One group was stripped from a frog at the same time as the enucleated eggs; the others were stripped from the same frog 13 hours later, and activated at that time. The regular control group, activated and left 13 hours, would have been early blastulas if they had been fertilized at the time of activation. In no case was protein synthesis in the enucleated eggs significantly different from that in the controls.

On the basis of these observations, we conclude that, prior to hormone stimulation, the cytoplasm of the *R. pipiens* oocyte has the capacity for independent protein synthesis. It appears that the oocyte cytoplasm retains this capacity throughout the period of pituitary-induced maturation and well beyond the time when the egg is capable of being fertilized or artificially activated. We have been unable to demonstrate any contribution of the nucleus to this protein synthetic capability; in all cases, the enucleate cytoplasm is as capable of supporting protein synthesis as the intact egg.

From this study we cannot say that the nucleus has no role in the control of synthetic events during this period. We do not know whether the enucleate and the nucleate oocytes had identical patterns of protein synthesis. Whatever function the nucleus may have, however, its presence is not essential to the continuation of protein synthesis.

There are two possible explanations for our observations: (i) Long-lived templates may be synthesized during oogenesis and function throughout the period of maturation and early cleavage. (ii) Templates of cytoplasmic origin may be synthesized *de novo* during maturation. Of course both of these mechanisms could function concomitantly. However, Davidson *et al.* (*1*) calculated that, in *Xenopus*, the template RNA synthesized during hormone-induced maturation (*2*) is only a small fraction of the amount already present in the full-grown ovarian oocyte. If this is generally true for amphibians, it is likely that the capacity for protein synthesis we observed in enucleate oocytes is due to the presence, in the cytoplasm, of long-lived templates synthesized and accumulated during oogenesis.

R. E. ECKER
L. D. SMITH
Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois
S. SUBTELNY
Department of Zoology, University of Iowa, Iowa City

References and Notes

1. E. H. Davidson, M. Crippa, F. R. Kramer, A. E. Mirsky, *Proc. Nat. Acad. Sci. U.S.* **56**, 856 (1966).
2. D. D. Brown, in *Developmental and Metabolic Control Mechanisms and Neoplasia* (Williams and Wilkins, Baltimore, 1965), p. 219.
3. V. Glisin, *J. Exp. Zool.* **157**, 115 (1964).
4. R. Briggs, E. Green, T. J. King, *ibid.* **116**, 455 (1951); J. A. Moore, *Exp. Cell Res. Suppl.* **6**, 179 (1958); R. Briggs, J. Signoret, R. R. Humphrey, *Develop. Biol.* **10**, 233 (1964).
5. L. D. Smith and R. E. Ecker, *Science* **150**, 777 (1965).
6. L. D. Smith, R. E. Ecker, and S. Subtelny, *Proc. Nat. Acad. Sci. U.S.* **56**, 1724 (1966).
7. T. A. Dettlaff, L. A. Nikitina, O. G. Stroeva, *J. Embryol. Exp. Morphol.* **12**, 851 (1964).
8. S. Subtelny and C. Bradt, *Develop. Biol.* **3**, 96 (1961).
9. K. R. Porter, *Biol. Bull.* **77**, 233 (1939).
10. R. E. Ecker and L. D. Smith, *Biochim. Biophys. Acta.* **129**, 186 (1966).
11. M. Steinberg, *Carnegie Inst. Wash. Yearbook* **56**, 347 (1957).
12. R. E. Ecker and L. D. Smith, in preparation.
13. Supported by the AEC and by PHS research grant CA 08700-03 awarded to S.S. We thank Mrs. Joan Steinhorst for technical assistance.

2 February 1968

Ouabain and Streptomycin: Their Different Loci of Action on Saccular Hair Cells in Goldfish

Abstract. *Ouabain, when applied to the periotic spaces, that is, to the basal end of the saccular hair cells, suppressed microphonic potentials of the inner ear in goldfish. In contrast, streptomycin produced such an effect only when it was applied directly into the endolymph, that is, to the hair-bearing ends of the hair cells.*

In glandular and epithelial cells in which the luminal and basal surfaces are usually functionally and morphologically quite different, drugs could affect the cells differently depending on whether they were applied from the inside (luminal side) or outside (*1*). The results indicate that ouabain, an inhibitor of (Na + K)-activated adenosine triphosphatase, stops active transport of Na^+ in the frog skin, gall bladder, goose salt-gland, and other organs when it is applied to the side of cells toward which Na^+ is actively transported (*2*). We have studied the effect of locally applied ouabain on the activity of hair cells in the goldfish sacculus, that is, the inner ear. We took microphonic potentials as an indication of hair-cell activity and placed special emphasis on the different effects of the drug when administered on the luminal and on the extraluminal surfaces. We also studied the effect of streptomycin and kanamycin, two well-known ototoxins, for, when applied systemically, they cause severe degeneration of hair cells (*3*). Local application of streptomycin in the lateral line organ causes an immediate abolition of microphonic potentials (*4*).

A goldfish (about 12 cm long) was anesthetized with ethyl *m*-aminobenzoate (MS 222 Sandoz Ltd., Basel) and placed in a fish chamber (*5*). The dorsal part of the skull was removed, and the exposed brain was largely sucked away to disclose underlying structures. Before recording, we immobilized the fish with an intramuscular injection of gallamine triethiodide (about 1 μg per gram of body weight). The gills were continuously supplied with running water through a mouthpiece. Microphonic potentials were recorded through a low-resistance micropipette electrode (tip, 1 μm) filled with 3*M* KCl. When such an electrode was inserted across the macula into the saccular lumen, we recorded negative microphonic potentials whose frequency was twice that of the sound (*6*). For sound stimulation, tone pips were applied through a loudspeaker placed about 30 cm from the fish. An arrangement was made so that the sound wave appeared, on repeated sweeps, at the same place, that is, with the same phase relations, on the oscilloscopic screen. This permitted the use of a digital computer (ATAC-401, Nihonkoden Kogyo Co.) for averaging microphonic potentials.

Drugs or ions were applied locally to the sacculus. For an extraluminal application, 0.03 to 0.1 ml of test solution was dropped onto the base of the skull so as to submerge the saccular macula under the solution. To keep the concentration constant, we sucked the fluid away from the base of the skull every 5 minutes and replaced it with fresh solution. An intraluminal application was made through a micropipette (tip, about 20 μm) inserted into the saccular capsule. The micropipette was connected to a microsyringe through polyethylene tubing, and the solution was driven out by pressure. The infusion rate was most often 0.1 to 0.3 μl/min. A fairly rapid exchange of the saccular endolymph may be expected

at this rate of infusion, for the volume of the sacculus is estimated to be as small as 1 to 2 μl. An infusion rate faster than 0.5 μl/min often caused irreversible damage. The fluid injected into the sacculus leaked out through ruptured portions of the semicircular canals.

The effects of ions or drugs administered extraluminally were quite different from those of drugs applied inside the lumen (Fig. 1, A–C). In all three cases shown, we applied drugs or ions successively intra- and extraluminally to the same preparations. When 120 m*M* KCl was administered intraluminally, it had no effect on the microphonic potentials (Fig. 1A). However, when applied from the outside, it immediately suppressed the microphonics (*7*). The suppression by KCl was reversible. The effect of NaCl was not impressive. An intraluminal administration of 120 m*M* NaCl only partially reduced the size of the microphonics. An extraluminal application also gave no definite change. The relatively mild effect of intraluminally injected NaCl is at variance with the result in the guinea pig cochlea, where an injection of isotonic NaCl into the scala media caused a rapid fall in the microphonics (*8*). The difference is perhaps attributable to the different ionic compositions of the endolymph in the two animals; K^+ constitutes almost all of the cation in the endolymph of the guinea pig, whereas in the goldfish about equal portions are contributed by K^+ and Na^+ (*9*).

Effects of ouabain (G-strophanthin) were similar to those of KCl; namely, the microphonics were markedly reduced only upon its extraluminal application. Ouabain (5×10^{-4} g/ml) was applied first intra- and then extraluminally (Fig. 1B). An intraluminal application did not cause any change in the size of microphonics even at a concentration as high as 2.5×10^{-3} g/ml. The microphonics began to decrease a few minutes after the extraluminal application and reached a steady low level of 20 to 40 percent of the initial value within 20 to 30 minutes. An almost similar reduction was produced by concentrations ranging from 2.5×10^{-5} to 5×10^{-5} g/ml. The effect was irreversible, and the microphonics did not reoccur after the ouabain was washed out.

Streptomycin (dihydrostreptomycin sulfate) and kanamycin (kanamycin sulfate) suppressed microphonics only when applied intraluminally. When 5×10^{-4} g of streptomycin per milliliter was applied, the microphonics decreased in size quite soon after an intraluminal application; they were totally abolished within 20 minutes. Lower concentrations of the drug often produced a partial reduction, but in two cases the microphonics were abolished by a concentration of 5×10^{-5} g/ml. Kanamycin had an effect similar to that of streptomycin, but the liminal effective concentration was higher, 2×10^{-4} g/ml. At this concentration the microphonics were reduced to 10 to 20 percent of the initial value within 20 minutes. The effect of streptomycin applied within the lumen was irreversible in all cases studied, but considerable recovery took place when kanamycin was applied, especially at near-threshold concentrations of the drug. Extraluminal application of streptomycin and kanamycin did not produce any detectable change in the microphonics at the highest concentrations so far tested, 5×10^{-3} and 2×10^{-2} g/ml, respectively.

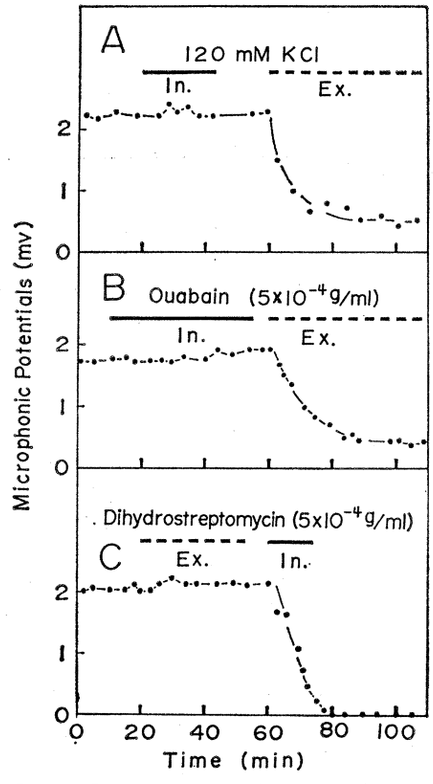

Fig. 1. Time course of change in the amplitude of microphonic potentials produced by local administration of ions and drugs. Thick continuous and dotted lines show periods during which drugs or ions were applied to the saccular macula intra- and extraluminally, respectively. Rate of intraluminal infusion was 0.15, 0.2, and 0.1 μl/min in A, B, and C, respectively. Sound stimuli were tone pips at 600 Hz (95 db SPL), delivered once every 2 seconds. Points represent averages of 50 responses.

The effect of ouabain suggests that hair cells in the fish's inner ear are the sites where membrane transport of Na^+ and K^+ is very active and that the function of these cells depends heavily on the integrity of this transport. That ouabain was effective only when applied extraluminally suggests further that the active transport is carried out mainly on the basal side of hair cells where Na^+ is continuously pumped out of the cell interior. According to a scheme postulated by Davis on the action of hair cells, microphonic potentials are derived from the preexisting resting polarization of the hair cell by a change in the ohmic resistance of a mechanosensitive portion of the cell membrane (*10*). As a corollary, he also assumes that there is a steady electrical leakage through the control element when it is mechanically at rest. From this point of view, the operation of the active transport mechanism at the basal part of hair cells seems to be a requisite for maintaining the resting polarization of the cell in the face of steady leakage current through the hair-bearing end of hair cells. It is also supposed that the requirement for Na^+ extrusion at hair cells would be more acute in lower forms, such as fish, than in mammals, for a substantial fraction of cations in the endolymph is contributed by Na^+ in the former, and this fact seems to indicate that both K^+ and Na^+ participate in carrying the steady leakage current through the hair-bearing end of hair cells. The effect of ouabain on the mammalian hair cell seems more difficult to analyze, because structures other than hair cells, such as stria vascularis and tegmentum vasculosum, are also susceptible to ouabain (*11*).

Streptomycin and kanamycin abolished the microphonics only when administered intraluminally. Wersäll and Flock (*4*), on the basis of their experiments on the excised lateral line organ, proposed that the primary action of streptomycin is on the surface membrane of hair cells, but it is not possible to tell from their experiments whether the whole surface membrane of the hair cells or only the membrane on the hair-bearing end was susceptible to streptomycin. Our results suggest that the latter is the case. Perhaps drugs such as streptomycin and kanamycin have a special affinity for the mechano-

sensitive part of hair cells. The ineffectiveness of extraluminally administered streptomycin and kanamycin might be attributable to the presence of some barrier which prevents the access of these drugs to the basal part of hair cells. However, in view of the fact that the molecular weight of kanamycin sulfate (582.6) is smaller than that of ouabain (728.77), the possibility seems remote.

S. MATSUURA
K. IKEDA
T. FURUKAWA

Department of Physiology, Osaka City University Medical School, Abeno-ku, Osaka, Japan

References and Notes

1. D. W. Fawcett, *Circulation* **26**, 1105 (1962).
2. V. K. Johnsen, *Acta Physiol. Scand. Suppl.* **145**, 87 (1957); J. Diamond, *J. Physiol.* **161**, 442 (1962); S. Thesleff and K. Schmidt-Nielsen, *Amer. J. Physiol.* **202**, 597 (1962); M. R. Hokin, *J. Gen. Physiol.* **50**, 2197 (1967); I. M. Glynn, *Pharmacol. Rev.* **16**, 381 (1964).
3. A. J. Duvall and J. Wersäll, *Acta Otolaryngol.* **57**, 581 (1964); J. E. Hawkins and H. Engström, *Acta Otolaryngol. Suppl.* **188**, 100 (1963); J. Farkashidy, R. G. Black, T. D. R. Briant, *Laryngoscope* **73**, 713 (1963).
4. J. Wersäll and A. Flock, *Life Sci.* **3**, 1151 (1964).
5. E. J. Furshpan and T. Furukawa, *J. Neurophysiol.* **25**, 732 (1962).
6. T. Furukawa and Y. Ishii, *ibid.* **30**, 1377 (1967); *Jap. J. Physiol.* **17**, 572 (1967).
7. I. Tasaki and C. Fernández, *J. Neurophysiol.* **15**, 497 (1952).
8. T. Konishi, E. Kelsey, G. T. Singleton, *Acta Otolaryngol.* **62**, 393 (1966).
9. C. G. Johnstone, R. S. Schmidt, B. M. Johnstone, *Comp. Biochem. Physiol.* **9**, 335 (1963); T. Naito, H. Uyematsu, T. Nakamura, K. Horii, T. Miyazaki, *Japan. J. Otol.* **68**, 1628 (1965).
10. H. Davis, *Cold Spring Harbor Symp. Quant. Biol.* **30**, 181 (1965).
11. W. Kuijpers, A. C. Van der Vleuten, S. L. Bonting, *Science* **157**, 949 (1967); Y. Tanaka and P. G. Brown, *Ann. Oto-Rhino-Laryngol.*, in press.
12. We thank Dr. H. Grundfest for reading the manuscript.

15 April 1968

Resistance Changes in Lipid Bilayers: Immunological Applications

Abstract. *The electrical resistance of a bimolecular lipid membrane in 0.1 molar NaCl decreases if antibody and complement are present on one side of the membrane and the homologous antigen is added to the other side. The reaction occurs within minutes and requires less than 0.1 microliter of antiserum.*

Immunological as well as enzyme-substrate reactions occurring on one surface of a lipid bilayer can lower the electrical resistance of the membrane (*1*). In addition, occasional proteins (*2*), valinomycin (*3*), and a group of related antibiotics can selectively induce an increased flux of cations, with a subsequent resistance decrease, whereas alamethicin can lower the resistance by nonselective transport of cations (*4*). We now report the effect of an antigen-antibody reaction, occurring across the membrane, upon the transmembrane electrical resistance. We have shown that when either antiserum or antigen alone is added no change in resistance is observed. When antiserum is added to one side of a membrane and the homologous antigen is added to the other side, a very significant decrease in the resistance is consistently observed.

All membranes were formed with a lipid solution containing 2.5 mg of sphingomyelin per 100 μl of a mixture of α-tocopherol, chloroform, and methyl alcohol (5:3:2). No traces of cholesterol, stearate, or palmitate could be demonstrated in the sphingomyelin which migrated as a single component on thin-layer chromatography (*5*, *6*). The α-tocopherol (*6*) had a light amber hue, an indication that some oxidation may have taken place. The lipid stock solution was kept in a nitrogen atmosphere at −80°C to reduce oxidation and evaporation.

A polyethylene cup (Beckman, 1.6 cm high and 2.0 cm in diameter) formed the septum which provided a support for the membrane and separation between the aqueous solutions. When not in use, the cup was stored in *n*-decane (*6*). The circular orifice in the wall of the cup, across which the membranes were formed, had an area of 0.0132 cm^2. Immediately before an experiment, the cup was thoroughly cleaned with Kimwipes, so that only a trace amount of decane was available for incorporation in the lipid structure (*7*). To make a membrane, a small amount of the lipid solution was applied to a thin sable brush, size 0000, which was then streaked across the orifice (Fig. 1) to deposit a sheet of lipid solution. As the chloroform and methyl alcohol diffused into the aqueous phase, bimolecular regions started to form locally (*8*); after a few minutes, a major part of the membrane area would assume the bilayer configuration. A discussion of the reproducibility and time course of the membrane resistance is given after the experimental data.

The aqueous medium was a 0.1*M* NaCl solution with 5 m*M* histidine (*6*) at *p*H 7.3. We found that concentrations of NaCl and KCl equal to or greater than 0.3 mole/liter made formation difficult and gave unstable membranes. The transmembrane electrical resistance was monitored with two fiber-junction electrodes (Beckman, No. 41239) at an applied voltage of less than 20 mv. The offset-voltage between the two electrodes was considered as part of the voltage source. The preferred temperature for membrane formation was 37° ± 1°C.

We found that the thinning procedure became unpredictable when the temperature went below 28°C, and the membranes consistently broke between 40° and 41°C. When the current density exceeded 4×10^{-8} to 10×10^{-8} amp cm^{-2}, the membranes would often break without any apparent mechanical or thermal stimulation. If only a small number of sites carry ions across the membrane, one can visualize a local increase in temperature, caused by the dissipation of (16 mv) (4×10^{-8} amp cm^{-2}) = 9.2×10^{-6} cal min^{-1} cm^{-2} in the membrane. Because lipid is a poor conductor of heat, the released energy may raise the temperature locally above the critical 41°C and hence weaken the membrane structure so that it breaks. When the difference in hydrostatic pressure between the two chambers exceeded 0.4 cm, the membranes would burst. Also, air currents in the room could give fluctuating resistance values.

In general, the resistance of a membrane increased for about 30 minutes after formation and then remained virtually constant for at least 96 hours if the temperature was held constant at 37°C. We found a transient decrease in resistance immediately after addition of a protein to either side of the membrane. The decrease was typically 10 to 20 percent of the resistance (R) value before addition of the protein, and the duration of these transients did not exceed 20 seconds. As Fig. 1 shows, deviations from a smooth curve of the relation between the logarithm of R and time were frequently encountered after addition of one of the proteins. There was no correlation between these devia-