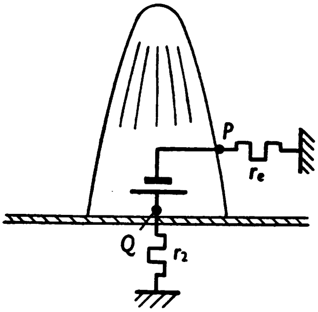

Fig. 7. Electrical circuit of a lateral-line organ (schematic).

$$V_1 = V_i r_e/(r_e + r_1),$$

where r_1 stands for $r_i + r_2$. From the influence of r_e on V_1 (p. 142) one can calculate r_e/r_1. Indeed, one obtains two equations for this ratio. The fact that both equations give the same result indicates that we are really dealing with a simple 'physical' circuit. One finds $r_1 = 2r_e$. Inserting this in the formula given above the final result is that the total electrical energy dissipated equals $3V_1^2/r_e$ joule/sec. (For the voltages the *effective* voltage was always used.) From measurements on a model we found that r_e was equal to about 30,000 Ω. In Fig. 4, fish 11, we see that V_1 equals 0·28u volt (u stands for the amplitude in cm.) for 48 cyc./sec. The quotient of mechanical energy E_m and electrical energy E_e now becomes $E_e/E_m = 0{\cdot}0055$. For fish 10 we find for the same quotient about 0·01, but fish 11 represents better the average of the fishes we have studied. At lower frequencies (and the same amplitude u) the mechanical energy decreases proportionally to n^2, whereas the decrease of V is less pronounced (p. 150). So we find that the quotient E_e/E_m equals about 0·025 for fish 11 at 14 cyc./sec.

We conclude from our calculations that the electrical energy does not exceed the mechanical energy. This is an important result, since it means that the organ need not provide any of the energy: the processes considered could be passive, i.e. simple physical processes. Because of the possible experimental errors, the proof is not complete. Moreover, we do not know the zero-line in Fig. 5 (this is a consequence of the properties of a.c. amplifiers). We have tacitly assumed the mean voltage to be zero. Assuming that the zero line goes through the upper tops of the curves—and this cannot be decided easily—the electrical energy would come out three times larger. Furthermore, there may be some short-circuiting in the organ itself, so that part of the electrical energy could have escaped from the measurements.

The cupula as the source of the voltages

It was suggested in the introduction that the microphonics might arise from piezo-electric properties of the cupula. We will prove now that this hypothesis is not compatible with the present measurements.

The main property of the microphonic activity of the lateral line which gives difficulties is its wave-form. The electrical response of the cupula would have the same frequency as the water-current] if it arose from piezo-electric properties or other simple properties. It would be possible to explain the *double* frequency, however, as follows.

If the top of the cupula is bent to the left, this part of the cupula is compressed, whereas the right-hand part is stretched. This means that voltages could be generated in the two halves which are in opposite phase. In order to attain the double frequency, a rectifying action of the epithelium has to be assumed. We then obtain what is called in electronics a full wave rectifier. Wave-forms from a circuit of this well-known type are given in Fig. 5. The resistances and capacities of this circuit were adapted to the problem. It is clear that even the flat tops appear in the model, together with the phase shift for lower frequencies (see Table 1). In order to obtain one single peak from a deformed cupula we would have to assume that the resistance of the epithelium changes after the passage of an electric current. This change of the resistances on one side is also simulated in curves 15 and 16 of Fig. 5.

We shall not go into further detail since we believe that this model is not compatible with all our observations. There are two serious objections against it.

(*a*) One must expect that close to the base of the cupula there would be some difference in wave-form between the front and the back parts since one side was compressed when the other side is stretched, and vice versa. But even when the electrode was brought into the cupula the same wave-form was obtained everywhere.

(*b*) Even when the greater part of the cupula was removed we obtained the same double wave. But such a low cupula is not bent. Its flat top is displaced *parallel* to its base. So our explanation would not apply to this case.

Therefore we abandoned the hypothesis that the cupula generates the microphonics.

The hairs of the sense cells as the source of the microphonics

The objections made above do not apply to the following explanation. Moreover, it has the advantage that it suggests more relations between the various labyrinth organs. Finally, we consider it to be of importance that the new model is very simple.

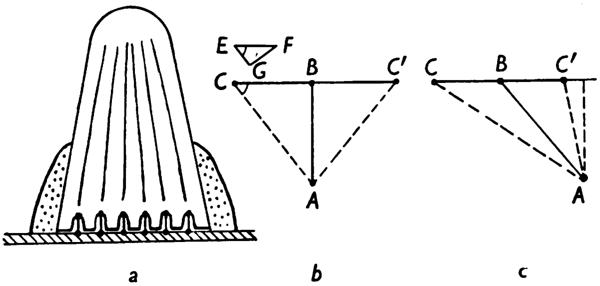

Fig. 8. Illustration of the mechanics of the hairs (see text).

Our idea is that the voltages are related to the *tension in the hairs* on the sense cells*, and that this tension is accompanied by a negative voltage at the upper side of the organ. Of course this idea is not new, but we believe that the following development is.

Fig. 8 gives a schematic drawing of the cupula with the wall and the hairs. When the cupula vibrates we saw on p. 146 that part of its motion is a gliding of the cupula over the epithelium. A vibration up and down is impossible *a priori*, since the upward motion would be accompanied by the creation of a vacuum. As a consequence of the gliding motion the hairs become longer both for displacements to the right and to the left (Fig. 8*b*). We see that AC as well as AC' is longer than AB. So we get a negative peak twice in one vibration.

This model also gives a very simple explanation of what happens when the cupula is given a constant displacement superimposed on the vibration (Fig. 8*c*). Now the hair is stretched only once in one period. When the constant displacement is larger than the amplitude of the vibration the hair is not stretched again in position C'.

As a further check we made some measurements on the effect of superposing a weak vibration with a high frequency on a vibration with low frequency (say, 480 cyc./sec. on 48 cyc./sec.). The high frequency was applied by pressing the

---

* When we speak about 'hairs' in this section we disregard all confusion which still exists here from a histological point of view.

moving system of a loudspeaker against the tank. In Fig. 8 this vibration is indicated by $EF=a$, while we shall denote the displacement by the low frequency by $u=BC$. One easily finds that the amount by which the hair is stretched is au/l, where $l=AB$ stands for the length of the hair. This means that the effect of the extra vibration is proportional to the deviation BC. This, indeed, is what we found by experiment (Fig. 9). The effect of the high frequency alone is very small and it has the double frequency. Superimposed on the other vibration its effect is very much larger in the extreme positions and it becomes very small again in the equilibrium position. The frequency of the output of this component is now equal to the frequency of the stimulus. The variation of the output is clearly demonstrated in Fig. 9, curve 4.

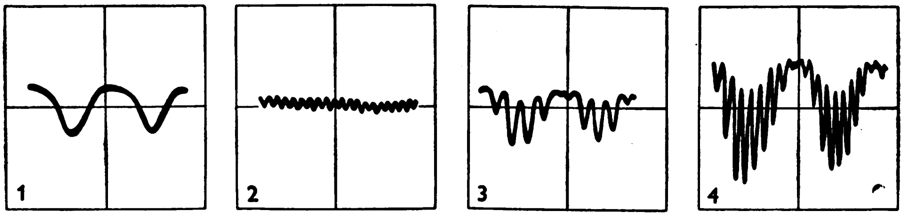

Fig. 9. Curve 1: output for 48 cyc./sec. Curve 2: 480 cyc./sec. Curve 3: these two stimuli together with the same amplitudes as in curves 1 and 2. Curve 4: 400 cyc./sec. superimposed on 20 cyc./sec. in a similar way.

Closer inspection of the data shows some complications. The constant deviation which was necessary to suppress one of the negative peaks was larger than the amplitude of the vibration (p. 145); there is a phase shift between the electrical output and the mechanical motion at low frequencies (Table 1); and finally one would expect the output for small amplitudes to go to zero faster than the first power of the amplitude (Fig. 4). We believe that these facts, together with the flat tops of the curves, can be explained by assuming that the hairs can slip, i.e. that they are not connected rigidly to the cupula. This will be the subject of further study.

Functional significance of the microphonic potential

Combining the findings of the foregoing sections with some results obtained in other investigations it is possible to say something about the functional significance of the microphonics. First we have to assume that a displacement of the cupula from the equilibrium position has an excitatory (and not an inhibitory) effect. The second assumption is that the organ can be stimulated electrically by a negative electrode which is located *above* the organ. This is a well-known fact in labyrinth physiology. One of the present authors recently checked this and with special care by stimulating the ampullae of the semicircular canals of the pike electrically. We intend to check the validity of these two assumptions for the lateral line organs studied in this article, but we can safely assume that the result will be positive.

Then we can prove first that the microphonics cannot be generated in a structure which is above the structure which can be stimulated electrically. For this 'generating organ' would have to be negative on its lower side in order to give a stimulating voltage, whereas we find it to be negative at the *upper* side where we pick up the microphonics normally. Of course we tacitly assume here that the microphonic effect is not completely meaningless. From all measurements of the microphonic activity of the labyrinth one can safely conclude that it is at least as important as the electrical phenomena are for nerve activity.

Consequently, the microphonics must be generated in the same organ which can also be stimulated electrically. This is the situation which is found in the excitation of nerves. The nerve is excited by the cathode and at the same time excitation causes negativity at the outside. In order to obtain a well-defined model (see also de Vries, 1948*a*) one can assume that the membrane between hair and sense cell is similar to the membrane which surrounds a nerve, but with a special sensitivity to mechanical excitation. The first step in the excitatory process would be a migration of (sodium) ions into the cell, thus creating the negativity one really observes.

It is very important that the model is compatible with the conditions derived from a discussion of Brownian motion (de Vries, 1948*a*, *b*, *c*). For the effect of Brownian motion at one cell would be distributed electrically over the whole epithelium so that it could not give much trouble. In the case of a displacement of the cupula all cells would act together in pumping—more or less actively—sodium ions downward. Close to the absolute threshold the energy could be concentrated on the cell with the smallest electrical resistance (see also the introduction).

Application to the cochlea

When the ear is stimulated by sound the cochlear partition bends up and down in the direction of the arrow in Fig. 10. When the tectorial membrane moves relative to the organ of Corti, the only possibility is a gliding motion. When the cochlear partition bends in the direction of the arrow, the tectorial membrane will glide over the organ of Corti to the *left*. This means that the hairs of the *outer* cells will be stretched. Since they are already in an inclined position they will be stretched only once every period. It is known that the microphonic activity has the same frequency as the incident sound.

Moreover, the sign of the voltage is predicted correctly. An outward motion of the stapes causes an upward displacement of the cochlear partition and a stretching of the hairs. Consequently (see the foregoing section), we expect the voltages indicated in Fig. 10. It is known that the round window, which is connected to the lower half of Fig. 10, becomes positive when the stapes moves outward.

Undoubtedly there is some meaning in the fact that the *inner* hair-cells are inclined in the opposite direction. They will be compressed when the outer hairs are stretched and vice versa. Up to now no special attention has been paid to this, and it would take us too far to discuss here all the possible implications of this situation. It may perhaps be important for the localization of sounds as well as for the explanation of pathological cases. It is possible that also in the lateral line organs a proportion of the hairs are in an inclined position. Then some of the cells could react to forward displacements, whereas other cells could detect displacements in the opposite direction. On the average, however, the hairs are perpendicular to the epithelium.

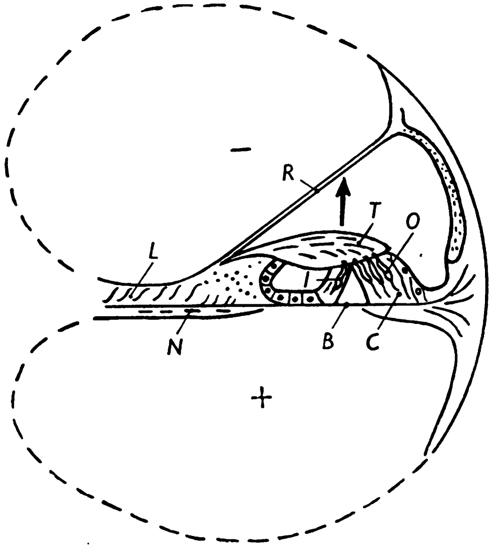

Fig. 10. Schematic drawing of the cochlea, with tectorial membrane *T*, inner and outer hair-cells (*I* and *O*) in the organ of Corti, *C*.

Finally we might point out that the arrangement of the outer cells is appropriate to protect them against damage. Even in uncivilized life the most important sources of damage are accompanied by an inward motion of the stapes. This, however, does not stretch the hairs.

At present it is not possible to say very much about the semicircular canals. We only wish to mention that Vilstrup (1950) has found—by more difficult experiments than we had to do on the lateral line—that also in the semicircular canals the cupula glides over the epithelium.

SUMMARY

1. The microphonic activity of the lateral line of fishes is similar to the microphonic activity of the labyrinth organs including the cochlea. It is emphasized, however, that the lateral lines are more suited for quantitative evaluations. After a short description of the anatomy and the histology of the organs

studied, measurements of the microphonic activity and of the mechanical properties of the motions of the cupula are given.

2. The most striking property of the electrical response is that its frequency is twice the frequency of the exciting water-current (frequencies between 14 and 600 cyc./sec.).
3. Between 14 and 48 cyc./sec. the output depends only on the amplitude of the cupula and is independent of the frequency.
4. The voltages above and below the organ are of opposite sign.
5. The electrical energy produced by the organs is less than the mechanical energy dissipated.
6. Since the voltages do not depend on the temperature (between 1 and 26° C.) it is very improbable that they are of nervous origin.
7. Other results disprove the hypothesis that they are generated in the cupula.
8. The measurements give convincing evidence that the voltages are related to the tension in the hairs on the sense cells. All details of the experiments, including the 'double frequency', can be explained by this assumption.
9. This explanation is found to be also compatible with what is known about the microphonic activity of the cochlea.
10. The measurements make it possible to give the microphonic activity its plàce in the chain of processes in the labyrinth and in the lateral line.

We have to thank Drs J. W. Kuiper for his help in the last part of the measurements, and the 'Organisatie voor Zuiver Wetenschappelijk Onderzoek' (Z.W.O.) for a grant which partly supported the present work.

Note added in proof. Recently von Békésy (1951) has studied the d.c. potentials in the cochlea. It was found that in the epithelium the potentials were about 100 mV. negative with respect to the potentials in the surrounding fluids. The smaller d.c. potentials which could be derived from the surrounding fluids decreased when the cochlea was stimulated by sound. This, and other details of that work, are in excellent agreement with the model developed above (page 155, line 10).

REFERENCES

von Békésy, G. (1951). *J. acoust. Soc. Amer.* **23**, 576.
Dijkgraaf, S. (1933). *Z. vergl. Physiol.* **20**, 161.
Groen, J. J. & Jongkees, L. B. W. (1948). *J. Laryng.* **62**, 231.
Leydig, F. (1850). *Arch. Anat. Physiol., Lpz.*, p. 170.
Leydig, F. (1851). *Arch. Anat. Physiol., Lpz.*, p. 235.
Schulze, F. E. (1861). *Arch. Anat. Physiol., Lpz.*, p. 759.
Schulze, F. E. (1870). *Arch. mikr. Anat.* **6**, 62.
Vilstrup, Th. (1950). *Ann. Otol., etc., St Louis*, **59**, 46.
de Vries, Hl. (1948*a*). *Experientia*, **4**, 205.
de Vries, Hl. (1948*b*). *Physica*, **14**, 48.
de Vries, Hl. (1948*c*). *Nature, Lond.*, **161**, 63.
de Vries, Hl. (1949). *Acta oto-laryng., Stockh.*, **37**, 334.
de Vries, Hl. (1950). *Acta oto-laryng., Stockh.*, **38**, 262.
Zotterman, Y. (1943). *J. Physiol.* **102**, 313.