the velocity of the stapes in the normal animal; or, to be more precise, that it is proportional to the displacement of the basilar membrane, which is proportional to the pressure at the oval window, which is, in turn, proportional to the velocity of the stapes. The time difference between the square waves in the first and third turns reflect the traveling-wave delay between these two points. In normal cochleas the CM reflects the output of the outer hair cells. Thus the output of the outer hair cells is proportional to the displacement of the basilar membrane, as we proposed earlier in this report.

In Fig. 2b are microphonic recordings from a cochlea of a kanamycin-treated animal. At the third turn—where both types of hair cell were in acceptable condition (Fig. 1)—the recording was a square wave having the appropriate time delay of approximately 0.9 msec relative to the stimulus peaks. This response is virtually indistinguishable from that in the normal cochlea. In contrast, the electrode pair at the first turn registered a pulse coincident with the peaks of the stimulus, and every second pulse was of opposite polarity. The additional, later peaks are uncanceled remote CM from the intact portions of the cochlea and are of no significance for our purposes *(17)*. When the responses from the normal and kanamycin-damaged first turns are compared, then the latter appears approximately proportional to the derivative of the former. Thus, inner hair cells produce a CM that is proportional to the rate of change of basilar membrane displacement, this in accord with our above-mentioned suggestions *(18)*.

In summary, comparison of CM output from inner and outer hair cells of the mammalian cochlea reveals that the two groups of hair cells possess different dynamic properties. The primary response of the outer hair cells is proportional to the displacement of the basilar membrane, and that of the inner hair cells is proportional to the velocity of the basilar membrane. The cause of these differences can be found in the relation between cilia and the tectorial membrane for the two groups. Apparently, the cilium of the inner hair cell is freestanding, and thus its stimulus is derived from viscous fluid drag; but some cilia of outer hair cells are attached, and their stimulus is the relative displacement of tectorial and reticular membranes. The existence of a dual system that is capable of providing information about both the signal and its first derivative can yield significant improvement in the data-processing capability of the peripheral auditory system.

PETER DALLOS
M. C. BILLONE
J. D. DURRANT
C.-y. WANG, S. RAYNOR
Auditory Research Laboratory and
Departments of Electrical and
Mechanical Engineering,
Northwestern University,
Evanston, Illinois 60201

References and Notes

1. E. G. Wever, *Ann. Otol. Rhinol. Laryngol.* **80**, 786 (1971).
2. ———, *J. Morphol.* **122**, 307 (1967); *ibid.* **123**, 355 (1967); *ibid.* **130**, 37 (1970); *ibid.* **132**, 277 (1970); *Copeia* **1**, 139 (1971).
3. H. Engstrom, H. W. Ades, J. E. Hawkins, Jr., *J. Acoust. Soc. Amer.* **34**, 1356 (1962); R. S. Kimura, *Acta Oto-Laryngol.* **61**, 55 (1965); G. Bredberg, *ibid.* (Suppl. 236), 1 (1968); D. Lim, *J. Acoust. Soc. Amer.* **50**, 90 (1971); private communication.
4. H. Spoendlin, *The Organization of the Cochlear Receptor* (Karger, Basel, 1966), pp. 6–22; C. Smith and T. Takasaka, *Contrib. Sens. Physiol.* **5**, 129 (1971); S. Iurato, *Z. Zellforsch. Microsk. Anat.* **53**, 259 (1961).
5. M. C. Billone, thesis, Northwestern University (1972).
6. G. von Békésy, *Experiments in Hearing* (McGraw-Hill, New York, 1960), pp. 485–500, 703–710.
7. W. S. Rhode and C. D. Geissler, *J. Acoust. Soc. Amer.* **42**, 185 (1967).
8. H. Davis, B. H. Deatherage, B. Rosenblut, C. Fernández, R. Kimura, C. A. Smith, *Laryngoscope* **68**, 596 (1958).
9. C.-y. Wang, thesis, Northwestern University (1971); ——— and P. Dallos, *J. Acoust. Soc. Amer.*, in press.
10. A. Kohonen, *Acta Oto-Laryngol.* (Suppl. 208) (1965); J. E. Hawkins, *Ann. Otol. Rhinol. Laryngol.* **68**, 698 (1959).
11. By "intact" inner hair cells we mean cells that appear normal under the phase-contrast microscope. Light microscopy can not reveal possible subtle morphological changes. Consequently, without electron microscopic verification, we must accept the possibility that some of the inner hair cells that appeared normal with light microscopic observation had actually sustained some damage.
12. In the classic experiments of von Békésy (*6*), in which he demonstrated that by directly stimulating the cochlear partition with a vibrating electrode an electrical output is obtained which is directly proportional to partition displacement, the electrical signal must now be interpreted as being produced by the dominant outer hair cells.
13. H. Engstrom, H. W. Ades, A. Andersson, *Structural Pattern of the Organ of Corti* (Almqvist & Wiksell, Stockholm, 1966), pp. 13–33, 114–131.
14. To indicate the consistency of these histological results, we offer the following considerations. We have processed 32 cochleas from kanamycin-treated animals (not all were used in this experiment). If three animals that suffered severe damage to both hair cell populations are excluded, then in the remaining 29 preparations only 6.9 percent (mean) of outer hair cells in the basal half of the cochlea were intact, while in this same population 89.2 percent of the inner hair cells remained present. Thus in guinea pigs it is possible to generate selective destruction of outer hair cells in the basal half of the cochlea.
15. G. Jako, K. Hickman, H. Adkins, *J. Acoust. Soc. Amer.* **40**, 1263 (1966).
16. P. Dallos and J. D. Durrant, *ibid.* in press.
17. The electrical output of the inner hair cells is at least 30 db less than that of the outer hair cells. Because of this, extreme care must be exercised in recording from a region of the cochlea containing only inner hair cells when there are intact outer hair cells in some other region. Under such circumstances a single intracochlear (or round-window) electrode would pick up potentials that are overwhelmingly derived from the remote normal regions of the cochlea. Such potentials would not reflect local conditions and would provide no information about the electrical output of the inner hair cells. A pair of differential electrodes carefully balanced to reject remote potentials, as used in this experiment, provides the only means of obtaining the characteristics of a small local potential in the presence of a relatively large remote response.
18. Experiments like those described in this report were performed on five guinea pigs treated with kanamycin. In two of these animals the displacement component was dominant even in the first turn. Subsequent histological study revealed that these animals had mild damage to outer hair cells, and thus the response was produced by outer hair cells remaining in the vicinity of the recording electrodes. In three animals that had lesions similar to that in Fig. 1, the electrophysiological results agreed with the description in this report. Steady-state measurements of cochlear microphonic phase were performed on an additional three kanamycin-treated guinea pigs, and the phase data from these animals is consistent with the dynamic measurements presented here.
19. Supported by various NIH grants. The piezoelectric transducer was supplied by R. Sachs of Industrial Research Products, Inc.

12 May 1972

Systemic Absorption of Intrauterine Copper

Abstract. *A comparison of the various effects, in rats, of intrauterine insertion of copper-64 or copper-67 wire with the effects of intraperitoneal injection of copper sulfate solutions has shown that copper ions, dissolved from the wire, are locally active contraceptively and, in part, systemically absorbed.*

The efficacy of intrauterine devices has been greatly increased by incorporating copper into their structure (*1*, *2*). Dissolved ions of the metal appear to be the actual contraceptive agent, since approximately 25 percent of a copper wire placed in a human uterus disappears within a year (*3*) and metals with low solubility, like platinum, or silver in the presence of chloride ions, are not very effective contraceptively (*2*).

The possibility that systemic toxicity may result from copper absorbed through the uterine mucosa must be considered in view of reports which indicate that 20 to 25 mg of copper are removed from these devices per year (*3*). To investigate this possibility, we

inserted radioactively labeled copper wire (*4*) into the uteri of rats and measured the concentratiton of the isotope in various organs as a function of time.

Adult Holtzman-strain rats weighing 200 to 230 g were placed under intraperitoneal barbital anesthesia. The uterus was exposed via a small midline, ventral incision under sterile conditions, and a copper wire was inserted in the left horn as described by Chang, Tatum, and Kincl (*2*). In experiments 1 to 3, the animals were allowed 7 to 10 days to recover from surgery and were then mated. The day a vaginal plug was found was considered to be the first day of pregnancy. The animals were killed on the 9th or 15th day of gestation and implantations, or embryonic resorptions, were counted.

In experiment 1 we repeated the procedures described by Chang, Tatum, and Kincl (*2*). In agreement with their results, no implanted blastocysts were found in any of the uterine horns containing copper in 18 rats.

In experiment 2, copper wire was coated with semipermeable collodion or impermeable enamel before intrauterine implantation. Table 1 shows that there were no pregnancies in the horn containing the collodion-coated copper wire, whereas enamel coating reduced, but did not eliminate, pregnancies.

In experiment 3, 12 rats were given intraperitoneal injections of 0.6-ml portions of copper sulfate (1 mg of copper per milliliter) for 3 days, while a second group of 12 matched rats received 0.6-ml portions of a 0.9 percent solution of sodium chloride daily. All rats were then allowed to mate, and those that became pregnant (eight from each group) were injected for another 5 days. Twelve to 18 implants were observed in each of 16 rats, indicating that the injected copper had no contraceptive effect.

In experiments 4 and 5, about 1.5 cm of ^{64}Cu wire or ^{67}Cu wire, respectively, were implanted into nonpregnant rats, which were then killed at intervals (Fig. 1). Our results, although preliminary, afford some insight into the mechanism by which copper-containing intrauterine devices act contraceptively and indicate that some of the metal is absorbed and transported from the uterus via the blood.

Experiment 2 shows that the contraceptive effect of copper is due to copper ions, since it is abolished by coating the wire with impermeable enamel, but is not much diminished by a coating of collodion, which is permeable to copper ions. Furthermore, if a property of the wire, such as conductance, were the effective contraceptive agent, the marked discrepancy in activity between copper and other metals (*2*) would hardly be expected.

Table 1. Comparison of the effects of semipermeable and impermeable coatings of copper wires on implants in the rat uterus; *N*, number of rats. The number of implants is given as the mean plus or minus the standard error.

Coating	*N*	Number of implants	
		Left horn	Right horn
Collodion	10	0	7.4 ± 0.3
Enamel	8	4.8 ± 0.5	7.8 ± 0.4

A small proportion of intrauterine metallic copper was consistently absorbed and widely distributed throughout the rat within hours after implantation. From 18.5 to 96 hours after insertion of a ^{64}Cu wire, about 0.4 percent of the radionuclide was found in the serum, liver, kidneys, and broad ligaments (Fig. 1). The small amount of radioactivity found in the right (control) horn probably represented that in the perfusing blood; the precise site of deposition of the larger amount in the left horn, after removal of the wire, was not determined.

Experiment 5 was carried out with ^{67}Cu since this has a fivefold longer half-life than ^{64}Cu, which permitted us to make observations over a longer period. The results of the studies with ^{64}Cu were confirmed, and, in addition, measurements of radioactivity revealed no ^{67}Cu present in the carcass. By 8 days most of the plated ^{67}Cu had probably been removed. This may be the reason for the smaller amounts of ^{67}Cu found in liver, kidneys, and uterus between 120 and 192 hours.

There are no data for humans from which we could estimate how much of the dissolved intrauterine copper wire is lost with uterine secretions or menstrual blood and how much, if any, is absorbed. Our results indicate that at least some of the copper dissolved may be absorbed into the systemic circulation and deposited in several organs. The amounts of copper used in the devices, 100 to 150 mg, are roughly equal to the total body content of this metal. Sufficient absorption could significantly increase the body content of copper, particularly if the devices are used for a decade or more, although it is unlikely that concentrations of copper approaching those seen in Wilson's disease will be reached in women without this disorder of copper balance (*5*). Such normal individuals maintain a zero copper balance even when they ingest amounts of dietary copper far in excess of the usual maximum daily intake of 5 mg. There is evidence, however, which suggests that parenterally absorbed copper may not be controlled by the genetically transmitted homeostatic mechanism. Thus, the hepatic concentration of copper almost doubled in a patient with chronic renal disease who was subjected to a 2-year period of hemodialysis in which copper-containing dialysis membranes were used (*6*). Since

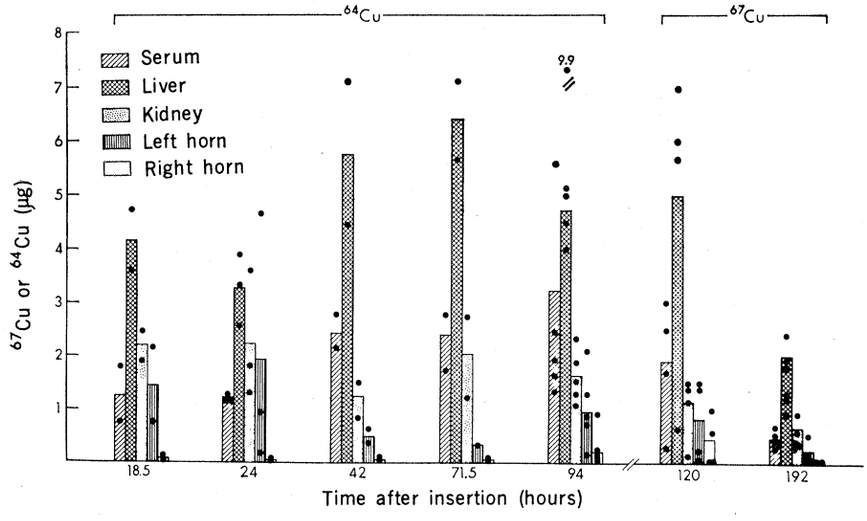

Fig. 1. Total content of ^{64}Cu and ^{67}Cu in serum and organs of rats at various times after the insertion of a radioactive-copper wire into the left uterine horn. The height of each bar indicates the mean content of radioactive copper.

copper-containing intrauterine devices are of such promise, it is imperative to extend studies of the fate of the copper before their use can be safely prescribed. Novel techniques will be required for these studies in human beings since, except possibly in rare instances, radioactive copper cannot be used, and the quantitative estimation of whether excess copper accumulation occurs in tissues requires chemical, not histochemical, analysis of serial biopsy samples (*7*).

TATI OKEREKE
Department of Obstetrics and Gynecology, Albert Einstein College of Medicine, Bronx, New York 10461
IRMIN STERNLIEB
ANATOL G. MORELL
I. HERBERT SCHEINBERG*
Division of Genetic Medicine, Albert Einstein College of Medicine

References and Notes

1. J. Zipper, M. Medel, R. Prager, *Amer. J. Obstet. Gynecol.* **105**, 529 (1969); J. A. Zipper, H. J. Tatum, M. Medel, L. Pastene, M. Rivera, *ibid.* **109**, 771 (1971); C. C. Chang and H. J. Tatum, *Contraception* **1**, 265 (1970).
2. C. C. Chang, H. J. Tatum, F. A. Kincl, *Fert. Steril.* **21**, 274 (1970).
3. H. J. Tatum, quoted by G. K. Oster, *ibid.* **23**, 18 (1972).
4. Copper-64 (half-life, 12.8 hours) wire, with approximately 100 mc, was prepared by exposure (by Union Carbide, Tuxedo, New York) of 75 mg of electrolytic copper wire, 100 cm long and 0.01 cm in diameter, to a neutron flux of 4×10^{13} cm^{-2} sec^{-1} for 2.5 hours. A solution of $^{67}CuCl_2$ (^{67}Cu half-life, 61.8 hours), prepared by irradiation of enriched ^{67}Zn in a fast neutron flux (Oak Ridge National Laboratory, Oak Ridge, Tennessee), contained 0.25 mc of ^{67}Cu and less than 2 μg of Cu per milliliter, with traces of ^{64}Cu, ^{65}Zn, and ^{60}Co as contaminants. To 1 ml of the $^{67}CuCl_2$ solution were added 1.7 ml of $CuSO_4$ solution (10 mg/ml), 5 ml of 1.0*N* sulfuric acid, 0.5 ml of 1.0*N* nitric acid, and 42 ml of water. Four 40-gauge copper wires (General Electric, Schenectady, New York), 0.008 mm in diameter and insulated by Formvar except for two 1.5-cm lengths separated by 5 cm, served as cathodes. These were placed at 0.5 cm from a central platinum wire, 0.2 mm in diameter, which served as the anode. Plating was generally carried out for 4 hours with a current of 6 ma at 2.4 volts, at room temperature. Subsequently, the wires were washed with a dilute solution of nonradioactive copper and then with water, and the two uninsulated portions were cut apart. About 50 to 175 μg (mean, 109 μg) of ^{67}Cu was plated on each exposed 1.5-cm segment of wire. The radioactivity of each wire was measured before insertion in a well-type scintillation counter with a gamma spectrometer. After the wire was removed from the uterus, its radioactivity and that of the serum, liver, kidneys, uterine horns, broad ligaments, and standards were determined. In the experiments with ^{67}Cu, the radioactivity present in the rat carcasses was also measured by using a large-sample counting system (Tobor model 4351, Nuclear-Chicago).
5. I. H. Scheinberg and I. Sternlieb, *Annu. Rev. Med.* **16**, 119 (1965).
6. B. H. Barbour, M. Bischel, D. E. Abrams, *Nephron* **8**, 455 (1971).
7. S. Goldfischer and I. Sternlieb, *Amer. J. Pathol.* **53**, 883 (1968).
8. Supported in part by NIH grant AM-1059. The copper wire was a gift from Dr. William W. Wareham, Phelps Dodge Magnet Wire Corp., Fort Wayne, Indiana. The ^{67}Cu was obtained through a grant from the Copper Research Association, Inc., New York.
* Requests for reprints should be sent to I.H.S.

19 May 1972

Hemoglobin Adaptation for Fast and Slow Water Habitats in Sympatric Catostomid Fishes

Abstract. *The oxygen equilibria of* Catostomus insignis *hemoglobins are* p*H dependent.* Catostomus clarkii *hemoglobins have some components (20 percent) whose oxygen equilibria are independent of* p*H because the alpha chains have* NH_2*-termini that are blocked and the beta chains lack the "usual" COOH-terminal histidine. Since the Bohr effect is normally a beneficial phenomenon, the maintenance of some hemoglobins without a Bohr effect must provide a physiological advantage that is habitat specific. The intrastream ecological preferences of these sympatric catostomids suggest that the hemoglobins without the Bohr effect confer an ecological advantage in a swift water habitat.*

There are 70 species of fish from the family Catostomidae. Twenty species of one genus (*Catostomus*) inhabit the western montane regions of the United States; 14 of these species are members of the subgenus *Catostomus*, while six represent the subgenus *Pantosteus* (*1*). Fishes are distributed so that only one species of a subgenus inhabits a given geographical region (that is, allopatric), although each species of one subgenus is usually found living with a member of the other subgenus (that is, sympatric) (*1*).

The subgenera *Pantosteus* and *Catostomus* can be distinguished from one another by the presence or absence, respectively, of cathodal components in the electrophoretic patterns of hemoglobins at *p*H 8.6 (*2*). I expected the characterization of the cathodal hemoglobins to be important in correlating structure and function of the hemoglobins with fish ecology. A representative pair of sympatric species, *Catostomus* (subgenus *Catostomus*) *insignis* and *Catostomus* (subgenus *Pantosteus*) *clarkii*, were collected and hemoglobins were separated by ion-exchange chromatography (see Fig. 1). Fractions were characterized by gel electrophoresis according to the method of Smithies (*3*, *4*).

The molecular weights, which ranged from 66,300 to 68,600, of single chromatographic components were determined by sedimentation equilibrium (*5*). Hence, hemoglobins from either *C. clarkii* or *C. insignis* have approximately the same molecular weight, which corresponds to the tetramer of mammalian hemoglobins. The electrophoretic banding multiplicity, therefore, cannot be attributed to polymerization through the formation of interchain disulfide bonds or to dissociation of subunits.

The ability of hemoglobin to bind oxygen is usually modified by the *p*H of its microenvironment. Within physiological range, the hemoglobin's affinity for oxygen is directly proportional to *p*H. Consequently, oxygen affinity is higher at the lungs (or gills) and lower at the tissues (lactic and carbonic acids lower the tissue *p*H). The dependence of hemoglobin oxygen affinity on *p*H is known as the Bohr effect.

Oxygen equilibria of chromatographic components were performed by the method of Riggs and Wolbach (*6*). The anodal hemoglobin components from *C.* (subgen. *P.*) *clarkii* and *C.* (subgen. *C.*) *insignis* had slightly different oxygen equilibrium curves but all anodal components demonstrated substantial sensitivity to *p*H changes (large Bohr effect). The cathodal components from *C. clarkii*, however, did not have a Bohr effect (see Fig. 1E).

The NH protons in the imidazolium ring of COOH-terminal histidine in the β chains and NH_2-termini of the α chains are known to be largely responsible (75 percent) for the Bohr effect in mammalian hemoglobins (*7–9*). Consequently, the NH_2-termini and COOH-termini of the hemoglobin components were examined for each of the species.

The α and β chains of the anodal components, fractionated by a method similar to that of Clegg *et al.* (*10*), from both species and the globins of the cathodal components from *C. clarkii* (*11*) were subjected to NH_2-terminal analysis by a modification of Edman's phenylisothiocyanate procedure (*12*). The phenylthiohydantoins of the amino acids were identified either by the methods of Jeppsson and Sjörquist (*13*) or by gas chromatography [procedure of Pisano and Bronzert (*14*)].

Carboxypeptidase A (CPA) and B