Paparella: Volume I: Basic Sciences and Related Principles

Section 2: Physiology

Part 1: Ear

Chapter 7: Introduction to Inner Ear (Fluid) Physiology

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It is difficult to study the physiology of the inner ear because the tissues that make up the auditory labyrinthine system are suspended in fluid, and any attempt to enter the fluid system causes the different kinds of fluids to mingle, thus destroying the normal function. Fortunately, some of the lower forms, especially the guinea pig, have a bony capsule for the inner ear, which protrudes into the middle ear space. The bone of this capsule is thin and somewhat transparent, which has made this particular animal a favorite one for experimental work, and, although there are gross structural differences related to the nature of skull design, there is probably little species difference so far as the basic physiology is concerned. Many of the experiments upon which our present knowledge of inner ear physiology is based have been carried out on the lower forms but, for the most part, the results can safely be inferred as true for humans as well.

A consideration of the physiologic processes of the ear is essentially a consideration of the properties of the fluids that surround the various tissues, and there are at least four purposes that these fluids serve:

1. The fluids provide the nutrients to, and remove the catabolic products from, those cells whose only contact with the blood is through the surrounding fluids.

2. The fluids provide the proper chemical (ionic) environment for energy transformation (vibration to nerve impulse) to take place.

3. The fluids are responsible for the relay of vibrations from the footplate of the stapes to the energy transforming elements.

4. The fluids control the pressure distribution within the system, if, indeed, there is any pressure.

A careful consideration of these purposes raises many questions of a more specific nature. What is known about the chemical constituents of the various fluids and what is the nature of exchange between blood and the fluids? What are the dynamic properties of these fluids? How do they circulate and does a hydrostatic pressure difference exists? In what way do these fluids contribute both to the mechanical properties of the inner ear and to the energy conversion process?

Actually, there are as yet no clear-cut answers to these questions, but progress is being made.

Fluid Components and Their Source

Fluid Components

If there is any area concerning the physiology of the inner ear about which more facts are known it is probably that concerning the chemical composition of the fluids, despite the fact that the area occupied by these fluids is extremely small and methods of analysis without contamination are difficult. There are many reviews in which listings of the various components in endolymph and perilymph can be found; see especially Fernandez (1967) and Maggio (1966).

Electrolytes. The most numerous investigations are those determining the electrolytes of the fluids. The earliest work was carried out by Kaieda (1930), who pooled the fluids of freshly killed sharks and found sodium, potassium, and chloride to be about twice as concentrated in endolymph as in perilymph.

In 1954, microchemical methods specifically adapted for the purpose were employed by Smith and co-workers (1954) in determining the electrolytes of the labyrinthine fluids as well as of serum and of cerebrospinal fluid in guinea pigs. Perilymph was withdrawn by piercing the round window with a Pyrex micropipette. Endolymph was taken from both the utricle and cochlea. Samples of cerebrospinal fluid were taken from the fourth ventricle, and blood was removed from the heart for serum analysis. The results are shown in Table 1.

Table 1. Electrolytes in Fluids of the Guinea Pig (mEq/L)

	Perilymph	Endolymph (Utricular)	CSF	Serum
Na	150.3	15.8	152	138.6
K	4.8	144.4	4.2	-
Cl	121.5	107.1	122.4	93.9.&

The remarkable thing about these findings is that endolymph, with its relatively high potassium content, resembles intracellular more than extracellular fluid. However, Rauch and Köstlin (1958) describe parotid gland secretions as also having a high potassium content.

Smith and co-workers were able to confirm their microchemical analyses by flame photometry in a few of the larger endolymphatic samples, although they state that the precision of measurements was less than with the chemical measures. These classic experiments were followed by others, and with some variation they have now been confirmed many times (Rauch and Köstlin, 1958; Rauch, 1963; Silverstein, 1966; and Ulrich et al, 1966).

Rauch and Köstlin (1958) reported, for the first time, the concentration of electrolytes in the inner ear fluids of humans. These do not differ in any great respect from the values given for the guinea pig. Rauch (1964) later reported these figures for living human beings in greater detail and added values for other electrolytes not previously determined. The histograms of Figure 1 summarize the results.

Proteins. In many different animals it has been shown that the protein content of perilymph is less than in blood serum and more than in endolymph and cerebrospinal fluid (Jensen and Vilstrup, 1954; Smith et al, 1954; Vilstrup and Jensen, 1954; Citron et al, 1956; Antonini et al, 1957; Miyake, 1960; Brosch, 1964). For humans Rauch and Köstlin (1958) reported total protein in mg pet 100 mL as serum, 7000; spinal fluid, 10 to 25; perilymph, 70 to 100; and endolymph, 20 to 30. As techniques were improved, many analytic methods were applied to determine the specific proteins present; Chevance and associates (1960) have summarized a series of their reports. Fritsch and Jolligg (1966), Palva and Raunio (1967), and Beck and Holz (1965) also reported on human perilymph and endolymph. The techniques have been applied to humans in diseased states (Silverstein and Schuknecht, 1966). The problem of contamination with erythrocytes and serum is a major one, and the results are not specific enough to give a concise list.

Other Characteristics

Some time before satisfactory methods were developed for analyzing the chemical constituents of the inner ear fluids, some of the more readily determined characteristics were measured. Generally, the interest was not based on curiosity concerning the measured physical attribute but on a desire to learn more about the metabolic processes occurring through the medium of the inner ear fluids, and to establish the characteristics by which these fluids differ. The major difference in working with the inner ear fluids is the paucity of material available. Maggio (1966) gives volume measures for cat, dog, and human estimated by calculating the area of cochlear partitions in histologic preparations. He reports for the cat that the volume of perilymph is 24.9 cu mm and the volume of endolymph is 2.91 ± 0.1 cu mm. It is interesting to note, however, that by sampling all the perilymph and endolymph that could be extracted by means of the capillary action of glass tubing, the quantities were very much smaller. From the cat he was able to withdraw only 6.77 cu mm of perilymph and 1.46 cu mm of endolymph. From the dog he was able to withdraw considerably more: 17.8 cu mm of perilymph and 6.1 cu mm of endolymph.

The total calculated volume of perilymph in humans is given as 78.3 cu mm, with only 2.76 cu mm for endolymph (two specimens).

Despite the small volumes of fluid, such characteristics as osmotic pressure, refractive index, specific gravity, viscosity, pH, and certain electrical properties have been determined.

Osmotic Pressure. Aldred and colleagues (1940) determined the osmotic pressure of blood, cerebrospinal fluid, perilymph, and endolymph by using a thermoelectric method employing especially small thermocouple loops. The round window of the cat was exposed and a sample of perilymph collected by piercing the membrane with a micropipette maneuvered by a micromanipulator. The round window membrane was then removed and another micropipette inserted through the basilar membrane after cerebrospinal fluid flowing into the area through the cochlear aqueduct was sucked away and the basilar membrane left dry. The fluid then rising in the pipette was considered to be endolymph.

Cerebrospinal fluid was collected from the cisterna magna, and a sample of blood was collected from the carotid artery.

The total osmotic pressure recorded by this method was expressed in equivalent percentages of NaCl (gm NaCl per 100 gm H_2O), subject to error of ± 0.005 per cent. The figures obtained were: blood, 0.994; cerebrospinal fluid, 1.017; perilymph, 1.046; and endolymph, 1.058.

Ledoux (1941) later used what was considered to be an improved thermoelectric method for very small samples, with results somewhat less than those listed above when expressed relative to the osmotic pressure of plasma.

Refractive Index. The first measures of the refractive index of the labyrinthine fluids were made by Szász (1923), who compared the refractive index of samples of cerebrospinal fluid obtained by cisternal puncture with that of perilymph samples obtained from inside the round window of dogs. The values he obtained were 1.335147 for labyrinthine fluid (perilymph) and 1.334270 for cerebrospinal fluid. From these results, he considered perilymph and cerebrospinal fluid to be different, but there is sufficient overlap in the spread of readings from his 17 tests to indicate chance alone could account for the differences in the means.

Ledoux (1941) also measured the refractive index in both dogs and cats, but his samples were too few and the overlap in readings too great to be of any significance.

Miyake (1953), in a study of the refractive index of cerebrospinal fluid in patients with nerve deafness, found it to be somewhat different from normal in all cases. The refractive index, as determined by "Pulfrich's refractometer", was said to be 1.33409 in the normal individual. He also made determinations in healthy rabbits of the refractive index of labyrinthine fluid, presumably perilymph, but this is not stated. This value he gives as 1.33546. not much different from the earlier measures. On the basis of his results Miyake decided that perilymph and cerebrospinal fluid are not the same.

Specific Gravity and Viscosity. So far, these characteristics have been determined only for pigeons, and the results do not agree very well (Rossi, 1914; and Money et al, 1966). The specific gravity and viscosity appear, within the limits of the experiments, to be greater for endolymph than for perilymph.

pH. Kaieda (1930) measured the pH of the inner ear fluids of the shark and obtained a value of 7.41 for perilymph, 7.23 for cerebrospinal fluid, and 7.36 for endolymph.

Ledoux (1943) determined these values in the cat as: endolymph, 7.82; perilymph, 7.87; cerebrospinal fluid, 7.45; and plasma, 7.33.

Because the transfer of the minute volumes of fluid, as carried out by Ledoux, could lead to diffusion of carbon dioxide out of the fluid, resulting in slightly high pH determinations, Misrahy and associates (1958) developed an electrochemical microelectrode technique to determine the pH of guinea pig endolymph and perilymph in situ. The active microelectrode was made by drawing soft glass capillary tubing to a tip of 10 to 12 microm and filling this with a specially prepared mixture of an antimony-Cerroseal alloy. The reference electrode was a glass micropipette with a 3-microm tip filled with a 0.9 per cent KCl solution into which a silver-silver chloride wire was placed. The pH of the perilymph exposed to the atmosphere was found to be 7.8 to 8.0 and that of the endolymph in situ 7.3

to 7.5. These values are lower and opposite to those found by Ledoux but are probably more reliable. Misrahy and co-workers found the pH to be very sensitive to changes in carbon dioxide tension, suggesting to them the possible presence in the endolymph of a bicarbonate buffer system.

Rauch and Köstlin (1958) give the pH in humans as perilymph 7.2; endolymph, 7.5; serum, 7.35; and spinal fluid, 7.35.

Blood-Endolymph Relationship

Until the chemical analyses of endolymph and perilymph were made it appeared entirely logical to assume that the stria vascularis is a special structure secreting endolymph. Corti, in 1851, made the statement, "One will be tempted to suppose a certain relationship between the vascular band (stria vascularis) in question and the secretion of endolymph". The supposition was further encouraged by the thought that one function of the fluids must be to separate the organ of Corti, sensitive to the slightest vibration or pulse, from its blood supply and, consequently, the fluid must convey the necessary nutrients to the sensory cells. But when it was found that endolymph has a high potassium content it was suggested that perilymph would be a more suitable solution for surrounding the hair cells and unmyelinated fibers within the organ of Corti, and then it was suggested that this must be an entirely different fluid. Engström suggested the name "cortilymph" (1953, 1960).

Whatever the specific nature of these fluids may be, it is obvious that, somehow, a source of nutrients and oxygen must move from a blood supply through fluid to the sensory cells. Complicating the possibilities for metabolic exchange in the vicinity of the hair cells, however, is the fact that two of these vascular areas, the stria vascularis and spiral prominence, border the endolymph, whereas the third lies beneath the basilar membrane as an arcade of capillaries within the tympanic lamella. The artery to the cochlea arises from the basilar artery within the cranial posterior fossa. It is, therefore, as Anson and associates (1966) have noted, completely separate from the blood supply of the otic capsule.

The arterial supply and venous drainage have been described, along with a review of the early literature, by Smith (1951) nd Scuderi and Del Bo (1952). Smith gives the description for the guinea pig ear. The cochlear artery (*arteria cochlearis propria*), along with the nerve fibers, enters the modiolus and gives off many branches as it spirals through the loose connective tissue between the nerve and bony wall to the apical turn. Relatively large primary branches give rise to coiled secondary branches that radiate either out over the scala vestibuli in the bone between two turns, or radiate toward the limbus and osseous spiral lamina, as shown in Figure 2.

The arteriole passing out over the scala vestibuli does not divide into terminal branches until it enters the spiral ligament. Except for one or two branches from each arteriole that turn in a spiral direction, all others descend into the spiral ligament, where they divided into four groups.

Group 1. Small branches given off from the arteriole as it enters the spiral ligament course in a spiral direction, usually above Reissner's membrane, and leave the area either by descending through the spiral ligament to the venules below or by turning upward to join the

collecting vein from the turn above.

Group 2. There is usually one branch from the arteriole that provides the capillary network of the stria vascularis. These capillaries follow a twisting course spirally between the epithelial cells with considerable interconnection and finally drain into a large venule that descends to the collecting venous system at the lower edge of the spiral ligament.

Group 3. A main branch descends behind the stria vascularis to enter the spiral prominence. It continues as a small vessel coursing in a spiral direction just beneath the epithelial cells. As it proceeds in this spiral course, it is drained by many vessels that descend into the collecting venules.

Group 4. The rest of the terminal branches descend in the depths of the spiral ligament to the collecting venules, making some interconnections in the region below the attachment of the basilar membrane.

The secondary branches coiling toward the osseous spiral lamina usually divide into two groups: one going to the limbus (Group 5), and the other to the plexus below the basilar membrane (Group 6).

The vessels entering the limbus remain in the connective tissue base of the limbus. After a succession of loops they descend to the upper bony plate of the spiral lamina, continuing toward the vein.

The vessels making up the plexus below the basilar membrane are extensions of vessels coursing outward either through the bone of the osseous lamina or directly on the surface of the nerve. After descending through the nerve they continue out below the fibers of the basilar membrane. Here, they first turn in a spiral direction to form an interrupted group below the inner pillars. There are many radial connections that eventually either descend directly to the posterior spiral vein or join the limbus vessels. A second group of vessels extend out in a spiral path below the tunnel. In some animals and in some turns, these "outer" spiral vessels may not be present (Smith, 1954). The two spiral groups may be connected by capillary bridges, but each has direct arterial and venous connections from the modiolus. Both groups of spiral vessels remain below the basilar membrane and are separated from the perilymphatic space by cells of the tympanic lamella and a thin layer of basilar membrane cells. Pericapillary spaces around these vessels have been described (Hawkins, 1968).

The various branches return to the modiolus after traversing either the bone or nerve channel and collect together in venules receiving branches from the spiral ganglion to end in the posterior spiral vein.

There are, then, three areas supplied with sufficient capillaries to provide nutrients and oxygen to the hair cells: the stria vascularis, the spiral prominence, and the osseous lamina vessels. A description of this vascular system in humans has been presented in a monograph by Axelsson (1968).

Perlman and Kimura (1955) reviewed earlier work (Krejci and Bornschein, 1951; Seymour and Tappin, 1951; Scuderi and Del Bo, 1952; and Weille et al, 1954) involving histologic and direct observations of the vessels of the spiral ligament and stria vascularis during stimulation of the cervical sympathetic ganglion, during direct application of epinephrine and norepinephrine, and following anaphylactic shock, all of which had varying results.

Their own technique for studying the vessels of the stria vascularis was an improvement over those of earlier workers. The bone over the spiral ligament of the fourth turn of the guinea pig was removed and the area was illuminated by an air-cooled 1000-watt light through a quartz rod. The animal's head was held rigidly and motion pictures were taken through a microscope with magnifications of from 90 to 165 times. Occasionally, magnifications of 260 and 390 times were used.

The blood flow appeared remarkably stable, and no spontaneous constriction or dilatation could be observed. Cutting the cervical trunk above the stellate ganglion or electrical stimulation of the stellate ganglion, cervical trunk, superior cervical ganglion, vertebral artery, basilar artery, and anterior inferior cerebellar artery did not produce visible changes in the observed strial vessels or in the rate of blood flow. Asphyxia and anaphylactic shock did produce changes.

Kimura and Perlman (1956, 1958) demonstrated, histologically, profound degeneration in the structures of the scala media following surgical obstruction of the inferior cochlear vein and the anterior inferior cerebellar artery. The hair cells were most vulnerable and, interestingly, the inner hair cells degenerated before the outer with arterial obstruction, whereas the outer hair cells showed disintegration before the inner hair cells following venous obstruction. Structures of the spiral ligament also degenerated. In a later experiment, Perlman and associates (1959) showed a decrease in the electrical response of the cochlea on temporary obstruction of the internal auditory artery.

Alford and co-workers (1965) showed that the injection of small plastic beads produced microscopic intravascular occlusion of the terminal branches of the arterial supply. The occasionally produced loss of hair cells in the presence of an apparently normal stria vascularis.

Although arterial and venous obstruction experiments have shown a direct relationship between blood flow to the structures of the spiral ligament and degeneration of the sensory epithelium, they do not show how the vascular areas are related to the fluids of the scala media.

Lawrence (1966) carried out an experiment the results of which indicate the capillary area responsible for the fluid exchange necessary for the maintenance of the hair cells. With the aid of an operating microscope and using aseptic conditions, entrance high up in the scale vestibuli of a selected turn was made by a sterile hand-held drill honed to a triangular point of less than 100 microm. A small probe of approximately 25 microm at the tip was then inserted by hand through the opening and through the bony modiolar wall in those instances in which interruption of blood supply to the vessels of the basilar membrane was desired. By back-lightning the cochlea, the vessels descending into the spiral ligament were seen and

avoided. In other animals, these latter vessels alone were interrupted.

A period of recovery of from 4 to 16 weeks followed the above procedure, at which time the animals were killed and prepared for histologic examination. It was found that occlusion of the vessels passing from the modiolus over the scala vestibuli to the stria vascularis resulted in its degeneration but had no effect upon the organ of Corti. Occlusion of only the modiolar vessels going to the capillary loops beneath the basilar membrane resulted in loss of hair cells in the presence of a histologically normal stria vascularis and spiral prominence, indicating these vessels as the source of nutrients for the organ of Corti. Cortilymph is most likely supplied by these vessels. The structures on the endolymphatic wall of the spiral ligament may maintain the ionic content of the endolymph essential to the energy transformation process.

Some supporting evidence for the importance of these spiral vessels in maintaining the organ of Corti is found in the examination of the cochleas of newborn Shaker-1 mice, which are known to lose their auditory function in a few days after the third to fourth week following birth (Grüneberg et al, 1940). Kikuchi and Hilding (1967) noted in these mice that the spiral vessel beneath the tunnel of Corti undergoes involution and loses its lumen along with subsequent degeneration of hair cells and neural elements in the organ of Corti. At this stage and until well into the second month after birth, the stria vascularis appears almost normal by electron microscopy.

It is also interesting to note that vasomotor innervation is supplied to the spiral vessels by unmyelinated fibers that accompany the myelinated cochlear fibers. The unmyelinated fibers descend to pierce the basilar membrane and terminate on the vessels (Hawkins, 1968). Somewhat similar observations have been made by Terayama and associates (1966).

Balogh and Koburg (1965) describe a special arrangement of cochlear blood supply in the "tractus arteriosus", which spirals about the cochlear nerve in the modiolus. In this region the cochlear artery supplies a number of capillary twigs to a band of tissue containing epithelial cells resembling those of the choroid plexus. Because of this similarity they have called this region the *cochlear plexus* and have ascribed to it a secretory role in the formation of fluid of the perivascular spaces of the modiolus passing along the nerve fibers to the spiral ganglion and possibly reaching the interior of the organ of Corti. There are other papers concerning this cochlear plexus (Müsebeck, 1965).

Many attempts have been made to trace substances from the blood capillaries into the fluids of the inner ear in order to determine the possible pathway from blood to fluid. Yamamoto and Nakai (1964) have reviewed some of the earlier papers appearing in Japanese journals and refer to the assertion that neither the stria vascularis nor the spiral ligament constitutes a pathway for metabolites from blood vessels to the endolymphatic sac or perilymphatic spaces. They cite the experimental work of Nomura (1961), who observed the small vessels of the spiral ligament and stria vascularis after intracardial injection of 5 to 10 mL of 1 per cent trypan blue solution and concluded that in the stria vascularis a barrier exists between it and blood.

Yamamoto and Nakai (1964) attempted to determine whether such a barrier exists. Iron dextran particles (19 nanom) were injected into blood vessels and into the endolymphatic

and perilymphatic spaces of guinea pigs. Following injection the animals were allowed to survive for various periods of time. They were then decapitated and the ears examined by electron microscopy.

The iron dextran particles injected into the blood vessels appeared in the stria vascularis and the spiral ligament in 10 minutes after injection and remained there for a subsequent 10 hours, but no particles were observed in the endolymph. Thus, there appears to be a barrier to the passage of these small particles even though, when used in the glomerular infiltration test, they easily enter glomerular cells through endothelial cells and the basement membrane.

The authors offer a discussion of related works, many of which are not referred to here, and conclude that this barrier probably exists for some specific substances but not for all substances. They bring out the observations made by other that the radioactive form of several elements has been observed to move into the fluid spaces from the blood (Hayashido, 1950; Ledoux, 1950; Rüedi, 1951; and Portmann et al, 1960).

From the experimental results described so far, certain conclusions seem to have evolved. It would appear that the organ of Corti does have a blood supply, the arcade of spiral vessels beneath the tunnel and basilar membrane, and that this supply serves at least one function: providing for the exchange of metabolites within the sensory epithelium. The structures along the wall of the spiral ligament serve other purposes, the most likely of which, and the one for which there is the most evidence, being control of ions and perhaps other substances like water and inorganic substances necessary for the energy transforming properties of the sensory epithelium.

Blood-Perilymph-Cerebrospinal Fluid Relationship

Interestingly, many of the studies on blood-endolymph relationship employing the movement of radioactive ions indicated considerable difference between perilymph and cerebrospinal fluid in the accumulation of this material. Choo and Tabowitz (1964) noticed that 15 to 24 hours following intraperitoneal injection of radioactive sodium (22Na), the rise of concentration in the perilymph was somewhat less than that in the cerebrospinal fluid, an observation that had also been made by Rüedi (1951). In another report (1965) they found, following intraperitoneal injection of radioactive potassium, 42K, that for up to 15 hours following the injection, the 42K concentration was slightly higher in perilymph than in cerebrospinal fluid. During the period of 15 to 48 hours the perilymph concentration increased five-fold, whereas the cerebrospinal fluid concentration increased two-fold.

Schreiner (1966), using radioactive substances, showed evidence that perilymph originates from the perilymphatic space itself.

These observations introduce the possibility that the blood-perilymph exchange is more predominant than the cerebrospinal fluid-perilymph exchange. Kley (1951) has emphasized this on the basis of his results from accumulation of fluorescein in perilymph after injection into the bloodstream.

The many differences in chemical and physical characteristics between perilymph and cerebrospinal fluid are themselves sufficient evidence that these fluids are not the same, even though the cochlear aqueduct is a channel connecting cerebrospinal fluid to the perilymphatic space of the scala tympani in the region of the round window. Those investigators collecting samples from behind the round window in the guinea pig and cat have always mentioned the necessity of taking a sample quickly before cerebrospinal fluid flowing through the cochlear aqueduct contaminated the area. And yet these samples showed the two fluids to be chemically different.

Studies of radioactive electrolyte concentration in the two fluids after injection into the blood or intraperitoneally have also shown a difference.

There seem to be, then, several possibilities for the source of perilymph: (1) It can be an ultrafiltrate of blood arising from some appropriate vascular area in the inner ear itself. (2) It can arise from endolymph, its chemical constituents being determined by the properties of the membranes separating the two fluids. (3) It can be a direct product of cerebrospinal fluid, perhaps acquiring a different chemical nature because the exchange between the two fluids is slow.

The commonly accepted view is that the aqueduct provides an open connection between the two fluids. This stems primarily from early anatomic studies, especially in the lower animals, in which there is no doubt about the patency of the duct. (For review see Grünberg, 1922; Karlefors, 1924; and Gerlach, 1939). However, this has been difficult to establish in primates, in which the duct is long and narrow; in some places in the course of the duct, especially near its junction with the scala tympani, the membranous lining almost fills the entire space. Karlefors (1924) reported patients in whom hearing was present before death, but in whom subsequent histologic examination of the temporal bones showed places where the lumen of the aqueduct was occluded. Uyama (1933) blocked the aqueduct in rabbits and, although he found a bulging of Reissner's membrane into the scala vestibuli, the organ of Corti was everywhere present and of good appearance. Although he concluded that the bulging of Reissner's membrane indicated that perilymph comes from cerebrospinal fluid and that the aqueduct was the pathway, one could reason that the bulging was produced by an overproduction of endolymph resulting from his method of occluding the duct.

The most frequently used method of investigating the communication of perilymph with cerebrospinal fluid is that of injecting into the perilymphatic space or the subarachnoid space identifiable material so that later histologic examination would reveal the distribution of this foreign matter. The use of the method is over 100 years old, dating back to Schwalbe (1869), who used techniques similar to those employed today, although Cotugno reported in 1774 the patency of the bony canal, which he demonstrated by forcing mercury through it. Subsequent investigations are reviewed by Lempert and associates (1952). The results of present-day experiments seem to depend on the species of animal used.

The experiments of Altmann and Waltner (1947) demonstrate this difference. They injected a mixture of iron ammonium citrate and potassium ferrocyanide solution into the cisterna magna of rabbits, cats, and monkeys. Immersing the tissues that had been removed from the killed animal for a period of time in hydrochloric acid produced a precipitation of particles of ferric ferrocyanide. In the rabbits and cats, the particles were found in the scala

tympani and often in the scala vestibuli, whereas in only one of nine monkeys was the precipitate found in the cochlea itself, although it was present in the cranial end of the cochlear aqueduct. Svane-Knudsen (1958) has carried out similar experiments on guinea pigs, with results comparable to those of nonprimates.

Schuknecht and Seifi (1963) injected avian erythrocytes into the subarachnoid space of the posterior fossa of cats. Subsequent histologic examination of the temporal bones of these animals, which were allowed to live for progressive periods of time following injection, revealed many erythrocytes caught in large quantities in the connective tissue network of the cochlear aqueduct and, in many ears, in the perilymphatic spaces. From these results, the investigators concluded that there is a flow of fluid from the subarachnoid space to the perilymph. It is to be noted, however, that when they blocked the aqueduct for periods up to 10 months no changes appeared in the cochlea, from which they conclude that the aqueduct is not necessary for maintenance of perilymph volume.

That there is some exchange between perilymph and cerebrospinal fluid, and that this exchange is in either one or both directions in animals lower than primates, is attested to by what has come to be known as the "Schreiner phenomenon" (1961). Schreiner found that after introducing radioactive phosphorus into the perilymph of one ear, the radioactivity of the perilymph of the opposite ear 1 hour later was considerably higher than that of either serum or cerebrospinal fluid obtained by suboccipital puncture. This phenomenon has been further investigated by Krochmalska and co-workers (1967).

Experiments with primates give different results, as Altmann and Waltner have shown. Lempert and associates (1952) injected an aqueous suspension of colloidal carbon into the cisterna magna of seven monkeys. These animals were sacrificed at various periods of time following injection, and none was found to have carbon particles in the perilymph.

Ritter and Lawrence (1965) reported a study in humans. Eighteen patients, scheduled to undergo stapes surgery for deafness due to otosclerosis, had injected into their spinal fluids, at various time periods before surgery, indigo carmin dye or a radioactive protein (radioiodinated serum albumin). In none of these patients did the perilymph, at the time of surgery, show detectable coloring by the dye or any radioactivity. On the other hand, there are occasional reports of excessive amounts of cerebrospinal fluid escaping from the oval window when the stapes is removed during human stapes surgery.

It is glaringly evident that the patency of the cochlear aqueduct varies considerably among different species, within a species, and even at various stages of life in humans (Lawrence, 1965). During fetal life and in the newborn, the aqueduct is wide open, whereas in the human adult and the monkey, the duct passes through a long bony channel. Considering these anatomic variations and the inconsistency of experimental evidence, it would seem most likely that the actual passage of fluid through this duct is not what gives perilymph its chemical and physical characteristics.

There is a vascular area associated with the perilymphatic spaces that has been suggested as a possible source of at least the special properties of perilymph. This area (Group 1 in Fig. 2) is to be found in the thin portion of the spiral ligament "above" the attachment of Reissner's membrane, in which the capillaries branching from the radial

arterioles arching over the scala vestibuli run more or less longitudinally. Hawkins (1968) gives this serious consideration, founded on his excellent microscopic studies. He described these capillaries as being surrounded by well-defined pericapillary spaces often interconnected by avascular channels, with the capillary pressure high enough to move fluid by filtration outward into the perilymph.

This is not a new idea. Just about every investigator who has compared perilymph with cerebrospinal fluid has come to this conclusion, and Mygind (1948) was on of the earlier writers to insist that perilymph arises from the inner ear. The perilymph is presumably resorbed in the lower spiral ligament near the basilar membrane in the scala tympani (Svane-Knudsen, 1958; and Kirikae et al, 1961).

Perilymph appears to be a unique fluid, acquiring its properties from the blood but connected with the cerebrospinal fluid through a duct that need not necessarily be patent in the sense of a direct fluid flow through it. Perhaps the connective tissue system is one of regulating pressures and controlling the slow circulation of the fluids, which brings up the consideration of the mechanical relationships of the fluids.

Dynamic Properties of the Fluids

There are tissues in the inner ear, such as Reissner's membrane and cells of the organ of Corti, that are immersed in fluids and could not survive if there were not replenishment of fluid contents and removal of waste. Also, study has shown the concentrations of various electrolytes and proteins to be very different on opposite sides of the separating membranes, so there must be movement of water or transport of ions to maintain the normal condition. The fluids, in a sense, must flow, but this need not necessarily be in the sense of a source, a conduit, and a sink. There may be constant exchange throughout the fluid system, but this may always be in delicate balance so that there is no buildup of pressure or failure in the circulation. There has been much speculation concerning pressure and circulatory relations in the fluids of the inner ear, and there has also been considerable contradictory evidence. Some patterns of significance are beginning to emerge, however.

Fluid Pressure

Except for those ducts that extend into the cranial cavity, and the round window membrane exposed to the middle ear, the entire fluid system of the inner ear is encased in bone. If the fluids were under any pressure other than that necessary to fill the inner ear, one might expect an excessive bulging of the round window membrane or perhaps a distention of the endolymphatic sac. Capillary pressures would have to be high to interact with fluid, or the capillaries would be taking in fluid. If relative pressure difference within the inner ear fluids is necessary, one might expect deviations from this normal situation to result in a change in function (hearing level).

Hansen (1968) took up the question of the relation between cerebrospinal fluid and hearing for pure tones. His material consisted of consecutively selected patients from the Department of Neurosurgery on whom, for a period of about 3 years, the pressure of the cerebrospinal fluid in the lateral ventricles of the hemispheres was measured in connection with intracranial ventriculography. Otologic and audiometric examinations were carried out

in the Department of Otolaryngology. The distribution of cerebrospinal fluid pressures ranged from the categories 0 to 200 mm H_2O to 1000 to 2000 mm H_2O. In 108 patients, no correlation between hearing and a raised intracranial pressure could be established.

Klockhoff and co-workers (1966) have reported changes in the acoustic impedance of the middle ear with what the investigators have described as increases in craniolabyrinthine pressure. This increase in intracranial pressure was produced by compressing the cervical veins by a pressure cuff around the neck of the subject. As the pressure in the cuff reached 30 mm Hg, the impedance shift reached a maximum and the subject usually reported an attenuation of the carrier tone (550 Hz), presumably indicating a diminished sound transmission. Further observations on this type of impedance change were made on cats and guinea pigs, and the authors believed that the effect was due to a direct transmission of intracranial pressure to the fluids of the inner ear. It is unfortunate that hearing tests were not carried out in these animals and that careful observations were not made of the blood vessels of the tympanic membrane and intratympanic muscles that might affect impedance.

Many investigators have demonstrated that intracranial pressure can be transmitted to the labyrinthine fluids in animals lower than the primate (Szász, 1926; Meurman, 1929; Hughson, 1932; Kobrak et al, 1933, 1940; Ahlén, 1947; Filippi, 1950; and Krejci and Bornschein, 1951). Most of these have been experiments in which the changes in labyrinthine pressure were measured by a capillary manometer, by changes in the round window, or by similar observations.

Following the lead of Hughson (1932), Allen and various associates carried out a series of experiments in which the effects of cerebrospinal fluid pressure changes were determined by observing the changes in the magnitude of the cochlear potentials.

Allen and Habibi (1962) recorded, by means of a silver wire foil electrode on the edge of the round window, the electrical potentials arising from the cat's ear when it is stimulated by sound. Alterations in the cerebrospinal fluid pressure were produced by inserting a fine polyethylene tube into a small dural slit made through a lumbar laminectomy. They observed a quickly reversible reduction in the amplitude of the electrical response with increases of cerebrospinal fluid pressure. A prolonged pressure, the electrical response sustained small permanent loss. The reversible loss was attributed to an impairment in the sound transmission system, whereas the permanent loss, they thought, might be caused by changes in some metabolic factor, such as anoxia resulting from compression of blood vessels.

On the other hand, Gulick and co-workers (1962) found the decrease in the electrical response of the cochlea to be very slight when up to 50 cu mm of perilymph was removed from the scala tympani. However, there was a slow progressive degeneration in the response if the perilymph was allowed to leak out continuously.

Later Kerth and Allen (1963) compared the perilymphatic fluid pressure changes with those made in the cerebrospinal fluid. They attempted to verity the observations of Kley (1951), who exposed the cochlear aqueduct in guinea pigs and observed the flow of fluid into the scala tympani. The flow, although very slow, still continued after plugging of the aqueduct. Kerth and Allen, however, observed no increase in perilymphatic pressure with increases in cerebrospinal fluid pressure after blockage of the aqueduct.

Feldman and Allen (1966) made a histologic examination of the cochlear structures of seven cats that had been intravitally perfused while the cerebrospinal fluid pressure was elevated 11 cm Hg above normal. There was no evidence of injury or alteration in any cochlear structure as a result of the pressure increase. Because of this they contend that the electrical changes seen in earlier experiments must have been the result of reversible mechanical alterations.

Following these observations Allen (1964) proposed a theory of pressure balance, which seems quite reasonable (Lawrence, 1965). He suggests that the endolymphatic sac functions to transmit the increased intracranial pressure to the endolymph so as to equalize pressure increase to the perilymph through the cochlear aqueduct, thus preventing changes in position of Reissner's or the basilar membrane (Fig. 4). He points out that this kind of mechanism must be operating to account for the constancy of auditory function during changes in cerebrospinal fluid pressure accompanying the many shifts in bodily position during a person's normal activities. However, as described later, other experiments in primates and in human temporal bones indicate that changes in perilymphatic pressure alone may not have a very profound effect.

In freshly removed human temporal bones, Békésy (1960) determined the effects of static pressure increase of the perilymph on the movement of the stapes. A needle was cemented into an opening made in the bony wall of a semicircular canal and the pressure of the perilymph was increased through a water-filled tube connected to the needle and to a syringe, the plunger of which was weighted and continuously rotated so as to produce a smooth pressure. He found that at a pressure of four to seven atmospheres the round window would break but that the stapes was not visually affected. After the preliminary measures, the volume displacement of the round window for frequencies of 300 to 1000 Hz was measured. No changes were observed as the pressure was increased until damage occurred to the inner ear and bone, allowing fluid to escape.

The effect of increases in perilymphatic pressure has been determined in the monkey, with the electrical potentials of the ear serving as an indicator. Lempert and co-workers (1949) sealed a No. 25 hypodermic needle into an opening made in the external semicircular canal so that measured air pressure could be applied to the fluid. The electrical response of the ear was recorded from a platinum foil electrode in contact with the round window membrane. Increases of perilymphatic pressure up to 50 mm Hg had no effect on the magnitude of the cochlear potential.

As pointed out before, there is quite a difference among species in the size of the lumen of the cochlear aqueduct. Allen's measurements were made in the cat, and pressure increases went directly through the aqueduct to the scala tympani. In these instances, increases in perilymphatic pressure had an effect on the electrical response of the ear. Perilymphatic pressure increases through the semicircular canals may be a different situation. Békésy's measurement may not have been sensitive enough, and the pressure may have been more evenly applied or more equally distributed in the Lempert experiments than in the Allen experiments. Any interference that might occur in inner ear function must come about through unequal pressure within the fluid systems of the inner ear, but it is unlikely that such a condition can occur naturally; Reissner's membrane is much to delicate to sustain much of a pressure gradient.

However, there are studies that have reported differences in static pressure between the endolymph and perilymph in normal ears. Weille and associates (1958, 1961) used a specially designed electromanometer, monitored the position of the tip by recording the DC potential within the scala media, and obtained measurements indicating that the pressure of perilymph in the living guinea pig is greater than pressure of the endolymph, but the results are much too variable to be quantified.

It is of interest that Henriksson and co-workers (1966), in a study of the effects of pressure variations on the frog labyrinth, noted that when the bony perilabyrinthine capsule was removed, the perilymphatic wall could be seen bulging somewhat outwardly, regaining its shape after having been slightly compressed by some instrument. When the membrane was penetrated and the perilymphatic fluid sucked away, the saccule seemed to lose its shape and become flatter, indicating a small or nonexistent pressure gradient between endolymph and perilymph.

Circulation

Corti (1851), in describing the stria vascularis, suggested that it might be the source of fluid in the inner ear. Until that time it had not been known that there is a completely separate fluid system in the auditory part of the labyrinth as well as in the nonauditory part. Then, in the same year, Reissner (1851) described the membrane which now bears his name and divides the scala vestibuli from the scala media. The nature of this closed membranous system gave the suggestion that endolymph provides the nutrients to the organ of Corti, being secreted by the stria vascularis and flowing along the cochlear duct, eventually to be absorbed by the endolymphatic sac. This interpretation was classified by Lawrence and co-workers (1961) as "longitudinal flow".

However, it was also suggested by many that the endolymph is not only secreted along the cochlear duct but is also absorbed by other structures along the duct. This was characterized as "radial flow".

We have seen earlier that nutrients for the organ of Corti do not come from endolymph but rather from cortilymph provided by the spiral vessels of the osseous spiral lamina. But it is important to see what the endolymph does and how it is replenished.

Very little experimental work had been done on the circulation of endolymph until that of Guild (1927). Through a small pipette Guild injected a solution of potassium ferrocyanide and iron ammonium citrate into the scala media of several living guinea pigs. After the lapse of various time intervals the animals were sacrificed and preserved for histologic examination. The acid in the fixation fluid precipitated Prussian blue granules in sites along the scala media. The temporal bones of these animals were then sectioned and mounted serially so that the location of the granules could be studied with the microscope. In 16 of 20 animals, the blue granules were found in the walls of the endolymphatic sac. From this, Guild concluded that the flow of endolymph was from the stria vascularis down the scala media through the ductus reuniens to the sacculus, ending finally in the endolymphatic sac after passing through the endolymphatic duct.

Some time later Anderson (1948) carried out an experiment in which he injected trypan blue intraperitoneally into a series of guinea pigs. This dye was later found in the endolymphatic sac but never in the endolymph, a condition which he explained as resulting from a concentration of stain too low to be observable. The dye is gradually absorbed in the sac, and this investigator thought the longitudinal flow of endolymph was indicated. It is possible, of course, that trypan blue did not show up in the endolymphatic fluids for other reasons.

Altmann and Waltner (1950), like Guild, injected iron salt solutions of various concentrations into the subarachnoid space of rabbits and monkeys. The iron salts were later found in the endolymph, in various areas of the scala media, and in the endolymphatic sac. The authors consequently favored a longitudinal flow.

Lundquist and associates (1964) injected a colloidal solution of 0.25 per cent silver into the basal turn of the cochlea of guinea pigs. These animals were killed 24 hours after the injection and the endolymphatic sac was prepared for electron microscopic examination. The silver granules were found in significant amounts only in the endolymphatic sac, where the cells of the epithelial wall appeared to have a considerable capacity to act as macrophages.

Ishii and co-workers (1966) injected foreign protein (peroxidase) into the cochlear duct; after 2 days this material was found phagocytized in cells floating free in the endolymphatic sac but not in the lining cells or elsewhere in the membranous labyrinth. They made other observations with radioactive carbon-labeled foreign protein and believed that all their findings were in agreement with the concept that endolymph flows from the cochlear duct to the endolymphatic sac. Koburg and associates (1967), following similar experiments, came to the same conclusion.

These experiments, however, have not gone without their critics. Seymour (1954), in a lengthy report, said that his studies on the histology of the saccus endolymphaticus in human and animal specimens had convinced him that the function of the sac is to secrete endolymph.

Van Egmond and Brinkman (1956) injected guinea pigs subcutaneously with a 0.5 per cent solution of trypan blue. This was later found in the endolymphatic sac. In two animals, a small quantity of blue mucous fluid was found in the vicinity of Bast's utriculymphatic valve. They claimed that this filling of the ductus endolymphatics was an indication that the flow of endolymph is in the direction of sac to sacculus - a longitudinal flow in the opposite direction from that suggested by Guild.

Controversy of equal magnitude has concerned the other end of the system. Smith (1957) says that morphologic evidence, including her electron microscopy observations, leaves little doubt that the stria vascularis and spiral prominence participate in the production of endolymph. Johnson and Spoendlin (1966) support this view, and they add that there may be several exchange mechanisms between endolymph and stria vascularis.

Chou and Rodgers (1962) determined by cartesian diver respirometry the rate of oxygen consumption by tissues lining the membranous labyrinth and came to the conclusion that the stria vascularis and the walls of the utricle and saccule are all involved in the

formation and maintenance of endolymph.

That the situation is not simply a secretion of the endolymph within the scala media with a flow toward the endolymphatic sac was questioned by many investigators, and theories of more complicated flow patterns evolved. Borghesan (1957) has said that the stria vascularis cannot secrete endolymph because its histologic structure is inadequate. However, he does infer that perhaps the stria secretes crystalloids while the spiral prominence secretes the plasma, which, when these substances get together, make up endolymph. He proposes a theory of circulation that falls in the category of radial flow (Lawrence et al, 1961). Borghesan believes that the endolymph, once formed as described, is absorbed by Hensen's cells, where it is transferred to the hair cells. This endolymph, containing catabolites, pours into the tunnel of Corti, flowing into the internal spiral sulcus, where it is absorbed through the interdental furrows of the limbus. Borghesan has published many other papers, supporting this idea with histologic observations.

It is fairly safe to say that just about every separate structure found along the walls of the membranous labyrinth has been allotted by many different investigators the attribute of either secreting or absorbing endolymph. These are all listed in table form in Rauch (1964) and need not again be reviewed.

The problem of longitudinal flow was further complicated by the observation made by several investigators that blockage of the endolymphatic duct did not produce any evidence of accumulated endolymph in the scala media, nor did it produce any other changes. Lindsay (1947) found no changes in the scala media of the monkey after obliteration of the endolymphatic sac and duct. Lindsay and colleagues (1952) and Schuknecht and Kimura (1953) found that cats suffered no histologic or functional injury following loss of the endolymphatic duct and sac. Whereas these observations were rather disconcerting to those interested in determining the pattern of circulation of the endolymph, they did promote investigation into the other possibilities. And then, blocking the endolymphatic duct in the guinea pig was found to produce hydrops in the labyrinth (Kimura and Schuknecht, 1965) and combined theories have evolved.

Of all the radial flow theories, that of Naftalin and Harrison (1958) has been the most thorough in considering the necessity of accounting for the unique distribution of cations within the fluids. Their theory of fluid exchange is shown in Figure 3. These authors suggest that the fluid flow proceeds from perilymph through Reissner's membrane to endolymph, with the stria vascularis acting as a selective absorbing site. A function of Reissner's membrane is to prevent the flow of potassium from the scala media to the scala vestibuli. The stria vascularis, by an ion exchange system analogous to that of the renal tubular cell, extracts sodium and exchanges potassium for it. Potassium cannot pass Reissner's membrane except by relatively slow equimolar exchange for sodium so that the amount of potassium builds up in the scala media until the required concentration is reached. The concentration of potassium in the endolymph is dependent upon the ratio of the volume of endolymph to the plasma flow through the stria vascularis per unit time, and equilibrium is determined by the structural characteristics of Reissner's membrane. Support for these speculations was later provided by Rauch and associates (1962, 1963) in experiments described below.

These contrasting points of view, the longitudinal flow and the radial flow theories, typify the confusion that was widespread regarding the circulation of inner ear fluids years ago. In 1961, Dobbing wrote a review on the blood-brain barrier in which he recommended that the use of foreign material for determining such things as the direction of cerebrospinal fluid flow be discarded. He arrived at this conclusion after a review of all the relevant experimental evidence, which seems to show that such procedures do not give valid results. These substances, injected into the body tissues, are not normal solutions and the reaction of tissues may be different from what it would be under normal conditions.

With this in mind Lawrence and co-workers (1961) attempted in a series of experiments to obtain some indication of direction and rate of endolymph flow without introducing any foreign matter. It was first necessary to determine whether the *pars superior* (semicircular canals and utricle) and *pars inferior* (cochlea and saccule) are capable of surviving alone; ie whether the flow of endolymph from both parts is toward the endolymphatic duct so that each system can exist without the other, and to determine to what extent restricted parts of the system are independent of others.

It had already been reported by Wever and colleagues (1956) that single semicircular canals in the monkey can be destroyed without the remainder of either the vestibular or cochlear portion degenerating. Kristensen (1960) reported the same results. Actually, such evidence dates back to 1842, when Flourens demonstrated that when separate parts of the vestibular labyrinth were destroyed, the others remained functional.

In the Lawrence experiments (1961), sensitivity to vibrations of the organ of Corti in cats and guinea pigs was determined by recording cochlear potentials before and after various time lapses following selective rupture of the membranous walls of the labyrinth. The animal's internal ears were then examined histologically. The experiments demonstrated that surgical injury to particular parts of the scala media did not result in complete deterioration of the organ of Corti either toward the apex or toward the base. In fact, the region of injury seems to remain confined to that particular spot unless damage is done to the bone of the walls of the cochlea or vestibule, in which case the entire area may fill with bone. There are also indications from earlier work (Lawrence and Yantis, 1957) that, in the case of injury to Reissner's membrane, it can repair itself, thus reestablishing the continuity of the scala media.

Because perilymph is toxic to the organ of Corti, one might expect a general deterioration following breakdown of Reissner's membrane. Davis (1955) showed that injections of artificial perilymph into the scala media abolish, in the region of injection, the electrical response of the organ of Corti. Békésy (1960) has reported that, as he looked through Reissner's membrane into the organ of Corti, if perilymph flows through a break in Reissner's membrane, thus contaminating the endolymph of the scala media, the hair cells, normally appearing as oil-like droplets, become opaque and the organ of Corti becomes nonfunctional. On the basis of this evidence, and assuming a longitudinal flow of endolymph, one would expect that a tear in Reissner's membrane would allow the perilymph to flow toward the base within the scala media, destroying the organ of Corti from the region of the Reissner's membrane tear all the way basally to the ductus reuniens.

There is very clear evidence that, should Reissner's membrane break, perilymph enters the scala media rather than endolymph flowing out in the scala vestibuli. By means of a

special stain (Lawrence and Clapper, 1961), which stains endolymph darker than perilymph, Lawrence has shown (1960, 1964) that a rupture in Reissner's membrane allows perilymph to flow into the scala media, and that the area of fluid mingling remains confined to the region of the rupture, which had been produced from within by excessive vibration of the cochlear partition.

Another set of experiments was performed (Lawrence et al, 1961) to measure the flow of fluids by recording the cochlear potentials through time, by means of implanted electrodes, as a small surgical tear was produced in Reissner's membrane of the second turn of the guinea pig cochlea. No marked decrease in the electrical response to high frequencies, which have their locus of maximum activity in the basal turn, was observed in the basal turn over a period of 4 hours following rupture of Reissner's membrane in the second turn. This would indicate that perilymph, in toxic contraction, was not carried basally over the organ of Corti by a longitudinal flow of endolymph.

This evidence suggests that the circulation of endolymph is local *and* radial and that any specific area of the organ of Corti is nutritionally independent of adjacent areas. Secretion and absorption of nutrient and waste material appear to take place continuously along the length of the scala media. Of interest is how specific this local circulation might be.

Small local surgical lesions were made in Reissner's membrane in a series of guinea pigs, and the animals allowed to recover for from 1 to 10 weeks, at which time they were killed and their inner ears examined histologically (Lawrence, 1966). All the animals showed very localized degeneration of the hair cells of the organ of Corti. The smaller the opening in Reissner's membrane, the more restricted the extent of degeneration. These observations were confirmed and the degenerated areas studied by electron microscopy by Duvall (1968; Duvall and Rhodes, 1967). It has also been shown that vital stains injected into the scala media through a small opening in Reissner's membrane remain localized (Duvall and Tonndorf, 1962; and Tonndorf et al, 1962).

There was some question as to whether these exposures through the bone, to provide access to Reissner's membrane so it could be slit, might not have interfered with the blood supply crossing the bony bridge between turns to the stria vascularis of the operated turn. Further experiments (Lawrence, 1966), however, demonstrated that it is actually the toxicity of perilymph that causes the hair cells to degenerate. These same experiments revealed the importance of the basilar membrane and spiral vessels to the maintenance of the hair cells.

Since Kimura (1967; Kimura and Schuknecht, 1965) reported that he had successfully produced an overaccumulation of endolymph, or hydrops, in guinea pigs by blocking the endolymphatic duct, one must now interpret the normal flow of endolymph as being very slow toward the endolymphatic duct and sac. But there is constant chemical exchange between endolymph and the structures of the spiral ligament as well as with perilymph across Reissner's membrane. The organ of Corti has its own lymph provided by the spiral vessels of the basilar membrane. This supplies the needed oxygen and nutrients to the structures between the tectorial membrane and the basilar membrane. These substances are supplied continuously over the length of the basilar membrane from base to apex, so the flow of cortilymph may be said to be localized in the manner of any fluid bathing a body tissue.

The function of endolymph is most likely that of providing the proper ionic content for the electrochemical functions of the organ of Corti. The exchange responsible for the balance of components within the endolymph also takes place throughout the scala media all along the surfaces of Reissner's membrane and the spiral ligament, with a slow drainage of this fluid toward the endolymphatic duct. So the flow is longitudinal, but the exchange or balance of chemicals is radial or continuous along the scala media.

Specifically, the exchange between endolymph and stria vascularis and spiral prominence on the wall of the spiral ligament may be for the maintenance of the potassium and sodium content of the endolymph. Evidence for the transport of ions through Reissner's membrane is based largely on experiments by Rauch and associates (1962; 1963). When 42K is injected into the scala vestibuli, it rapidly appears in endolymph, exceeding the concentration of 42K in the perilymph of the scala vestibuli in about 2 minutes. After injection of 42KCl into the scala tympani, virtually no 42K appears in the endolymph as long as the isotope does not diffuse into the scala vestibuli. After injection of 24NaCl into the scala vestibuli there is a moderate increase of 24Na in the endolymph, but this does not reach more than one-third to one-half the concentration in the perilymph of the scala vestibuli. The 42K ion exchange is four to five times more rapid. Reissner's membrane, therefore, is permeable to certain ions: potassium can enter endolymph from perilymph against the concentration gradient at a rate that is four to five times higher than that of sodium. A review of these results is shown in Figure 4.

After injection of 42K into the endolymph, it not only appears in the endolymph but also in considerable quantity in the stria vascularis; apparently potassium is resorbed back through the stria vascularis (Rauch, 1963). However, Rauch never proved that ions within the perilymph did not travel through the spiral ligament to the endolymph rather than across Reissner's membrane. An, in a clever experiment, Yamashita and co-workers (1977) demonstrated that transport across Reissner's membrane may not be involved at all. They perfused the entire perilymphatic space of guinea pigs with paraffin oil. After 2 hours, endolymph removed from the second turn scala media showed no changes in ionic content as determined by flame photometry. Perhaps, then, Reissner's membrane serves only to separate endolymph from perilymph.

Johnson and Spoendlin (1966) interpret the function of the stria vascularis on the basis of observations of Erulkar and Maren (1961), who showed that tissues of the cochlear partition possess a very high concentration of carbonic anhydrase. This chemical is best known for its activity in the kidney, where it accelerates the reversible reaction between carbon dioxide, water, and carbonic acid. Erulkar and Maren gave cats acetazolamide (Diamox), which inhibits carbonic anhydrase and decreases the rate of the above reaction. The dosage was sufficient to cause great inhibition of cochlear carbonic anhydrase, along with a marked decrease in potassium concentration in the endolymph.

Johnson and Spoendlin examined by electron microscope the cochleas of guinea pigs after Diamox administration. They found a decreased number of vacuoles in the stria vascularis and a decrease in potassium in the endolymph, which suggested that the stria vascularis serves to secrete potassium, with the observed vacuoles acting in some unknown fashion to carriers.

Studies of adenosine triphosphatase activity within the stria vascularis support the notion that this structure is important in endolymph metabolism and active in sodium and potassium transport (Nakai and Hilding, 1966; and Iinuma, 1967). And Hilding (1965) has demonstrated what he has called "cochlear chromaffin" cells deep in the spiral ligament, which he believes may influence the production of endolymph through control of the blood supply by release of epinephrine or norepinephrine.

The role of endolymph appears to be the control of ions in fluid "above" the tectorial membrane. These ions, and the associated resting potentials, are probably there to make possible the conversion of vibratory energy into a nerve impulse. In addition to this, the fluids must convey the vibrations to the right spot for this conversion to take place.

Fluid-Dependent Mechanical Properties of the Inner Ear

Mechanical Response to Vibration

The conveyance of vibrations to the basilar membrane is obviously a property of fluids. The nature of interaction between the membrane and the fluids is complicated and has been the subject of debate for many years. In fact, this problem has been one of considerable importance because Helmholtz (1885), invoking Mueller's doctrine of specific energies of nerves (1837) to account for frequency analysis in the peripheral ear, set up the requirement that the fluids conduct the vibrations to the right spot or, as Helmholtz described it, that the right spot be activated by the vibrations in the fluids. An excellent review of the interplay between fluids and place of energy conversion can be found in E. G. Wever's book *Theory of Hearing* (1949) and in a later review of the traveling wave theories by Wever and Lawrence (1954).

Helmholz, in his so-called *resonance theory,* which is mostly of historic interest, proposed specific resonators along the basilar membrane that, for different frequencies, activated specific nerves. Careful anatomic observations, however, have failed to indicate structures capable of isolated resonance, and even if there were such resonators, once put into sympathetic vibration the activity would continue after termination of sound stimulation. In order to give a resonator the characteristic of rapidly ceasing its vibration, it must be "damped", ie the density of the medium in which the vibration occurs must be increased. However, when this happens the resonator responds, although with less sensitivity, to a broader band of frequencies, thus negating the purpose of specific resonators.

Also of historic interest is the *frequency theory* of Rutherford, described in 1886 to the members of the British Association for the Advancement of Science. Rutherford believed that there is no analysis of complex vibration in the cochlea, but that all vibrations, regardless of frequency, amplitude, or complexity, are directly portrayed by nerve impulses to the brain. The entire length of the basilar membrane, along with the hair cells, is involved in every tone. Rutherford ignored earlier experiments that showed impulses higher than about 1400 per second to be impossible in nerve fibers; he said that because this is the auditory nerve it must have unique properties.

If any error had been made in the nerve conduction-velocity experiments, it probably was in overestimating the firing rate. The fastest single fibers cannot repeat pulses of more

than 1000 per second, and the auditory nerve fibers do not appear any different from other nerve fibers in this respect. Present-day computer analysis of auditory nerve firing patterns demonstrates that single fibers do not necessarily fire on each wave of a stimulating sound but some may drop out while others fire. Thus, the total sum of firings may add up to a very rapid rate for the higher frequencies. Wever and Bray (1937) had proposed this much earlier as the *resonance volley theory*, but not strictly in defense of Rutherford's notion of basilar membrane action.

At the beginning of the 20th century, another class of theories arose, which purported to establish some form of peripheral analysis of sound without separated resonators. These are the so-called *traveling wave* theories, based upon the concept of a displacement wave progressing along the basilar membrane. There have been many traveling wave theories, but Békésy's (1928) has received the most support. His work on this subject has been so thorough and illuminating that, among many other awards, he won the 1961 Nobel Prize in Medicine or Physiology.

There is one fact that puzzles many auditory theorists today. The theories just described require mechanical motion and Békésy made his observations of this motion at 134-dB sound pressure level (SPL). However Békésy (1960) also calculated that the fluid motion of the organ of Corti at threshold is only 10^{-11} cm, which is the same order of magnitude as that separating electrons in the electron cloud of an atom. At the threshold of hearing the mechanism that transforms a vibration into a nerve impulse must be of molecular dimensions. There is a group of suppositions called *tube-resonance theories* that rely more on the fluid columns in the various fluids than on the mechanics of the basilar membrane and give better possibilities for explaining transductions at molecular levels.

The latest of these tube-resonance theories has been proposed by Naftalin (1967). He first constructed a very sensitive vibration-detecting probe using a Rochelle salt piezoelectric bimorph wrapped in layers of silicone rubber, copying the structure of a pacinian corpuscle. By means of a small filter paper strip attached by a special arrangement to this structure he could determine the acoustic energy distribution in small cavities. He then constructed several models, each a modification of an earlier one, so as finally to imitate the essential characteristics of the internal geometry of the cochlea. For fluid he used tap water. His experimental results showed that sounds of various frequencies were distributed along the "basilar gap". He found further that this differentiation with frequency became sharper when a thin wedge-shaped gel, in imitation of the tectorial membrane, was added to the model.

In these tube-resonance theories, it is the geometry of the fluid-filled tubes and the characteristics of the walls that determine the linear analysis of sound, whereas in Békésy's theory, it is the hydrodynamic action of the fluids on the basilar membrane at the area of maximum vibration. In any case, there is one more act to follow: that of transforming this differential vibration into a stimulus for the dendrites of the complexly distributed cochlear nerve fibers. Békésy describes a shearing motion between the tectorial membrane and the hair cells acting upon the hairs, presumably triggering an energy conversion process. There are many theories, but we do not yet know what this process may be, although it is becoming obvious that the electrical characteristics of the fluids play an important part.

Energy Conversion

Until and after the time of Helmholtz it was thought that the nerve fibers were stimulated directly by the mechanical action of some element put into vibration by a sound wave. Although Helmholtz knew of Corti's description of the cellular elements on the basilar membrane, he attached no importance to the hair cells, probably because Corti himself thought they were attached to small rods and beat on the nerve endings like drum-sticks on a drumhead. Helmholtz described the hair cells as grouped "like a pad of soft cells on each side of Corti's arches", serving only as a "peculiar auxiliary apparatus".

In 1892, Gustav Retzius described the termination of the nerve fibers on the base of the hair cells and suggested that the fibers receive their stimulation from these cells, which are the final receptors of vibrations conveyed through the fluids. Then, in 1930, Wever and Bray described the electrical AC potentials that arise from the cochlea when it is stimulated by sound, and subsequent experimentation has shown that this response is absent when the hair cells are not present. Although we do not know specifically what the stimulus to the nerve endings is, the evidence seems to point to this electrical AC output as part of sensory cell activity.

Of great importance has been the discovery by Békésy (1960) of the DC resting potentials within the cochlear partition. He described the endolymph as normally resting at + 50 mV and the organ of Corti at - 40 mV with respect to the perilymph. These have subsequently been shown both to be at a higher values - near 80 mV. These resting potentials are essential to many theories but not to all.

Davis (1953) has proposed, as one possible model, that the DC voltages provide a current flow through hair cells of the organ of Corti and through the tissues outside the scala media. Bending of the hairs causes a change in the electrical resistance at the hair-bearing end of the hair cells, modulating the current flow to produce the recorded AC potentials.

Another type of theory, proposed by Dohlmann (1959) and based on his observation of mucopolysaccharides in the endolymph and tectorial membrane, suggests a release of potassium ions as the membrane is pushed up and down by the vibratory motion. Dohlmann objects quite strenuously to the notion that bending hairs, through shearing action, can produce an alternating electrical current. He believes that the mucopolysaccharides with bound potassium ions are the most likely source of electrical changes. Reviewing some of the work on electron microscopy. Dohlmann concludes that the hairs of the sensory cells protrude into fine canals in the substances of the tectorial membrane and that this meshwork is soaked through with a secretion containing sulfomucopolysaccharides and potassium. Upon movement of the membranes, the positive potassium ions move in the direction of displacement. The hairs of the hair cells (which Dohlmann calls antennae), surrounded by the large molecules and the freed ions, are exposed to the electrical charges so that movement toward the hairs increases the negative charge. Dohlmann further assumes that there is a high concentration of potassium inside and outside the cell so that varying charges could not change the permeability of the cell membrane, because this would overload the cell with potassium and kill it.

There are other theories of this nature (Lawrence, 1967). Vinnikov and Titova (1964), for example, have proposed an elaborate cytochemical theory in which the energy transformation takes place by chemical activity within the hair cell.

One of the problems of great concern has been that of reconciling the conventional view of traveling waves along the basilar membrane and shearing forces within the organ of Corti with the small amplitudes of vibration encountered at the threshold of hearing. Békésy combined the calculated amplitude of stapes vibration with figures from his experiments on the volume displacement of the round window to show that, at the threshold of hearing, the amplitude of movement of the basilar membrane is 10^{-11} cm (0.001 Å). Naftalin (1965) points out that the thickness of the membrane of the hair process is 25 to 30 Å for the outer layer. He continues:

"From X-ray diffraction studies the axis repeat unit of a protein backbone is of the order of 3.6 Å. The H atom has a radius of, on the average, 0.53 Å, but we are now in the realm of probabilities. The 'front' of a membrane, having as its outside layer a peptide or C-chain backbone, is a cloud of electron orbitals. If we draw this 'front' and give the outside C-chain a definite position we have a probability cloud extending outwards from any fixed part of the structure a distance of at least 0.5 Å, but to complicate matters this electron fog will be more or less extensive at any given point varying rapidly with time. This minimum for depth of 0.5 Å to the 'front' is *at least* 50 times greater than the specified, calculated distance for the whole body movement of the ossicles acting as *mechanical levels* or of the movement of the basilar membrane to make the hair processes bend mechanically .. A whole body, even of microscopic size like a hair process, can never be said to have moved translationally through less than the average distance of the electron fog which constitutes its front". For this reason Naftalin has looked for a molecular process and has pinned his attention on the tectorial membrane.

From a careful analysis of the constituents of the tectorial membrane, Naftalin and co-workers (1964) conclude that this membrane does not simply lie in endolymph, bathed and permeated by the substances found in the endolymph, but is an independent medium with its own ionic composition. Compared with endolymph, the potassium content is low, but magnesium is high in the tectorial membrane. However, the ease with which magnesium can be washed out from fresh tectorial membrane suggests that the magnesium concentration gradient between endolymph and tectorial membrane may be maintained by metabolic activity in the scala media and, further, that this gradient allows for a mobility of distribution of electrical charges. Furthermore, the tectorial membrane is sensitive to hydration changes, indicating that it is osmotically sensitive - a gel with a very high water content held in water-structured form. The possibility is suggested that the acoustic wave energy within the fluids produces a transient change in pressure within the tectorial membrane, causing oscillatory-osmotic pressure changes by "ion shuttling". The metastable state of the protein-metal complex in the tectorial membrane gel is probably maintained by the endolymphatic DC potential, and because this membrane is in direct contact with the hairs of the sensory cells, "the orientation of otherwise random molecular vibrations by the acoustic compression-relaxation wave provides the trigger for the transfer of the signal from the gel to the hair processes". Presumably the hair cells relay this to the nerve endings.

Naftalin has elaborated on this theory (1965) and Lawrence (1965) has searched for physiologic evidence. It does not seem to improbable that the tectorial membrane may act as a polarized piezoelectric semiconductor to amplify the acoustic signal. Lawrence (1965, 1967, 1968) has reported a plateau of zero potential between the negative of the cells of the organ of Corti and the positive of the endolymph and has localized this to the tectorial membrane or subtectorial space. The importance of the tectorial membrane as an acoustic amplifier such as described by Gibson (1965) is obvious in the face of the concern over the amount of energy available at auditory threshold levels. Davis (1957) has also discussed the necessity for such an amplifier.

The resting DC potential of the inner ear may not, then, just be a consequence of ion distribution but may be very important for the energy conversion process of the ear. The situation is unique in biologic systems because endolymph has a high K+ content, much like intracellular fluid, yet it has a high positive potential fluid, unlike the interior of a cell. No doubt, the fluids play an important part in the maintenance and distribution of these potentials, but nothing very certain has yet been established.

There is, first of all, the question of whether the DC resting potentials are the same in all turns. Misrahy and colleagues (1958) reported a DC potential near the round window as high as 120 mV but near zero in the upper turns. Gisselsson (1960) and Suga and co-workers (1964) report that the endocochlear DC potentials are almost equal in all turns from base to apex.

Tasaki and Spyropoulos (1959) observed that the DC endolymphatic potential was of normal magnitude in waltzing guinea pigs, which have no organ of Corti. They also drained the fluids from the ear and found a strong positive DC potential on the surface of the stria vascularis, from which they conclude that the stria vascularis is the source of the positive DC potential and that it is maintained by some oxidative process. Davis and co-workers (1955) and Smith and colleagues (1958) arrived at the same conclusion.

On the other hand, Johnstone (1965), following a study of the effects of anoxia on the endolymphatic potential with subsequent microanalysis of the endolymph, concluded that the endolymphatic potential is a function of the differential K+ gradient between the scala media and plasma. However, at an earlier date, Johnstone and associates (1963), following an analysis of the fluids, had decided that the sodium concentration in the endolymph is low enough to allow the possibility that the positive endolymphatic potential is a Na+ diffusion potential.

Actually the source and method of maintenance of the resting potentials are still a mystery. Wever (1966) has discussed the various viewpoints.

The experiments of Yamashita and co-workers (1977) in which the perilymph was replaced by paraffin oil without changes in the DC endolymphatic potential or the K+ content even after a time lapse of 2 hours, would indicate that Reissner's membrane is not involved in the physiologic state of endolymph. If the endolymphatic potential is one of K+ diffusion, it must be between plasma and the scala media fluids.

Kuijpers and Bonting (1970) in a series of experiments studied the effects of ouabain and anoxia on the endolymphatic potential. Ouabain produced a concentration-dependent depression of the potential and, as had been demonstrated earlier, anoxia, if maintained over a period of time, reduced the potential to a negative value. They have concluded that the endolymphatic potential is composed of two components: a negative potential determined by the K+ gradient between endolymph and perilymph (see Yamashita et al, 1977) and plasma and a positive potential due to an ouabain- and anoxia-sensitive electrogenic K+ pump, represented by the Na+-K+ ATPase system of the stria vascularis.

It has also been shown that the high K+ of the endolymph is necessary for the generation of the AC potentials (Konishi et al, 1966, 1968).

In recent years, since this chapter was originally written, a great deal of research has been carried out on the mechanical movement of the basilar membrane and the transduction process. So much has been published that it is beyond the scope of this review on inner ear fluid. The reader is encouraged to pursue the subject.