Paparella: Volume I: Basic Sciences and Related Disciplines

Section 4: Biochemistry

Chapter 23: Biochemistry of the Labyrinth

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Biochemical Characteristics of Inner Ear Fluids

Composition of Inner Ear Fluids

Many investigators have analyzed inner ear fluids, and varying values of sodium, potassium, and protein concentrations in these fluids have been reported. Despite the discrepancies among the results obtained, the general consensus is that endolymph has a higher concentration of potassium than of sodium and that perilymph has a higher concentration of sodium than of potassium (Smith et al, 1954; Bosher and Warren, 1968; Juhn, 1973; Makimoto and Silverstein, 1974). Differences in the concentration of sodium and potassium between cochlear and utricular endolymph have also been observed (Rodgers and Chou, 1966).

The total protein content of perilymph and endolymph in various species has been determined by many investigators (Ledoux, 1950; Citron et al, 1956; Lawler et al, 1967; Silverstein and Griffin, 1970; Juhn, 1971). The greatest difference between plasma and inner ear fluids is the low protein content of the latter. However, the protein content of perilymph is higher than that of cerebrospinal fluid (CSF), and perilymph also seems to have more protein than endolymph (Makimoto et al, 1978). The protein content in perilymph is reported to be elevated in certain pathologic conditions, especially in acoustic neuroma (Silverstein and Schuknecht, 1966). Palva and Raunio (167) studied the patterns of protein in serum, CSF, and perilymph by disc electrophoresis. They could identify prealbumin, albumin, transferrin, and haptoglobulin bands in each fluid. The large molecular substances, such as group-specific proteins, gamma globulins, and beta1-lipoproteins, had much weaker bands in the perilymph than in either serum or CSF. On the other hand, perilymph contained two weak bands in the prealbumin region that appeared to be neither serum nor CSF, one of which had the same mobility as one of the prealbumin bands found in brain tissue. Chevance and co-workers (160) isolated 13 different protein fractions in human perilymph by immunoelectrophoresis. Their results did not support the proposition that the perilymph is a mere dialysate of the CSF. Scheibe and associates (1972) used immunoelectrophoresis to analyze guinea pig perilymph. They reported that five components (albumin, alphaglobulin, beta1 globulin, beta2 globulin, and gamma globulin) were detectable; however, gamma globulin and especially beta2 globulin bands were barely visible in some but not in all samples.

Rauch (1964) and Mouhgrabi (1966) studied the amino acids in guinea pig perilymph and found that the amino acid pattern of perilymph was similar to that of serum. On the other hand, Crifo and Crifo (1971) reported differences in amino acid content between perilymph and CSF

or serum. They observed a higher content of glutamic acid in perilymph than in serum. Miyamoto (1978) reported differences in amino acid composition of endolymph and perilymph in the guinea pig, and also reported a higher concentration of glutamic acid in the inner ear fluids than in serum.

Thalmann and co-workers (1982) determined the levels of 19 amino acids in utricular endolymph, vestibular and cochlear perilymph, and cerebrospinal fluid in guinea pigs. Aspartate and glutamate were significantly higher in endolymph than in perilymph. All other amino acids analyzed were significantly lower in endolymph. Amino acid levels in vestibular perilymph and perilymph of the scala vestibuli are virtually identical, and these levels are all higher in perilymph of the scala vestibuli than in cerebrospinal fluid.

Thalmann (1985) has recently pointed out that the true concentration of certain substances, including amino acids, can vary a great deal in perilymph sampled without blocking the cochlear aqueduct. He described a new technique for microsampling using concentric pipettes with an oil seal. This technique allows the sampling of perilymph from the cochlea in small volumes without requiring blockage of the cochlear aqueduct.

Changes in the enzyme activity of body fluids are known to reflect the presence of local or systemic pathologic conditions. Several enzymes have been identified in the inner ear fluids (Antonini et al, 1957; Rauch, 1964; Silverstein, 1966; Schindler and Schneider, 1966; Juhn et al, 1974; Palva and Raunio, 1967; Lotz and Kuhl, 1968), including lactate dehydrogenase (LDH), malate dehydrogenase (MDH), alkaline phosphatase, and phosphohexoisomerase (PHI). In general, enzyme activity in perilymph has been shown to be less than that in blood but higher than that in cerebrospinal fluid. The distribution of LDH isoenzymes in the cochlear tissues and perilymph has been studied (Palva and Raunio, 1967; Lotz and Kuhl, 1968; Kluyskens and Verstraete, 1969). All five LDH isoenzymes were identified in endolymph and perilymph. The pattern of distribution of isoenzymes in the labyrinth suggests an independent nature of perilymph compared with serum or CSF. The source of LDH in perilymph seems to be the cellular lining of the labyrinthine tissue. An elevation of LDH and MDH concentrations in perilymph in animals with middle ear infections has been reported (Rauch, 1965). An increased level of LDH isoenzyme 4 in certain tumors (meningioma or cholesteatoma) of the pontocerebellar angle has also been observed (Kluyskens and Verstraete, 1969). Although enzyme analysis of the cochlear fluids seems to offer a possibility of biochemical diagnosis of labyrinthine lesions, further studies are necessary before this can be adopted as a means for differential diagnosis of the various pathologic conditions of the inner ear.

The importance of glucose for normal cochlear function was demonstrated by Koide (1958), who reported a decrease in cochlear microphonics in hypoglycemic cats, a decrease that disappeared following the injection of glucose (Koide et al, 1960). Juhn and Youngs (1972, 1976) observed that the glucose concentration in animal perilymph parallels the serum glucose concentration, with a constant time lag, following experimentally induced hyperglycemia or hypoglycemia.

The possible significance of mucopolysaccharides in the inner ear fluids and tissues has been discussed by many investigators. The presence of hyaluronic acid and hexosamine in the inner ear fluids has been reported (Vilstrup and Jensen, 1954; Makimoto et al, 1967). Hyaluronic acid is a characteristic component of tissues with a large water content, such as vitreous humor or synovial fluid. Since the hyaluronic acid-protein complex produces a colloid osmotic pressure in biologic fluids, a colloid osmotic pressure gradient would be expected to play an important role in influencing the diffusion of fluid and crystalloids from blood capillaries to the cochlear fluids. The presence of mucopolysaccharide components in the cochlear sections of the membranous labyrinth has been reported (Juhn and Niederwieser, 1968, 1970; Lotz and Kuhl, 1970). These versatile macromolecules within the inner ear tissues and fluids seem to play an important role in metabolic and excitatory mechanisms of biopotentials (Dohlman, 1960).

Lipids are essential constituents of animal cells and are used metabolically by all living organisms. In the human body, they are concentrated in adipose tissue, cell membranes, and brain and nervous tissues. A small amount of triglyceride was identified in perilymph by thin-layer chromatography (Schiff and Christiensen-Lou, 1967). The quantitative analysis of fatty acids and triglycerides has also been reported (Schindler and Wolf, 1968); the concentration of both lipids in perilymph is higher than that of CSF. The role of lipids in the inner ear fluids needs to be clarified further. Concentrations of prostaglandins (PGs) in the perilymph and CSF of chinchillas and guinea pigs were measured (Jung and Juhn, 1984; Escoubert et al, 1985). The researchers found that levels of a 6-Keto-PGF1alpha (stable metabolites of PGI2) in perilymph significantly decreased after aspirin treatment. Concentrations of leukotrienes (LTs) in the perilymph of chinchillas were measured before and after salicylate administration. Levels of LTB4 and LTC4 were found to be elevated after treatment with salicylate (Jung et al, 1989).

The organ of Corti contains a fluid termed cortilymph by Enström (1960). Four possible origins of cortilymph have been suggested (Rauch, 1964): (1) CSF, (2) the vas spirale basale, (3) Hensen's cells, and (4) the canaliculae perforantes of the spiral limbus. Based on studies of sodium and potassium concentration, Rauch (1964) suggested that the composition of the fluid corresponds to that of perilymph. On the other hand, based on studies of its embryologic development, Engström contends that the organ of Corti is an epithelial organ of ectodermal origin and does not communicate with the perilymphatic system, which is of mesodermal origin. However, a system of fluid channels connecting the scala tympani with the organ of Corti has been reported (Schuknecht et al, 1959). Tonndorf and co-workers (1962) observed the passage of dyes injected into the scala tympani through the basilar membrane. Following injection into the scala tympani, movement of thorium dioxide into the "cortilymph space" by free diffusion through intercellular spaces has been reported by Ilberg (168). The existence of communication routes between the scala tympani and cortilymph has also been observed by the accumulation of inulin (Masuda et al, 1971). The mode of exchange of fluids between the perilymph of the scala tympani and the cortilymph space has not been clarified.

Endolymphatic sac fluid is reported to have a high protein concentration; this may be due to the loss of its water by osmotic forces or by some other mechanisms existing between the endolymphatic sac wall and the surrounding blood vessels (Silverstein, 1966).

The characteristic compositions of inner ear fluids are summarized in Table 1.

Table 1. Characteristic Composition of Inner Ear Fluids

Endolymph	Na < K	Low protein
Perilymph	Na > K	Low protein
Cortilymph	Na > K	
Endolymphatic sac fluid	Na > K	High protein.

Function of Inner Ear Fluids

The most important factors in normal hearing and vestibular function may be intact morphology and maintenance of a microhomeostasis of the inner ear fluids. As stated previously, endolymph has a unique composition, namely, a higher potassium concentration than other extracellular fluids. It is generally agreed that the positive endocochlear potential observed in the cochlear duct is most likely due to potassium transport into the scala media from the stria vascularis, the suggested source of these ions. In the vestibule, it has been suggested that the dark cells are responsible for maintenance of the high concentration of potassium in the vestibular endolymph.

The primary role of inner ear fluids is to provide a proper, unique ionic environment for optimal generation of the biopotentials that are necessary for adequate inner ear auditory function.

The second function of the inner ear fluids seems to be to provide a homeostatic pressure equilibrium in the inner ear. Hydrostatic variations of CSF are conveyed through the cochlear aqueduct into the perilymphatic spaces. At the same time, this CSF pressure is applied to the walls of the endolymphatic sac, which brings about an equalization of pressure on both sides of Reissner's membrane.

Third, the inner ear fluids appear to serve as a means for transport of nutrients to the end-organs and also for removal of any metabolic waste products from the cochlea. Dye, pigment, or silver particles injected into the cochlea have been observed to accumulate in the endolymphatic sac. Perilymph has been reported to be the major route for the supply of oxygen to the hair cells of the organ of Corti.

Finally, the inner ear fluids serve as relay media for the vibrations transmitted from the footplate of the stapes to the energy transforming centers in the inner ear. If indeed there exists a longitudinal flow of perilymph toward the subarachnoid space, as suggested by the studies of Haug and co-workers (178) and Kaupp and Giebel (1980), such a flow could play a role in the removal of metabolites and neurotransmitters from the vicinity of the hair cell-eight nerve synapse.

Regulatory Mechanisms for Inner Ear Fluids

Blood-Labyrinth Barrier. In a discussion of inner ear fluid dynamics, the concept of the blood-labyrinth barrier must be considered. The observed differences in composition of the inner ear fluid components (perilymph, endolymph, cortilymph, endolymphatic sac fluid) suggest the existence of efficient regulatory mechanisms or functional transport processes for the maintenance of the observed concentration gradients. Collectively, these mechanisms or processes can be designated the blood-labyrinth barrier and may include all phenomena that prevent, reduce, delay or even facilitate the penetration of any substance into the inner ear fluid system. The penetration may occur by simple diffusion, ultrafiltration, osmosis, lipid solubility, special tissue affinity, or metabolic activity of inner ear tissues (Juhn et al, 1981). For example, the blood vessels of the spiral ligament are surrounded by layers of pericytes and fibrocytes, and the endothelial cells lack fenestration (Takahashi and Kimura, 1970). The slow diffusion from the capillaries to the extracellular space of the spiral ligament, together with the time needed for passage through the layers of connective tissue and the perilymphatic space, could account for the slow transport of certain substances into the perilymph and these factors comprise the blood-perilymph barrier (Juhn et al, 1976).

CSF-Labyrinth Barrier. The differences in chemical composition between CSF and the inner ear fluids suggest the existence of regulatory mechanisms for maintaining these differences. These mechanisms can be termed the CSF-labyrinth barrier and may include some of the same processes suggested for the blood-labyrinth barrier.

Maintenance of Microhomeostasis of Inner Ear Fluids

The maintenance of a constant ionic environment in the labyrinth seems to depend on the following three conditions: (1) energy-dependent ion pumps, (2) constant blood circulation, and (3) the blood-CSF-labyrinth barrier. In order to maintain a high potassium (K) and low sodium (Na) concentration in the endolymph, an energy-dependent cation pump must exist to pump sodium ions out and potassium ions in. The Na-K activated adenosine triphosphate (Na-K-ATPase) hydrolyzes adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and inorganic phosphate, thus liberating energy that is used by this pump system to move the monovalent cations against the concentration gradient in the inner ear. The highest activity of Na-K-ATPase has been demonstrated in the stria vascularis (Kuijpers and Bonting, 1969) as compared with the other structures of the cochlear tissues. This enzyme has also been found in the dark cells of the vestibule. Since the most efficient ATP production depends on an adequate supply of oxygen, it is obvious that a rapid and constant blood flow is essential for the proper functioning of this pumping system. Under ischemic conditions, it has been reported (Thalmann et al, 1972) that there is a decrease in ATP concentration in the stria vascularis and a decrease of bioelectric activity (Okumura, 1970), and that changes occur in the ionic composition of the endolymph (Konishi et al, 1978) and in the morphology of the hair cells (Spoendlin, 1969).

The blood-labyrinth barrier certainly contributes to maintenance of constancy in the composition of the inner ear fluids. Because the inner ear fluids are in contact with the

surrounding tissues and are connected directly or indirectly to CSF and blood, it is highly probable that there is a continuous exchange of ions and metabolites between them. To examine the response of the inner ear fluids to changes in blood and CSF composition, certain test substances have been injected into either the blood or the CSF. When radioactive sodium was injected into the blood, the sodium entered the perilymphatic space more slowly than it entered CSF or aqueous humor. It reached equilibrium in about 12 hours (Juhn et al, 1976). This experiment demonstrates the existence of a blood-labyrinth barrier in the pathways for net ion transport into the labyrinth.

The osmotic relationships between body fluid and inner ear fluids have been studied. Bosher and Warren (1971) reported that alterations in body fluid osmolality, produced by intraperitoneal injection of water or hypertonic glycerol, were accompanied by changes in the osmotic pressure of the inner ear fluids in neonatal rats. Juhn and co-workers (1976) also demonstrated that the osmolality of perilymph paralleled the serum osmolality in guinea pigs, although with a time lag.

These studies demonstrated that the osmolality of perilymph responds to variations of serum osmolality and that a shift of water takes place through the blood-labyrinth barrier when the blood osmolality is either increased or decreased. Therefore, the blood-labyrinth barrier appears to be freely permeable to water.

The effect of intracisternally injected albumin on the composition of perilymph was also studied (Juhn and Guzowski, 1973). An increase in perilymph protein followed the increase in CSF protein, suggesting a potential flow of CSF into the scala tympani through the cochlear aqueduct. However, the maintenance of a high protein concentration in the perilymph suggested a slow excretion or backflow of perilymph into CSF. When the osmolality of chinchilla CSF was increased by intracisternal injection of a hypertonic sodium chloride solution, the osmolality of perilymph immediately increased, almost parallel to the changes in CSF osmolality (Juhn, 1977). Thus, a change in osmolality of CSF induces changes in the perilymph osmolality, at least in animal studies. Osmotic changes in CSF probably have less influence on perilymph osmolality in the human because of the differences in morphology of the cochlear aqueduct; however, the possibility still exists that some modification of perilymph osmolality can result from changes in the osmolality of CSF in humans.

A possible relationship involved in the osmotic equilibrium existing between the inner ear fluid and the surrounding tissues is shown. Under normal conditions, osmotic balance seems to be maintained by a sequence of water shifts. However, whenever any condition exists that destroys this homeostasis, an above normal accumulation of water in the inner ear fluid compartments can result. Because the inner ear membranes separating the fluid-containing compartments of the inner ear seem to be permeable to water and because there are mechanisms that can cause the accumulation of ions or metabolites in any one of these compartments, the resulting osmotic imbalances should result in water movement, which would cause a distention of the membrane that separates these fluid compartments. How biologic substances are able to accumulate in only one of two adjoining inner ear compartments still needs to be thoroughly

investigated.

Biochemical Aspects of Inner Ear Tissue

Stria Vascularis, Reissner's Membrane, and Spiral Ligament

The stria vascularis is extremely active metabolically and is believed to be involved in ion transport and generation of the positive DC endocochlear potential (EP). The stria vascularis is composed of three types of cells: (1) a row of apical or marginal cells that face the endolymph of the cochlear duct; (2) an intermediate row of pale, stellate cells (intermediate cells), classified as melanocytes by Hilding and Ginzberg (1977); and (3) a row of basal cells that connect the stria vascularis to the spiral ligament (Rodrigues-Echandia and Burgos, 1965). Intraepithelial blood capillaries are prominent, and pigment granules are abundant in the intermediate cells.

The marginal cell is a large columnar cell with a convex free surface bearing sparse, short microvilli. Near the free surface, the cells are bound firmly by zonulae occludens and a few small desmosomes. Prominent infoldings of the lateral cell membranes, which are more numerous toward the base of the cell, form a system of laminar processes or narrow cytoplasmic compartments that contain mitochondria. Groups of these processes interdigitate with one another or with the projections of the other two strial cell types. The remarkable infoldings of the lateral and basal cytoplasm of the marginal cells, leading to a great increase in the area of cell surface, in intimate relation with clusters of mitochondria suggest specialization for ion transport (Rodriguez-Echandia and Burgos, 1965).

The intermediate cells are irregular in shape and generally stellate. These cells have large, pale cytoplasmic processes that divide into smaller projections that intercalate with marginal cells or basal cells or come into direct contact with the basal lamina of capillaries. Because all stages of melanogenesis have been found in intermediate cells, they can be classified as melanocytes (Hilding and Ginzberg, 1977).

Basal cells are flat cells with large ascending prolongations that penetrate between lateral borders of intermediate and marginal cells. Basal cells form cuplike structures that nearly isolate marginal cells from adjacent cells. In addition, basal cells are also in extensive contact with the capillary surface. The structural appearance of the basal and intermediate cells suggests a function similar to that of glial cells of the central nervous system. Elements of the spiral ligament are in direct contact with the basal cells of the stria, without any intervening basal lamina. The spiral ligament cells have an oval or somewhat irregular shape and possess a pale cytoplasm with few organelles. Thick bundles of filaments, without apparent periodicity, are noted in the extracellular space.

Studies using radioactive tracers have shown that Na+ and K+ pass through Reissner's membrane (Choo and Tabowitz, 1964, 1965; Rauch, 1966). Fernandez (1967) and Citron and Exley (157) suggested that endolymph may be produced by exchange of inorganic ions across Reissner's membrane and by other tissues in the cochlear duct, in addition to the stria vascularis.

Prazma (1969) suggested that Reissner's membrane contributes to the ionic content of perilymph and to the EP, based on the effects of G-strophantin and 2,4-dinitrophenol in guinea pigs. Hinojosa (1971) found unidirectional transport of ferritin across Reissner's membrane from perilymph to endolymph.

On the other hand, Kuijpers and Bonting (1969) found a very low degree of transport ATPase (Na+-K+-ATPase) activity in Reissner's membrane; thus, they proposed that it played a minor role in maintaining cochlear cation gradients. Konishi and Mendelsohn (1970) attributed the effect of ouabain on EP (decrease) and on ionic content (rise in sodium, decline in potassium) of endolymph to blockade of active transport in the stria vascularis. Yamashita and co-workers (1977) studied the contribution of Reissner's membrane to the ionic content of endolymph and EP in guinea pigs by replacing perilymph with paraffin oil. Under these conditions, there was no significant alteration of endolymphatic K+ or Na+, and EP was maintained over a 2-hour period. They concluded that the stria vascularis plays the major role in the production of endolymph, the maintenance of its ionic gradients, and the generation of EP. Reissner's membrane was found to have no visible ouabain-binding sites, with the possible exception of the portion adjacent to the spiral limbus (Drescher and Kerr, 1985).

Konishi and associates (1978) and Konishi and Hamrick (1978) studied cochlear ion transport in the guinea pig by perfusing the perilymphatic spaces with radio-labeled Na+, K+, and Cl-. Endolymph took up 42K and extruded 22Na against concentration gradients, but no differential concentration of chloride was found. The active processes as well as diffusion of chloride from perilymph to endolymph were all inhibited by local application of ouabain or by anoxia. They concluded that K+ is actively transported from perilymph to endolymph, that Na+ is extruded from endolymph to perilymph, and that chloride influx from perilymph to endolymph is driven by the EP. Schön and Jung (1983) used the data of Konishi and co-workers (1978) and their own data to construct a two-compartment model of the guinea pig cochlea. For the basal turn of the cochlea, they calculated a circulating flux between endolymph and perilymph of 190 pMol/min/mm of cochlea, whereas a value of 100 Pmol/min/mm was calculated for the flux from the perilymphatic spaces out of the cochlea.

The production of energy is required for ion transport. High energy phosphate compounds such as ATP can be hydrolyzed to liberate this required energy. Iinuma (1967) has demonstrated the presence of membrane ATPase in the stria vascularis and spiral ligament. Intense ATPase activity in the strial blood vessels, spiral prominence, and spiral ligament and moderate activity in the strial cells was demonstrated histochemically (Ishii and Nomura, 1968) and ultrastructurally to the basolateral marginal cell extensions (Kerr et al, 1982; Mees, 1983). This "polarized" cellular distribution of enzyme activity in epithelial cells is said to be typical of ion-transporting epithelia (Drescher and Kerr, 1985). The positive DC EP seems to be generated by an electrogenic pump in the stria vascularis. This pump probably is powered by energy generated by ATP hydrolysis via the Na+-K+-ATPase system. This results in high potassium, low sodium concentration and the anoxia- and ouabain-sensitive EP (Dallos, 1975). The primary fuel for generation of energy for ion transport and the EP seems to be glucose (Marcus et al, 1978). Ischemia blocks the oxidative metabolism of the stria vascularis and results in depletion of high

energy phosphates (ATP and phosphocreatine), which fall in proportion to the decline in EP (Thalmann et al, 1972).

Adenosine 3,5-cyclic phosphate or cyclic adenosine monophosphate (the "second messenger" in the action of many hormones and drugs) has been detected in the stria vascularis, as well as the enzyme responsible for its formation, adenylate cyclase (Thalmann et al, 1977; Paloheimo and Thalmann, 1977). The exact role of cAMP or of adenylate cyclase in the stria is not known.

A new hypothesis was suggested associating cAMP metabolism with hydrops (Juhn et al, 1982, 1985). Adenylate cyclase is present in cochlear tissues, with the greatest activity reported to be present in the stria vascularis, followed by the organ of Corti and spiral ligament according to the studies of Ahlstrom and co-workers (1975). However, these levels are substantially lower than that reported in brain and kidney. Feldman and Brusilow (1976) reported that the injection of purified cholera toxin (an adenylate cyclase stimulant) into the scala media caused an increase of endolymph volume, and they presented this as a model for endolymphatic hydrops.

A more recent study by Feldman and colleagues (1979) showed that the normal endolymphatic pressure in the Hartley guinea pig was 0.12 cm of water when measured by a micropressure transducer connected to a micropipette inserted into the scala media. The injection of cholera toxin into the scala media through the basilar membrane resulted in a significant increase in the endolymphatic pressure of more than 10-fold, 2 hours after injection. Biochemical studies of the effects of cholera toxin on the cAMP levels in tissues lining the cochlear duct have been reported by Thalmann and colleagues (1982). They found that the perilymphatic application of cholera toxin did not alter the level of cAMP in these cochlear tissues. The addition of theophylline (a phosphodiesterase inhibitor that prevents the breakdown of cAMP) to the perfusate containing cholera toxin resulted in dramatic increases in the cAMP content in all three tissues, and these changes occurred with both perilymphatic and endolymphatic perfusion. The most striking elevation in cAMP content was found in Reissner's membrane, suggesting that this tissue may play an important role in cochlear fluid homeostasis (Thalmann et al, 1982). Schacht (1982) found that adenylate cyclase of the mammalian cochlea is located in the infolding of the stria vascularis basolateral membrane, which is analogous to the configuration found in the mammalian nephron. Zenner and Zenner (1979) reported that the purified membranes from the inner ears of guinea pigs contained adenylate cyclase that functionally couples with membrane receptors for vasopressin and beta receptors for isoproterenol or epinephrine, respectively. Both types of hormones stimulate the production of cAMP by activating adenylate cyclase. cAMP has been shown to be an intracellular mediator of salt and water absorption in the toad urinary bladder and renal collecting tubules. In addition, it enhances secretion from the intestinal epithelium, salivary glands, pancreas, and renal tubules. It has also been demonstrated that cAMP can alter membrane permeability as well as electrolyte secretion by epithelial cells. Production of cAMP can be stimulated by biogenic amines, various hormones (including antidiuretic hormone or vasopressin), and purified cholera toxin. These substances could stimulate adenylate cyclase to increase the production of endolymph, and could possibly explain the production of endolymphatic hydrops by excessive activity of adenylate cyclase and excess cAMP production

(Juhn et al, 1982, 1985).

The cerebrospinal fluid secretion also appears to be controlled by adenylate cyclase in the choroid plexus (Nathanson, 1979). Recently, Schacht (1985) has characterized adenylate cyclase of the mouse stria vascularis. In the presence of the regulatory nucleotide GTP, the enzyme was stimulated by isoproterenol and epinephrine with a half-maximal effect near 10 micromolar, and stimulation was blocked by propranolol. These findings are consistent with the presence of adrenergic beta2 receptors on the strial enzyme complex. The enzyme was also activated by forskolin, an activator of this enzyme complex. Schacht casts doubt on the physiologic role of adenylate cyclase in the stria vascularis when he found that vasopressin did not act on the strial enzyme. However, the enzyme in other labyrinthine structure (for example, endolymphatic sac) may have different properties. As discussed earlier, antidiuretic hormone is known to increase the water permeability of various epithelial cells by activating adenylate cyclase and increasing the intracellular content of cAMP. At the luminal membrane of the renal collecting tubule, antidiuretic hormone decreases water excretion by increasing the water permeability of these epithelial cells. After intravenous infusion of antidiuretic hormone in experimental animals, the electrolyte concentration of serum and perilymph decreased (Juhn et al, 1985). Membranes of the inner ear contain adenylate cyclase, as mentioned earlier, which may be coupled to membrane receptors for vasopressin, perhaps not in the stria vascularis but rather the endolymphatic sac as discussed previously. Our recent studies that clearly demonstrate the presence of prostaglandins in inner ear fluids and tissues suggest that these local hormones may possibly be involved in the regulation of inner ear fluid dynamics (Juhn et al, 1985). It was shown that inner lining tissue of cochlea can convert precursor, arachidonic acid into prostaglandins and thromboxanes in the cyclo-oxygenase pathway and hydroxyeicosatetraenoic acid (HETE) in the lipoxygenase pathway (Jung and Juhn, 1984).

There appears to be a close relationship between antidiuretic hormone and prostaglandins. Antidiuretic hormone can stimulate adenylate cyclase activity and cAMP accumulation, resulting in increased water permeability. However, antidiuretic hormone also enhances prostaglandin E2 synthesis, resulting in a decrease of adenylate cyclase activity and an inhibition of water flow (Zusman, 1981). It thus appears that antidiuretic hormone and prostaglandins are involved in the regulation of water permeability and water flux in many tissues, perhaps including the inner ear. Any changes that cause a shift in the water balance may disrupt homeostasis, resulting in membrane distention pressure changes and inner ear dysfunction.

Carbonic anhydrase has been localized in inner ear tissues by microdissection and by direct biochemical assay as well as histochemical techniques. Erulkar and Maren (1961) showed that the concentration of carbonic anhydrase increases from the base to the apex in whole tissues of the cat cochlea. Drescher (1977) found that the highest specific activity of the cochlear enzyme was found in the spiral ligament fraction of the lateral wall. The activity of carbonic anhydrase in the stria vascularis was also found to be high. The biochemical findings of these studies have been recently confirmed by histochemical studies. A method specific for carbonic anhydrase was used by Lim and co-workers (1983), who showed that in the chinchilla, the concentrated stain for carbonic anhydrase was localized in the spiral ligament cells, with somewhat less intense

staining in the stria vascularis. Watanabe and Ogawa (1984) also found in the guinea pig that carbonic anhydrase was present in the fibrocytes of the spiral ligament as well as in the strial intermediate cells, apical vesicles of the strial marginal cells, between marginal cells, and in endothelial cells of the stria vascularis capillaries. The role of carbonic anhydrase is important in the stria vascularis and saccule for the production of bicarbonate in endolymph (Maren et al, 1975; Sterkers et al, 1984). Carbonic anhydrase in the spiral ligament may furnish bicarbonate ion for the rapid formation of perilymph (Drescher and Kerr, 1985).

Endolymphatic Sac and Duct

The endolymphatic sac, the final portion of the endolymphatic system, is connected by narrow ducts to the utricle and saccule and indirectly to the cochlea. The lumen of the sac appears to contain free macrophages and cell debris. This portion of the sac has been shown to have a rapid protein turnover and was shown to be rich in enzymes that are associated with protein degradation (Ishii et al, 1966). The luminal fluid has a very high protein concentration - 5 gm per 100 gm of fluid (Silverstein, 1966). A major function of the endolymphatic sac appears to be removal of cellular debris and macromolecules from the endolymph. Phagocytosis of injected tracers by lining cells of the sac has been clearly demonstrated.

Based on morphologic studies, the endolymphatic duct and sac have been suggested to constitute pathways for endolymph outflow. These structures may participate in the regulation of the amount of fluid and the ionic composition in the endolymphatic compartment by drawing endolymph through the epithelial lining to the surrounding blood vessels and periductal lymphatics. Morphologic studies also indicate that the endolymphatic duct and sac possess selective membrane properties with a high permeability to water and ions. Thus, these structures may represent fluid transporting epithelia with an ability to transfer water and ions. Recently, Friberg and associates (1985) reported on the presence of the so-called lateral intercellular spaces in the epithelium of the endolymphatic sac, and it was suggested that these spaces may form a pathway for transepithelial water flow in the endolymphatic sac. The protein content of the endolymphatic sac is remarkably high (Friberg et al, 1985), probably owing to resorption of endolymph. Protein may be concentrated in the sac and cleared by the phagocytic activity of free-floating macrophages through the epithelial cell lining into the perisaccular connective tissues. The endolymphatic sac is also filled with the gel-like intrasaccular materials and their role in the regulation of endolymph volume or osmolality are not yet clearly defined. However, it is conceivable that the endolymphatic duct and sac are actively involved in the resorption of water and ions of the endolymph and that they may serve as filters for the membranous labyrinth. The endolymphatic sac, with its free-floating cells that participate in phagocytosis, may also serve as an important immune defense organ of the inner ear (Friberg et al, 1984). If phagocytic cells (macrophages, mast cells, lymphocytes) receive antigenic stimuli, they can produce potent vasoactive substances (prostaglandins, leukotrienes, and histamine) and change the vascular permeability. An elevation of intracellular osmotic pressure can draw water from the endolymph and subsequently elevate the osmotic pressure of the endolymph. If an elevation of osmotic pressure takes place only in endolymph, this would draw water from perilymph and endolymphatic hydrops may occur.

Tectorial Membrane

The exact chemical nature of the tectorial membrane and its possible role in the hearing mechanism are still not clear. According to Naftalin (1967, 1977), the tectorial membrane surface protein, ie a protein gel secreted over the surface of the secretory hair cells in the organ of Corti is thus an essential component of the transducing mechanism that converts the acoustic signal to electrochemical energy.

Iurato (1960) analyzed the tectorial membrane chemically and reported that the total nitrogen content is 15 per cent, indicating that the tectorial membrane is composed principally of proteins. He also determined the amino acid and carbohydrate composition in the tectorial membrane. Based on the absence of hydroxyproline, Iurato suggested that the protein found in the tectorial membrane is not collagen. The absence of hexuronic acids, which are essential components of mucopolysaccharides, was also reported. However, earlier work by Belanger (1953) and Friberg and Ringertz (1956) using an isotope (35S) demonstrated the presence of acid mucopolysaccharides in the tectorial membrane. Later, Saito and Daly (1970) reported that the tectorial membrane and Reissner's membrane contained 0.1 per cent of mucopolysaccharides per unit of dry weight.

Naftalin and co-workers (1964) also performed a chemical study of the tectorial membrane and reported that this membrane contains a noncollagenous protein that has a minimal amount of hexosamine in the molecule. By direct chemical analysis, they reported that the concentration of potassium was intermediate between the high concentration in the endolymph and the extracellular value in the perilymph. The concentrations of calcium and magnesium were considerably higher than values in the surrounding fluids, since calcium is much more firmly bound to the protein than magnesium. Ross (1974) confirmed this ion distribution by x-ray analysis of rat tectorial membrane.

It has been suggested that the gelatinous materials in the tectorial membrane may be secreted by the epithelial cells of the cochlea and that its metabolism may be regulated by the interdental cells and possibly by the action of substances secreted by various epithelial cells of the cochlear duct, as occurs in the vestibular gelatinous membrane, otolith, and cupula (Iurato, 1967; Dohlman, 1971).

Recent studies have attempted to more precisely define the chemical and functional properties of the tectorial membrane. As Steel (1985) has stated, "If the molecular structure of the tectorial membrane were known, then its behavior and response to mechanical or chemical changes in its environment might be predictable".

Ultrastructural studies of the tectorial membrane have yielded interesting new data. Instead of simply being a structure composed of fibrils imbedded in an amorphous ground substance as was thought earlier, the careful evaluation of this substance under the electron microscope has revealed another class of fibril (Kronester-Frei, 1978). These two classes of fibrils have been named type A and type B. Type A fibrils are straight, do not branch, and run in bundles. Type

B fibrils are more irregularly aligned and may branch. The latter type of fibrils occurs throughout the tectorial membrane and occurs either as loosely packed or tightly packed structures. Tightly packed type B fibrils form the cover net and Hensen's stripe (Steel, 1985).

Until recently, the study of tectorial membrane proteins was carried out only with the crude method of paper chromatography. Steel (1985) has recently utilized sodium dodecyl sulphate polyacrylamide gel electrophoresis to characterize protein subunits of the tectorial membrane. She found that the most intensely staining bands (with Coomassie blue) corresponded to molecular weights of 145, 155, and 165 kilodaltons. She was able to resolve up to 22 individual bands in some gels. Recent analysis of the carbohydrate content of the tectorial membrane using gas-liquid chromatography revealed that the total carbohydrate represented about 2.5 per cent of the dry weight of the tectorial membrane (Steel, 1985). Steel (1985) reported that the mouse tectorial membrane contained glucose, galactose, mannose and N-acetylglucosamine as determined by gas-liquid chromatography.

Another group of investigators used a very sensitive technique, namely, fluorescent lectin binding, to identify carbohydrates present in the tectorial membrane of histologic sections of rat and guinea pig cochleas (Loyzaga et al, 1985). They found each of the latter three sugars found by Steel to be present, but instead of glucose found fucose to be present. Radioautographic studies confirmed the presence of glucosamine and fucose in the neonatal rat tectorial membrane. There is evidence to suggest that the tectorial membrane may act as a separate phase, maintaining an electrochemical character independent of that of surrounding fluids and tissues. Lim (1977) found that the tectorial membrane has a lower potassium content than endolymph. In vitro, the tectorial membrane maintains a potential difference relative to the bathing fluid (Steel, 1983). Thus, the tectorial membrane may function as a reservoir for important ions involved in transduction, such as potassium and calcium. The shrinking and swelling of the tectorial membrane on exposure to various electrolyte solutions (Kronester-Frei, 1978; 1979) suggest the possibility that mechanical stimulation of the tectorial membrane may release calcium into the subtectorial space, which could influence hair cell function (Steel, 1985). Contrary to all previous studies, Thalmann and co-workers (1986) have reported finding collagen in the tectorial membrane. These investigators have found that the predominant proteins of the guinea pig tectorial membrane virtually superimpose with purified collagen standards when these materials are subjected to two-dimensional polyacrylamide gel electrophoresis with an extended alkaline range. Peptide mapping of cyanogen bromide fragments of the protein of tectorial membrane from guinea pig results in a pattern closely resembling that of type II collagen (Thalmann et al, 1986). These findings may have important implications for explaining the function and properties of the tectorial membrane.

Organ of Corti

Figure shows the structural components of the tectorial membrane and the organ of Corti. Cytophysiologic investigations have shown that exposure to sound induces resonating vibrations in structures of the middle ear and cochlear canal and results in equivalent transformations of mechanical into chemical energy. This initiates a series of characteristic dynamic changes in the

hair cells of particular cochlear turns. The organ of Corti consists of these sensory cells of hearing and their supporting cells.

According to Vinnikov and Titova (1963), cochlear potentials are, in fact, no more than the expression of the underlying biochemical events evolving entirely within the hair cells under the effect of sound. Undoubtedly, these energy-supplying metabolic processes do actually constitute the basis of perception and excitation as well as of impulse transmission by the hair cells.

It has been suggested that the hair cells are the generators of microphonic potential. The microphonic potential is dependent on the metabolic activity of the cochlea, in particular the sensory hair cells (Tasaki and Fernandez, 1952; Tasaki et al, 1954; Tasaki and Davis, 1955; Tasaki, 1957).

Since the hair cells have no direct blood supply of their own, it has been postulated that the hair cells receive their supply of oxygen and nutrients from endolymph. However, the occlusion of the modiolar vessels leading 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. Based upon this experiment, Lawrence (1965; 1966) suggested that these loops of vessels beneath the basilar membrane furnish fluid that exchanges with the organ of Corti. However, Okumura (1970) observed the decrease of cochlear microphonic potential when the oxygen tension in the perilymph was decreased. He concluded that the major oxygen supply of hair cells was from the perilymph.

It has been reported that if perilymph is allowed to flow into the endolymphatic space through surgical tears produced in Reissner's membrane, degeneration of the outer hair cells and nerve fibers and also atrophy of stria vascularis occur (Lawrence, 1965; Duvall and Quick, 1969).

Chou and Vosteen (1971) studied the changes of the cochlear microphonic potential after replacing the perilymph by various media of different electrolyte composition, which contained metabolic inhibitors with or without energy-rich phosphate compounds. They observed that the ionic composition of the perilymph was not the only factor affecting the cochlear microphonic potential. The results of their experiments showed that when available energy normally supplied by the process of oxidative phosphorylation in the cells was abolished by 2,4-dinitrophenol, the addition of energy-rich phosphoenolpyruvate could provide the energy required for maintaining the microphonic potential.

The activity of oxidative enzymes in the inner ear has been demonstrated by many investigators (Vosteen, 1960; Gerhardt, 1967; Spoendlin and Balogh, 1963; Vinnikov and Titova, 1964). Most of the investigators observed the high content of oxidative enzymes in the outer and inner hair cells and nerve endings that surround the bases of the hair cells.

Koburg and Plester (1962) have injected amino acids labeled with radioactive carbon atoms into animals. They found the incorporation of the labeled carbon atoms to be most

apparent in the outer hair cells. This result also suggests that metabolic activity is higher in the outer hair cells. Within the mitochondria, succinate dehydrogenase and NADH-diaphorase (NADH-cytochrome C reductase) were reported to be distributed on the outer surface of cristae (Nakai and Hilding, 1968). The richer distribution of oxidative enzymes in the outer hair cells was suggested to indicate the higher metabolic rate in the outer hair cells (Koide et al, 1964).

Submicroscopic localization of NADH-cytochrome C reductase was studied by Lim (1970). This enzyme was found in the endoplasmic reticulum of the outer and inner hair cells, the epithelial cells of the stria vascularis, Reissner's membrane, and the connective tissue cells of the spiral ligament and limbus. The strongest reaction was observed in the mitochondria of the outer hair cells (Lim, 1970).

Hansen and Thomsen (1963) reported predominantly uniform glycogen content in the outer hair cells and a decrease of the glycogen content from the apical to the basal turn. This may indicate a qualitative difference in metabolism or a requirement for an energy reserve. It may also mean that the perception of deep tones requires more energy than that of high tones.

Matschinsky and Thalmann (1967) also reported that the organ of Corti and stria vascularis are well endowed with carbohydrate stores. Glycogen content of the organ of Corti is 10-fold higher than that found in average human brain tissue but is comparable to that of average rabbit retina.

Nakai and Hilding (1967) studied the ATP-ase distribution in the organ of Corti. They speculated that since this enzyme plays an important role in transport across the cell membranes, the way it is distributed within the organ of Corti should be related to the way that hair cells obtain vital substances. These authors observed the ATPase activity in the surface of most cells in the organ of Corti, the entire surface of the supporting cells, the basement membrane of the spiral vessel, and on the outer membrane of tunnel crossing nerve fibers and the endolymphatic layer of Reissner's membrane cells. No enzyme activity was observed along the side of hair cells where they are in contact with endolymph or between adjacent nerve endings. However, the activity was present in the synaptic space between nerve endings and hair cells.

Kerr and associates (1982) also reported finding elevated levels of Na-K-ATPase localized to synaptic regions beneath the inner and outer hair cells, the vicinity of the inner and outer spiral bundles, to unmyelinated fibers crossing the tunnel, terminal nodes of Ranvier in the habenula perforata, and fibers crossing through the osseous spiral lamina (Kerr et all, 1982). Numerous enzyme sites were visualized at the basal pole of inner and outer hair cells by light microscope autoradiography of labelled ouabain binding (Drescher and Kerr, 1985). Ultrastructural study has shown that the outer hair cells do not usually have cytochemical evidence of Na-K-ATPase. Rather, the activity was found in the nerve endings. It is not clear yet whether the ouabain binding in the inner hair cell is associated with the hair cell itself or with neural elements (Drescher and Kerr, 1985).

Acetylcholine is one of the neurohormones. It is readily hydrolyzed to choline and acetic acid by the action of the enzyme acetylcholinesterase (AChE), found not only at the nerve endings but also within the nerve fiber. Martini (1941) demonstrated that acetylcholine was present in the inner ear perilymph. Later, Gisselsson (1950) detected cholinesterase in both the perilymph and endolymph of the cochlea by incubating measured volumes of these fluids with acetylcholine of known concentration and comparing the contractions of both the leech dorsal muscle and the front rectus muscle when immersed in the solution. The magnitude of the contraction of the indicator muscles in the solution yielded evidence that acetylcholine esterase was present in the cochlear fluids.

Churchill and co-workers (1956) demonstrated that the efferent system of the organ of Corti has marked AcHE activity. Schuknecht and associates (1959) observed that AChE at the base of hair cells in the organ of Corti in the cat disappeared when the olivocochlear bundle was sectioned in the medulla. The neurochemical behavior of crossed and uncrossed olivocochlear fibers was demonstrated as identical by Desmedt and LaGrutter (1963). It was concluded that the presence of AChE in the cochlea was dependent on the integrity of the olivocochlear bundle. Ishii and Balogh (1968) also studied the efferent innervation of the organ of Corti in the cat by demonstrating specific AChE activity in this structure. They also reported that the AChE activity was poorly visualized around the inner hair cells but was very strong in the inner spiral bundle of nerve fibers throughout the cochlea. Hiraide (1970) also found essentially similar efferent innervation using an alkaline phosphatase staining method.

Kaneko and Daly (19968) reported the localization of AChE at the ultrastructural level in the bottom of the outer hair cells and on the tunnel radial fibers of the organ of Corti. They observed that in the bottom of only the outer hair cells, vesiculated nerve endings showed the AChE activity on the plasma membrane. Kaneko and Daly (1969) also reported that AChE is located on the endolymphatic surface of outer hair cells and not on the supporting cells in the organ of Corti of the guinea pig cochlea. This finding might suggest that a cholinergic mechanism is present on the endolymphatic surface of the hair cells.

Recent exciting studies have provided new insight into the cellular basis of hearing. Ultrastructural and biochemical studies of hair cells and innovative electrophysiologic techniques have helped to shed light on the mechanoelectrical transduction process and cellular events occurring within hair cells during stimulation of the stereocilia. The hair cells have many stereocilia at their apex. The core of each stereocilium has closely packed microfilaments (Pickles, 1985; Hudspeth, 1985; Tilney and DeRosier, 1985; Flock, 1985; Drenckhahn et al, 1985). The stereocilia and the cuticular plate contain actin filaments with a specific structure, and these filaments provide the rigidity to allow the participation of stereocilia in transduction (Pickles, 1985; Flock, 1985; Tilney and DeRosier, 1985). Myosin is found in the cuticular plate. In muscle cells, this protein interacts with actin to cause contraction. Tropomyosin has been found in the hair cell at the point in the cuticular plate where the stereocilia roots are inserted (Flock, 1985). The presence of these contractile proteins in the receptor regions of hair cells has evoked the hypothesis that these proteins may help the hair cells to move or "kick back" after acoustic stimulation and to supply the "negative damping" of the basilar membrane, which could

account for such phenomena as spontaneous and stimulated otoacoustic emissions (Hudspeth, 1985). Another cytoskeletal protein, tubulin, has been demonstrated to combine with actin to form rigid arches inside Deiters' cells and pillar cells that encase the outer but not the inner hair cells (Flock, 1985). Direct measurements of the compliance of sensory hairs (Strelioff and Flock, 1984) have shown that the stiffness of the stereocilia is about twice as great for displacement in the excitatory direction (toward the basal body in the guinea pig cochlea) as in the inhibitory direction. This asymmetry of stiffness could be explained by the differences in cytoskeletal proteins. The protein fibrin is present in inner hair cells (cuticular plate and stereocilia) (Flock, 1985) and may be responsible for cross linking actin filaments (Pickles, 1985). The cytoskeletal protein spectrin, which is one of the components of the erythrocyte membrane cytoskeleton, was demonstrated by antierythrocyte spectrin antibodies to satin weakly in the cuticular plate of the rat organ of Corti (Drenckhahn et al, 1985). It appears likely that the thin filaments that connect the cuticular plate actin filaments to the apical plasma membrane of hair cells are composed of a spectrin-like protein (Drenckhahn et al, 1985). A detailed model showing the location and proposed function of cytoskeletal proteins can be found in Drenckhahn's work (1985). The contractile properties of isolated mammalian cochlear hair cells have recently been demonstrated in vitro (Brownell et al, 1985; Zenner et al, 1985).

The relationship of recently identified horizontal and vertical cross-links between stereocilia on electron microscopy has led to the development of plausible theories of the cellular basis of hearing, and a mechanism may contribute to the opening and closing of ion channels in the hair cell (Hudspeth, 1985). Voltage-clamp measurements of the effects of substitution of various ions in the fluid bathing the hair bundle indicate that potassium readily traverses the channel. However, the channel is permeable to other cations as well. Although the chemical nature of the ion channels is unknown, current mapping experiments reveal that the transduction channels occur at or near the distal tips of stereocilia (Hudspeth, 1985). These channels open and close very rapidly. An excitatory stimulus causes shearing between adjacent stereocilia, and the cross-links may play a role in stretching open potassium channels, causing the channel to spend more of its time open, resulting in a larger potassium current flowing through the cell. Hudspeth (1985) has explained the ionic basis for electrical resonance by results of single saccular hair cell recordings using patch clamp techniques. A biphasic response is seen after depolarization. The early inward current appears to be carried by a calcium conductance. The second, delayed component is carried by potassium. Thus, the hair cell appears to have calcium-sensitive potassium channels. Electrical resonance, at least in vestibular hair cells, may result from an interaction of inward calcium currents and outward potassium currents (Hudspeth, 1985).

According to Retzius (1884) and Kolmer (1927) the number of sensory cells found in the organ of Corti ranges between 17,000 and 23,000. Enström (1965) reported that approximately 3500 bottle-shaped inner hair cells form a single row on the modiolar side of the inner pillars and that 13,000 to 20,000 outer hair cells are arranged in three or four rows on the outside of the outer pillars. Bredberg (1968) estimated the number of sensory cells and reported that the number of outer hair cells of the fetal cochlea averaged 13,400 cells (range 11,200 to 16,000) and the number of inner hair cells averaged 3400 cells (range 2800 to 4400).

Cochlear Aqueduct

Many questions are still awaiting answers concerning the connection between labyrinthine fluids and the CSF space, ie the patency of the cochlear aqueduct. It has been assumed that the cochlear aqueduct is a communicating link between the perilymph of the scala tympani and the CSF. Anson (1965) and Anson and co-workers (1964) studied the cochlear aqueduct in human adult temporal bones and concluded that the aqueduct is filled with tissues, although it might be possible for fluids to pass through it. On the other hand, Lempert and associates (1952) and Ritter and Lawrence (1965) described the minute size (less than 10 mm) and the narrowness of the aqueduct and concluded that there was no evidence that fluid could flow through it. Waltner (1948) even described a barrier membrane that he found at the mouth of the aqueduct. Based on their study of subarachnoid hemorrhage, Holden and Schuknecht (1968) suggested that the cochlear aqueducts are wide enough to admit erythrocytes into the inner ear in at least 50 per cent of the cases.

Silverstein and associates (1969) reported that the total protein concentration in perilymph was elevated two to three times compared with control values 5 days following the surgical obstruction of the cochlear aqueduct. The elevation of total protein concentration of perilymph remained for about 4 months after the blockage of the cochlear aqueduct. They did not observe any changes in sodium, potassium, and glucose concentrations in perilymph. These authors speculated that CSF flows through the cochlear aqueduct, after which water is probably absorbed into the veins of the perilymphatic space. They also speculated that a continuous flow of CSF through the cochlear aqueduct may help keep the perilymph from accumulating metabolic waste products and contaminants. After the surgical obstruction of the cochlear aqueduct, a degree of stagnation in the perilymphatic fluid may occur.

Juhn and Guzowski (1973) observed that the total protein concentration in cat perilymph increased abruptly 1 hour after intracisternal injection of albumin solution. There was considerable decrease of total protein in the CSF by 2 to 3 hours, although protein concentration in the perilymph remained constant during the 3 hours. This may indicate that a flow of CSF occurs into the scala tympani or that the backflow of protein from perilymph into the subarachnoid space may be quite slow.

Intracisternal injection of ouabain in chinchillas resulted in a decrease in sodium and an increase in potassium concentration in perilymph, which paralleled changes in CSF, although with a time lag (Juhn and Pearce, 1977).

Further studies are necessary to elucidate the physiologic role of the cochlear aqueduct in the functioning of the inner ear. The structural and functional alterations of this canal in various pathologic stages of the cochlear and vestibular organs need to be investigated.

Vestibular Organs

The vestibular labyrinth consists of five major structures, namely, the utricle, saccule, and three semicircular canals. These structures intercommunicate with each other as well as with the auditory portion of the labyrinth.

Bairati and Iurato (1960) concluded from the studies of the ultrastructural characteristics of the planum epithelial cells in the rat ampullae that these cells are associated with the production of endolymph by a filtration process. Dohlman and co-workers (1959) demonstrated the secretory role of the planum semilunatum in pigeons using autoradiography with 35S.

Kimura and associates (1964) studied the morphologic characteristics of the major epithelial lining of the ampullae and reported that the planum semilunatum possesses some of the characteristics that have been related to filtration and secretion in other areas of the body, such as the kidney, pancreas, ciliary body, choroid plexus, and stria vascularis. They also reported that the dark cells (the special epithelial lining found on both sides of the cristae) demonstrate some features that are suggestive of secretory and resorptive function. These cells show certain morphologic similarities to those in the distal renal tubules and the striated ducts cells of the parotid gland, both of which are known to selectively reabsorb sodium. These findings have been substantiated by Nakai and Hilding (1968), who also reported ATPase on the surface of the convoluted membrane of the dark cells.

The latter studies use the nonspecific lead ion capture method. More recent studies were reported by Burnham and Stirling (1984) using tritiated ouabain autoradiography combined with stereology. The highest "pump concentration" and the highest "membrane pump density" were found in vestibular dark cells. The highest pump concentrations were found in the vicinity of the basolateral extensions of the cells. The high pump concentration in the vestibular dark cell is apparently due to the increased membrane surface area provided by the dark cell basolateral membrane extensions.

Following denervation of the frog saccule, nearly a 50 per cent decrease of ouabain binding sites was observed in the macula (Burnham and Stirling, 1984). These studies found that enzyme sites were present in the hair cells of the frog saccule, which was in sharp contrast to the lack of detectable activity in guinea pig cochlear outer hair cells (Drescher and Kerr, 1985). Carbonic anhydrase was found to be present with great activity in the dark cells of the utricle and ampullae as well as the supporting cells of the sensory epithelium (Lim et al, 1983; Watanabe and Ogawa, 1984) but not in the sensory cells themselves.

Dohlman (1964, 1965) also reported the absorptive activities of dark cells. As the structural support for this assumption, he described the abundance of microvilli emerging from the cell surface into the endolymph, the wide intercellular spaces, and the highly clefted infoldings of the plasma membrane at the base of the cells.

The activities of a number of oxidative enzymes in tissues of the vestibular system were studied by Nomura and Balogh (1964). They reported high activity of these enzymes in the sensory epithelium of the crista ampullaris and macula sacculi. Their results indicated that the sensory epithelium has a marked capacity for anaerobic glycolysis, the presence of a citric acid cycle and an active hexosemanophosphate shunt, the existence of the cytochrome oxidation system, and the capacity for oxidative decarboxylation of alpha-keto acids to energy-rich acyl coenzyme A. They speculated that physiologic stimuli and toxic agents affect the sensory epithelium by interfering with its complex metabolism.

Through studies of the histochemical and histoenzymologic properties of the epithelial band of the crista ampullaris, Mira and Dal Negro (1969) reported that this epithelium possesses a strong metabolic activity evidenced by strong activities of oxidative enzymes. They also observed period acid - Schiff and alcian blue-positive mucoprotein-like substances in this tissue and suggested that these substances may participate in the formation of the cupula.

Ishiyama and co-workers (1969) studied the succinic dehydrogenase activity within the ampulla of the pigeon and observed heavy formazan precipitation within the planum semilunatum and moderate precipitation within the sensory epithelium, transitional zone, and septum cruciatum.

Hiraide (1971) also studied a number of various enzyme systems (oxidative, hydrolytic enzymes, and peptidase) in dark cells. He reported that these cells displayed almost the same degree of enzyme activities as cells of the stria vascularis. He also suggested that these cells may be engaged in high energy consumption and might contribute to the secretion and absorption of endolymph, similar to that of the stria vascularis. Based on enzyme histochemical studies of dark cells, he concluded that dark cells are capable of utilizing both aerobic and anaerobic glycolysis for energy production.

Evidence of acetylcholine accumulation in the vestibular receptors based on the use of cholinesterase inhibitors has been reported (Brunetti et al, 1964; Rossi et al, 1964). Iurato and co-workers (1971) studied the localization of AChE in the cristae ampullares, utricle, and saccule of the chinchilla. The reaction was positive on plasma membrane of efferent nerve fiber endings. The reaction product filled the synaptic gap between these endings and the efferent dendrites and nerve chalices but was absent at the junction between the hair cells and the efferent nerve endings and chalices.

Thalmann (1971) reported on the metabolic features of the auditory and vestibular systems. He pointed out that the purpose of both systems is the transduction of information obtained in a physical stimulus to a neural impulse. He observed that the organ of Corti has a carbohydrate store (glycogen) almost three times higher than that in the cristae. In the stria vascularis, glycogen levels are comparable to those in the vestibular structures. Thalmann also observed that in contrast to the carbohydrate stores, the reserve of immediately available chemical energy in the form of ATP and phosphocreatine is distributed rather evenly among the different labyrinthine tissues. He also suggested that the inner ear tissues may primarily utilize oxidative

energy metabolism.

The endolymphatic potentials of the vestibular labyrinth are much smaller than those of the cochlea. The ampullar endolymphatic potential was found to decline much more slowly than the cochlear endolymphatic potential in response to ischemia or asphyxia. The ampullar endolymphatic potential also became reduced and changed polarity following ethacrynic acid intoxication. However, higher doses were necessary than those needed to produce the same effects on the cochlear endolymphatic potential, and much longer time periods were required for attainment of maximum negativity of normal positive potentials (Kusakari and Thalmann, 1976).

Marcus and Marcus (1985) examined ion transport in the isolated nonsensory epithelium of the gerbil utricle. They found that the isolated nonsensory region accumulated rubidium (as a marker for potassium) in the endolymph to a level eight times that in the surrounding medium. However, since sodium also accumulated, it appeared that cells excluded from the preparation (sensory regions) may be involved in sodium reabsorption. This implies that the processes of sodium absorption and potassium secretion may be shared in some way between the two regions.

Although the chemical difference and independent nature of vestibular endolymph have been reported, further studies are necessary to clarify the origin and circulation of this fluid.

Biochemical Aspects of Otologic Disorders

Biochemical Aspects of Ménière's Disease

Ménière's disease has a characteristic triad of symptoms including (1) tinnitus, (2) fluctuating sensorineural hearing loss, and (3) episodic attacks of vertigo. These symptoms were first described by Prosper Ménière 1861. The deafness is sensorineural in type, often fluctuating, usually unilateral, and progressive. The deafness may recover in large measure between episodes early in the disease, but later each episode seems to cause some additional permanent impairment. The vertigo usually occurs in well-defined episodes. The definitive spell is often prostrating, frequently is accompanied by nausea and vomiting, and persists for a prolonged period of time. Spontaneous vestibular nystagmus is always present during the attack. Between definitive spells, there may be various kinds of adjunctive spells, such as motion intolerance, positional vertigo, falling attacks, and momentary ataxia on cornering, but the diagnosis is not tenable unless definitive spells are present with good health between them. During and briefly before a definitive spell, hearing in the affected ear may decrease and tinnitus may increase, and the condition may remain unchanged for a variable time after the spell. Since the time of Ménière's first description, a considerable amount of clinical and experimental investigative effort has been expended to attempt to unravel the cause and pathophysiology of this disease process.

The true incidence and prevalence of Ménière's disease is not known. An estimate of the incidence in Sweden was published by Stahle and co-workers (1979) as one case in every 2163 persons in the population, or 46 cases per 100,000 population. Cawthorne and Hewlett (1954) estimated an incidence of one case for every 636 persons, and Naftalin and Harrison (1958)

reported incidences of one case per 1000 persons in Great Britain. Conservative estimates by Stahle and associates are that Ménière's disease is at least four times more common than otosclerosis.

Ménière's disease is usually unilateral, although Balkany and colleagues (1980) reported that the incidence of bilateral involvement with Ménière's disease in the literature varies from 2 to 78 per cent. In their own small series of patients, they report a bilateral incidence of 46 per cent.

In spite of a substantial amount of clinical observation and experimental work in animals, the pathogenesis of Ménière's disease remains unknown. The most significant histopathologic finding has been endolymphatic hydrops, which may be the underlying factor in Ménière's disease. Factors that may be responsible for the formation of hydrops are shown in Table 2.

Table 2. Factors Possibly Responsible for Endolymphatic Hydrops

Mechanical obstruction of endolymphatic duct
Biochemical or physiologic malfunctions
- Hypoxia (vascular insufficiency)
- Metabolic or endocrine disturbances
- Immunologic reactions
- Infection.

Many possible causes of the dilatation of the membranous labyrinth in Ménière's disease have been proposed: vascular spasm of the capillaries of the stria vascularis, vasodilatation of the strial capillaries by histamine, or disturbances in the production and absorption of inner ear fluids; however, to date none of these mechanisms has been confirmed to be a cause of Ménière's disease.

The theory of Naftalin and Harrison (1958) deals with the mechanism of ion exchange in the labyrinth and accounts for the formation of hydrops by faulty perilymph production or drainage. These authors suggested that the flow of fluids in the inner ear proceeds from the perilymph through Reissner's membrane to the endolymph, with the stria vascularis acting as the absorbing site. Reissner's membrane acts only to prevent the outflow of potassium ions from the scala media back to the scala vestibuli and, by its impermeability to large molecular weight substances, prevents the proteins of the perilymph from entering the endolymph. Through an ion exchange system, analogous to that of the renal tubule, the stria vascularis extracts sodium and exchanges it for potassium. Since potassium does not pass through Reissner's membrane toward the perilymph, except possibly by a slow equimolar exchange of potassium for sodium, the concentration of potassium within the scala media builds up until the required concentration is reached. Thus the ionic content of the endolymph is controlled by the stria vascularis, which promotes the buildup of potassium. A breakdown of this mechanism could produce fluid imbalance. Naftalin and Harrison proposed that the dilatation of the membranous labyrinth in Ménière's disease could be due to a decrease in perilymph production. This could come about

by a thickening of the fibrillar material of the basement membrane, such as that seen in early glomerulonephritis, or by an imbalance of innervation, leading to constriction of arterioles.

In Ménière's disease, the sudden stimulation of the balancing mechanism might be mediated through a physicochemical event, for example, an osmotic change or reversible ion and water shift. Therefore, the low molecular weight ions of inorganic substances are more effective in regulating osmotic pressure than the organic ones, which are higher in molecular weight.

West and colleagues (1966) commented that the small molecules in plasma and tissue fluid, such as glucose, amino acids, urea and salts, freely diffuse back and forth through the blood capillaries and exert about the same total osmotic pressure in both fluids. However, plasma and lymph proteins do not freely diffuse through the capillary walls, and since the protein concentration of plasma is much higher than in lymph, the osmotic pressure of plasma exceeds the osmotic pressure of lymph by the difference in the protein concentration. This is estimated to average about 22 mm of mercury and represents the "effective osmotic" pressure or oncotic pressure of plasma.

When hypertonic solutions are ingested, water passes from the intracellular fluid to expand the extracellular volume until osmotic balance is attained. If this process is not compensated by the intake of water, severe intracellular dehydration may occur and may lead to imbalance of the central nervous system and other tissues. Osmotic disturbances associated with hemodialysis may cause inner ear changes and hearing impairment. It has been argued that in the course of dialysis the abnormally severe osmotic strains to which cells are subjected may have a serious damaging effect (Rizvi and Holmes, 1980).

Arslan (1969) reported a biphasic vestibular spontaneous picture provoked by the introduction of a saturated solution of sodium chloride into the middle ear. He demonstrated that the changes in osmotic pressure between perilymph and endolymph are the essential pathogenic factors of all clinical, histologic, and electrophysiologic manifestations.

The first phase represents the passage of water from the perilymphatic space to the middle ear due to differences in osmotic pressure. A passage of water from the endolymph to perilymph also may take place, and collapse of the endolymphatic canal may occur. The second phase illustrates the compensation of water loss from perilymph by the ultrafiltration mechanisms and the consequent bulging out of the endolymphatic canal.

Endolymphatic hydrops may also be caused by overproduction or underabsorption of endolymphatic fluid, or by a combination of both. Wullstein and Rauch (1961) reported that the electrolyte content of the endolymph was normal in Ménière's disease. This seems to lead to the conclusion that the disturbance is localized in the resorbing apparatus.

Fibrosis of the perivascular tissue in the pars rugosa in patients with or without Ménière's disease was reported by Hallpike and Cairns (1938).

Ultrastructural analysis of the human endolymphatic sac and duct imply that these structures are involved in fluid transport (Friberg et al, 1984; Bagger-Sjöback et al, 1986; Wackym et al, 1986; Friberg, 1986). The endolymphatic duct and sac contain clear intercellular spaces (lateral intercellular spaces) that are believed to form a pathway for transepithelial endolymph flow (Friberg, 2986; Friberg et al, 1985). Three mechanisms of endolymph transport across the epithelium were postulated by Wackym and associates (1986): (1) passive transcellular water outflow; (2) active transcellular ion exchange with passive transepithelial outflow of water; and (3) native transcellular vascular transport. The endolymphatic sac appears to have a secretory property. Mice subjected to hemilabyrinthectomy were found to have initial collapse of the sac lumen, followed later by ballooning of the sac, which became filled with a darkly staining homogenous substance that was shown histochemically to be proteoglycan.

A mechanical blockage of the endolymphatic duct can produce hydrops through an overaccumulation of endolymph, as Kimura and Schuknecht (1965) have shown. Histopathologic studies of the temporal bone or biopsies of the endolymphatic sac in patients with Ménière's disease have disclosed characteristic pathologic changes in the endolymphatic sac and epithelium and its surrounding perisaccular connective tissue (Arenberg et al, 1970; Shambaugh et al, 1969; Gussen, 1974). Yuen and Schuknecht (1972) and Ikeda and Sando (1984) found that patients with Ménière's disease had a reduced width of the endolymphatic duct. Other abnormalities, which have been confirmed by electron microscopy, include perisaccular fibrosis (Schindler et al, 1979; Lim and Glasscock, 1981; Yasawa and Kitahara, 1981), decreased vascularity of the sac (Schindler et al, 1979), and loss of rugae in the midportion of the sac (Galey et al, 1980). A recent study found that the basal lamina of the endolymphatic duct was darkly stained, irregular and richly folded, and appeared to contain very small striated fibrils (Fitzgerald O'Connor et al, 1985). These changes may result in a reduction of the size of the resorptive surface area of the sac. Therefore, a disturbance of endolymph resorption may result in hydrops formation or induce the symptoms of Ménière's disease. However, this does not preclude the possibility that the other factors may be responsible for the formation of hydrops in the inner ear.

A prominent finding in Ménière's disease is vacuolation of vestibular sensory cytoplasm. However, this type of degeneration seems to be a nonspecific indication of an ongoing destructive process, because vacuolation of sensory cells in the vestibular apparatus has also been observed in experimental animals after administration of ototoxic drugs (Wersall and Hawkins, 1962). Zechner and Altmann (1969) insisted that this fibrosis, noted by themselves and other investigators, might indicate a disturbance of resorptive function of the endolymphatic sac and play an important role in the pathogenesis of Ménière's disease.

A rupture of Reissner's membrane due to increased endolymphatic pressure and subsequent contamination of endolymph with perilymph was suggested as the cause of hearing impairment and initial attacks of vertigo in Ménière's disease (Lawrence and McCabe, 1959). Schuknecht and co-workers (1962) suggested that the rupture of labyrinthine membranes is the histopathologic finding that most reasonably explains the episodic nature of Ménière's disease. They also speculated that hearing loss and depression of vestibular responses are not the result of degenerative changes in the sense organ or ganglia, but are probably the result of chemical

alterations in the endolymph or fine structural alterations affecting the mechanical action of the sense organs.

Dohlman and Johnson (1965) introduced a solution of potassium chloride into a squirrel monkey's labyrinth through the oval window. This resulted in nystagmus. The nystagmus ceased after the ear cavity was flushed with normal saline. They suggested that an attack of Ménière's disease may be the result of the rupture of Reissner's membrane, allowing the endolymph to depolarize the nerve endings.

Silverstein (1970) reported that the perfusion of the perilymphatic space of cats with artificial perilymph did not result in nystagmus, whereas perfusion with artificial endolymph induced nystagmus lasting one to two and one-half hours. When the artificial endolymph was collected after cessation of nystagmus, the sodium and potassium concentrations in the fluids had dramatically changed to those found in perilymph. According to Silverstein, the development of nystagmus in those circumstances is caused by increased potassium concentration in the perilymph, which may alter the excitability of the vestibular nerve or sensory cells of the vestibular labyrinth. Subsequent lowering of the potassium concentration in perilymph to a normal level reduced this excitability.

The possibility that alterations of the body fluids may play a part in the pathogenesis of Ménière's disease has been suggested. Although dietary electrolyte control does not seem to have any effect on the underlying lesion in Ménière's disease, Naftalin and Harrison (1958) recommended the stabilization of the water and electrolyte fluctuations by dietary means to prevent gross swings in the hormonal control of homeostasis. However, direct evidence of the possible deleterious effects of body fluid changes upon the inner ear fluids has not yet been established.

Juhn (1971) observed the independent nature of perilymph and CSF fluid cations (Na^+, K^+) under the condition of abrupt deletion of sodium and elevation of potassium in the blood after intraperitoneal dialysis with ammonium chloride solution.

Metabolic dysfunction, especially of endocrine origin due to allergy and to fluid and electrolyte disturbances, has been suggested as an etiologic factor in Ménière's disease (Jordan, 1952; Shambaugh, 1959, 1952; Godlowski, 1960; Weille, 1968). Godlowski (1962, 1968) stated that Ménière's disease may be a local expression of a disturbance of an immunometabolic nature. The lesion in the temporal bone could be a specific instance of pathologic processes involving the whole organism.

It is well known that the adrenal cortical hormones participate in electrolyte balance and glucose metabolism in the body. Aldosterone, an important mineralocorticoid produced by the adrenal cortex, maintains the level of sodium chloride in the body by stimulating the reabsorption of sodium ions in the kidney tubules and by decreasing the loss of sodium through the sweat glands. At the same time, the excretion of potassium by the kidney is increased. Any fall in the level of circulating aldosterone results in a fall of sodium chloride and a corresponding loss of

water. Cortisol, the chief glucocorticoid, regulates the general metabolism of carbohydrates, proteins, and fats on a long-term basis. Cortisol acts as an insulin antagonist and converts amino acid residues into glucose, which elevates blood glucose as needed. Cortisol increases the breakdown of tissue protein to amino acids and mobilizes fat from the depots for conversion into ketone bodies.

Henkin and his associates (1967) reported an unusual combination of auditory disturbances associated with adrenal cortical insufficiency. They also reported that there was no recovery of auditory performance in these patients after treatment with deoxycorticosterone acetate. Goldman (1962) reported that 68 (90 per cent) of 75 patients with Ménière's disease responded to treatment with whole adrenocortical extract (1962).

Parkin and Tice (1970) observed a decrease of hearing during a hypoglycemic state and speculated that the cause of hearing impairment was a decrease in glucose fuel presented to the organ of Corti for energy production. Currier (1971) proposed that dizziness (or vertigo) and hypoglycemia are frequently associated symptoms and suggested the possible participation of changes in the hypothalamo-pituitary adrenal axis, with disturbed carbohydrate metabolism.

Reversible hearing loss in hypothyroidism in humans was reported by Ritter and Lawrence (1960). It seems reasonable to assume that these hormones may play a significant role in maintaining the level of responsiveness of the sensory system to incoming stimuli. The relationship between biochemical and auditory distortion appears to have potential research applications that may contribute to a general auditory theory (Lassman and Doyle, 1970).

Powers (1972) studied the role of metabolic error in patients with Ménière's disease by using the thyroid function test, the 5-hour glucose tolerance test, and adrenal-pituitary function tests. He suggested that abnormal carbohydrate metabolism and hypoendocrine function seem to be primary or contributing factors in Ménière's disease.

McCabe and Wolsk (1961) injected a high-potassium, low-sodium solutions into the guinea pig cochlea to produce a graded increase in intracochlear pressure. They observed a decrease of cochlear potentials proportional to the increased pressure.

Based upon experimental results with frogs, Henriksson (1968) suggested that the pressure within the endolymphatic sac had little effect on the sensory cells directly, but the mechanical disturbances of the sensory cells must be held responsible for the dizziness in patients with Ménière's disease. Thus, a pathophysiologic explanation for the repeated attacks of vertigo would involve distention of the membranous labyrinth due to increased pressure, with subsequent pressure reduction and return of the membranous labyrinth to its normal size.

Investigation of the pressure relationships of the labyrinthine fluids was reported by Weille and colleagues (1958). They found indications of a greater pressure in perilymph than in endolymph. On the other hand, Henriksson and associates (1966) reported that the saccule flattened when perilymphatic fluid was removed, indicating a rather small or nonexistent pressure

gradient between endolymph and perilymph.

More specific patterns of degeneration of the labyrinth were observed in a study of the macula utriculi in patients with Ménière's disease undergoing labyrinthectomy (Rosenhall et al, 1977). This degeneration appeared to start with a separation between type I sensory cells and their nerve chalices, leading to formation of a large cystic space, which probably interfered with neural function to a large degree.

A recent ultrastructural study of the utricle from a patient with Ménière's disease demonstrated that the basal lamina lining the columnar epithelial cells was markedly thickened and had a fibrillar structure (Fitzgerald O'Connor et al, 1985). Their findings could indicate a drastic alteration of the permeability of the labyrinth, which could markedly disturb fluid homeostasis and cause endolymphatic hydrops.

Ménière's disease may have as its pathologic correlate hydropic distention of the membranous labyrinth; however, endolymphatic hydrops is a pathologic diagnosis and, therefore, Ménière's disease should not be called endolymphatic hydrops unless the pathology is proved. Other conditions such as lues and leukemia may cause similar symptoms and may include membranous labyrinth distention, but such cases should not be termed Ménière's disease. Obviously, endolymphatic hydrops is the result of an imbalance between secretion and absorption of endolymph, with changes in permeability phenomena. In any examination of the basis of hydrops, the following questions must be answered:

1. What structures are primarily responsible for secretion and absorption of the cochlear fluids

2. What are the driving forces for water and electrolyte movements

3. What substances are transported passively or actively

4. How is the composition of the endolymph modified in its passage through the endolymph system

Furthermore, one must consider the ionic, electrophysiologic, and metabolic properties of endolymph and its surrounding structures in all of the three major parts of the inner ear: (1) the cochlea, (2) the vestibular system, and (3) the endolymphatic duct and sac.

In each of these structures, the resting potentials and ionic concentration differ significantly. Accepting that endolymphatic hydrops is due to increased fluid pressure in scala media, two possible mechanisms can be postulated: (1) blockage or malfunction of the outflow system (endolymphatic duct) and (2) increased water inflow due to osmotic imbalance or hypersecretion.

A mechanical blockage of the endolymphatic duct can produce hydrops by an overaccumulation of endolymph (Kimura and Schuknecht, 1965). Willbrandt and Stahle (1981) reported that one of the predisposing factors for blockage of the endolymphatic duct or sac could be decreased pneumatization around the sac, or decreased sac size, as seen in patients with Ménière's disease.

Johnstone and Robertson (1981) suggested the importance of chloride leakage into endolymph, which leads to a rise in osmotic pressure in the scala media. They showed that if potassium leakage develops in the cochlear duct, the endocochlear potential declines and potassium efflux from the endolymph slows down. The net result is almost no change in the potassium content of the endolymph. If there is a slowing of the chloride pump, the chloride concentration would increase in the endolymph. However, it is not known which factors could be involved in chloride maldistribution.

Gussen (1982) has proposed that venous insufficiency can create back pressure that reduces the ability of the endolymphatic duct and sac to reabsorb fluid. This process would result in a gradual increase in endolymph volume and pressure.

Klockhoff and Lindblom (1966) have reported substantial hearing threshold improvements following the administration of glycerin in patients with Ménière's disease, and have advocated the glycerol test as a simple and rapid diagnostic aid for differentiating cases of reversible Ménière's disease from those of irreversible Ménière's disease, or of perceptive deafness without the endolymphatic hydrops component. Angelborg and Agerup (1975) have reported a decrease in the intracochlear and cerebrospinal fluid pressure in guinea pigs 10 and 20 minutes after glycerin infusion and have related these changes to transient effects in hearing in Ménière's disease. Carlborg and Farmer (1983) reported a reduction of CSF and perilymphatic hydrostatic pressure following the administration of osmotic agents, regardless of whether the cochlear aqueduct was blocked. Angelborg and associates (1982) reported that ethanol and mannitol were much less effective than glycerol in increasing hearing ability. Yoshida and co-workers (1985) showed a clear difference in the ability of hyperosmotic solutions to reduce hydrostatic pressures in endolymph and perilymph of guinea pigs. Glycerol and urea produced a substantial endolymphatic pressure reduction. However, mannitol did not significantly reduce endolymphatic pressure compared with CSF pressure. Juhn and colleagues (1976, 1979) reported that the systemic administration of osmotic agents such as glycerol and urea caused perilymph osmolality changes parallel to changes in serum osmolality but with a distinct lag period. Their studies demonstrated that the blood-perilymph barrier appears to be permeable to water in either direction, and this may explain the occurrence of positive glycerol tests in patients with Ménière's disease.

The prevailing hypothesis of the attacks of Ménière's syndrome is that the rupture of the membranous lining of the endolymphatic space results in a subsequent leakage of potassium into the perilymphatic space. A potassium solution of this concentration (150 mEq/L) causes neural paralysis. Thus, the cochlear and vestibular nerve fibers, which are normally bathed in perilymph, are subjected to the toxin action of endolymph (Schuknecht, 1982). Another aspect of the

triggering mechanisms for Ménière's attacks may be either the "diffuse leakage hypothesis" proposed by Jahnke (1977), which is related to permeability changes of the transepithelial ion transport system, or the metabolic osmotic hypothesis proposed by Juhn (1977).

The inner ear must maintain a delicate homeostasis in order to preserve a high sensitivity to acoustic and vestibular inputs. Juhn and colleagues (1982, 1985) have conducted extensive studies on the transport characteristics of the blood-perilymph barrier to determine how the inner ear fluid composition changes under physiologic, pharmacologic, and other experimental conditions. Radioactive ions (sodium, chloride, and calcium) were found to penetrate into perilymph more slowly than they pass into CSF or aqueous humor. They found that an intravenous infusion of glucose in the chinchilla over a wide range of serum values, from 130 to 943 mg/dL, resulted in a constant perilymph to blood glucose ratio of about 45 per cent. The transport of albumin from blood into perilymph was very small after intravenous injection of radioactive albumin. Osmotic agents such as urea, glycerol, or mannitol were found to cause hypertonicity of the plasma, resulting in an efflux of water from the perilymph and an increase in perilymph osmolality (Juhn et al, 1982, 1985). A new hypothesis was suggested implicating an involvement of metabolic disorders (hormonal imbalances) in hydrops formation (Juhn et al, 1986). Adenylate cyclase is present in cochlear tissues with the greatest activity reported to be in the stria vascularis, followed by the organ of Corti and spiral ligament in the studies by Ahlstrom and colleagues (1975). However, these levels are substantially lower than that in brain and kidney.

The mechanical aspects of fluid exchange in the inner ear appear to be controlled by the cochlear aqueduct and the endolymphatic duct. Although there is not a large volume of fluid movement in the ear that might produce an outpouring of fluid into large cavities, as in the capillary and lymphatic systems of the general body circulation, blockage of these ear fluid channels may produce overaccumulation of fluid if absorption does not take place elsewhere with the membranous system. By blocking the endolymphatic duct, Kimura and Schuknecht (1965) produced a distention of Reissner's membrane in the guinea pig. One of the most interesting aspects of this experiment was the frequent atrophy of the sensory cells, stria vascularis, and spiral ganglia in the apical regions regardless of the methods used to block the duct.

In spite of the consistency of hydrops production in this experiment, Kimura (1982) points out that hydrops may also be due to (1) blockage of the endolymphatic duct by some other factors associated with the obliteration, (2) mixing of endolymph and CSF, (3) irritation resulting from opening of the endolymphatic duct and sac, (4) irritation resulting from the presence of a foreign substance, (5) reduction of the total fluid space by surgical elimination of the endolymphatic duct and sac, (6) elimination of the secretory area (endolymphatic sac), (7) elimination of the phagocytic center in the endolymphatic sac, (8) a damming effect from mechanical blockage of the fluid resorption site in the endolymphatic duct, or (9) a vascular lesion at the operative site. It is still not clear whether the hydrops itself produces the symptoms in Ménière's disease or whether it is merely some concomitant event resulting from a more basic disturbance in perilymph and endolymph production and absorption, or from a disturbance in the distribution of ions, or from some local cell damage in transducing systems.

The problem of species differences in the production of hydrops by sac obliteration is a cause for concern about the validity of an animal model and, furthermore, the validity of the theory of longitudinal flow of endolymph is in doubt. Kimura and associates (1980) experimentally obliterated the ductus reuniens in the guinea pig. They found that the cochlea developed hydrops, the saccule collapsed, and the utricle remained normal. When the endolymphatic duct and sac were blocked in ears in which the ductus reuniens had been obliterated two months earlier, endolymphatic hydrops was shown in the cochlea and utricle and also in the saccule, which demonstrated evidence of collapse following the first procedure. Therefore, these experimental results did not refute the theory of absorption of endolymph in the endolymphatic duct and sac in the guinea pig but supported it. The same conclusion was reached after experimental cryosurgical destruction of the horizontal cristae of guinea pigs without entering the membranous labyrinth; increased fluid density and phagocytic activity of cellular debris were demonstrated in the endolymphatic sac. Attempts to reduce hydrops or prevent occurrence of hydrops were made by surgical fistulization of the endolymphatic walls of the horizontal canal, common crus, utricle, or saccule, and by a common crus-subarachnoid shunt with a polyethylene tube. All these procedures resulted in closure of the opening, and the hydrops remained; collapse was rare, although some successes were achieved by interrupting the horizontal canal. Attempts to block the endolymphatic duct and to apply various methods to prevent the development of hydrops have so far failed except in a few specimens in which the scala media was perforated.

Pulec (1972) reported from studies of 120 patients with Ménière's disease that multiple but specific etiologies of the disease were found in 36 per cent of the patients: allergy in 14 per cent; congenital or acquired syphilis in 7 per cent; adrenal or pituitary insufficiency in 6 per cent; myxedema in 3 per cent; stenosis of the internal auditory canal in 3 per cent; acoustic or physical trauma in 3 per cent.

Several recent studies have suggested that hormonal disturbances may play a role in auditory disturbances and may be related to Ménière's disease. De Fronzo and associates (1983) reported that catecholamines directly influenced the sodium transport epithelial cells. A variety of epithelial cells can be affected by an increased level of circulating catecholamines, especially cells in the kidney and cochlea. Rarey and co-workers (1981) found norepinephrine in the lateral cochlear wall of the rat and speculated that its local production and release may play a role in the regulation of fluid and ionic balance. The effects of catecholamines on the central nervous system's homeostasis have also been described, revealing that circulating catecholamines can influence the permeability of the blood brain barrier.

Matsunaga and associates (1981) investigated the release of catecholamines into the plasma of patients with Ménière's disease compared with that of healthy controls. After standing erect for 5 minutes, both groups had increased plasma norepinephrine levels. However, the increment in plasma norepinephrine was significantly greater in the group with Ménière's disease than in the normals. This excess of circulating catecholamines, whether it is stress related or due to increased autonomic tone, may have significant effects on regulation of inner ear fluid volume and composition. Antidiuretic hormone, or vasopressin, is known to affect the distal renal tubules

and collecting duct and to facilitate the reabsorption of water. An increase in its secretion can thus result in a general increase in extracellular fluid volume throughout the body. Of direct relevance to the auditory system is the observation by Angelborg and associates (1973) that patients with Ménière's disease had hyperosmolality in serum. A lower level of plasma renin has been reported in patients with Ménière's disease, and it was suggested that this result indicated abnormal expansion of the extracellular fluid volume (Arenberg and Goodfriend, 1979).

Morgenstern and associates (1982) reported the effect of blockage of the endolymphatic duct and sac of guinea pigs according to the model of Kimura and Schuknecht. Three months following the induction of endolymphatic hydrops, the endolymphatic DC potential and potassium concentration in endolymph were different from the control. On the other hand, protein concentration of endolymph was significantly decreased in animals with the hydrops compared with their controls (80 ± 5 mg/dL versus 120 ± 8.5 mg/dL, respectively). By 12 months after endolymphatic sac obliteration, the endolymphatic DC potential was significantly lower in the hydrops ear compared with the control ear; however, the potassium concentrations of endolymph for the experimental and the control ear were equal. The authors postulated a decreased activity in the stria vascularis or decreased resistance of Reissner's membrane. They investigated the function of the endolymphatic sac by using anoxia or ethacrynic acid as active pump inhibitors. During transient anoxia, the DC potential decreased and it recovered after reoxygenation. Ethacrynic acid injection resulted in an increase in potassium activity in the endolymphatic sac fluid and a decrease in the potassium-sodium ratio in the epithelial and subepithelial tissues of the endolymphatic sac. The researchers suggest that these findings indicate that an active energy-consuming transport of ions exists in the endolymphatic sac that is inhibited by anoxia or ethacrynic acid.

Schuknecht (1982) has summarized the pathogenesis and pathology of Dand suggests the following sequence of events:

1. Developmental hypoplasia, trauma, or viral labyrinthitis, causing reduced resorptive function of the endolymphatic sacs.

2. A gradual overaccumulation of endolymph, causing hydrops and distortion of the membranous labyrinth.

3. Ruptures of the membranes surrounding the endolymphatic system, with contamination of perilymph by neurotoxic endolymph, resulting in the episodic vertigo and fluctuating hearing.

4. Healing of ruptures, followed by recurrence of the entire process.

5. Progression of the disease, resulting in permanent alterations in the biochemical and morphologic features of the membranous labyrinth and persistent dysequilibrium or hearing loss, or both.

"In the final analysis, Ménière's disease may be but one expression of a symptom complex that results from dysfunction of the endolymphatic sac. The disease may have its etiologic basis in developmental hypoplasia, trauma, or inflammatory disease, of which subclinical viral labyrinthitis evolves as a possible most common cause" (Schuknecht, 1982).

Ross and associates (1982) reported evidence that Na,K-ATPase plays a role in regulation ion transport into the scala media. Perturbations in the Na,K-ATPase activity in the inner ear not only could disturb certain aspects of fluid balance but could also account for the sensory disturbances experienced by patients with Ménière's disease, since high levels of the enzyme have been demonstrated on cochlear nerve fibers, especially near the foramina nervosa and within the organ of Corti.

Spring (1982) has reviewed the general mechanisms of salt and water transport by epithelial cells. Fluid absorption by Necturus gallbladder (and possibly other leaky epithelia) may be explained by the following steps:

1. Sodium chloride enters the epithelial cell across the apical membrane by a carrier-mediated process in which the co-transport of chloride is driven by the sodium gradient. This entry process appears to be the principal mode of salt movement associated with transepithelial transport and is the rate-limiting step for transepithelial fluid transport.

2. The entry of sodium chloride into the cell maintains a cellular osmolality slightly higher than that of the mucosal bath (about 2 mOsm). Water flows from the mucosa-bathing solution into the cell, driven by the gradient osmolality from the mucosal bath to cell.

3. Sodium is transported out of the cell across the base of the lateral membrane by the sodium, potassium-ATPase. The mechanism of chloride exit from the cell is uncertain. The transport of sodium chloride into the basolateral interstitial space increases its osmolality by about 1 mOsm above that of the cell. Water moves from the cell to the basolateral interstitium, driven by the gradient in osmolality.

4. A small hydrostatic pressure (about 3 cm water) develops in the basolateral interstitial space and drives the fluid across the submucosal connective tissue.

Sterkers and colleagues (1982) postulate that the endolymph originates from perilymph, based on kinetic studies of the entry of water and electrolytes into endolymph and perilymph after intravenous administration of radioactive tracers in rats. A compartmental analysis using perilymphatic perfusion of tracers indicated that perilymph rather than plasma may be considered the precursor of endolymph. Since the cochlear epithelium was found to be freely permeably to water, an alteration of electrolyte transport across the membranous labyrinth may be involved in the pathophysiology of Ménière's disease.

The possibility exists that Ménière's disease represents an autoimmune disease. Yoo and colleagues (1982) found that patients with Ménière's disease had higher antibody titers to type

II collagen than controls, suggesting that autoimmunity to type II collagen may play a role in the etiology of Ménière's disease. Further evidence to support the autoimmune theory was provided when Yoo and associates (1983) were able to induce endolymphatic hydrops in guinea pigs by immunizing them with native bovine type II collagen. Histopathologic evaluation revealed distention of Reissner's membrane, mild degeneration of spiral ganglion cells and the organ of Corti, and dilated capillaries in the stria vascularis.

The presence of a longitudinal flow of endolymph has been inferred from the studies of Kimura and co-workers (1980), which show that endolymphatic hydrops develops in guinea pigs following ablation of the endolymphatic sac on blockage of the ductus reuniens. Two tracer studies have suggested significant longitudinal flow of endolymph. Giebel (1982) used fluorescent rhodamine in endolymph to measure flow. Because the dye appeared in the endolymphatic sac within 10 minutes of injection, he estimated the rate of longitudinal flow to be 40 mm/hour. Proeschel, Sellick, and Johnstone (1984) used iontophoresis of ototoxic substances into endolymph and followed the reduction of cochlear responses to monitor direction and rate of flow. They estimated that endolymph flows toward the basal turn at a rate of 0.5 mm/min. More recent studies by Salt and associates (1986) used a nontoxic tracer monitored by ion-selective microelectrodes. The spread of tracer was found to occur predominantly by passive diffusion. The rate of longitudinal endolymph flow was estimated to be less than 0.01 mm/min toward the basal turn.

Several studies by Morizono and colleagues (1985) have further investigated endolymphatic hydrops in an animal model. Guinea pigs with surgically induced endolymphatic hydrops were found to have a reduction of the EP. On the other hand, the concentrations of sodium, potassium, and chloride in the scala media and the scala tympani were not significantly altered. Since the EP was reduced without a concomitant change of ionic concentrations, it was suggested that only the electrogenic portion of the EP was altered in animals with experimental hydrops (Cohen and Morizono, 1984). A time-dependent, progressive elevation of compound action potential threshold in these animals was also observed (Morizono et al, 1985).

Moreover, a reduced degree of compound action potential amplitude and extent with simultaneously delivered low-frequency biasing tones was observed in animals with endolymphatic hydrops (Morizono and Sikora, 1984). A qualitative relationship between the degree of hydrops and the extent of compound action potential biasing was reported, and these effects were invoked in the normal animal subjected to positive or negative pressure applied to endolymph. These findings suggest that the pressure differences that can cause auditory functional changes in endolymphatic hydrops can be quite small (less than a few cm of water) (Morizono et al, 1986). A recent human study by Tran Ba Huy (1984) confirmed the animal studies of Morizono. He found normal concentrations of sodium, potassium, and chloride in endolymph obtained from three of four patients with Ménière's disease by labyrinthotomy. The EP was found to be low in one of two patients.

Abnormal carbohydrate metabolism has been implicated in studies of the serum insulin levels by Updegraff (1977) and Mangabeira Albernaz and Fukuda (1984). In a series of 100

patients, evaluated by the latter investigation because of a suspicion of metabolic inner ear disorder, 82 patients were found to have abnormal glucose or insulin values, or both, in a 5-hour glucose tolerance test with simultaneous insulin measurements. Hyperinsulinemia was the most frequent abnormality found. These patients, including patients with Ménière's disease with abnormal glucose metabolism, responded well to a diet with a reduced intake of low-molecular-weight carbohydrates. The possibility of a systemic abnormality of fluid and solute regulation in Ménière's disease, leading to an abnormal expansion of the extracellular fluid volume, has been suggested by Arenberg and Goodfriend (1979) who found low plasma renin concentration in patients with Ménière's disease. Some patients with Ménière's disease could have abnormalities of the kidneys or adrenal glands. Rats subjected to nephroadrenalectomy were found to have significant increases in osmolality, sodium, and potassium concentrations in perilymph (Urquiza et al, 1983).

Biochemical Aspects of Ototoxicity

A more complete discussion of ototoxicity is found in Volume II.

There are a number of morphologic and functional studies on the inner ear after the administration of ototoxic drugs (aminoglycosides, diuretics, salicylates). According to Hawkins (1976), "Ototoxicity may be defined as the tendency of certain therapeutic agents and other chemical substances to cause functional impairment and cellular degeneration of the tissues of the inner ear, and especially of the end organs and neurons of the cochlear and vestibular divisions of the 8th cranial nerve".

In general, certain aminoglycoside antibiotics may produce a permanent effect by damaging the hair cells. On the other hand, diuretics and quinine appear to cause reversible disturbances in the labyrinth. Certain ear drops, including those containing chloramphenicol, can also cause permanent damage to the labyrinth if the dose is high and the exposure is prolonged (Morizono and Johnstone, 1975).

Ototoxic Agents

Aminoglycoside Antibiotics. Certain aminoglycosides, such as streptomycin and gentamicin, seem primarily to produce vestibular toxicity. On the other hand, agents such as kanamycin, neomycin, and amikacin appear to be more toxic to the cochlea. Among the commonly used aminoglycosides that are given systemically, the evidence of clinically observed cochlear toxicity seems to follow the order, from most to least frequent, of kanamycin, amikacin, gentamicin, and tobramycin (Brown and Feldman, 1978; Rybak, 1986). The semisynthetic aminoglycosides, netilmicin and dibekacin, have been found to be much less ototoxic (Brummett et al, 1978; Parravicini et al, 1983; McCormick et al, 1985; Lerner et al, 1983; Aran et al, 1982).

Toxicity in patients may be demonstrated by audiometry, by vestibular tests, or by both methods. The use of antibiotic serum levels as guidelines to minimize toxicity may prevent detectable cochlear or vestibular damage. Risk factors that may increase the likelihood of

ototoxicity should be considered (Lerner and Matz, 1978). The use of audiometric and caloric testing have been suggested if symptoms of ototoxicity (auditory or vestibular) develop, if nephrotoxicity occurs, or if elevated peak serum levels of antibiotic are achieved (above 10 to 12 microg/mL for gentamicin or tobramycin; above 35 to 40 microg/mL for kanamycin or amikacin). Temporal bone studies show damage to the outer hair cells of the basal turn progressing in an apical direction in patients with kanamycin ototoxicity (Benitez et al, 1962).

The pattern of damage to the organ of Corti in experimental animals is similar to that seen in humans. The outer hair cells seem to be more sensitive to damage by aminoglycosides than are the inner hair cells. Physiologic disturbances were indicated by reduced cochlear microphonics with reduced N1 response.

Measurements of drug concentrations in experimental studies have revealed a prolonged half-life for these antibiotics in perilymph compared with that in serum. For example, kanamycin has a half-life of 80 to 90 minutes in serum but 12 hours in perilymph. Multiple-dose experiments have shown accumulation of aminoglycosides in perilymph to concentrations considerably higher than those measured after single doses (Stupp et al, 1967). However, more recent studies by Schacht (1986) have shown that aminoglycosides do not accumulate in the perilymph but rather in cochlear tissues.

Kaku and co-workers (1973) have shown that cochlear succinic dehydrogenase and reduced DPN-diaphorase in guinea pigs were more strongly suppressed by a large dose of kanamycin administered over a short period of time than by a moderate amount given over a long period. Sato and associates (1969) reported inhibition of cochlear respiration using microrespirometry in kanamycin-treated guinea pigs. Iinuma and co-workers (1967) found that administration of kanamycin to guinea pigs was associated with a decrease in membrane ATP-ase activity in the stria vascularis, but the ouabain-sensitive component showed an increase in activity. The ATP-hydrolyzing system as a whole did not yield any common results. Saito and Daly (1971) demonstrated a decreased quantity of acid mucopolysaccharides in the stria vascularis and spiral ligament in guinea pigs subjected to kanamycin intoxication. This effect was attributed to complexing of the drug with mucopolysaccharide, which reduced its synthesis. Tachibana and co-workers (1978) have shown that acidic glycosaminoglycans in the lateral wall of the cochlear duct are reduced after kanamycin intoxication. Postma and associates (1976) found that loss of outer hair cells due to the administration of tobramycin was most prevalent in the areas that have the least glycogen.

Tachibana and associates (1976) have demonstrated an inhibitory effect of kanamycin on glycolysis in cochlear and renal tissues and correlated these effects with ototoxicity and nephrotoxicity.

A series of studies by Schacht and associates on the biochemical effects of neomycin on the cochlea and kidney reveals an interference with polyphosphoinositide metabolism in the guinea pig kidney tissue (Schibeci and Schacht, 1977) and in cochlear tissues (Schacht, 1974; Stockhorst and Schacht, 1977; Schacht et al, 1977; Schacht, 1986). In cochlear tissues, the same

concentration of neomycin that inhibited phospholipid metabolism was found to reduce cochlear microphonics by perilymphatic perfusion of guinea pig cochlea in parallel preparations (Nuttal et al, 1977). Jung and co-workers (1987) showed that serial treatment with kanamycin at the ototoxic dose lowered concentration of prostaglandin in the perilymph in chinchillas. Lowering of prostaglandins in the perilymph may be one of the mechanisms of aminoglycoside ototoxicity.

Diuretics. The loop diuretics - ethacrynic acid, furosemide, and bumetanide - have been associated with temporary, or in some cases, permanent hearing loss. Ototoxicity usually occurs in patients with renal failure.

Matz and co-workers (1969) demonstrated outer hair cell damage in a patient with ototoxicity caused by ethacrynic acid. Wigand and Heidland (1970) demonstrated reversible hearing loss with administration of furosemide. Bumetanide was substituted, and the patient's hearing returned to normal but the diuretic effect was retained (Bourke, 1976).

Hair cell damage has been associated with ethacrynic acid (Federspil and Mausen, 1973) but not with furosemide (Federspil and Mausen, 1973). Bumetanide (Santi and Duvall, 1979) and furosemide (Quick and Hoppe, 1975) appear to cause reversible edema in the stria; with all diuretics, electrophysiologic measurements have demonstrated a dose-related decline in the endocochlear potential (EP), with secondary effects on cochlear microphonics and N1 response.

Experiments (Rybak et al, 1979) have shown that furosemide enters the perilymph after systemic injection. However, no accumulation of furosemide in perilymph was found after multiple doses, suggesting that temporary disturbances of hearing correlate with presence of the drug. When the drug is eliminated, it appears that hearing returns.

Biochemical investigations into the mechanisms of diuretic toxicity have suggested that dysfunction of energy consumption processes possibly may play a role in ethacrynic acid toxicity (Thalmann, 1973). Prostaglandin does not seem to mediate furosemide ototoxicity because concentrations of 6-keto-PGF1alpha did not change significantly after intravenous injection of furosemide (Jung et al, 1988).

Bosher (1977) proposes that the action of ethacrynic acid on the cochlea may be used as a model for cochlear pathology. He summarizes these actions in three major processes: (1) inhibition of strial transport enzymes, (2) concurrent alterations in membrane permeability (reduced transport of sodium into endolymph and reduced maximum anoxic diffusional potential), and (3) possibly a decrease in the available energy supply due to late mitochondrial inhibition, resulting in a prolonged recovery time. The complex actions of ethacrynic acid on the cochlea resemble the effects of most damaging agents acting on the cochlea, including spontaneous pathologic states.

Other Ototoxic Agents. Tinnitus, vertigo, and in some cases, temporary hearing loss have been reported after clinical use of salicylates and other nonsteroidal anti-inflammatory drugs such as indomethacin, naproxen, fenorpofen, and ibuprofen (Brown and Feldman, 1978; Rybak, 1986).

These drugs are thought to induce inner ear ischemia, perhaps by blocking prostaglandin synthesis, as demonstrated by an initial depression of N1 followed by reduction in cochlear microphonic responses. Indeed, it was demonstrated that treatment with salicylates and other nonsteroidal anti-inflammatory drugs decreases the level of prostaglandin PGI2 (as measured by 6-keto-PGF1alpha) in the perilymph (Jung et al, 1984, 1985; Escoubet et al, 1985). It was also shown that hearing loss induced by salicylate has a negative correlation with the levels of PGI2 in the perilymph. For example, as hearing loss increases, levels of PGI2 in the perilymph decrease and as hearing loss is recovered, levels of PGI2 in the perilymph recover to normal high level (Jung et al, 1988).

Ataxia and hearing loss are characteristic findings of Minamata disease produced by alkyl mercury compounds. Outer hair cells of the middle coil of the cochlea are selectively damaged. Chronic mercury poisoning reduces the concentrations of succinic dehydrogenase, nonspecific esterase, and protein-bound sulfhydryls (Westernhagen, 1969).

Organic solvents such as benzene, gasoline, ethanol, methanol, and propanol can produce cochlear lesions and sensorineural hearing loss (Brown and Feldman, 1978). Nitrogen mustard, 6-aminonicotinamide, and cis-diaminedichloroplatinum (II) have been associated with tinnitus and, in some cases, high-frequency sensorineural hearing loss, which may or may not disappear when the drug is stopped. Animal studies show morphologic changes similar to those observed after aminoglycoside administration (Cummings, 1968).

Interactions of Ototoxic Agents. It has been found that hearing loss can occur when a loop diuretic and an aminoglycoside antibiotic are given together in doses that would not be expected to cause ototoxicity if either drug were given alone (Mathog and Klein, 1969; Quick, 1976). Noise exposure seems to enhance the ototoxicity of aminoglycoside antibiotics, but not that of loop diuretics (Vernon et al, 1977). Pathologic changes were greatest when noise exposure was concurrent with, or preceded by 1 week of, kanamycin treatment (Marques et al, 1975). Aspirin was found to potentiate the temporary threshold shift induced by noise trauma (McFadden and Plattsmier, 1983).

The threshold levels of ototoxic drugs in perilymph that cause functional disturbance are being evaluated. The serum concentration associated with the threshold level of accumulation of the drug needs to be further established. The serum level of the drug depends on several factors - namely, dosage, duration and frequency of administration, and excretion rate. Establishment of toxic serum levels, based on functional studies and inner ear fluid kinetic studies in animals, will be an important step for the prevention of ototoxicity in the future.

Biochemical Aspects of Acoustic Trauma

Noise-induced damage to the inner ear can be identified as sensorineural deafness and sometimes becomes an object of public concern.

There are numerous studies that correlate the physiologic and morphologic changes in animals exposed to acoustic trauma under laboratory conditions (Eldredge et al, 1961; Beagley, 1965). Biochemical aspects of noise trauma have been reviewed by Drescher (1976).

There is ample proof that the lesions produced in acoustic trauma are located predominantly in the organ of Corti; specifically, these lesions are the destruction of sensory cells and a breakup of the supporting cell attachments. Electron microscopic studies of the cochlea after experimental sound exposure have been reported (Spoendlin, 1958; Engström et al, 1970; Lim and Melnick, 1971; Bohne, 1976). Spoendlin (1958) found swelling of the mitochondria and dissolution of their lamellae in the outer hair cells as well as in the associated nerve endings as a first sign of structural damage. Similar findings in the mitochondria were also described by Koide and co-workers (1960), although only in the apical part of the outer hair cells and not on the nerve endings. Engström and Ades (1960) also reported mitochondrial swelling in nerve endings after exposure to intense sounds. The time required for healing of the noise-damaged inner ear was found to be proportional to the extent of initial injury (Bohne, 1976).

Nucleic Acids and Protein Synthesis

Many authors have reported the decrease of both ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) in outer hair cells and ganglion cells in acoustic trauma. The protein level and RNA level in the cytoplasm decreased after noise exposure first in outer hair cells and then, following increased sound intensity, in the inner hair cells (Beck and Beickert, 1958). This was followed by formation of vacuoles within the cytoplasm. In the final stage, changes in the cell nuclei, including nuclear swelling, pyknosis, and atrophy, were observed. Beck and Beickert (1958) also recorded the microphonics from the animals and correlated structural changes with biochemical changes (a marked inhibitory effect on protein metabolism) and functional alteration (the decline of microphonics). Anichin (1966) reported that the decrease in RNA is greater following intermittent sounds than following steady sounds. Nakamura (1967) investigated changes of nucleic acid activity in the cochlea resulting from white noise exposure. Pyknosis and decreased clearness of chromatin granules in the nuclei of the outer hair cells appeared in all cases after exposure to white noise at 120 dB for 10 minutes. DNA synthesis in the organ of Corti increased temporarily immediately after noise exposure. Thereafter, Nakamura found low rates of DNA synthesis up to 14 days, then the rates returned to normal. From these histochemical and morphologic studies of hair cells, it was concluded that the limit of reversible changes following acoustic trauma resulting from white noise exposure was 120 dB for 10 minutes or at 130 dB for 3 minutes. Nakamura (1967) believes that these exposures represent the upper limit for the effect to be reversible, in agreement with Nakamura (1964), who also contends that 110 dB for 90 minutes is another point on the just-tolerable exposure continuum.

Enzymes in the Cochlear Tissues

Many investigators (Spoendlin, 1958; Koide et al, 1960; Enström and Ades, 1960) have shown a structural change in the mitochondria after lengthy exposure to sound. This fact suggests dysfunction of the respiratory enzymes bound to mitochondria. The reduction in the activity of

enzymes that participate in energy production processes after acoustic stimulation results in a decrease of synthesis of energy-rick compounds (eg ATP). Thus, sensory cell degeneration can be attributed to the deficiency of energy supply.

Under normal conditions, succinic dehydrogenase activity is strongest in the stria vascularis and spiral ligament. The lactate dehydrogenase activity is highest in the hair cells, and low lactate dehydrogenase activity is observed in the stria vascularis and spiral ligament. Although decrease of succinic dehydrogenase in hair cells is observed under conditions of anoxia and noise exposure, there is little change in lactate dehydrogenase activity. This may imply that when aerobic metabolism is damaged, anaerobic metabolism may supplement the energy need of hair cells.

Vosteen (1957) observed changes in succinic dehydrogenase activity in the organ of Corti after stimulation by pure tones with an intensity range of 75 to 85 dB for 2 days. He reported that a drop in enzyme activity occurred after such prolonged and continuous sound exposure.

A decrease of acetylcholinesterase activity in the guinea pig cochlea following a 3-hours exposure to sound of 90 dB was reported by Conti (1961).

Vinnikov and Titova (1963) reported the reduction of succinic dehydrogenase activity in outer hair cells after exposure to sound of 95 dB for 1, 2, 4, or 6 hours. Hayata and co-workers (1967) studied succinic dehydrogenase and phosphorylase levels in the cochlea of guinea pigs exposed to white noise. The initial decrease of succinic dehydrogenase in outer hair cells was found after exposure to white noise with an intensity of 100 phons for 3 hours. After a 24-hour exposure, numerous damaged outer hair cells showed complete disappearance of enzymatic activity. Phosphorylase activity did not significantly change after a 24-hour exposure to intense noise, the same authors found that the first and second rows of outer hair cells were more sensitive than the third row, the lower turn was damaged sooner than the upper turn, and the inner hair cells were less vulnerable.

Nakamura (1964) reported a pattern of a decrease (after an initial temporary increase) in the activities of many different enzymes, eg succinic dehydrogenase and DPN-diaphorase. He also reported a prophylactic effect from papaverine hydrochloride, nicotinic acid, vitamin B, nylidrin hydrochloride, thioctic acid, ATP, and chlorpromazine.

Takahashi (1967) studied oxygen consumption in the organ of Corti by observing succinic dehydrogenase activity following exposure to noise. He reported that in the group exposed to white noise for 120 dB for 30 minutes, activity of succinic dehydrogenase began to rise immediately after exposure to the noise and attained a maximum activity after 24 hours. In the group exposed to white noise of 120 dB for 2 hours, a temporary rise in the activity of succinic dehydrogenase following the exposure did not appear, but the value dropped immediately after the exposure until about the seventh day, and no sign of recovery was noted thereafter.

It was inferred from the results just mentioned that the amount of succinic dehydrogenase participating in metabolic activities in the organ of Corti was transiently increased by noise stimulation immediately after exposure to noise. It then tended to decrease and, if reversible, to slowly regain its former level. However, if the process was not reversible, the activity level continued to fall, and at the same time, the organ of Corti suffered irreversible damage.

Ishida (1978) reported an increase of lactate dehydrogenase activity in perilymph 15 hours after the noise exposure (115 dB, 10 to 50 kHz), and he speculated that the increase may be due to the leakage of lactate dehydrogenase from the hair cells.

Jung and associates (1986) reported concentration of prostaglandins (6-keto-PGF1alpha) in the perilymph 4 and 12 hours after the noise trauma for the permanent threshold shift (110 dB for 12 hours) and 12 hours, 1 week, and 1 month after the noise trauma for the temporary threshold shift (95 dB for 4 hours). The concentrations of prostaglandin in perilymph after noise trauma for the permanent threshold shift increased more than five times of normal levels. After the noise trauma for the temporary threshold shift, the levels of prostaglandin increased more than four times that of normal in 12 hours, then dropped down to normal levels in 7 days, and in 1 month, the hearing returned to normal. It was speculated that a large increase of prostaglandin in the perilymph may be due to depletion of prostaglandin from the cochlear tissue.

Oxygen Supply and Consumption

The correlation between hypoxidosis and acoustic trauma has been studied. Tonndorf and co-workers (1955) reported that a moderate oxygen deficiency may potentiate the amplitude shift of cochlear microphonics after high-intensity sound exposure (130 dB) and also may protract the recovery period. Misrahy and associates (1958) reported a decrease of endolymphatic oxygen tension following hypoxia and sound exposure. It is assumed that oxygen tension within the endolymph depends on the oxygen supply delivered by the stria vascularis and on the oxygen consumption of the hair cells. Since the transformation of sound within the hair cells is an energy-consuming process, it is conceivable that the oxygen consumption may increase because of an increased acoustic load, thus decreasing the oxygen tension in the endolymph.

Jansen (1967) reported a generalized peripheral vasoconstriction in response to noise. Schnieder (1970) observed a sharp reduction in the clearance rate of dye from the perilymph in guinea pigs exposed to noise. Hawkins (1971) studied the vascular changes in the inner ear tissue after noise exposure. He reported the evidence of vasoconstriction and loss of capillaries in the spiral ligament above the attachment of Reissner's membrane after 8 hours of exposure at 118 to 120 dB sound pressure level (SPL). However, mechanisms causing vasoconstriction in the spiral ligament need further study.

On the other hand, according to Perlman and Kimura (1962), during strong (120 dB) acoustic stimulation, vessels of the stria vascularis did not reveal either vasoconstriction or dilation, whereas at the same time a marked reduction in microphonic response was recorded. They also reported that when the acoustic exposure was severe enough to produce a decrease or

permanent loss of cochlear microphonics, an increase of blood flow rate in the vessels of stria vascularis could be observed. The cause of this increased flow rate may be attributed to the local increase in CO_2 due to increased metabolism.

Glycogen

Since the organ of Corti is remote from a blood supply, it is reasonable to assume that anaerobic glycolysis may have an important role in its metabolism, with glycogen as the main source of energy. Many investigators have studied the distribution of glycogen in the inner ear. Ishii and co-workers (1969) demonstrated the distribution pattern of glycogen in various species, using histochemical methods at the light and electron microscopic level. After exposing guinea pigs to 110 dB of white noise for 30 minutes, they noted a quantitative decrease in the number of glycogen granules; in 12 to 24 hours, these granules aggregated to form larger granules. Finally, 24 hours after the exposure to white noise, the distribution pattern of glycogen reverted to normal. Borzoli and Boriani (1958) reported a diminution of glycogen content of the hair cells after exposure to sound (sound pressure level of 50 dB, for 30 minutes). The results of these studies should indicate the release of chemical energy after acoustic stimulation and restoration of the depleted glycogen stores by glucogenesis, suggesting that glycogen serves as an energy source in the hair cells.

Protein Patterns

Beck and Holz (1965) reported that new protein fractions that seem to be gamma globulins and have low molecular weight were observed in the guinea pig perilymph after sound exposure. No new protein fraction could be observed in endolymph after noise exposure. Scheibe and co-workers (1975) reported a small increase in the protein concentration of the perilymph after noise exposure (140 dB for 1 hour) in guinea pigs.

Ionic Changes

Nakashima and associates (1970) reported that intense acoustic stimulation increased the Na+ concentration and decreased the K+ concentration in the endolymph. They observed a decrease of Na+ and an increase of K+ concentration in the perilymph of the scala vestibuli after similar acoustic stimulation. Bohne (1976) reported interruption of the continuity of the reticular lamina by noise exposure and damage to the cells in the organ of Corti by the flow of endolymph, which contains high levels of potassium.

A few chemical substances have been reported to have a beneficial effect on functional damage caused by the noise exposure. A favorable effect of ATP, adenosine monophosphate, and adenine on the restitution of cochlear microphonics after acoustic load and hypoxia has been reported (Faltynek and Vesely, 1964, 1967). The authors used ATP for the treatment of patients with sudden deafness and reported improvement in hearing in most of these patients.

Kellerhalls (1972) found constriction of strial capillaries with tight packing of blood cells in noise-exposed guinea pigs and correlated this finding with the fact that dextran-treated animals had fewer missing hair cells. He also demonstrated that dextran seems to enhance perilymph production and postulated that any drug that enhances perilymph production may help to prevent inner ear damage from acoustic trauma (Kellerhalls, 1974).

Bohne (1976) summarized the theories of noise damage to the inner ear and listed four categories: (1) mechanical, (2) metabolic exhaustion, (3) vascular change, and (4) ionic change. She feels that the last mechanism is the most likely cause of injury, based on perfusion studies with artificial endolymph.

Biochemical Aspects of Otosclerosis

Otosclerosis is a disease or disorder of the bony capsule of the labyrinth. The bony changes seem to vary according to the duration of the disease. At first, the normal bone is absorbed and replaced by spongy vascular osteoid tissue. The most common site of disease is the promontory in the region of the anterior margin of the oval window, and in advanced cases, the stapes becomes ankylosed in position by a mass of spongy new bone. A conductive hearing impairment results from the decreased mobility of the stapediovestibular articulation, and sensorineural hearing losses have been attributed to this disease.

The focus originates within the endolymphatic layer, and as the lesion enlarges, it spreads across the annular ligament to encroach upon the stapes footplate. During its initial stages, the disease process resembles that in osteoporosis. The immature cartilage bone typical of the otic capsule is resorbed, and new vascular bone is formed. A large increase in the number of osteoblasts and osteoclasts indicates that otosclerosis is not simply an alteration of old bone but rather involves active remodeling activity (Reydon and Smith, 1968). The early appearance of this new bone is "spongy" because of numerous channels that accommodate vascular and cellular elements. Indeed, Gussen (1969) suggests that otosclerotic bone is the normal secondary membrane bone formed by other skeletal structures during remodelling. Only later in the disease does calcification occur.

The pathologic picture in otosclerosis generally seen by the surgeon may be the result of a disease of several years' duration, since it only when the otosclerotic focus interferes with stapes mobility sufficiently to cause clinical symptoms that an individual seeks professional help.

Although the extensive work of numerous investigators has partially clarified the morphology and morphogenesis of the disease process in otosclerosis, the critical question still remain: What are the biochemical processes underlying the otosclerotic changes? What is the physiologic trigger that initiates the abnormal sequence of metabolic events

Bone is a highly specialized form of connective tissue, consisting of two main parts: (1) an organic protein matrix and (2) inorganic minerals. The organic matrix is primarily composed of collagen fibers embedded within an amorphous gel of mucopolysaccharides that form a

cementing ground substance. Within the ground substance are the cellular components of bone that are associated with specific functions: osteoblasts, associated with bone formation; osteocytes, associated with bone maintenance; and osteoclasts, associated with bone resorption. The organic matrix is called osteoid.

Mineral in bone exists mainly in the form of hydroxyapatite, a complex crystal lattice with the general formula of Ca10(PO4)6(OH)2. The skeleton contains other ions, eg sodium, magnesium, strontium, and citrate. The ground substance consists of the extracellular and interfibrillar component of all connective tissue and is characterized chemically by its content of sulfated polysaccharides containing hexosamines, or amino sugars.

Throughout life, skeletal bones undergo a constant remodeling, which entails a turnover of their components. This is a dynamic process that involves resorption of both organic and inorganic substances of the calcified tissue, with subsequent formation of new bone (Armstrong and Singer, 1965).

The bone of the otic capsule and ossicles differs from other bones in the body in many respects. The developmental anatomy of the auditory ossicles and labyrinthine capsule has been studied intensively by Anson and co-workers (1959; 1962). The ear represents the only place in the body where bone derived from cartilage does not develop further after the time of ossification during fetal life. In all other bones, the primary cartilage bone undergoes secondary erosion and subsequent replacement by lamellar haversian bone. Differences in chemical composition between the long bone (femur) and the auditory bones have also been observed; the otic capsule appears to have lower concentrations of hydroxyproline than do either auditory ossicles or the femur (Juhn, 1987).

Intercellular enzymatic changes in bones having active otosclerosis have been reported by many investigators (Chevance, 1964; Ardouin and Wegmann, 1961; Alberti and Tarkannen, 1963). Chevance (1964) demonstrated a positive histochemical reaction for the sulfhydryl group in the region of otospongiotic foci. This may be an index of the osteolytic activity occurring in the focus. This study reveals that the alteration in the enzymatic activity in the osteocyte seems to be the primary factor in the development of an otosclerotic focus (Chevance et al, 1970).

Using histochemical techniques, Alberti and Tarkannen (1963) have demonstrated high activities of leucine amino peptidase and nonspecific esterase in otosclerotic osteocytes, in the walls of blood vessels that penetrate the focus, among the perivascular fibroblasts, and in the mucosa overlying the focus. The presence of leucine amino peptidase indicates the presence of immature fibroblasts in fibrous tissue.

Arslan and Ricci (1961) suggested alterations in the ground substance of otosclerotic foci as being localized manifestations of a generalized mesenchymopathy. Ricci (19620 demonstrated an abnormal accumulation of mucopolysaccharides and increased amounts of alkaline phosphatase in otosclerotic foci. He considered alterations of ground substance to be the primary factor in otosclerosis. Changes in both the ground substance and the collagen fibrils of the matrix have

been reported (Albernaz and Covell, 1961). These authors found a large increase in the amount of acid mucopolysaccharide in active otosclerotic foci. Soifer and co-workers (1969) studied hexosamine, hydroxyproline, and proline in ossicles and cortical bone from otosclerotic and nonotosclerotic patients. They tried to determine whether biochemical changes in the ground substance of fibrillar content occur in the matrix of bone from patients with otosclerosis. No differences were found between otosclerotic and nonotosclerotic individuals with respect to the hexosamine or the hydroxyproline and proline content of ossicles and cortical bone. These results indicate that no quantitative changes in the amount of total mucopolysaccharide or in the fibrillar content of the bone matrix occur as a result of otosclerotic process. Soifer and co-workers suggested, however, that there may be structural differences between the collagen fibrils of bone from otosclerotic patients and those of bone from nonotosclerotic individuals.

Linck and associates (1967) reported a slight decrease in calcium content in the otosclerotic footplate. This means that even the completely mature otosclerotic bone is unable to reach the high degree of mineralization that characterizes the labyrinthine capsule bone. However, the otosclerotic bone calcium content was similar to the skeletal bone calcium content. These authors also reported a highly significant increase in the fluoride content of otosclerotic footplate. Compared with meatal bone, the fluoride content of otosclerotic bone was increased nearly three-fold. This can be considered a consequence not merely of the heightened remodeling activity but also of some structural and chemical properties of the otosclerotic bone.

Evans and Henkin (1969) studied the mineral content of otosclerotic ossicles (mallei and incudes) with photon beam absorption. They reported that the mean mineral content of otosclerotic incudes and mallei was significantly lower than normal. However, when Soifer and co-workers (1970) compared the calcium and phosphorus concentration in the stapes and the cortical bone from individuals with otosclerosis with that of nonotosclerotic controls, they could not observe any significant differences.

As a result of their microradiographic studies, Roberto and associates (1971) reported that the otosclerotic stapes is less mineralized than the normal bone.

The determinations of certain enzyme activities in otosclerotic foci have been performed. Albernaz and Covell (1961) found large amounts of succinic dehydrogenase and cytochrome oxidase in osteoclasts, fibroblasts, and blood vessel walls of otosclerotic foci, suggesting a high level of respiratory activity.

Soifer and co-workers (1969) reported a considerable elevation of alkaline phosphatase activity in otosclerotic stapedes and suggested that there may be an increase in the cellular metabolic activity of this ossicle in otosclerosis.

On the other hand, Maurer (1961-1962, 1967) studied bone samples from otosclerotic patients and evaluated calcium, phosphorus, and alkaline phosphatase activity. A decrease in the alkaline phosphatase activity in involved bone was observed. It was suggested from these data that the primary change in otosclerosis is in the collagen fibrils. Holdsworth and associates (1973)

reported finding aberrant enzyme activity (phosphofructokinase) in otosclerotic stapedes. These authors suggest that otosclerotic bone appears to be adapted to a primarily aerobic metabolism.

Biochemical changes in perilymph in otosclerosis have been studied by many investigators (Wullstein et al, 1960; Rüedi et al, 1965; Silverstein and Schuknecht, 1966; Schindler and Schnieder, 1965; Schindler et al, 1966; Rauch and Plester, 1965); there were no significant differences in the levels of sodium and chloride in the perilymph between control subjects and otosclerotic patients. The level of potassium in the perilymph of those with otosclerosis was, however, well above control levels in the perilymph of those without otosclerosis. A low level of calcium was also reported. Increased protein levels were determined in the perilymph of otosclerotic patients. These authors suggested that this is probably the result of venous congestion of the spiral vessels, with an attendant transudation of blood serum into the perilymph. This increase, however, cannot be considered a special characteristic of otosclerosis, since high protein values can be found in many diseases of the inner ear that arise from a different cause, eg infections, neurovegetative disturbances, Ménière's disease, tumor of cranial nerve VIII, and other disorders. The only characteristic biochemical change in otosclerosis is an increase of the alkaline phosphatase concentration in perilymph above the levels found in normal perilymph.

Rauch and Plester (1965) reported an elevation of the malate dehydrogenase concentration in both the serum and perilymph of patients with severe hearing loss secondary to otosclerosis that was directly proportional to the enzyme levels. They postulated that general elements of stress affecting the deaf person were responsible for the observed elevations. Silverstein and Schuknecht (1966) also reported perilymph malate dehydrogenase values in otosclerotic patients to be approximately twice the concentration in serum. No correlation between the magnitude of hearing loss and that of malate dehydrogenase elevation could be demonstrated.

Many authors have reported studies of tissue and body fluids of patients suffering from otosclerosis, eg in veins (Soifer et al, 1965), skin (Vyslonzil, 1956), serum (Fowler, 1948; De Jorge et al, 1965), and urine (Herrmann and Maurer, 1955; Maggio, 1953). Most of the authors found no significant variations between otosclerotic patients and control subjects. However, De Jorge and co-workers (1965) found that the serum of otosclerotic patients had low inorganic phosphorus levels, high inorganic sulfur levels, and tendencies toward high calcium and low alkaline phosphatase activity. From these factors, they suggested that otosclerosis may be a metabolic bone disease. Soifer and associates (1965) reported that average lactate dehydrogenase activity in vein tissue from patients with otosclerosis was 30 per cent higher than normal. Pal'chun and Gamov (1969) reported the results of serum protein fraction of otosclerotic patients determined by electrophoresis. They found a statistically significant reduction of beta globulin fraction in the serum of otosclerotic patients. This was considered to be a general reaction of protein metabolism associated with the otosclerotic process.

An accumulation of radioactive sulfate in the otosclerotic ossicles was reported by Ardouin and Wegmann (1961). A positive sulfur balance caused by the retention of sulfate in otosclerotic patients was reported (Paiva and De Jorge, 1969). They suggested that a particular kind of sulfate thesaurismosis may be a possible factor in the pathogenesis of otosclerosis.

Puhakka (1971) studied the crystalline structure and the mineral content of auditory ossicles using x-ray crystallography, microradiography, and quantitative chemical analysis. He reported that the calcium, phosphorus, and magnesium content in otosclerotic bones was significantly lower than in normal bones. There were no significant differences in sodium content. He attributed the abnormal crystalline structures he observed in the otosclerotic bone to alterations in the organic component of these bones.

Certain clinical observations suggest the involvement of endocrine disturbances in the process of otosclerosis. Cawthorne (1955) reported that otosclerosis is found more than twice as frequently in women as in men. Deafness due to otosclerosis sometimes seems to be initiated or made worse by pregnancy. Wullstein and co-workers (1960) and Larsson (1960) reported that otosclerotic deafness often coincides with puberty and that the incidence of onset is also increased with the age of the individual.

It is known that the dynamic processes involved in bone metabolism are under hormonal control. Parathyroid hormone and thyrocalcitonin act in an antagonistic manner to provide the control system that regulates calcium availability. Parathyroid hormone and vitamin D act to stimulate bone resorption. Thyrocalcitonin and phosphate inhibit its resorption. It is also known that androgens and estrogens are involved in bone metabolism and that adrenocortical hormones activate bone resorption. Osteoporosis, a softening and demineralization of bone, is commonly found in patients with Cushing's syndrome and in patients who take large doses of adrenal corticoids.

The measurement of levels of urinary steroids in patients with otosclerosis has been reported with conflicting results; studies include measurements of androgens (Herrmann and Maurer, 1955; Vyslonzil and Klein, 1961), estrogens (Vyslonzil, 1963; De Jorge et al, 1965), and 17-ketosteroids (Maggio, 1953; Soifer et al, 1970). It is also interesting to note that a significant number of patients with otosclerosis were diagnosed in a group of acromegalics (Richards, 1968). The specific function of hormones in the pathogenesis of otosclerosis needs much more investigations.

Several enzymes with potential toxicity for the organ of Corti are known to play an active role in bone remodeling. Chevance and co-workers (1972) suggested that lysosomes are involved in the extension of the otosclerotic lesion. More specifically, they postulated that lysosomal hydrolases, such as acid phosphatase, cathepsin, hyaluronidase, and collagenase, form the front line of an advancing focus. They support their hypothesis by demonstrating acid phosphatase activity at the periphery of active otosclerotic foci. It is conceivable that these enzymes could affect the organ of Corti or the stria vascularis, causing sensorineural hearing loss directly if the foci were to invade the endosteal layer in the area of the spiral ligament or indirectly by diffusion through the perilymph and basilar membrane. It is also possible, however, that sensorineural losses are the result of interference in venous drainage within the cochlea due to vascular shunt formation as postulated by Rüedi and Spoendlin (1966).

Histologic changes in the ground substance of bone having otosclerotic foci have been observed, and differences may occur in the proportions of the specific types of mucopolysaccharide molecules. Roberto and co-workers (1971) found that in otosclerotic stapes, the active foci are less mineralized than the parts not affected by the disease. In the active foci, they observed a great number of enlarged lacunae surrounded by areas of low mineralization. A close relationship between the deposition of minerals in otosclerotic bone and collagen fibrils embedded in the ground substance has been reported (Lim and Saunders, 1977).

Jensen and associates (1979) reported that in 63 patients with surgically proven otosclerosis, the bone mineral content was normal in skeletal bones, further supporting the concept that otosclerosis is a localized rather than a systemic skeletal disorder. More recent studies by Pedersen and co-workers (1984) of iliac crest biopsies have confirmed the above findings. Mann and colleagues (1980) performed a histochemical and ultrastructural study of otosclerotic stapes. They observed chondrocytic chondrolysis, which strongly suggested a role for cartilaginous remnants in the otic capsule, perhaps releasing proteolytic enzymes. These substances may cause structural changes of the otic capsule. Another lysosomal enzyme, cathepsin B, was found to be one order of magnitude higher in the otosclerotic stapes footplate than in the superstructure, which was not affected by the disease (Ribari et al, 1983. Ribari and Sziklai (1983) have fractionated noncollagen proteins extracted from separately pooled superstructure and stapes footplates of surgically removed stapes from otosclerotic patients. Two fractions were obtained, one of which contained acidic, low-molecular-weight proteins not detected in the superstructure and temporal cortical bone. The absence of this low-molecular-weight protein in the superstructure was said to prove that otosclerosis was localized to the footplate and did not involve the superstructure. In the immature stages of otospongiosis, especially with cochlear involvement, high concentrations in the perilymph of other lysosomal enzymes (acid phosphatase, beta glucuronidase, collagenase, and leucine aminopeptidase) accompanied by a low concentration of alkaline phosphatase have been reported (Petrovic et al, 1985). However, when the disease reaches the mature or otosclerotic stage, the concentration of these enzymes falls, whereas the activity of alkaline phosphatase increases significantly (Petrovic et al, 1985). Moreover, the pH of the perilymph is said to change from acidic to more alkaline. Treatment of a patient with immature otospongiosis for 3 to 6 months is said to decrease the concentration of acid phosphatase and the other acidic enzymes mentioned above while increasing the alkaline phosphatase.

These authors state that, in organ culture, otospongiotic bone produces 4 to 12 times more prostaglandin E2 and 3 to 6 times more cyclic AMP than mature otospongiotic bone (Petrovic et al, 1985). Guinea pigs that were subjected to perilymphatic perfusion with trypsin were found to have a dose-dependent damage to hair cells in the organ of Corti. Another group of animals inoculated with trypsin into the bulla had decreased cochlear microphonic responses, with 32 per cent having a severe hearing loss across all frequencies (Chevance and Niaussat, 1979).

An interesting new potential animal model for the study of otosclerosis has been found. The inbred LP/J mouse spontaneously develops abnormal bony lesions that are grossly and histologically similar to human otosclerotic lesions (Chole and Henry, 1983). These investigators

found chalky white lesions having a greater ratio of cells to bone matrix than surrounding bone. Transmission electron microscopy showed the active regrowing lesions in the LP/J mouse in areas of osteoblastic activity where calcospherites were being deposited on poorly organized collagen fibers surrounded by amorphous ground substances. These lesions are typical of otosclerotic lesions studied in human substances (Lim and Saunders, 1977). More recent and detailed histologic evaluation of the lesions in the animal model have provided further insights into comparing this disease with its human counterpart (Chole and Henry, 1985). This inbred mouse was found to develop abnormal bony lesions of the middle ear resembling human otosclerosis, which developed after puberty and involved only the ossicles and otic capsule. Cochlear hair cell loss was progressive throughout the life span of these animals. Although there are certain differences from the human counterpart, this model may be useful in studying details of the otosclerotic process.

Another interesting way of inducing otosclerosis in animals was reported by Yoo and associates (1982). They produced otosclerosis-like bone lesions in rat otic capsules by inducing immunity to type II collagen. They suggest an autoimmune etiopathogenesis for otosclerosis (Yoo, 1984).

Histologic evidence of cochlear otosclerosis without oval window involvement was presented by Balle and Linthicum (1984). They suggest that sodium fluoride and vitamin D may help improve the hearing in cochlear otosclerosis.

An intriguing clinical study was reported by Brookes (1983). He studied 10 cases of bilateral cochlear deafness and found that each patient had low vitamin D levels. Replacement therapy improved two of four patients. He speculated that vitamin D deficiency may underlie otosclerosis and other types of hearing loss, and that localized demineralization of the cochlea may cause secondary morphologic changes.

Skin biopsies from patients with otosclerosis and osteogenesis imperfecta tarda did not differ from normal in collagen fiber structure by light or electron microscopy (Balle et al, 1984). In contrast to otosclerosis, patients with osteogenesis imperfecta have been found to have reduced bone marrow content in their long bones (Pedersen, 1985) and slightly elevated serum alkaline phosphatase. Nine of fifteen stapes from patients with osteogenesis imperfecta exhibited a localized focus in the footplate with histologic similarity to the early otosclerotic lesions. Unlike otosclerosis, osteogenesis imperfecta is a generalized systemic disorder of collagen metabolism, with the localized changes in the footplate representing part of the general systemic changes (Pedersen, 1985). In a related bone disease, calcitonin has been reported to arrest the deterioration of hearing in Paget's disease, and may even reverse sensorineural hearing loss in some patients with this condition (Solomon et al, 1977).

Sodium Fluoride and Otosclerosis

It is generally accepted that fluoride forms a chemical compound with the hydroxyapatite crystals of bone, the fluoride ion replacing the hydroxyl ion. The resulting fluoroapatite is less

soluble than the hydroxyapatite that it replaces. The rate of deposition of fluoride in teeth and bone is proportional to the fluoride intake from food, inhalation, medication, and most often, drinking water.

Shambaugh and Petrovic (1967) proposed theoretical reasons why large doses of sodium fluoride might favor recalcification and inactivation of the spongy, actively growing otosclerotic focus. Petrovic and Shambaugh (1968) also have reported that sodium fluoride brings about a reduction in bone resorption and a promotion of bone calcification. They reported that sodium fluoride has a promoting effect on otosclerotic bone calcification in organ culture and suggested that sodium fluoride along with phosphates may be useful in the treatment of patients with secondary osteoporosis, Paget's disease, and possibly the osteoporotic stage of otosclerosis.

Linck and co-workers (1967) found that the normal stapes, footplate, and crura contain more fluoride and calcium than does meatal bone. They discovered a reduced calcium content in otosclerotic footplate compared with the normal footplate. Fluoride content in otosclerotic bone was significantly greater than in meatal bone. Thus, they suggest that sodium fluoride in optimum doses promotes calcium deposition in bone by depressing bone resorption, probably also inducing a stimulating action on bone calcification.

Jowsey and associates (1968) have shown that increased bone formation is dependent on fluoride intake, not on the amount of fluoride already incorporated into bone, suggesting that fluoride acts to directly stimulate bone formation. Shambaugh and Causse (1974) postulate that toxic enzymes liberated by the otosclerotic foci may account for sensorineural deterioration in otosclerotics and that sodium fluoride appears to act partly to inhibit enzyme production and partly to decrease osteoclastic resorption and to increase calcification within foci to arrest the process. Although the current fluoride regimens used (Causse et al, 1974) appear very promising, a thorough understanding of fluoride's effect on bone would be extremely beneficial in improving the effectiveness of therapeutic programs.

Bretlau and co-workers (1981) determined calcium to phosphorus ratios of otospongiotic stapes in sodium fluoride-treated patients and compared these data with those from stapes from untreated otosclerotic patients using a transmission scanning electron microscopy technique with an energy dispersive x-ray elemental analyzer. They found that the sodium fluoride-treated otospongiotic stapes had a significantly higher calcium to phosphorus ratio (Bretlau et al, 1981).

Chevance and co-workers (1976) showed an inverse correlation between the alpha-antitrypsin activity of perilymph of patients and progression of bone conduction hearing loss. This adds support to their earlier conclusions correlating progression of inner ear deafness with activity of hydrolytic enzymes in perilymph (Chevance et al, 1972; Causse et al, 1972). Causse and associates (1980) postulated that toxic enzymes liberated by the otosclerotic focus may account for the sensorineural deterioration in otosclerosis, and that sodium fluoride appears to act as an enzyme inhibitor and as an inhibitor of osteoclastic resorption, thereby increasing the calcification within otospongiotic foci to arrest the process. More recent studies by this group (Causse et al, 1982) have utilized samples of perilymph obtained during stapedectomy. Three selected enzymes

were studied - trypsin, alpha-1-antitrypsin, and alpha-2-macroglobulin. They proposed a theory that sensorineural hearing loss occurs when the equilibrium between trypsin and the other substances that act as antienzymes (alpha-1-antitrypsin and alpha-2-macroglobulin) is disrupted and relative trypsin activity increases. Sodium fluoride seems to reduce overall enzyme values, causing fluoride equilibrium and favoring the arrest of cochlear deterioration in otospongiosis. Further clarification of the role of these enzymes in otosclerotic patients and more data are necessary before establishing these enzymes and enzyme inhibitors as indices for the severity of progression of otosclerosis.

Sodium fluoride is said to improve otosclerotic lesions through three actions: (1) direct inhibition of trypsin; (2) overall reduction in enzymatic values within perilymph microfoci in the otic capsule, shown by cytochemical studies; and (3) a direct or indirect conversion of active foci into inactive ones by interfering with the calcium to phosphorus ratio (Causse et al, 1985). In addition to sodium fluoride, diphosphonates appear to act also as enzyme inhibitors in the otic capsule and perilymph, reducing the intensity of reabsorption phenomena and, thereby, promoting a stable secondary new bone formation within otospongiotic foci (Causse and Causse, 1985; Stutzmann and Petrovic, 1985). The use of sodium fluoride for otosclerosis is controversial (Snow, 1985). Causse and Causse (1984) have even recommended prophylactic treatment of children of otospongiotic patients with sodium fluoride to prevent or control otosclerosis. Karjalainen and colleagues (1982) found that, in Finland, artificially elevated fluoride levels in drinking water increased the fluoride content of bone compared with the fluoride content in bone found in residents of a low-fluoride area. There did not appear to be any difference in the incidence or the age of occurrence of otosclerosis. However, the severity of the hearing loss appeared to be less in the residents who drank fluoridated water.

A recently published double-blind, placebo-controlled study of patients with otospongiosis showed that sodium fluoride treatment can stabilize otospongiotic lesions to retain calcium relative to phosphorus (Bretlau et al, 1985). The study of 95 patients showed a significantly worse deterioration of hearing in the placebo group than in the actively treated group (40 mg of sodium fluoride administered daily). This supported the view that sodium fluoride can change otospongiotic-active lesions to more dense, inactive otosclerotic lesions. This may be related to the antienzymatic activity of sodium fluoride as discussed above. In the study mentioned earlier, 400 munits of vitamin D, 500 mg of calcium gluconate, and 20 mg of sodium fluoride were contained within enteric-coated tablets given twice a day to the active group. The placebo group was given a similar type of tablet without sodium fluoride but containing the same dose of calcium gluconate and vitamin D. Thus, it appears that vitamin D alone is not sufficient to provide the full therapeutic benefit equal to that obtained with sodium fluoride plus vitamin D and calcium (Bretlau et al, 1985).

Immunologic Aspects of Otologic Disorders

Immunologic disturbances recently have been recognized to have a relationship to many causes of auditory dysfunction (Stephens et al, 1982). A particularly fascinating group of immunologic diseases have been classified as autoimmune diseases affecting the inner ear. It is

believed that Lehnhardt (1958) first postulated that an autoimmune disease could affect the inner ear because of clinical observations of patients with recurrent bilateral sudden deafness. An approach to classifying various immunologic disorders has been proposed that is based on the degree of tissue specificity and immunologic mechanisms involved. A summary of autoimmune diseases that can cause sensorineural hearing loss is shown in Table 3 (Stephens et al, 1982). The mechanisms of auditory dysfunction include several proven and several postulated mechanisms. It seems that the most widely documented effects of autoimmune diseases resulting in sensorineural hearing loss are mediated by a vascular means (Stephens et al, 1982) (Table 4). Many of the autoimmune diseases do cause vasculitis, resulting in a variety of secondary degenerative changes. Massive intracranial hemorrhage and hemorrhage into the cochlea and cochlear nerve may occur in conditions such as polyarteritis nodosa and Wegener's granulomatosis, resulting in acute loss of hearing. Other potential mechanisms that may be related to the hearing loss include the development of hypertension, with its simultaneous effects following renal involvement by this systemic condition; anemia; or hyperviscosity problems arising either directly or indirectly from the condition. Many autoimmune disorders may lead to renal failure, and an inner ear disorder can accompany such a state. Such a disorder may be reversed by successful renal transplantation (Mitschke et al, 1973). Iatrogenic hearing impairment relates to the fact that the autoimmune diseases are often treated with potentially ototoxic drugs. These include aminoglycoside antibiotics, salicylates, antimalarials, and gold compounds.

Table 3. Autoimmune Diseases that can cause Sensorineural Hearing Loss

Auto-antibody diseases
- Autoimmune sensorineural hearing loss
- Vogt-Koyanagii-Harada syndrome

Immune complex diseases
- Rheumatoid arthritis
- Relapsing polychondritis
- Systemic lupus erythematosus
- Disseminated vasculitis
- Ulcerative colitis

Cell-mediated disease

Multiple sclerosis.

A specific antibody action against a particular tissue has been postulated in the Vogt-Koyanagi-Harada syndrome. It has been suggested that this action occurs in cochlear tissue derived from neural crest. The Vogt-Koyanagi-Harada syndrome becomes manifest as a bilateral inflammation of the eye, characterized by a granulomatous uveitis with systemic disturbances of pigmentation and sensorineural hearing loss. This condition occurs most commonly in Japan, although it is not known why. The hearing loss from this condition may vary from mild fluctuating cochlear loss to complete deafness. This is usually accompanied by tinnitus and vertigo which was unresponsive to caloric stimulation (Maxwell, 1963; Seals and Rise, 1967). Maxwell (1963) has suggested that hearing loss in this disease may result from serous labyrinthitis secondary to pigment destruction in the cochlea.

Table 4. Primary Disseminated Vasculitides

Polyarteritis nodosa
Wegener's granulomatosis
Giant cell arteritis
Temporal arteritis
Cogan's syndrome
Behçet's syndrome
Auto-immune sensorineural hearing loss.

Relapsing polychondritis is an episodic, generally progressive disease of cartilaginous structures throughout the body. It is said to cause auricular chondritis in 89 per cent of patients and inner ear damage in 46 per cent (McAdam et al, 1976). The immunologic mechanisms involved are still not clearly understood, but both immune complexes and cell-mediated responses appear to be involved. Cody and Sones (1971) have shown that these patients may have either conductive or cochlear hearing loss. The cochlear hearing loss may result from vasculitis of the labyrinthine artery or its cochlear branch. Recently, a number of other autoimmune diseases have been associated with relapsing polychondritis. Other such diseases include systemic vasculitis, rheumatoid arthritis, systemic lupus erythematosus, Reiter's syndrome, Behçet's syndrome, polymyalgia rheumatica, Hashimoto's thyroiditis, and others (Michet et al, 1986). More than half of a series of patients at the Mayo Clinic with relapsing polychondritis presented with symptoms referable to the ear: auricular chondritis, hearing loss, or vertigo (Michet et al, 1986). In a series of 112 patients, hearing loss occurred in 33 patients and was bilateral in 15. Serous otitis media occurred in 5 patients, with sensorineural hearing loss occurring in 28 remaining cases, not all of whom were documented. In 8 of 13 documented cases of sensorineural hearing loss, there was improvement with corticosteroid therapy (Michet et al, 1986).

A recent prospective study of 30 patients hospitalized with exacerbation of systemic lupus erythematosus found an 8 per cent incidence of substantial, previously undetected hearing loss without attributable cause, in spite of the fact that nearly every patient was receiving immunosuppressive therapy at the time of testing (Bowman et al, 1986).

Because of semantic and diagnostic confusion in the literature, there is some difficulty in differentiating between the auditory dysfunction due to polyarteritis nodosa and that due to other conditions, particularly Wegener's granulomatosis. Peitersen and Carlsen (1965) reported two patients with temporal arteritis and Wegener's granulomatosis in addition to two patients with polyarteritis nodosa, presenting with hearing impairment as a first symptom. They also referred to 3 out of 19 patients with polyarteritis who had a hearing impairment. Weaver (1972) described a case of total bilateral deafness in a 30-year-old white male who he diagnosed as having Wegener's granulomatosis. He considered Wegener's granulomatosis and polyarteritis nodosa as equivalent collagen diseases. He stated that ear involvement in Wegener's granulomatosis is fairly common, and when the ear is involved, the frequency of sensorineural hearing loss is high. Gussen (1977) described the temporal bone of a patient with polyarteritis nodosa that had perivascular infiltration of a labyrinthine artery, fibrosis, bone formation and a

hydropic changes in the cochlea. A temporal bone study of another patient who died 7 months after the onset of polyarteritis nodosa was reported by Jenkins and associates (1981). They found loss of the organ of Corti in the hook portion of the basal coil, absence of the tectorial membrane, atrophy of the stria vascularis with obliteration of the scala tympani by fibrosis, and new bone formation. Hydrops was present in the scala media, and a marked decrease in the spiral ganglion cells and nerve fibers was seen. The vestibular structures were normal, but small vessel arteritis was found in the dural and subarcuate vessels (Jenkins et al, 1981).

Wegener's granulomatosis is a necrotizing vasculitis involving primarily the respiratory tract but also the kidneys, joints, and other parts of the body. Ear involvement has been reported to occur in 38 per cent of patients (Wolff, 1974). Sensorineural hearing loss was reported to occur in 30 per cent of patients with Wegener's granulomatosis who have hearing loss (Watanuki et al, 1975). McDonald and DeRemee (1983) reported that, in a series of 108 patients, 24 had involvement of the ear. Of these 24 patients, 19 had serous otitis media, 9 had sensorineural deafness, and 5 had chronic otitis media.

Behçet's syndrome is a triad of oral and genital ulcers and eye lesions that has subsequently been found to involve the skin, joints, blood vessels, and the central nervous system (Lehner and Barnes, 1979). There is considerable evidence for the presence of circulating immune complexes in Behçet's syndrome and also a cell-mediated response (Lehner and Barnes, 1979). These may then result in a more generalized type of vasculitis. Brama and Fainaru (1980) showed a hearing loss in excess of 25 dB in 9 of 16 patients tested, with nystagmus or reduced caloric responses in 5 patients. Their audiometric investigations suggested cochlear lesions that they thought were most likely caused by perivascular infiltration of the cochlear blood vessels.

Animal experiments have documented the existence of immunopathologic alterations of the inner ear (Beickert, 1961; Terrayama and Sasaki, 1968; Arnold et al, 1976; Weidauer et al, 1977). Quick and associates (1973, 1975) showed a surprising antigenic similarity between the cochlea and the kidney. Rat cochlea injected with antiglomerular basement membrane antibody were found to have a membrane-like exudate in the perilymphatic compartment of the cochlea. These studies suggested an immunologic cause of hearing loss in patients with kidney transplants.

Cogan's syndrome consists of nonsyphilitic interstitial keratitis and audiovestibular disorder progressing to profound hearing loss. Edström and Vahlne (1976) presented the findings from a case of Cogan's syndrome. A transient depression of cell-mediated immunity was found, along with signs of complement consumption, suggesting the presence of immune complex disease in association with ocular and cochlear vestibular findings.

Kumagami and co-workers (1976) induced endolymphatic hydrops in rabbits by injecting protein antigens, to which the animals had been sensitized, into the inner ear through the facial canal. Harada and colleagues (1984) also noted the development of hydrops in 4 out of 11 guinea pigs immunized with rabbit stria vascularis and Freund's adjuvant. On the contrary, no hydrops was found in complement-deficient guinea pigs treated in a similar manner.

In 1979, McCabe described a series of 18 patients with autoimmune sensorineural hearing loss. Typically, the progression of deafness in his series occurred over weeks or months rather than over hours, days, or years. Typically the disease is bilateral but asymmetric. The only positive laboratory test available at that time was a positive lymphocyte inhibition assay. In cases in which some residual hearing remained, therapy with immunosuppressive drugs (cyclophosphamide and corticosteroids) was beneficial. Yoo and associates (1982) demonstrated an important finding of the presence of antibodies and serum of antibodies to type II collagen in the serum of patients with otosclerosis and Ménière's disease. Hughes and associates (1983) studied two patients with Cogan's syndrome to look for autoimmune inner ear disease, using both cellular and humoral immune tests. They found that both patients had positive lymphocyte migration inhibition and lymphocyte transformation tests when hearing loss was acute. They concluded that (1) the vestibular and auditory symptoms of Cogan's syndrome are autoimmune in origin; (2) the autoimmune process is mediated through cellular rather than humoral pathways; (3) systemic steroids may suppress positive test results; and (4) test results are more likely to be positive when symptoms are acute. Hughes and co-workers (1983) also studied 10 patients with bilateral Ménière's disease for possible autoimmune inner ear disease using tests of cellular and humoral immunity. In order to study lymphocyte migration inhibition, lymphocyte transformation, and serum study for immune complexes, lymphocytes from these patients were exposed to inner ear antigenes. Two patients had positive lymphocyte transformation tests; two patients had an abnormal cellular immune response to skin antigen stimulation; and one patient had abnormal circulating immune complexes. Controls had negative responses to the tests mentioned earlier. These preliminary findings suggest that some of Ménière's disease may be of autoimmune origin.

McCabe (1983) subsequently expanded his series to 41 cases of autoimmune inner ear disease, and more recently, McCabe and McCormick (1984) expanded the series to 54 cases. In the more recent publication, McCabe and McCormick (1984) described in detail the procedure for the leukocyte migration inhibition tests, and the details for the preparation of the inner ear membrane antigen and control antigen.

Harris (1983) studied guinea pigs' inner ear responses to keyhole limpet hemocyanin (KLH) challenge. Following intradermal immunization, there was a rising serum anti-KLH titer, whereas perilymph had a very small titer only after 7 weeks, which was 0.9 per cent that of serum. Following inner ear perfusion with KLH, higher titers of perilymph antibody were detected. These experimental results suggested that (1) the blood-labyrinth barrier is analogous to the blood-brain barrier with respect to immunoglobulin equilibrium; (2) the inner ear is capable of responding to antigen challenge; and (3) the inner ear is an effective route for systemic immunization. These studies were corroborated in a recent report by Mogi and colleagues (1985). Subsequent experiments by Harris (1984) demonstrated that the inner ear was the source of the antibody in the previous study. Animals were systemically immunized to bovine serum albumin (BSA) as a serum reference. During inner ear immunization with KLH, Harris was able to demonstrate an increase in anti-KLH perilymph antibody levels without a corresponding increase in anti-BSA levels. These findings suggested that the increased vascular permeability was not a factor responsible for the increased perilymph levels of anti-KLH antibody and that local production of antibody had occurred within the inner ear.

A more precise clinical test demonstrating antibodies directed specifically at inner ear tissue has been described (Arnold et al, 1985). This important study consisted of three parts: (1) an immunohistochemical study of normal human temporal bone; (2) the leukocyte mitogen stimulation test on fresh blood from patients with Cogan's syndrome, Ménière's disease, and progressive bilateral sensorineural hearing loss of uncertain origin; and (3) the serum of patients with bilateral sensorineural hearing loss was tested for antibodies against mesenchymal structures of the human ear using deparaffinized healthy human temporal bones. The immunohistochemical methods showed the presence of immunoglobulin A and the secretory component exclusively in the epithelium and in the lumen of the human endolymphatic sac, especially in the proximal portion. Plasma cells with cytoplasmic IgA were commonly found in many perisaccular region. IgG was found in many perisaccular cells, in many perisaccular lymphatics and in the epithelium, mainly in the distal part of the endolymphatic sac. The lymphocyte mitogen stimulation test did not provide any indication of the presence of cellular immunity in fresh blood of patients with Cogan's syndrome, Ménière's disease, or progressive sensorineural hearing loss. In the serum of 15 patients with bilateral sensorineural deafness (Ménière's disease, bilateral sudden sensorineural deafness, or progressive sensorineural hearing loss), the investigators were able to detect antibodies against mesenchymal structures of the inner ear. IgG antibody affinity to the basement membrane of strial capillaries, the dark cell area, the spiral ligament, and spiral lamina could often be demonstrated. In addition, IgA antibodies were found in the serum of three patients with bilateral recurrent sudden deafness, and in two patients with Cogan's syndrome, antibodies against the epithelial cells of healthy cornea and inner ear tissue could be demonstrated (Arnold and Gebbers, 1984; Arnold et al, 1985).

The importance of making a diagnosis of autoimmune hearing loss or Cogan's syndrome lies in the fact that patients with these conditions may respond to corticosteroids or immunosuppressive agents, or both, with an improvement in hearing (Haynes et al, 1981; McCabe, 1979, 1983). Another recent study of potential clinical importance was reported by Harris and colleagues (1985). Three hundred and eighty patients who had unilateral acoustic tumor surgery had preoperative and postoperative audiograms performed on the nonoperated ear. Of these patients, 1.3 per cent had a significant (greater than or equal to 20 dB) hearing loss in the non-operated ear. This occurred in a mean time of 15 days following surgery. These findings suggested a syndrome of sympathetic cochleolabyrinthitis caused by autoimmune sensitization against cochlear tissue antigens at the time of acoustic neuroma surgery. These interesting findings should provide a stimulus to further study of inner ear immunology, an area that has been overlooked by investigators in the past. The fact that a number of patients have responded to corticosteroid therapy for sudden idiopathic deafness suggested that a number of these patients may indeed be suffering from some type of autoimmune sensorineural hearing loss.

Another type of autoimmune disease that may be associated with sensorineural hearing loss responsive to corticosteroids is ulcerative colitis (Weber et al, 1984). It has been postulated that colonic and extracolonic manifestations of ulcerative colitis are caused by both tissue deposition of circulating immune complexes and T-cell lymphocyte-mediated cytotoxicity (Weber et al, 1984).

More recent studies by Harris and co-workers (1985) have shown a parallel rise of antibody titers over a 3-week period in guinea pigs immunized by either inner ear or peritoneal routes of antigen presentation. These studies indicate that the inner ear is an effective route of antigen processing that results in the acquisition of systemic humoral immunity as well as cellular immunity. Furthermore, systemic immunity was found to protect the inner ear. Guinea pigs previously immunized systemically against KLH displayed an intense immune response in the middle ear to local antigen challenge (Harris and Ryan, 1985). Antibody levels against KLH increased substantially during middle ear responses to KLH challenge, suggesting a local origin of KLH antibody. This local response either originated in the middle ear with transfer of antibody to the inner ear or the antibodies were produced by the inner ear in response to antigen diffusing across the round window membrane (Harris and Ryan, 1985). Yoo and associates (1985) have summarized their development of an animal model from collagen-induced autoimmune ear disease. Lewis and Wistar rats have been shown to develop sensorineural hearing loss with atrophy of the organ of Corti, spiral ganglion and vestibular degeneration, otospongiosis-like lesions in the tympanic annulus, and cochlear vasculitis. Both cellular and humoral responses to type II collagen were identified. This should provide a very useful animal model for the study of immunologically mediated ear disease in humans.

Apparently, there seems to exist some correlation between biochemical, physiologic, and morphologic changes in the labyrinth in various pathologic conditions. Further experimental and clinical studies are necessary to understand mechanisms involved in disorders of hearing and equilibrium.