ELECTRICAL POTENTIALS OF THE SUBTECTORIAL SPACE IN THE GUINEA PIG COCHLEA

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Abstract Precipitation of cobalt ions and iontophoretic marking by Alcian Blue were utilized in examination of physiological properties of the subtectorial space in the guinea pig cochlea. Cobalt ions injected into the scala media were sulfurated and observed as a black precipitation in cross sections. Precipitation was seen on the upper and the lower surface of the tectorial membrane, and on the reticular membrane. Alcian Blue was the most suitable dye for marking in the organ of Corti. Recording sites of potentials in the subtectorial space were identified by Alcian Blue marking. The potentials were similar to those measured in the scala media. These facts verify that the subtectorial space communicates with the scala media through the outermost margin of the tectorial membrane. Thus the sensory hairs of hair cells are bathed in the endolymph of high potassium concentration, and the condition for optimum sensitivity of their receptor function is maintained.

For an understanding of the receptor mechanism in the organ of Corti, it is of considerable importance to know whether or not the sensory hairs of the hair cells are exposed to an endolymph of high potassium concentration. About a half century ago Kolmer (1924) illustrated with excellent sketches a netlike structure at the outer margin of the tectorial membrane. A similar netlike structure, called the marginal net, was disclosed in a recent electron microscope study (Lim, 1972). Morphologically, the subtectorial space is believed to communicate with the scala media through small holes in the marginal net. However, Tonndorf et al. (1962) investigated the permeability of the intracochlear membranes employing a vital staining technique and suggested that the upper surface of the tectorial membrane was an endolymph-perilymph boundary. X-ray microanalysis showed that the ionic composition of the fluid beneath the tectorial membrane differed from that

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in the endolymph (Ross, 1974). The incompatibility between the experimental
and morphological considerations cited above points to the desirability of electro-
physiologic exploration of the subtectorial space in search of a clue to better under-
standing of the transducer mechanism in hair cells.

In the present study, a cobalt injection method was used to determine whether
or not a fluid transfer occurred through the partition between the subtectorial space
and the scala media. For the purpose of potential identification microelectrodes
were inserted into the subtectorial space or the inner sulcus, by penetrating the
organ of Corti from the pars tecta of the basilar membrane toward the scala media,
and the origin of potentials measured was localized with a refined technique of
electrode marking.

METHODS

Guinea pigs weighing between 250 and 500 g were used. The animals were
anesthetized with 30 mg/kg of sodium pentobarbital injected intraperitoneally,
and their heads were firmly fixed in a modified Hess type fixation instrument.
Tracheotomy was performed and muscle relaxation was induced with gallamine
triethiodide. The animals were maintained by means of artificial respiration.
The tympanic bulla was widely opened through a ventrolateral approach. A piece
of glass rod, specially made by Olympus Optical Co. (Tokyo), was implanted in
the basal turn of the right cochlea. The glass rod served as a light guide to produce
an effective intracochlear illumination.

For both potential recording and iontophoretic dye application glass micro-
pipettes with less than 1 μm tip diameter were filled with 1.5 M KCl solution which
contained 3% Alcian Blue 8GX. The resistance of the electrodes ranged from
40 to 100 MΩ. The reference electrode was a silver plate placed on the neck mus-
cles. The electrodes were inserted toward the scala media through the round
window and the basilar membrane. Under the intracochlear illumination de-
scribed above the point of the electrode insertion at the basilar membrane were
determined easily through the microscope. The potentials were led to a Nihon
Kohden MZ-4 preamplifier and recorded with a Toa EPR-3T pen recorder. The
a.c. component was led to a Tektronix 565 cathode-ray oscilloscope, and thence
through a Nihon Kohden ATAC 201 averaging data processor to a Yokogawa
3077 X-Y recorder.

Acoustic stimulation was delivered through a closed system composed of
a Mitsubishi Diatone TW-25 tweeter and a coupler tube to the external ear canal.
Stimulation between 2,500 and 3,000 Hz in bursts of 25 msec with 1 msec rise and
decay time was delivered once every second. The sound intensity could be ad-
justed by an attenuator in which zero dB was set to yield a sound pressure of 0.0002
dyn/cm² in the external ear canal.

A cobalt iontophoresis (Pitman et al., 1972) was initially used. However,
iontophoretically applied cobalt ions were insufficient for staining, so a pressure infusion method was resorted to. The infusion electrodes were glass capillaries of 2 to 10 µm in tip diameter, filled with 200 mM cobalt chloride. The electrode was connected through a polyethylene tube to a syringe containing mineral oil. Reading excursion of the meniscus at the oil-solution interface in the infusion micro-pipette, the cobalt chloride solution was slowly injected for over 2 to 5 min. Reissner’s membrane was ruptured at the apex to permit perfusion of the scala media with 1 ml of cobalt chloride solution. No noticeable change in cochlear potentials was produced by fenestration at the apex and the slow rate injection of artificial endolymph (KONISHI et al., 1966; TANAKA and BROWN, 1970). After infusion, potentials were observed for 15 min, and the animal were then decapitated. The cochlea removed from the temporal bone was immersed in 0.5% ammonium sulfide-Ringer solution until the preparation became black.

For marking any desired position of electrode a cathodal current pulse of $1 \times 10^{-7}$ A and 0.5 sec duration was passed through an Alcian Blue electrode at a rate of once per second for 1 to 2 min. The marking spot produced with Alcian Blue was too small to be detected with a stereoscopic microscope at ×60. Immediately after decapitation the cochlea was removed from the skull, widely fenestrated at the round window and apex, and then fixed for 12 hr at 4°C in 2.5% glutal aldehyde solution brought to pH 7.2 with cacodylate buffer. The basal end of the basilar membrane, the hook part, was carefully picked out from the membranous labyrinth of the cochlea. Under a microscope this material was observed as a surface preparation (ENGSTROM et al., 1964). It was then dehydrated in ethanol and embedded in Epon. The Epon embedded preparation was suitably trimmed and 1 to 2 µm sections were made with an LKB 8800 Ultrotome III. The sections of the hook part were cut across the hair cell row. The Alcian Blue spot was so small that it could be detected in only a few sections.

**RESULTS**

*Injection of cobalt into the scala media*

The infusion electrode was inserted through the pars pectinata of the basilar membrane into the scala media from which the endocochlear potential (EP) could be recorded. The cobalt chloride solution was injected from the electrode into the scala media. It was reported by TANAKA and BROWN (1970) that the injection of an artificial endolymph into the scala media produced a slight decrease both in the cochlear microphonics (CM) and EP. The infusion of cobalt ions did not induce any remarkable change in CM and EP during injection. This indicates that no immediate damages of the intracochlear membranes might occur. At 15 min after the injection, however, a decrease in CM and a depressing EP to half of its initial level were observed. Regarding this depression in cochlear potentials effects of potassium lack in the scala media and of cobalt ions themselves should be
Black precipitation appeared as a dotted line along the lateral margin of the tectorial membrane in the surface preparation (Plate I). Radial sections of this preparation revealed cobalt sulfide in the scala media surface of Reissner's membrane, the surface of the tectorial membrane and a part of the reticular membrane (Fig. 1). The cobalt ions seemed to be adsorbed on these surfaces, and were found uniformly over all of the upper and lower surfaces of the tectorial membrane. The cobalt deposit was definitely heavy at the outer margin of the tectorial membrane, and it was weak on the surface of the stria vascularis.

![Diagram of cochlea sections](image)

**Fig. 1.** Cross section of cobalt ion deposits in the third turn of cochlea. Cobalt ions were injected into scala media of basal turn and were sulfurated to produce black deposits. Deposits are evident on Reissner's membrane, tectorial membrane, reticular membrane and surface of stria vascularis. SM: scala media, IS: inner sulcus, TM: tectorial membrane, RM: Reissner's membrane, Str: stria vascularis. Tectorial membrane has been detached from its original position. This might be produced by histological procedures; see text. Scale: 50 µm.

*Sequence of potentials during penetration from the scala tympani to the scala media*

The microelectrode was inserted perpendicularly to the basilar membrane and advanced towards the scala media. The d.c. potential level indicating contact of the electrode tip with the round window was defined as zero. The d.c. potentials recorded in the scala tympani were usually either zero or a few millivolts positive. As the electrode tip penetrated and proceeded through the basilar membrane, two or three negative deflections were recorded after which the scala...
media was entered and EP was obtained (Fig. 2). The negative deflections have been referred to as the negative potentials of the organ of Corti. The negative potentials were thought to originate from cells when they were first recorded by Bekesy (1952). Until recently, however, there has been a controversy as to whether the source of the negative potential is intracellular or intercellular (Butler, 1965). Several attempts have been made to localize this negative potential (Tanaka et al., 1968; Tanaka et al., 1975; Bobbin, 1976). These studies suggested that the negative potential might be ascribed to intracellular origin.

Unstable, large, positive potentials (b) were occasionally recorded in the transition region between the negative deflection (a) and the stable EP (c), as shown in Fig. 2. Alcian Blue was expelled iontophoretically from the electrode to identify the location from which these unstable positive potentials were recorded. These potentials will be discussed in the next section.

**Alcian Blue marking of the subtectorial space**

Though this unstable positive potential probably indicated the immediate emergence of the electrode from the hair cell structure into the subtectorial space, it was difficult in surface preparations to determine the localization of the Alcian Blue spot in the vertical plane. To obviate this difficulty it was necessary to prepare cross sections of Corti's organ. When this was done, the stain spots could be detected in some of the sections and precisely localized.

Plate II illustrates an Alcian Blue spot located in the inner sulcus surface of the vestibular lip, indicating that the tip of the electrode had been in the inner sulcus.
The structural locations of stained spots could be clearly contrasted by Fuchsin counter staining as seen in Plate III which shows an Alcian Blue spot on the lower surface of the tectorial membrane. The location corresponds to Hensen's stripe, and the stained portion of this fibrous material is a little larger in size than neighboring cell nuclei. One frequent difficulty in making cross sections of the organ of Corti is detachment of the tectorial membrane from the hair cell structure. In the preparation shown in Plate IV, Alcian Blue spots appear both on the reticular membrane and on the tectorial membrane. The presence of the dye in two sites indicates that the tectorial membrane was not displaced from its original position when the iontophoretic marking was performed, thus verifying that the potential recorded from the subtectorial space was recorded under physiological conditions.

In 18 cases the Alcian Blue spots were detected on the surface surrounding the subtectorial space. The d.c. potentials at the marking points ranged in value from 38 to 88 mV with the mode at 60-70 mV (Fig. 3). The CM's responding to sound stimulation between 2,500 and 3,000 Hz were investigated for amplitude and phase. As seen in Fig. 4, the magnitudes of CM, expressed as the relative values.
of the measurement at the round window, are distributed between 0 and 2.5 with a peak at 1.0-1.5. Figure 5 demonstrates changes of the CM amplitude and phase encountered as the electrode progresses from the scala tympani to the subtectorial space and back to the scala tympani. In this figure, I refers to intracellular recordings and E to extracellular ones. The subtectorial CM was reversed in phase against the CM of the scala tympani. In other words, the phase of the subtectorial CM was the same as that recorded from the scala media. It has been known that the summating potential (SP) has a negative polarity at high frequencies when recorded in the scala media of the basal turn (Davis et al., 1958). SP’s were positive in polarity through the hair cell layer of the organ of Corti and negative in the subtectorial space. The Alcian Blue marking verified that the polarity change occurred at the interface between the hair cell layer and the subtectorial space.
DISCUSSION

The finding of cobalt in the lower surface of the tectorial membrane and the reticular membrane revealed the existence of a channel through which the cobalt bearing solution could have diffused into the subtectorial space. The potentials observed in the subtectorial space, the sites of which were identified by Alcian Blue marking, closely resembled those observed in the scala media. These results support Lim's morphological view (1972) that the marginal net allows passage of endolymph. Tonndorf et al. (1962) reported that the marginal fiber network, but not the subtectorial space, is stained with Alcian Blue introduced into the scala media. It is not inconceivable that the dye injected into the scala media was completely captured by the marginal fiber network, and thus, would not reach the subtectorial space. It seems reasonable to conclude that the subtectorial fluid is not perilymphatic but endolymphatic. In a quite recent X-ray microanalysis, Flock (1977) reported that the inner sulcus had the same high potassium content as the endolymph. This is in good agreement with our results. The perilymph reaches the tunnel of Corti and Nuel's space through the habenula perforata (Schuknecht and Seifi, 1963). The reticular membrane is the boundary between the endolymph of the scala media and the perilymph of the scala tympani as advocated by Tasaki et al. (1954).

In an electrode marking experiment, Alcian Blue spots were found on the lower tectorial surface, on the surface surrounding the inner sulcus and at the reticular membrane. Igarashi and Alford (1969) reported in their histochemical study that Alcian Blue could moderately or strongly stain the organ of Corti in sections of squirrel monkey temporal bones. Alcian Blue seems to combine with the negative charges of mucopolysaccharide and mucoprotein in tissue. This dye has several advantages in electrode marking: 1) The spot produced with Alcian Blue is minute and distinct because it does not readily diffuse. 2) The spot does not fade through ethanol dehydration and Epon embedding. 3) The dye is suitable for both intra- and extracellular marking.

DC potentials measured in the subtectorial space approximated the EP and the phase of CM was the same in the scala media and in the subtectorial space. Since no potential changes were recorded immediately before and after marking, there was negligible difference between the recording point and the marked spot. A question may be raised as to whether or not the tectorial membrane might have been detached before marking. The finding of halved spots, part in the reticular membrane and part in the tectorial membrane, verified that the potentials recorded from the subtectorial space were measured under normal structural conditions.

Lawrence (1967) reported that the unstable potential between EP and the negative resting potential in the organ of Corti was around zero. By the newly developed marking technique with Alcian Blue, we were able to locate more accurately the electrode tip, and have determined that the potential in the subtectorial
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space is positive in polarity. If this potential were in fact zero, then LAWRENCE’s support of the tectorial membrane boundary hypothesis (NAFTALIN, 1965) would be justified. However, the present discovery that this potential is positive places the boundary in the reticular membrane instead. High potassium concentration is necessary to maintain a high sensitivity of the hair cells (TANAKA, 1963; KONISHI et al., 1966; HASHIMOTO and KATSUKI, 1972). From the present study it is apparent that the sensory hairs of the hair cells are actually exposed to the endolymph containing potassium at a high concentration.

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EXPLANATION OF PLATES

Plate I. Surface view of cobalt ion deposits following injection into Scala media in basal
turn of cochlea (animal: G-815). Deposits are seen as a dotted line along outer margin
of tectorial membrane. Scale: 50 μm.

Plate II. Alcian Blue spot on an unstained section of the basal turn of the cochlea (animal:
G-741). Spot is located on inner sulcus surface of vestibular lip. Scale: 50 μm.

Plate III. Alcian Blue spot on lower surface of tectorial membrane in basal turn of cochlea
(animal: G-792). Section counter stained with Fuchsin. Scale: 50 μm.

Plate IV. Split Alcian Blue spot in subtectorial surface of basal turn cochlea (animal: G-772).
One part is on reticular membrane and the other on lower surface of tectorial membrane.
Section counterstained with Fuchsin. Scale: 50 μm.
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