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OTOPATHOLOGY IN TWO LINES OF THE DIABETIC
CHINESE HAMSTER, CRICETULUS GRISEUS

by

Jo A. Oostveen

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
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Department of Biomedical Science

Western Michigan University
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OTOPATHOLOGY IN TWO LINES OF THE DIABETIC
CHINESE HAMSTER, CRICETULUS GRISEUS

Jo A. Oostveen, M.S.

Western Michigan University, 1983

Recent evidence suggests that a bilateral, progressive sensorineural hearing loss is associated with diabetes mellitus in humans. The microangiopathy presumedly leading to this loss seems to be similar to microvascular changes leading to renal and retinal deterioration. A total of twenty-four pairs of temporal bones from two diabetic, and one non-diabetic lines of the Chinese hamster were processed and evaluated for their total auditory histopathology. Hematoxylin and eosin serial sections demonstrated a mean otopathological involvement 5.6 times greater for diabetic animals compared to non-diabetic animals. Capillary basement membrane thickening was observed in the auditory and vestibular systems 5.5 times more often for diabetics. The Chinese hamster appears to be an adequate animal model for studying the otopathological effects of the diabetic state.

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Jo A. Oostveen

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CHAPTER I

INTRODUCTION

Despite the contribution of more than a half a century of intensive investigation, diabetes mellitus is still a poorly understood and highly destructive disease. Diabetes, and its associated complications, still ranks as the sixth most common cause of mortality in the United States (Fajans, 1976). Considering its high incidence, mortality rates and socioeconomic impact on the worlds population, diabetes is a major public health problem.

Diabetes mellitus is characterized primarily as a metabolic disorder. In reality diabetes may be a group of diseases with heterogeneous etiologies. The main unifying characteristic is the development of an impaired glucose metabolism. Diabetes is caused by either insufficient insulin levels, or the production of incompetent insulin, or target organ reception difficulties. These have the effect of increasing blood glucose levels (Ellenberg, 1970). Insulin is a pancreatic hormone produced by the beta cells of the Islets of Langerhans. It functions to lower blood glucose levels. Therefore, in its fully developed clinical expression, diabetes is characterized by continuous or fasting hyperglycemia (excess glucose or sugar in the blood).

Long range complications of diabetes usually manifest in one of three forms: 1) macrovascular disease is usually expressed as atheroscleroses of the major vessels, or 2) microvascular disease (microangiopathy or disease of the capillaries and connecting vessels), or

3) neuropathy, or impairment of nerve conduction (Tepperman, 1981).

All of these manifestations can be found in most tissues of the body. Renal disease leading to kidney failure and death, as well as retinal deterioration leading to blindness, are the most commonly associated manifestations of the diabetic state. As intensive investigations into the various clinical expressions of diabetes evolve, it is being demonstrated that the diabetic state affects more than just the heart, kidneys, and eyes.

Otolaryngologists are aware that up to 40% of diabetic patients develop a hearing loss (Statloff, 1978). The type of hearing loss which is associated with diabetes mellitus, is bilaterally progressive and sensorineural in nature (Statloff, 1978). The etiology seems to be similar to that of retinal disease (Quick, 1973).

Because of the increased interest in hearing loss associated with the diabetic state, studies have been and are being conducted in an effort to explore this relationship. Accumulation of human diabetic temporal bones for the purpose of studying the auditory and vestibular systems is a lengthy, expensive and somewhat difficult undertaking. The acquisition of human temporal bones can occur only after a complete autopsy has been granted. This is even more difficult with young juvenile onset diabetics.

Since the vestibular system and cochlea are so delicate (composition is approximately 95% water), the time delays which are incurred in acquiring the specimens result in autolyses of some parts of these organs (Schuknecht, 1974). Because of these problems with human temporal bones, it has become necessary to search for an

animal model.

Until recently, the majority of studies of the effects of diabetes in animals was limited to metabolic alterations as a result of: 1) pancreatectomy, 2) chemical damage to the islet cells by alloxan or other chemical agents, 3) physical interference of the release of insulin from the beta cells, or 4) interference with the effect of insulin on the target organs. These methods did not, however, always produce alterations which closely resembled the early metabolic changes of spontaneously developing diabetes in man (Sirek and Sirek, 1970).

An alternative approach is to utilize the diabetic genetic line of the Chinese hamster which develops spontaneous diabetes similar to the condition in humans. The Chinese hamster (*Cricetulus griseus*) has proved to be an appropriate model for evaluating many aspects of the long range complications of diabetes mellitus (Sirek and Sirek, 1970). Early studies have shown the Chinese hamster to be an effective model for the study of auditory loss resulting from the diabetic state (McIntire and Benitez, 1981).

The Problem

Diabetes mellitus in humans and animals has been a topic of fascination for hundreds of years. It has been the subject of books, presentations, scholarly works, and much attention in general. With all of the research the details of how the pancreatic beta cell is abnormal in its secretion of insulin, or if it is in fact abnormal, are not known. One of the main reasons for the sparcity of detailed

knowledge is the subtle nature of diabetes. Clinical expressions which eventually are fatal are usually not present at the onset of the condition. Knowledge is, however, increasing about the long term complications and treatment of diabetes.

Manifestations of the disease range from the unrecognizable (silent complications) to the recognizable (ketoacidosis, acute hyperosmolar coma). Diabetes is a syndrome with a number of factors (genetic, environmental, viral). Among these factors are a genetic predisposition or inherited susceptibility, environmental agents, chemical agents and infectious agents in the environment, as well as nutrition, physical activity, and psychological stress on the individual. The expression of the diabetic syndrome in any particular individual is an integrated response to these multiple factors. A combination of any of these factors could lead to pancreatic beta cell injury and decompensation eventually leading to maturity or juvenile onset diabetes.

Diabetes mellitus is one of the most complex disease states (Fajans, 1976). Long term macrovascular disease (usually atherosclerosis) has a premature onset in diabetics and is nondistinguishable from the nondiabetic form. Atherosclerosis is a vascular change affecting the larger blood vessels, and is associated with an increase in coronary, cerebral, and peripheral vascular disease. It is characterized by a thickening of the inner lining of the artery (tunica intima), and atrophy of the arterial wall without endothelial proliferation (Naufal and Schuknecht, 1972).

Another common macrovascular disease associated with diabetes is

arteriosclerosis. Hypertension results from arteriosclerosis and is therefore not a direct consequence of the abnormal metabolic state. The major result of macrovascular changes is coronary artery disease. This complication accounts for up to 42% of the deaths related to the diabetic condition (Fajans, 1976).

The second long range complication of diabetes is microvascular disease. Due to the results of the present study, and many others, the primary cause of hearing loss seems related to changes in the capillary basement membrane material which is the hallmark of microangiopathy. Narrowing of the lumens of small arterioles, capillaries, and venules supplying various tissues occur because of either an increase in production, or a decrease in normal breakdown rates of the basement membrane lining these vessels. This microangiopathic vascular disease is most often detected as an increase in capillary basement membrane material.

Many experiments concerning the initial phases of development of basement membrane thickening have shown that it is normal at the onset of the disease. This is usually when it is first observed clinically in young diabetics. The basement membrane is normal in its pre-diabetic state, but over years of a diabetic's life, what were normal basement membranes become thickened, and can finally lead to death due to organ damage in the kidney, heart, or brain. The majority of the knowledge and information on basement membrane morphological changes accumulated has been from studies in the kidney, retina, and muscle.

Before one can understand the morphological changes manifested

in the capillary basement membrane of the diabetic, it is necessary to understand the morphology in the normal, pre-diabetic condition.

The basement membrane is the continuous sheet of material which is periodic acid schiffs (PAS) positive underlying epithelia or endothelia cells. It is also called basal lamina or basement lamina. Most importantly for understanding the present study, the basement membrane is found underlying the endothelia of capillaries.

The basement membrane is typically 200 to 500 Å in thickness, and is separated from the epithelial or endothelia cell layer by a lighter electron lucid zone approximately 100 Å thick. This area is composed of glycoprotein. The membrane itself is composed of tightly matted arrays of fine (30 to 40 Å) fibrils embedded in a finely granular matrix. The PAS technique stains the basement membrane itself, the lucid glycoprotein zone, and some of the reticular fibers on the connective tissue side.

Basement membranes belong to the collagen family of protein, but are amorphous, unlike classical collagens. They do, however, give diffraction patterns similar to collagens. The basement membrane is rich in mucopolysaccharides and possesses a high type IV collagen content. Ninety percent of the basement membrane is peptide and ten percent is carbohydrate (glucose and galactose).

The basement membrane serves to de-limit the area of connective tissue and provides a barrier between it, and areas of non-connective tissue elements and thus maintains orderly organization of organs and tissues. The basement membrane also represents a barrier which serves to protect passage of plasma proteins. The basement membrane

is the main filter for both size and charge of particle. Micro-vascular disease thus alters the morphology and functions of the basement membrane and the tissues they supply (Marquhar, 1978). The third feature of long term diabetic complications is neuropathy which encompasses any abnormal condition of the nervous system. Disordered nerve function results in sensory impairment. This abnormal condition and function may be due to a chronic metabolic disturbance of the nerves. In the case of diabetes, the nerves are exposed to high concentrations of glucose from the blood over a long time span.

Pathologic exams have revealed small blood vessel changes occurring in the auditory and vestibular systems of animals, very similar to those found in the kidney, brain, and muscle tissues (Statloff, 1978). Also atherosclerosis of the large blood vessels, possibly from elevated serum cholesterol and glucose, seem to develop within these systems prematurely. Vascular disease and polyneuropathy are well associated with progressive hearing loss (Makishima and Tanaka, 1971). These vascular changes result in compromised oxygenation and function of the supplied tissue (DeLorenzo, 1973). The brain is particularly vulnerable. Similar effects of vascular changes in the inner ear may be related to cerebral vascular disease.

Correlations between diabetes and a hearing loss depend on many factors, such as 1) duration of the diabetic condition, 2) severity of the condition, 3) sex, 4) age, 5) blood pressure, and 6) vascular status (Martin, 1981).

At the present time, there is considerable evidence that the major reason for hearing loss associated with the diabetic state is both macrovascular and microvascular disease (Martin, 1981). Manifestations of these diseases include thickening of the vessel walls within the capillary network supplying the inner and middle ear. The lumens become progressively more narrow leading to a decreased blood supply. In addition to decreased blood supply and resulting ischemia to the tissue they serve, chronic high glucose levels supplying the nerves lead to pathological changes in the nervous system of the ear (Schuknecht, 1974). A limited blood supply and a progressive sensorineural hearing loss are often found together. Effects of diabetic neuropathy have been documented within the human ear in the stria vascularis, the modiolas, and the eighth nerve, all associated with a thickened vascular capillary wall supplying the innervation to these areas (Martin, 1981). As the lumen of the internal cochlear artery becomes progressively more narrow leading to a decreased blood supply, there is evidence of loss of a percentage of neurons of the spiral ganglia of the cochlea, and the myelin sheath of the stato-acoustic portions of the VIII cranial nerve.

In addition to the vascular or neuropathic changes (sensorineural), hearing loss associated with the metabolic alteration of diabetes may result from an increased incidence of chronic infection of the middle ear (otitis media). It is a widely held axiom that the diabetic individual is more susceptible to bacterial and fungal infection (J.E. Johnson, 1970). Diabetics have a more difficult time coping with infection of any type in any tissue. Increased glucose levels,

ketoacidosis, vascular insufficiency, and neuropathy all contribute to changing the host defense mechanisms of the diabetic. The definite pathologic mechanisms of diabetes mellitus which predispose the individual to recurrent or chronic infections have a profound effect on the auditory and vestibular systems of the individual.

By using the Chinese hamster as a model to represent spontaneously developing diabetes it is the purpose of the present study to determine to what extent diabetes affects the vestibular and auditory systems in this animal model.

Significance

There are at least six million diabetics in the United States presently, and the number grows by 6% each year (Statloff, 1978). Diabetes is a more common disorder than ordinarily supposed, and greater public attention is being given to it. Because of the increased recognition of its prevalence, there is more interest in its widespread complications.

With time the fundamental biochemical, physiological, and pathologic concepts of diabetes are becoming clearer. Even though many facets of the disease are now completely understood, many changes which occur in the diabetic state are still unclear. One of these changes is the pathogenesis of capillary basement membrane thickening. It is evident that thickening does occur, and that the effects on the function of this membrane are profound. Diabetic changes have been observed in various locations of the body such as the kidney, heart, brain, peripheral nervous system, muscles, skin, retina, iris, .

pancreas, liver, placenta, and the inner ear (Osterby and Lundbaek, 1970; Schuknecht, 1974; Stary 1966).

These and other consequences of the long term complications of this metabolic disorder are great. The significance of the effect of diabetes on the auditory and vestibular function of the individual is clearly evident. It has already been shown that effects on different parts of the body from the retina of the eye, to the glomerulus of the kidney are similar. The knowledge gained of the pathophysiology of diabetes may well assist in the management of this serious disease.

CHAPTER II

REVIEW OF SELECTED LITERATURE

Reviewing the history of diabetes, insulin and its effects, is nothing less than a minihistory of biology and medicine (Tepperman, 1981). In the year c. a. 20A.D. the name "diabetes" was first introduced by Aretaeus. Many investigators following Aretaeus, also noted symptoms of the diabetic state. In 1679 Thomas Willis described a disease characterized by a "sweet taste of urine". It wasn't until 1788 that Crawley acknowledged the pathology of the pancreas in association with diabetes mellitus. A century later in 1869, Langerhans first described the pancreatic islet cells (Tepperman, 1981). Almost at the same time that Langerhans was describing and determining the function of the various islet cells of the pancreas, Jordae (1857) in Paris, suggested a relationship between diabetes and a hearing loss (Gladney, 1978). Jordae's treatise was the first of many to suggest such a relationship. The main features of the vascular supply to the inner ear were well known at the end of the 1800's since Siebenmann published his monograph on the human ear in 1894 (DeLorenzo, 1973).

About the time of Jordae, Langerhans, and Siebenmann, two other investigators, Todd and Bowman, first described the nature of the basement membrane (Kefalides, 1978). For over a hundred years knowledge and interest in this extracellular membrane remained dormant. The basement membrane was isolated and demonstrated to be an

extracellular matrix containing collagenous and noncollagenous glycoprotein components (Krakower and Greenspon, 1951).

Knowledge of the metabolic disorder of diabetes was increasing rapidly in the early 1900's when Banting and Best in 1921 published the famous article "The Internal Secretions of the Pancreas", using the dog as a model for the discovery of insulin (Tepperman, 1981). It seemed as if the relationship between the pancreatic islet secretions of insulin and the diabetic state was finally cemented. During the 1930's young diabetics began to survive due to supplemented insulin injections (Osterby and Lundbaek, 1970). Later in that decade the importance and severity of vascular disease in diabetes became apparent.

In 1955 Sanger received the Nobel prize for his elucidation of the structure of insulin. Sanger's work represented the first time that the amino acid sequence of a polypeptide was accurately determined. Insulin has since been shown to be essential for the synthesis and conservation of body carbohydrates, fats, and proteins (Tepperman, 1981).

Shortly after Sanger received his Nobel prize for the amino acid structure of insulin, two other researchers, Meier and Yerganian (1959) published the first reports about inbreeding producing diabetes in the Chinese hamster. The animals were captured near Peking and transported to Yerganians laboratory in Boston in order to use their cheek pouches for studies of cell implants (Sirek and Sirek, 1970). Because of several stress factors, mainly the new unnatural environment of the lab and the confinement, as well as several generations

of inbreeding, the diabetic state finally resulted. Further inbreeding of these hamsters would have resulted in the loss of the diabetic trait, so the hamsters were rebred into new lines of the Upjohn Company colony in 1967. This work was carried out by L. Butler, a geneticist from the University of Toronto. A joint program carried out by Gerritsen and Dulin at the Upjohn Company and Butler in Canada produced what is regarded as the most complete analysis of the genetics involved in the diabetic Chinese hamster (Sired and Sirek, 1970). Since that time, in the late sixties, the colony of Chinese hamsters have been characterized into several distinct true breeding genetic lines. Spontaneous diabetes in the Chinese hamster has been well described and provides a valuable model for diabetic research. In many respects the Chinese hamster closely approximates the human condition.

In the early 1960's, much later than Jordae's initial work, otopathologists once again became interested in the relationship between diabetes and a progressive hearing loss. Sparking the interest was a report by Cojazzi and Bötner (1950). Their work with alloxan induced diabetes in rabbits showed significant changes in the stria vascularis and degenerative changes in Scarpa's ganglia and the vestibular nerves in association with diabetes (Schuknecht, 1974).

In 1961 Jorgensen and Buch published a paper with excellent documentation of the otopathological correlation with diabetes. They examined 60 diabetic patients and found that 28 had a hearing loss. In this early report they found no distinct correlation between the duration of the diabetic state and the degree of hearing loss.

However the loss was more severe in the older patients, and in those with diabetic retinopathy. In their patients under 40 years of age there was a distinct relationship between increased hearing loss and nephropathy. No relationship was found between diabetic neuropathy, hypertension and the degree of hearing impairment. Hearing loss was bilateral and progressive. Of the 28, three had sudden onset hearing loss and nine experienced vertigo similar to Meniere's disease.

In 1961 Jorgensen noted histopathologic loss of ganglia cells, and changes in the stria vascularis. He also showed that there were PAS positive (capillary basement membrane) thickenings in the capillary walls of the stria "....which stood out in some preparations as thick cables having walls 10-20 times thicker than normal....". In his observations of 32 manifest diabetics (no pre-diabetics) these microvascular changes were not age related changes, only changes related to the duration of the diabetic state. In the aforementioned paper with Buch, Jorgensen found no correlation of hearing loss and duration of the diabetic state with living individuals, but when histological studies were evaluated, there was a definite relationship of increased otopathological findings with increased duration of the disease. It seemed that the observed pathology did not reflect the degree of hearing loss observed. Jorgensen did demonstrate severe PAS positive thickenings of the capillary basement membranes of the stria vascularis and postulated that there were hemodynamic changes secondary to the microangiopathy of the diabetic state. Costa (1967) found that alloxan induced diabetes in rats also produced stria vascularis capillary basement membrane changes which were similar to .

Jorgensen's report about human inner ears.

After Jorgensen's reports many other investigators subsequently demonstrated basement membrane thickenings in virtually all tissues of human diabetics (eye, brain, kidney, etc.), however prevalence and magnitude of thickening varied greatly.

Controversy continued as to whether capillary basement membrane thickening was a result of the diabetic state or occurred as a co-existing pathological condition. In 1968 Siperstein showed an increase in capillary basement thickening in muscle capillaries in 50% of pre-diabetic individuals (those with two diabetic parents and without glucose intolerance). He therefore concluded that basement membrane thickening was a co-existing abnormality, and not a complication of diabetes. Shortly thereafter, Williamson and Kilo demonstrated that basement membrane thickening was not evident in new juvenile diabetics, but developed later in direct relationship to duration of the diabetic state. They concluded that basement membrane thickening was related to duration and intensity of diabetes, and not to a co-existing inherited abnormality. Many recent studies including transplantation experiments of normal kidneys into a diabetic animal and resulting basement membrane thickening, point to the fact that this thickening especially in the renal glomerulus is a consequence of diabetes mellitus. The multifactorial pathogenesis of capillary basement membrane thickening is clearly evident as well as the fact that this thickening follows rather than precedes the onset of metabolic abnormalities associated with insulin insufficiency.

Several investigations in the 1970's also demonstrated a causal

relationship between insulin deficiency and auditory dysfunction. Gladney and Shephard (1970) showed the interrelationship between the metabolic and vascular components of diabetes. The biochemical mechanisms are operative prior to the development of the manifest diabetic state. Labyrinth dysfunction correlated with diabetes was reported in 19 cases. Working with four diabetic individuals Makiskima and Tanaka (1971) found a severe loss of cochlear neurons most pronounced in the basal turn. The blood vessels in the internal auditory canals, modioli, and stria vascularis showed hyalinization and narrowing. There was demyelination in 20% of the nervous supply. Severe atrophy of the stria vascularis was the most common finding. They concluded that the vascular lesions were thought to be one of the most important causative factors for neuronal degeneration leading to hearing loss.

In addition to Makiskima and Tanaka, during that same year, Korczyn studied 130 patients with Bell's palsy, and found 88 of the patients were diabetics. Bell's palsy involves a primary inflammation or neuropathy of the VII cranial nerve which includes the chorda tympani nerve and the main trunk of the complete facial nerve. Korczyn stated that Bell's palsy may be the first manifestation of diabetes and the high incidence of Bell's palsy in diabetics had been clearly established.

In 1972 Igarashi reported auditory dysfunction observed in about 50% of diabetics. PAS positive thickening of capillary walls in the stria vascularis was typical.

More recently Taylor and Irwin (1978) completed a study in which the hearing of 38 diabetic listeners was compared to the hearing of

39 non-diabetic listeners. They showed that the diabetic group had a greater proportion of hearing impairment.

In 1981 McIntire and Benitez using assorted lines of the Chinese hamster as an animal model showed that the summation of qualitative and quantitative aspects of the otopathology revealed a mean pathological involvement three times greater for the diabetics than the non-diabetics. The otopathological assessment involved over 28 types of middle ear, vestibular, and cochlear abnormalities.

The present study is an attempt to sort out the kinds of otopathological manifestations that are characteristic of each of the different genetic lines of the Chinese hamster. The histopathology of the middle ear, cochlear, and vestibular systems was assessed in hopes of showing the pathological manifestations which are characteristic of each of the three genetic lines of the Chinese hamster.

CHAPTER III

DESIGN AND METHODOLOGY

Animals

Temporal bones from twenty-four Chinese hamsters were processed and prepared for light microscopic evaluation. The animals were sacrificed in four groups of six animals each over a four month period. Each group contained two nondiabetic hamsters and four diabetics. The total number of animals consisted of eight normal non-diabetic and sixteen diabetic animals. All animals were male, which equalized any differences due to sex of the animal. Within each of the four groups, there were two animals from each of the three genetic lines. Therefore, there were a total of eight animals from each genetic line of the Chinese hamster. Two of the diabetics were also ketotic. Table one gives age related information for each animal, including age in months at the time of sacrifice, and length of diabetic and ketotic condition in months for each animal.

The Chinese hamster is characterized by inappropriate hyperglycemia in which a minimum of four gene pairs or alleles, all recessive, are involved. At least two gene pairs have to be homozygous abnormal for glucosuria and inappropriate hyperglycemia to occur. Three abnormal homozygous pairs must be present for a ketotic individual. Different genetic lines (or syndromes) are produced according to which 2 or 3 or the 4 possible homozygous abnormal genes are involved (Sirek and Sirek, 1970). Age of onset of diabetes is

TABLE 1
AGE DATA FOR CHINESE HAMSTERS

Animal Number	Sacrifice Date	Birth Date	Age in Months at time of Sacrifice	Diabetic Date	Time in Months Diabetic
1. M26-014	7-21-81	9-5-80	9.5		
2. M25-133		9-4-80	9.5		
3. XA25-142		5-1-80	14.7	7-8-80	12.4
4. XA25-133 a		4-24-80	15.0	7-8-80	12.4
5. AC22-086		6-10-80	13.4	8-5-80	11.5
6. AC23-025		4-24-80	15.0	7-11-80	12.3
7. M24-58	8-14-81	1-25-80	18.7		
8. M24-57		1-25-80	18.7		
9. XA25-24		1-25-80	18.7	3-4-80	17.3
10. XA25-31		1-25-80	18.7	3-4-80	17.3
11. AC21-94		2-7-80	18.2	8-4-80	0.3
12. AC22-26		1-30-80	18.5	4-3-80	4.3
13. M26-31	9-23-81	11-6-80	10.6		
14. M26-45		11-24-80	10.0		
15. XA26-217		11-4-80	10.6	5-7-80	4.5
16. XA26-118 b		8-27-80	12.9	10-7-80	11.5
17. AC22-133		10-8-80	11.5	12-10-80	9.4
18. AC23-110		11-26-80	9.9	3-11-80	6.4
19. M25-118	10-16-81	8-4-80	15.4		
20. M25-117		8-4-80	15.4		
21. XA26-189		10-15-80	13.0	11-5-80	12.4
22. XA26-233		11-18-80	12.1	1-7-81	10.3
23. AC22-134		10-8-80	13.3	12-10-80	11.2
24. AC23-55		7-3-80	16.4	10-7-80	13.3

Note: Mean Age in months at time of Sacrifice = 14.15
Mean time in months Diabetic XA hamsters = 12.26
Mean time in months Diabetic AC hamsters = 8.59
Mean time in months to onset of Diabetes XA hamsters = 2.2
Mean time in months to onset of Diabetes AC hamsters = 5.9

a Ketotic date 6-4-81 time in months ketotic = 1.6

b Ketotic date 7-6-81 time in months ketotic = 2.6

also variable. Capillary basement membrane thickening has also been observed in various tissues.

The Chinese hamsters are classified on the result of glucosuria evaluations with Tes Tape, and urine ketone levels from the Ketostix. All animals are tested twice monthly from 15 days of age. A non-diabetic animal has never had a positive test for glycosuria. A diabetic has had a positive glucosuria evaluation of 4+ for two of four tests within a two month period. A ketotic diabetic also has a 4+ glucosuria rating and a ketonuria rating of "large" for over a month (Gerritsen and Dulin, 1967).

Three genetic true-breeding lines of the Chinese hamster were evaluated. Each line has different characteristics, as shown in table two.

TABLE 2
CHARACTERISTICS OF CHINESE HAMSTER LINES

Genetic lines	M	XA	AC
Characteristics			
Diabetic	no	yes	yes
Insulin levels	normal	low	low
Acid Glycohydrolase activity	normal	decreased	elevated
Basement Membrane thickness	normal	thickened	normal
Onset of diabetes	none	4-6 weeks	8-10 weeks

The "M" is a normal non-diabetic line, which served as the control. "XA" and "AC" are two distinct true-breeding lines of diabetic animals. The "XA" animals are characterized by low insulin levels (diabetic), decreased acid glycohydrolase activity, a markedly thickened basement membrane, and onset of the diabetic state in 4 to 6 weeks. Conversely, the "AC" line has low insulin levels (diabetic), elevated acid glycohydrolase activity, normal capillary basement membrane thickening, and onset of diabetes in 8 to 10 weeks (Gerritsen, February, 1983).

Except for acid glycohydrolase activity, all of the other characteristics are self-explanatory. The acid glycohydrolases are part of the glycodisases (3.2). These hydrolases act on glycosyl compounds and effectively transfer glycosyl residues to oligosaccharides. Therefore they have a prominent role in glycoprotein degradation or catabolism. The general reaction for the acid glycohydrolases is as follows: glucoside (disaccharide) + $H_2O \rightarrow$ glucose + alcohol (Enzyme nomenclature 1975).

According to the work of A.Y. Chang of the Upjohn Company, there are four primary enzymes included in the acid glycohydrolases, each of which has a specific function within the capillary basement membrane.

α - glucosidase (3.2.1.20) hydrolyzes the terminal 1,4 linked α - D - glucose residues with the release of free α glucose. Specifically it exohydrolyzes these glucosidic linkages and therefore hydrolyzes oligosaccharides rapidly. The hydrolysis of β - D - galactosides to release β - glucose is accomplished by the β - glucodisases (3.2.1.21). Two galactosidases, α - galactosidases (3.2.1.22) and β - galactosidase (3.2.1.23) catalyze the following general reaction:

galactosides + $H_2O \rightarrow$ alcohol + galactose.

The "XA" hamsters follow a more typical diabetic reaction, namely, all four of the renal acid glycohydrolases decrease sharply with an insulin lack. α & β - galactosidase levels in the kidney, liver, and spleen were all lower in the "XA" animals than the nondiabetic "M" hamsters (Chang, 1978). The decreased level of these enzymes leads to a decreased catabolic activity of the disaccharide units in the glycoproteins of the capillary basement membranes. The depressed enzyme level may be one of the contributing factors leading to the accumulation of the disaccharide unit in the basement membrane. Therefore lowered enzyme levels may play a role in the development of microangiopathy in diabetes (Chang, 1978).

The "AC" Chinese hamsters are an oddity (Chang, 1978). They are a highly inbred line and exhibited the highest levels of α & β galactosidases. The α & β glucosidase levels are also elevated. Since the acid glycohydrolase levels are high, there is an increased level of glycoprotein degradation or catabolism. The acid glycohydrolase activities of these animals are high presumable for one of the following reasons. Either the mechanism by which hyperglycemia induces depression of these enzymes fails to operate in the "AC" line, or the "AC" animals are genetically predisposed to produce excessively high levels of the acid glycohydrolases. This would result in masking the hyperglycemia dependent depression of these enzymes levels. There are several other lines of the Chinese hamster and all show some line dependent variances. According to Chang and Greenberg (1978), the renal acid glycohydrolases levels are highly variable in the Chinese

hamster colony and have obvious roles in glycoprotein degradation.

There are two principal ways the liver secretes glucose into the blood. Glycogenolysis which is the breakdown of existing glycogen to glucose-6-phosphate, and, secondly, gluconeogenesis which is the utilization of pyruvate to make glucose-6-phosphate (Tepperman, 1981). Acid glycohydrolase is needed in glycogenolysis for the formation of glucose-1-phosphate and free glucose from the hydrolytic cleavage of 1,6 glucosides. Decrease in glucose use by the peripheral tissues, mainly muscle and adipose tissue, will result from a lack of insulin. This in turn leads to an elevation of liver and muscle glycogenolysis, as well as gluconeogenesis. Both add to the increase in blood sugar. These pathways tend to exaggerate the hyperglycemia state. An elevated acid glycohydrolase activity would favor the glycogenolysis pathway, increasing the glucose output. Likewise, a decrease in glycohydrolase activity would favor the glyconeogenesis pathway. Therefore wither higher or lower glycohydrolase activity leads to an exaggerated hyperglycemia state.

These characteristics of the Chinese hamster lines were known at the onset of the project. An objective was to see if these characteristics held true in the auditory histopathological evaluation.

Materials and Methods

The method used to prepare the temporal bones, containing the auditory and vestibular systems, is essentially the same as has been used to histologically prepare other tissues for over a century, namely treating the tissues by fixation, decalcification, dehydration,

infiltration with an embedding media, sectioning, staining and mounting on glass slides.

The animals were exsanguinated through the orbital sinus. Since many of the tissues were used in various other studies, intravital perfusion for a fast and complete circulatory delivered fixation was not possible. Temporal bones were removed in block and placed in Heidenhein-Susa fixative within 15 minutes of death. After 24 hours the temporal bones were changes to 10% neutral buffered formalin, where they remained until further processing. Processing was completed by a special non-distortion histological embedding technique (celloidin), so that serial representation was possible and anatomical spacial relationships were maintained in the natural position.

Before decalcification, the bones were washed for one hour to clear the excessive fixative. The bones then were decalcified in a mixture of eight parts of hydrochloric acid, ten parts formic acid and 82 parts distilled water. This decalcification solution was maintained at a constant temperature of 38°C for approximately eight hours (the time required for temporal bone decalcification). After decalcification, the bones were washed in running water for 15-30 minutes, and then placed in 5% sodium sulfate for 12 hours to neutralize the residual acid that remained in the bones. Dehydration was carried out with increasing concentrations of ethanol. One to two milliliters of a saturated solution of iodine was added to the 70% and 80% alcohol changes for the purpose of removing the mercury which remained in the specimens as a result of having used Heidenhein-Susa as the primary fixative. Infiltration took place with increasing concentrations

of celloidin (5%, 10%, and 15%). All dehydration and infiltration changes took place in sealed jars at 55°C. Total time elapsed for the process was approximately two weeks per group of bones.

The first group of bones (animals #1-#6) were exposed to temperatures of 70°C for 48 hours during the dehydration process due to a malfunction in the drying oven. The high temperature caused the bones to shrink in size and increased the amount of histological artifact.

After the specimens had been in the last celloidin change (15%) for a period of 48 hours, they were then transferred to the freezer which was set at approximately -15°C. At this time the specimens were placed in cone shaped pieces of filter paper for the purpose of maximizing the evaporation surface. The ether and absolute ethanol therefore evaporated more quickly than could be achieved through allowing evaporation only to occur through the top surface which would have resulted from remaining in the jars. The specimens hardened for two to three days and then each bone was trimmed and mounted on a wooden block. They were then exposed to chloroform for 24 hours to complete hardening of the celloidin mounting medium. The temporal bones were stored in 80% ethanol until sectioning.

Sectioning was accomplished on a sliding microtome. Serial sections were cut at 20 microns. The first and sixth sections, and every fifth thereafter was saved and stained with hematoxylin and eosin (H&E) for general histological evaluation. The second, seventh, and every fifth section thereafter was saved for periodic acid-Schiffs (PAS) reagent (allochrome) to demonstrate any increases in glycoprotein

material in the capillary basement membranes. An additional section from each set of five was saved for future use if needed. The fourth section of every series of five was also saved for possible autoradiological tests in the future. Therefore, for every five sections cut, four were saved and the fifth was discarded. Appendix A (hematoxylin and eosin) and appendix B (periodic acid-Schiffs) give the staining procedures used in the present study.

Sections were saved beginning at or near the cristae of the superior semicircular canal. After staining, and mounting these sections on glass slides, each slide had a weight placed upon the coverslip and was allowed to dry in this position for two weeks. A study of the vestibular and auditory systems which could be seen in part on any given slide were studied and placed in serial order from most superior to most inferior.

Qualitative and quantitative aspects of several pathological conditions for the middle ear, and vestibular and cochlear systems were evaluated. All assessments were blind. When evaluating the slides, the animal number and line was not known. For H&E assessment each animal's serial series was evaluated. Appendix C gives a sample qualitative serial assessment. From that assessment, the information was qualified to the form in appendix D. From these forms the completed otopathological quantitative assessment was made. Similarly, for PAS basement membrane assessment, each set of temporal bones was blindly evaluated. Appendix E gives a sample PAS qualitative serial assessment. The information on capillary basement membrane thickness was then transferred to the form in appendix F. When the PAS basement

membranes were evaluated an individual separate from the scorer was taught the identification of the particular structural features. He then placed each structure to be evaluated in the field of view. In this way the scorer did not scan any other parts of the specimens before evaluating the specific capillary basement membranes.

Simultaneous confidence intervals were constructed by the Bonferroni method for mean H & E otopathology. Welch's T analysis of variance was used because the variances were not the same for each type of hamster. Statistical significance at $p .05$ was determined between mean H & E middle ear pathology of diabetic and non-diabetic animals. In the case of mean basement membrane thickening, a significance of $p .05$ was determine between all three hamster lines.

CHAPTER IV

RESULTS

General Otopathology

The hematoxylin and eosin set of serial sections for each temporal bone, demonstrated a significantly higher total otopathologic involvement for the two diabetic lines over the non-diabetic line of Chinese hamster.

Following is a short description of each otopathological condition as it applies to this study. A glossary of auditory terms is included following the appendices. A complete understanding of these conditions, and how they relate to the study is essential to evaluate the results.

Middle Ear Conditions

- 1.) Round Cell Infiltration: collections and pockets of the following in any percentage; lymphocytes, macrophages, plasma cells, and polymorphonuclear leukocytes
- 2.) Fibroblasts and Cholesterol Crystals: collections of fiber producing, large, flat, spindle-shaped cells. Cholesterol crystals are demonstrated after preparation as empty needle shaped clefts. Cholesterol crystals are always associated with fibroblasts, but fibroblasts may occur without the presence of cholesterol crystals.
- 3.) Tympanic Membrane Thickening: thickening of the mucosal and submucosal layers
- 4.) Fibrous Attachment to the Tympanic Membrane: mucosal layer of the tympanic membrane is continuous with fibrosis in the middle ear cavity
- 5.) Fibrosis Around Ossicles: fibrosis around or attached to the malleus, incus and/or stapes

- 6.) Polyp Formation in the Middle Ear: polyps or collections of granulation material with or without its own blood supply throughout the middle ear except for the round window niche
- 7.) Cholesterol Granuloma: dense collections of cholesterol crystal granulation material within the middle ear. Granulomas are usually spherical and have a definite structural shape in contrast to pockets of extraneous cholesterol crystals. The granulomas are a more advanced stage than individual collections of cholesterol crystals
- 8.) New Bone Growth: extraneous bone growth, shown histologically as having greater hematoxylin uptake
- 9.) New Gland Formation: not actually new gland, but an increase in secretory function of the usually low cuboidal cells of the lining of the middle ear. Cells increase to high columnar, and also take on an active secretory appearance. These columnar cells form in collections around an artery within the middle ear.
- 10.) Hemorrhage: any nature of hemorrhage or lysed red blood cells within the middle ear
- 11.) Stapedial Cavity Inflammatory Cells: cavity consists of the area between the ossicle walls and inflammatory cells are basically round cells
- 12.) Fibrous Attachment within the Stapedial Cavity: fibrosis is continuous with the interior of the crura, footplate, or head of the stapes
- 13.) Thickened Footplate or Crura: new bone growth leading to thickening of the stapes footplate and/or crura. This growth is usually associated with areas of bone erosion or reabsorption
- 14.) Fibrous Attachment to the Stapedial Artery: the tunica adventicia is continuous with the fibrous material within the stapedial cavity
- 15.) Thickened Stapedial Artery: thickening of the tunica intima without endothelial proliferation
- 16.) Fibroblasts in the Stapedial Cavity: fibroblasts alone or fibroblasts and cholesterol crystals within the stapedial cavity, usually continuous with the ossicular surface

- 17.) Fibrous Attachment to the Round Window Membrane: the round window membrane is continuous with fibrosis in the niche
- 18.) Inflammatory Cells in the Round Window Niche: round cell infiltration limited to the niche area
- 19.) Fibroblasts in the Round Window Niche: collections of fibroblasts alone or fibroblasts and cholesterol crystals in the round window niche
- 20.) Polyp Formation in the Round Window Niche: polyp or collections of granulation material with or without its own blood supply or inflammatory cells in the round window niche

Cochlear-Vestibular Conditions

- 1.) Strial Atrophy: atrophy or decrease in size and resultant narrowing of the stria vascularis
- 2.) Precipitate: inflammatory cells and products of their inflammatory reaction in the perilymphatic areas of the cochlear and vestibular system. This includes fibrous or granular connective tissue proliferation, and possible protein coagulate due to fixation
- 3.) Hemorrhage: hemorrhage within the scala tympani or scala vestibuli (perilymphatic areas)
- 4.) Round Window Membrane Thickening: including those areas which showed epithelial thickening as well as cases in which the connective tissue layer showed increase thickening
- 5.) Reissner's Membrane Thickening
- 6.) Sensory Epithelium Changes: changes include a swelling of the hair and/or support cells of the various cristae or maculae as well as loss in population number of these cells

Table three shows the number of animals in each group (eight total for each group) which presented the pathological condition indicated. Shown in this table are the general otopathological differences between the three lines. In the non-diabetic "M" line, the

TABLE 3

NUMBER OF ANIMALS WHICH EXHIBIT
EACH OTOPATHOLOGICAL CONDITION

Condition - Middle Ear	XA Animals			AC Animals			M Animals		
	Sl	Mo	Se	Sl	Mo	Se	Sl	Mo	Se
Round Cell Infiltration	4	1	3	1	1	6	4	0	0
Fibroblasts & Chol. Crystals	1	0	0	1	2	4	0	0	0
Tympanic Memb. Thickening	2	1	3	1	4	3	4	0	0
Fibrous Attach. to T. Membrane	1	1	3	1	2	5	1	0	0
Fibrosis Around Ossicles	1	3	2	1	1	6	0	0	0
Polyp Formations in M.E.	0	2	1	1	4	2	0	0	0
Cholesterol granuloma	0	0	0	3	0	2	0	0	0
New Bone Formation	1	2	0	2	2	1	0	0	0
New Gland Formation	1	2	0	2	2	2	0	0	0
Hemorrhage	1	2	2	1	2	2	1	3	0
Stapedial Cav. Inflamm. Cells	2	3	3	1	1	6	0	0	0
Fibrous Attach. within S. Cav.	1	2	3	1	1	6	0	0	0
Thickened Footplate or Crura	3	0	3	3	3	1	0	0	0
Fibrous Attach to S. Artery	2	1	3	2	1	5	0	0	0
Thickened Stapedial Artery	0	3	3	1	1	6	0	0	0
Fibroblasts in S. Cavity	0	1	0	5	0	2	0	0	0
Fibrous Attach. to the R.W.M.	1	1	2	1	2	4	0	1	0
Inflamm. Cells in the R.W.M.	2	2	2	1	0	6	3	0	0
Fibroblasts in the R.W.N.	2	0	0	2	3	1	0	0	0
Polyp Form. in the R.W.N.	1	0	0	2	1	2	0	0	0
Condition - Cochlear & Vestibular									
Strial Atrophy	0	1	0	0	2	0	0	0	0
Precipitate	2	4	0	3	2	2	4	3	0
Hemorrhage	4	0	3	3	1	2	3	2	1
Reissner's Memb. Thickened	2	0	0	1	0	1	0	0	0
Sensory Epithelium Changes	6	0	0	8	0	0	8	0	0
Round Window Memb. Thickened	2	2	2	1	2	5	2	1	0

Note: Sl = slight
Mo = moderate
Se = severe

8 animals total in each group

most significant finding was hemorrhage and inflammatory cells within the cochlear system. Inflammatory cells include round cells and their products, usually protein coagulate. All of these findings, except the small round cell infiltration, could be due to fixation and preparation of the bone. The other major finding, sensory epithelium changes, occurred across all animal lines and was observed as edema of the hair cells and support cells. Since this condition was found in almost all animals, except two "XA" animals, possibly it was a result of histological technique. Three "M" animals had a slight inflammation in the round window niche. Since this particular area is in the most inferior recesses of the middle ear, a slight accumulation of inflammatory material is not uncommon. Hemorrhage which is often seen in the middle ear proper may be due to disruption of blood vessels at the time of removal of the temporal bones. It should also be noted that there were four animals with slight otitis media, and as would be expected these four cases also had a slight thickening of the tympanic membrane. In consideration of the foregoing factors which represent an account of the otopathological assessment of the "M" line, it can be stated that the non-diabetic line of animals in this study were relatively free of middle ear and inner ear pathology.

In the "XA" diabetic line a somewhat intermediate degree of severity of otitis media was seen along with its associated complications. All eight animals had otitis media, and they seemed to show a broad range from slight (50% of the animals) to severe (appro. 40% of the animals). The most characteristic finding in the "XA" line was the absence of cholesterol crystals. Only one animal (#9 XA25-24)

demonstrated any occurrence of cholesterol crystals. This animal will be discussed further, but the temporal bones of animal #9 (XA25-24) had a mean total otopathological involvement of 48, well above the 28.4 average of the "XA" line. This value of 48 clearly shows that animal #9 had a severe form of otitis media. Cholesterol crystals generally are associated with a chronic middle ear infection (Nager, 1972).

The presence of tympanic membrane thickening in the "XA" line and fibrous attachment to the tympanic membrane was seen in conjunction with otitis media. The inflammatory round cells seemed to occur in association with polyps in the three severe "XA" cases. These three severe cases also had new bone formation and an increase in the serous gland activity.

The stapedial cavity was the second most common site for inflammatory cells within the middle ear of the "XA" line. Only the superior aspects to the stapes had a greater incidence. The stapedial cavity reflected almost the same picture as the rest of the middle ear for this line of Chinese hamster. The same three animals showed severe inflammatory precipitate within the stapedial cavity, along with changes to the stapes due to severe chronic otitis media. The remaining five animals demonstrated similar changes to a lesser extent.

Two animals each or twenty five percent of the total "XA" group showed a non-existent, or slight, or moderate, or severe case of otitis media in the round window niche.

Precipitate and hemorrhage within the perilymphatic spaces of the cochlear or vestibular systems were slightly more severe in the

diabetic strains than the non-diabetic strains. Associated with the three cases of severe hemorrhage were two cases of slight thickening of the Reissner's membrane.

Possibly the most significant correlations between the diabetic state and otopathological involvement were evident in the "AC" animals. A chronic state of otitis media and its associated complications was consistently observed in these animals. The main histological finding associated with the "AC" line was the evidence of cholesterol crystals. Fifty percent of the animals had a severe cholesterol crystal condition, while forty percent demonstrated either moderate or slight accumulations of this severe manifestation of chronic otitis media. Cholesterol granulomas which are one of the final stages of cholesterol crystal involvement (Dota, Nakamura, Saheki, and Sasaka, 1963) occurred to some degree in over 62% of the "AC" animals.

Fibrous attachment to the tympanic membrane, as well as associated ossicular fibrosis and polyps, were expressed in almost 100% of the "AC" animals. Severe stapedia inflammation and erosion also occurred in 75% of the "AC" animals. Cholesterol crystals were evident in both the stapedia cavity and in the round window niche. These animals exhibited more severe cases of otitis media in the round window niche than animals of other lines. Severe round window membrane thickening was associated with inflammatory precipitate over 60% of the time.

To summarize table three, the "M" line was essentially normal in otopathological assessment. Both diabetic lines demonstrated acute to chronic inflammatory states and their associated pathological complications. The "XA" line had a moderate inflammatory destruction,

while "AC" exhibited severe otopathological conditions which included the accumulation of cholesterol crystals indicative of a chronic inflammatory state.

Table four gives cumulative values of the otopathology for each specific animal including both H & E and PAS preparations. Looking solely at the H & E values, each pathological condition was given a value of 1, 2, or 3, for a finding of slight, moderate, or severe, respectively. This was an attempt to quantitatively analyze each animal.

Nondiabetic "M" animals had a mean otopathological average of 6.75 with a range of 2 to 14. Total diabetic animals ("XA" and "AC") had an average value of 37.62 (range 4 to 60) or a 5.6 times greater pathological involvement than the "M" animals.

When considering the two diabetic lines separately the "XA" line had a mean otopathological involvement of 28.34 and range 4-54. This represents 4.2 times greater involvement over the "M" line.

The "AC" animals had a mean value of 46.9 and had a range of 17-60, or a 7 times greater pathological involvement over the non-diabetic animals. A significant finding showed that the values of "XA" and "AC" animals verses "M" animals were consistent with the findings in table three. Namely, for the diabetic animals the intensity and the frequency of occurrence was greater than the non-diabetic animals.

Age information is given in table one. The range of ages was from 9.5 to 18.7 months at the time of sacrifice. The average life span of a non-diabetic Chinese hamster is approximately 30 months and

TABLE 4

TOTAL OTOPATHOLOGICAL INVOLVEMENT AND PERCENT OF MAXIMUM
POSSIBLE CAPILLARY BASEMENT MEMBRANE THICKENING

Animal Number	Sum of H&E for Middle Ear	Sum of H&E for Cochlear-Vestibular	Total H&E Pathology	Sum for Extent of BMT	Maximum Possible for BMT Sum	% of Max.
1. M26-014	2	5	7			
2. M25-133	2	4	6			
3. XA25-142	43	11	54			
4. XA25-133	41	11	52			
5. AC22--86	8	9	17			
6. AC23-025	27	9	36			
7. M24-58	4	5	9	6	38	16
8. M24-57	9	5	14	2	38	5
9. XA25-24	42	6	48	23	40	57
10. XA25-31	15	2	17	22	40	55
11. AC21-94	51	5	56	26	40	65
12. AC22-26	50	7	57	31	40	77
13. M26-31	0	2	2	4	38	10
14. M26-45	2	3	5	5	38	13
15. XA26-217	3	1	4	25	40	62
16. XA26-118	23	4	27	26	40	65
17. AC22 133	45	1	46	34	38	89
18. AC23-110	50	6	56	32	38	84
19. M25-118	4	4	8	7	38	18
20. M25-117	0	3	3			
21. XA26-189	3	6	9			
22. XA26-233	13	3	16			
23. AC22-134	39	8	47	28	36	77
24. AC23-55	47	13	60	26	38	68

Note: Mean Non-diabetic Total H&E Pathology = 6.75 % of Max. CBM = 12.4
Mean Diabetic Total H&E Pathology = 37.62% % of Max. CBM = 68.1
Mean Total H&E Pathology for XA Animals = 28.37 % of Max. CBM = 59.7
Mean Total H&E Pathology for AC Animals = 46.87 % of Max. CBM = 76.6
BMT = Basement membrane thickening

for the diabetic hamster it is up to 25 months (Gerritsen, February, 1983). The average age in months for the 24 animals in this study was 14.2, while the average diabetic age for all 16 diabetic animals in the study was 10.42 months. There was no correlation between age and severity of pathological involvement.

Average age of onset of the diabetic state was 2.2 months for the "XA" animals, and 5.9 months for the "AC" animals. This information is basically consistent with the characteristic information listed in table two. The age in months of the animals in this study to the start of the diabetic state was greater than the average for each line in table two. The delayed age of onset of the "AC" animals over the "XA" animals in this study was consistent with data from all animals monitored in the Upjohn colony.

The following animals exhibited specific pathological findings, which need further description.

Animals # 1 through # 6 (the first group) were subjected to excessive heat during dehydration (70 C verses 55 C normal) for 48 hours due to equipment malfunction. The result was shrinkage of the total bone, cellular disruption, and varying amounts of histological artifact. Uptake quality of the stain varied with each of these "heat disrupted" bones. Assessment was carried out even though it was difficult.

Animals #1 and #2 (M26-014 and M25-133 respectively) were free from otopathology. Animal #3 (XA25-133) had abnormally high pathological involvement in terms of the average pathological involvement level for all 24 animals. Animal #4 (XA25-133) (one of the two ketotic animals) was similar. The otitis media was severe in these two animals.

Unilaterally the footplate was bowed toward the vestibule, possibly being secondary to the otitis media. Both exhibited polyp formations and other conditions indicative of a chronic inflammatory state. Characteristically neither animals # 3 or #4 had any cholesterol crystal formations or fibroblasts. In animal #3 all ossicles were eroded to some degree. Animal #3 at the level of the superior canal crista on the right had a polyp attached to the wall of the osseous portion of the cochlea. Areas of new bone formation were also shown bilaterally in both animals although some aspects of the new bone growth were atypical. Animal #3 contained a polyp in the round window niche, and animal #4 also had bilateral polyps similar to those observed in animal #3 on the right side of that animal.

Animals #5 (AC22-086) and #6 (AC23-025) had a slight degree of otitis media unlike the rest of the animals from this strain. These two bones were damaged by the heat more severely than the other four, and as mentioned earlier, this situation could have altered certain aspects of the original pathological condition. Animal #6 did possess an area of new bone formation on the right medial to the malleus and incus head.

In the second group, animals #7 (M24-58) and #8 (M24-57) both "M" animals, had little or no inflammation or other pathological deformities. Animal #7 had a slight hemorrhage on the right side and round cell infiltration around the malleus. Animal #8 although basically free of inflammation did have severe hemorrhage in the perilymphatic areas of the cochlea in the left ear mid-modiolar area. Hemorrhage also existed on the same side occupying between 20 to 70%

of the space on either side of the round window membrane. The round window membrane in this area above the posterior semicircular canal crista was ruptured with some white blood cells on both sides of the membrane.

Hamster #9 (XA25-24) bilaterally had a chronic state of middle ear inflammation. This included ossicular erosion and thickening. The incudo-malleolar joint was also eroded. Polyps at the posterior cristae were also evident. There were some areas of new bone formation on the left and the beginning of a unusual large spherical polyp which occupies approximately 30% of the total middle ear space just past the mid-modiolar area. Cells within the polyp were swirled in concentric circles. The area of new bone formation inferior to this polyp contained some fibroblasts in the process of changing to chondroblasts. The polyp which was located just superior to the stapes was observed to contain some cholesterol crystals. This material was continuous with fibroblasts within the crura area of the stapes. Going through the next few sections (from superior to inferior) it was seen that the new bone formation continued medial to the head of the stapes. There was a fibrous continuity between the new bone formation area and the head of the stapes, the footplate of the stapes was also eroded.

In contrast an early stage of inflammation was evident in animal #10 (XA25-31) also of the "XA" line.

Rounding out the second group, the two remaining animals #11 (AC21-94) and #12 (AC22-26), demonstrated severe bilateral otitis media, with full complications. Cholesterol crystals were severe

throughout both bones bilaterally. At the superior crista level right side, severe fibrosis around the ossicles was observed as well as the beginning of new bone formation between the incudomalleolar joint and the osseous part of the ampullated superior semicircular canal. Fibroblasts are in transition to surface type chondrocytes (flat). The next slide in serial order still at the superior crista level demonstrated a large area of the same new bone formation with a greater amount of fibroblasts in transition. It was approximately 20% of the size of the incus and malleus heads. The next slide in sequence on the right showed continuity of the new bone mass lying adjacent to the malleus. There was no connection to the medial wall. The fourth slide in the series from the superior on the right contained an excellent example of a cholesterol granuloma in the superior middle ear. The space between the crura of the right stapes was full of inflammation and approximately 60% filled with cholesterol crystals. The next five slides in sequence on the right showed the condition of the round window membrane and niche. Cholesterol crystals filled up to 80% of the niche. The niche also contained polyps. Some of the slides on the left at the round window membrane location showed excellent examples of spherical polyps with areas of new internal capillaries supplying the polyps. Fibrous attachment to the round window membrane was severe on the left, and some precipitate had crossed to the perilymphatic areas of the cochlea (scala vestibuli and scala tympani).

Hamster #12 (AC22-26) had severe otitis media. It showed cholesterol crystals in the middle ear and new bone formation in the

superior middle ear, medial to the tympanic membrane. At the mid-modiolar area of the left, Reissner's membrane was thickened. The round window niche also contained cholesterol crystals.

Both animals #13 and #14 (M26-31 and M26-45 respectively) were normal non-diabetic hamsters. Animal #13 from the "M" line had a normal condition of the middle ear, including the tympanic membrane, and the normal cuboidal secretory cells. The stapedial artery was normal in this animal. Animal #14, also from the "M" line exhibited normal organs of Corti at the left mid-modiolar area even though there was some compression of the osseous portion of the cochlea due to preparation. The round window niche included some precipitate, probably protein coagulate.

The "XA" bones #15 (XA26-217) and #16 (XA26-118) had moderate involvement. Animal #15 had part of the left ear missing and both sides showed a large amount of histological artifact. The otopathological quantitative involvement might have been different if the total temporal bone had been available for assessment. This animal did show a very early stage of otitis media.

Animal #16 from the "XA" line was the second ketotic hamster. Even though it was ketotic, its quantitative evaluation was about normal for the "XA" animals (27 as compared to an average of 28.37 for "XA" animals). This is paradoxical, since a ketotic animal should show significantly higher pathological involvement, even over others in its same "XA" line. Unfortunately, none of the "AC" animals were ketotic. Animal #16 did show inflammation around the malleus, and some macrophages. Some histological tearing was evident on the

left throughout this bone.

The two "AC" animals of group three #17 (AC22-133) and #18 (AC23-110) were consistent with general findings of the chronic otitis media for this line. Approximately 80% of the middle ear of animal #17 contained fibroblasts and cholesterol crystals. Areas of new bone formation and malleus erosion were evident on the right as well as active secretory cells. These collections of columnar cells were seen throughout the bone. Fibroblasts filled the stapes.

Animal #18 exhibited a severe bilateral chronic state of inflammation. Two distinct polyps were located between the ossicular heads and the medial wall at the ampullated position of the superior semicircular canal. Together the area of the polyps occupied approximately 40% of the area of the heads of the malleus and incus. They contained arterioles and venules which were continuous with the fibrous material that lined the medial wall. In addition on the right side there were several areas of cholesterol crystal formation, and areas of round cell infiltration, including lymphocytes, macrophages, plasma cells, and polymorphs. Severe tympanic membrane thickening on the right and fibrous attachments compressed the tympanic membrane near the medial wall of the middle ear. Adjacent to the beginning of the lateral process of the malleus lay a bony spur. The tympanic membrane exhibited extreme waviness to its course. Continuing inferiorly through the sequence of sections of the right side, this animal also had areas of collections of columnar secretory cells, many of which surrounded arteries. The stapes of this animal on the right was extremely disrupted. One hundred percent of the stapedial cavity

was filled with round cells. The crura and footplate were significantly thickened with fibrous attachments which were continuous with the tunica adventicia and inflammatory precipitate. The left side contained a much smaller number of cholesterol crystals unlike the more severely disrupted right side. The round window niche of this animal showed extensive pathological involvement greater than seen in any of the 24 animals. It was 100% filled with round cell infiltration and pockets of columnar cells. The round window membrane was severely thickened. Inflammatory cells were also in the perilymphatic area of the cochlea with some fibrous material and protein coagulate.

In the last group of six animals (#19 to #24) some of the bones were poorly trimmed at the time of removal. As a result parts of the auditory and vestibular systems of some animals were missing. Animals #19 (M25-118) and #20 (M25-117) were normal non-diabetic animals and the only otopathological involvement observed was on the left side of #19 which had some hemorrhage in the scala tympani. Animal #20 was badly trimmed, but had normal malleus and middle ear conditions as well as the conditions of the stapes and round window membrane.

Animals #21 (XA26-189) and #22 (XA26-233) both "XA" hamsters had beginning stages of otitis media. Animal #21 was the most severely trimmed and had hemorrhage throughout the perilymphatic areas of the cochlea on the right. Animals #21 and #22, both "XA" animals were almost equal to the average age (14.2 months average for diabetics), and therefore had equal time to develop any complications of the diabetic state. It is tempting to conclude that a genetic component could be a factor.

Animal #23 (AC22-134), an "AC" animal had a left ear that showed fibroblasts and cholesterol crystals throughout, while the right ear was 100% full of round cell infiltration. Some beginning cholesterol crystals on the right were noted at the level of the mid-modiolas. One unusual finding in this bone was fibrous attachment in the left scala vestibuli to Reissner's membrane. This attachment or fibrous matrix was evident throughout the perilymphatic areas of the scala vestibuli and scala tympani. Polyp formations existed in the round window niche on the left.

The last bone #24 (AC23-55) from the "AC" line had the highest quantitative sum for pathological involvement (60) of any of the 24 animals evaluated. Massive chronic inflammation filled the middle ear spaces of the bone bilaterally. In the superior aspects, cholesterol crystals and fibroblasts made definite changes in the ossicles. Cholesterol granulomas were apparent. Hemorrhage in the right ear within the cochlea was severe (100%) at the level of the lateral canal crista. The basilar membrane was slightly thickened on the right. The scala tympani was filled with a few macrophages and damage to the cochlea was evident (right mid-modiolar area). In the crista of the lateral canal there was disruption between hair and support cells. Hemorrhage was visible in both the basal and middle turns of the scala tympani. On the right at the mid-modiolar level, a middle ear polyp was attached adjacent to the osseous portion of the cochlea. There were definite areas of new bone formation. The stapes footplate was eroded and bulging into the vestibule. Inflammatory precipitate was seen in all perilymphatic area (scala tympani and scala vestibuli)

with possible areas of Reissner's membrane thickening. This thickening could have been due to sectioning in a tangential plane rather than at right angles to the membrane. Stapedial cavity inflammation and fibrous attachments with cholesterol crystals was observed, and the stapedial artery was significantly thickened in the tunica intima and media. New bone areas were evident. The left side (mid-modiolar) had one major area consisting of a very thick band of lymphocytes surrounding hemolyzed red blood cells. The interior of this aggregate seemed to be full of coagulated protein. On the left the round window membrane was severely thickened, and the cochlear perilymph was filled with fibrous material.

Capillary Basement Membrane Thickness

One complete set of serial sections from 15 Chinese hamsters was stained with periodic acid Schiffs (PAS) and examined for vascular basement membrane thickenss. The basement membrane glycoprotein component is strongly PAS positive and appears as a band of dark pink material the thickness of which can be assessed.

After the general otopathological assessment with the H & E set of serial sections was analyzed, a decision not to evaluate nine out of the 24 total for capillary basement membrane changes was made (see table 4). The first group of animals, #1 through #6, suffered varying amounts of histological artifact due to high heat during preparation. Even though general structural abnormalities could be evaluated, it was felt that any information obtained from the PAS stain would be questionable. Likewise animals #20 through #22 were obmitted because

of trimming difficulties. Therefore, out of the 24 total animals, 15 were stained with PAS and evaluated for capillary basement membrane changes. Out of these 15 hamsters, there were five "M", four "XA", and six "AC" animals.

All 15 pairs of temporal bones were stained with PAS for capillary basement membrane changes. Then all animals were blindly assessed as a group to give a qualitative evaluation. Appendix E gives a sample qualitative report of one animal. From this preliminary blind assessment a consideration of the range of basement membrane changes was obtained. Basement membranes from many normal non-study animals were also observed in an effort to be able to assess the basement membranes as "thinner" or "thicker". This was necessary since sophisticated quantitative measurements of actual basement membrane thickness was not possible. Following this preliminary assessment a second blind evaluation with the help of an individual other than the scorer was made. Four or five slides per animal which contained the specific structures to be evaluated were selected in advance of the final assessment. It was during this final blind assessment that quantitative values were assigned as shown in the sample in appendix F.

It can be seen in appendix F that capillaries from specific locations within the auditory and vestibular systems were evaluated. The selection criteria was in accordance to the literature in relation to the emphasis which is placed on specific capillary beds, that is the capillary beds supplying particular structures. In the stria vascularis 12 total cross sections (six from each ear) were assessed. All capillaries were from a mid-modiolar section of the cochlea and from the

middle turn of the cochlea. One modiolar capillary from the same mid-modiolar section was evaluated from each ear. In the three semicircular canal cristae (superior, lateral, and posterior), one capillary in cross section was assessed from either ear, whichever gave the clearest view of a capillary cross section. One capillary was also assessed from the macula of either the utricle or saccule from either side. The macular capillary was taken from the same slide which contained the assessed lateral canal cristae. Two capillaries from the central nervous system were also included, one from the pons and one from the cerebellum. Capillary selection criteria was the first clear complete basement membrane cross section of a capillary (not in tangential) in each location. This was a blind evaluation.

In all cases a normal capillary was distinguished by a very thin basement membrane. The capillaries were difficult to see because of the small amount of basement membrane material in the capillary walls. The moderately thickened capillaries were thickened over their normal counterparts by about twice the amount of basement membrane material. These capillaries were easily distinguished and stood out nicely. The severely thickened capillaries were even more evident due to the larger amount of PAS positive material in their walls, and contained approximately three times the amount of basement membrane material over their normal counterpart. No occluded capillaries were found, and at no time were they as thickened as reported in many studies (Costa, 1967; Jorgensen, 1961). The lumens became smaller in volume according to the extent of thickening. The capillaries in the present study were assigned a description of normal, moderate, or severe

depending on the extent of thickening evident in all of the animals.

A normal thickness capillary was given a value of zero, moderate a value of one, and severe capillary basement membrane thickening a value of two. Maximum total number of capillaries assessed from each animal was 20. In most cases there were less than the total number of 20 to evaluate for a variety of reasons. To compensate for this difference, the value given for capillary basement membrane thickness was based on the percent of maximum possible for each animal. In some animals all 20 capillaries were evaluated giving a maximum possible sum of 40 (20 times a severe value of two), while in others only 19 capillaries were assessed giving a maximum possible sum of 38, etc.

Total values for each animal were therefore converted to a percent value of maximum possible capillary basement membrane thickness. The average for the five "M" animals was 12.4%, for the four "XA" animals it was 59.7%, and for the six "AC" hamsters it was 76.6%.

Statistical Assessment

Simultaneous confidence intervals were constructed for mean H&E otopathology by the Bonferroni method (Neter and Wasserman, 1974) to assure family confidence coefficient was at least .95 and that the joint set of comparisons was exactly correct. Welch's T analysis of variance (Johnson and Leone, 1977; Welch, 1938), a two-tailed test was used because the variances were not the same for each type of hamster. Statistical significance at $p .05$ was determined between mean H & E middle ear pathology of diabetic and non-diabetic animals. No significance between cochlear-vestibular mean pathology was found.

In the case of mean basement membrane thickening, a significance of $p .05$ was determined between all three Chinese hamster lines. The variances were about equal for all three hamster lines so analysis of variance followed pairwise comparisons between each of the three genetic lines.

Discussion

Microvascular changes are one of the most specific major vascular diseases in diabetics. The lumens become narrowed because of hypertrophy or proliferation of the capillary basement membrane material. This occurs by excessive deposition of polysaccharides and lipids. This result was shown to some extent in the auditory and vestibular systems of at least ten hamsters in the present study.

As noted these broad vascular changes effect the retina, renal glomerulus, as well as all other major organ systems. They may precede the appearance of the clinically recognizable metabolic abnormality. The brain is particularly vulnerable. There is strong circumstantial evidence that the abnormal vascular pathology will also involve the auditory and vestibular systems.

It is postulated that these vascular changes lead to specific auditory loss. The hearing loss generally associated with diabetes is sensorineural, progressive, and bilateral. It is first noted in the high frequencies, gradually extending into the mid-frequency range. Neuronal degeneration in other studies has been shown in the basal turn of the cochlea, which accounts for the loss of hearing in the high frequency range. Some individuals have experienced unilateral

hearing loss or permanent low frequency loss associated with sudden onset deafness. This sudden onset type loss and its relationship to diabetes has not been proven.

The vestibular system may also be affected by microvascular disease and/or neuropathy. Dizziness is a complaint of many diabetics. A syndrome similar to the symptoms of Menieres disease may also be associated with diabetes. These symptoms include fluctuating sensorineural hearing loss, periods of vertigo, and tinnitus. Coats (1978) determined that there are no specific ENG findings with diabetes, but peripheral vestibular symptoms are generally detected.

All of these complications to the auditory and vestibular systems tend to be progressive in spite of adequate antidiabetic therapy and treatment.

Microvascular changes (capillary basement membrane thickening) were the most significant finding of the present study in the capillary networks supplying the end organs of the auditory and vestibular systems. Present day knowledge of the morphology, synthesis, maintenance, and breakdown of the normal basement membrane is limited, and that of the abnormal basement membrane is even more so. The main morphologic change associated with diabetes is an increase thickening of basement membrane material, and results to some degree in loss of the function of filtration and anchor of cell populations. Insulin and insulin antagonists acts directly on cell membranes and therefore their direct actions on the basement membrane in the auditory, vestibular, and central nervous system must be considered.

To understand the results on capillaries assessed in the present

study, it is necessary to have an appreciation of the biochemical changes in the diabetic state which lead to this thickening. Biochemical changes noted in addition to morphological changes described in the diabetic state are very limited. Findings include: 1.) an alteration of amino acid and/or carbohydrate composition, 2.) an increased affinity for serum proteins, 3.) an decreased cysteine content, and 4.) a disturbed ratio of disaccharide to heteropolysaccharide units.

In the previous references to the differences in the specific lines of the diabetic Chinese hamster, the basement membrane thickening appears to be attributable to a disorder of the biosynthesis in the matrix macromolecule of this collagen and/or associated glycoprotein components. The glycoproteins themselves contain substantial amounts of glycine, hydroxyproline, hydroxylysine, glucose and galactose as mentioned earlier. Chang (1978) found that post-translational modification of proteins increased in diabetics due to the excessive glucose levels. There are two types of carbohydrate units present. One is a heteropolysaccharide with no glucose, similar to other glycoproteins, while the other one is a disaccharide unit consisting of glucose and galactose linked to the hydroxylysine residues in the peptide chain. Chang also found an increase in hydroxylysine linked α -glucosyl - β -galactose disaccharide units in the glomerulus basement membrane. Assembly of the basement membrane requires several steps because of its complex structure. First is the synthesis of the peptide chain followed by hydroxylation of proline and lysine. The attachment of the monosaccharide residues to form both types of the carbohydrate units, the heteropolysaccharide and the disaccharide

units. Finally cross links are formed to establish the end product. Changes in the carbohydrate units will lead to the observed result of capillary basement membrane thickening in the present study's animals.

The disaccharide unit seems to be altered the most in the diabetic state. A decrease in the acid glycohydrolases found in most diabetics leads to an increase in the amount of the disaccharide unit relative to the heteropolysaccharide unit. This is due to the decrease in catabolic activity of the enzymes. In summary the acid glycohydrolases serve to degrade the disaccharide unit. Two other enzymes serve to assemble the disaccharide unit. These two highly specific glycosyltransferases (2.4) build the disaccharide unit by transferring a sugar from an oligosaccharide to another carbohydrate unit. Specifically, these two glycosyltransferases responsible for the attachment of carbohydrate units are 1.) glucosyltransferase which transfers glucose to galactose, and 2.) galactosyltransferase. Many studies have shown an elevated level of kidney glycosyltransferase activity in the normal diabetic state as well as in the alloxan and streptozotocin induced hyperglycemia. These both lead to an increased basement membrane synthesis. Therefore capillary basement membrane thickening can occur through an increased synthesis of the disaccharide unit (glycosyltransferase) and/or a decrease in the degradation of the disaccharide unit (acid glycohydrolases). In addition, the decreased cysteine content: (noted above) to less than $\frac{1}{2}$ normal level may indicate an alteration in crosslinking of the protein in the basement membrane.

Studies of the metabolism of diabetic kidneys in experimental.

animals, using cortical homogenates and isolated glomeruli, have demonstrated higher anabolic and lower catabolic enzyme activity which would fit the action of the two enzyme types. Biochemically, both an increase in glycosyltransferase activity and a decrease in catabolism rate (decrease in acid glycohydrolase activity) would both lead to basement membrane thickening observed in the present study ultimately leading to vascular changes in the auditory and/or vestibular systems of the Chinese hamsters.

The conclusion is clear as to the mechanism of altering the vascular walls constituting diabetic microangiopathy. The morphologic lesion of the diabetic vessels appears as an increased thickness of the basement membrane material. As noted this thickness could arise from increased synthesis, decreased breakdown, or both actions occurring simultaneously. Since the basement membrane is a glycoprotein structure, and capillary basement membrane thickness increased by a disturbance in the metabolism of the carbohydrate containing portion, biosynthesis of the basement membrane is undoubtedly related to glucose metabolism.

It is evident that glucose plays a central role in the biosynthesis of the monosaccharide component of glycoproteins. Glucose is the only free sugar in the blood and therefore the only monosaccharide which can be used for the synthesis of other sugar components and glycoproteins (including basement membranes).

Because the turnover rate of basement membranes is very slow, the overutilization of glucose leads to an increase in accumulation over time. This increase in material interferes with normal function and

would lead to the clinically recognizable small blood vessel lesions in the inner ear. Because of the slow turnover rate, capillary basement thickening would not be reversible upon lowering of blood glucose levels. After basement membrane is laid down it cannot be quickly metabolized and therefore even sporadic hyperglycemia over time will lead to capillary basement membrane thickening.

Functional changes from morphological and biochemical alterations in the diabetic state lead to functional changes in filtration. An increase in capillary basement membrane thickening leads to a decrease in the capillary surface area and the area available for filtration. Also if occlusion is complete, ischemia to the tissues results.

Some studies suggest that long term diabetic vessels are more penetrable than normal vessels, and in actuality become "leaky" (especially in the kidney). The whole question of correlation between morphology and function (before occlusion) awaits further investigation.

Impairment of hearing associated with diabetes mellitus has been primarily associated with capillary basement membrane thickening within the auditory and vestibular systems. There are several major capillary beds which supply the inner ear. These areas are found in association with the spiral prominence, the upper spiral ligament adjacent to the scala vestibuli, the scala tympani, and the stria vascularis. The stria vascularis consists of a band of tissue lying on the internal surface of the spiral ligament. It extends from the spiral prominence to Reissner's membrane. The stria is very dense with numerous capillaries passing mainly in a longitudinal direction. It is considered

a vascular epithelium and the major blood supply of the cochlear duct. It is for this reason that the stria vascularis was the primary site for assessment of capillary basement membrane thickening in this project.

The capillary network of the stria is interspersed among three different cell types. The basement membrane separates the capillary wall from the stria cell. The chromophilic cells are marginal cells and lie on the endolymphatic surface. They have large basal extensions which interdigitate with the second cell type, the chromophobic intermediate cells. The third cell type (basal cells) lie adjacent to the spiral ligament. The width and depth of the stria vascularis decreases gradually toward the apex.

Oxygen consumption studies by Chou (1962) show the stria vascularis to be the most metabolically active tissue in the body. There is an abundance of mitochondria in the cell types. The stria not only serves as a major vascularization tissue, but also serves to maintain the biochemical properties of the endolymph.

Mendelsohn and Roderiques (1977) showed that an increase in insulin leading to hypoglycemia had a direct effect on the potassium/sodium ratio in the endolymph. Normal cochlear endolymph has a high potassium and low sodium concentration. No other extracellular fluid has such a ratio. Results show that glucose is essential for maintenance of active transport. Hypoglycemia leads to a shift in endolymph concentration to low potassium and high sodium. Again glucose levels have a direct effect on the cochlea.

One aspect of this study which deserves further attention is the

high incidence of infection (otitis media) in the diabetics over the non-diabetics. This leads to a question as to the relationship of infection to capillary basement membrane thickening or metabolic alterations of diabetes directly.

It is generally believed that diabetics have more infections than non-diabetics, and that infections in diabetics are severe and difficult to manage (Silva and Fekety, 1976). Diabetes is also more severe in the presence of an infection because the inflammation influences the availability and utilization of the substrate. Therefore infections must be given a prominent place as a factor which affects carbohydrate tolerance.

There are conflicting studies as to diabetes affect on the body's cellular and humeral immune system, however it does seem that diabetics have a greater susceptibility to infections of various kinds. An infectious state increases the insulin requirement and therefore magnifies the diabetic state.

A general overview of the infectious state is necessary before the complications of the diabetic state can be understood. These complications can then be related to the auditory system.

The normal host defense mechanism begins with the acute reaction. This includes the release of antibodies and other chemical substances such as histamines and prostaglandins. Phagocytic leucocytes such as granulocytes (polymorphonuclear neutrophils) and macrophages invade the area. These ameboid type connective tissue cells must be able to move fast. Therefore any interference with their movement will alter the host defense mechanism. Acute events are an elevation in the

vascular blood flow, an increased vascular permeability, and infiltration of the tissue by leukocytes.

The chronic inflammatory state can arise directly from the acute stage, or arise without the acute stage. The two main features of the chronic stage are the presence of granulation tissue, and mononuclear cells which predominate. These agranular leukocytes are either lymphocytes or monocytes. Monocytes which account for 3 to 8% of the leukocytes can be distinguished by their kidney shaped nucleus. More importantly lymphocytes accumulate in the site of a chronic inflammation and comprise 20-35% of the white blood cells. Lymphocytes can change to plasma cells distinguished by a cartwheel nucleus. As the chronic state continues, fibroblasts arise as well as granulomas. Granulomas are aggregates of inflammatory cells, usually arranged concentrically. Components include macrophages, lymphocytes, and fibroblasts. Fibroblasts progressively lay down collagen which can distort or occlude the lumen of any duct or fill a space.

In the ear the inflammatory state may be caused by abnormal physical forces, allergy, or direct infection, as well as metabolic imbalances such as diabetes.

The acute or invasive state of secretory otitis media is characterized by the sudden appearance of non-purulent exudate. Transudation is an osmotic force which forces the fluid component of the blood out of the vessels into the middle ear. The leukocytes escape into the middle ear to form the exudate. The mucosa and the tympanic membrane becomes edematous and thickened, and macrophages are typically seen. The cell population which comprises the exudate changes from

predominantly macrophages to a population of lymphocytes and plasma cells. The exudate which tends to originally be serous, may also be hemorrhagic, mucoid, or purulent depending upon the nature of the inflammatory process and tends to accumulate in the various areas of the middle ear (Nager, 1972).

Time alone does not make an acute infection into a chronic inflammatory state. Chronic means that the inflammatory process has not been resolved and pathologic changes which occur in the middle ear may be irreversible.

The original flat cuboidal epithelium of the middle ear is transformed into high columnar epithelium (an active secretory state). This was dramatically shown in most of the "AC" Chinese hamsters since these animals exhibited the most severe state of otitis media. In particular animal #18 had islands of columnar epithelium which served to increase secretion due to an increase in surface area.

Partial resorption of the ossicles associated with an increase in osteoclastic activity may occur even though few osteoclasts were observed in the Chinese hamsters. Fibrous proliferation was characteristic in the present study, and was found in the animals with chronic otitis media.

Cholesterol granulomas were observed in 62% of the "AC" Chinese hamsters, and were always associated with a chronic state of inflammation. A keratoma results from the accumulation of keratin from squamous epithelium from the auditory canal. This is not the same condition as the cholesterol granulomas seen in the present study. The cholesterol granuloma is chronic granulation tissue containing

cholesterol crystals. In the presence of a chronic infection a cholesterol granuloma may develop rapidly. This type of tissue can increase the erosion of the bone by exerting pressure as it enlarges. Also additional enzymes are liberated, and granulation material can accumulate and become a polyp (Nager, 1972). Several polyps were found in the hamsters.

Cholesterol granulomas, polyps, and granulation material may fill the middle ear cavities as well as cause ossicular erosion.

Bone destruction by osteoclasts can occur simultaneously with new bone formation. Many times the ossicles, round window niche, and footplate become constricted or fixed.

In the presence of certain underlying diseases, such as diabetes mellitus, a middle ear infection may take an unusual course. The high blood glucose level may facilitate the growth of certain microorganisms. Ketoacidosis can adversely affect the host defense mechanisms by impairment of phagocytic movement of leukocytes. Ketoacidosis interferes with the bactericidal action of the blood and may lead to a decrease of host antibody production (J.E. Johnson, 1970).

The increase in capillary basement membrane thickening has profound effects. In the kidney the increase in basement membrane material actually allows for an increase in fluid flow, and in a sense they become leaky. Renal basement membranes are different in many regards, such as allowing for an increase in fluid passage, but the basement membrane thickening in most other locations such as the ear actually decrease filtration. This decreased blood flow to the tissues will hamper granulocyte movement and decrease tissue viability.

The tissues supplied will become poorly perfused and receive less oxygen. This allows certain microorganisms to gain a foothold in tissues which would normally not be susceptible (J.E. Johnson, 1970). It is reasonable to assume that an increase in capillary basement membrane thickening within the auditory and vestibular systems lead to an accentuation of the inflammatory state, ultimately leading to hearing loss.

The third long range complication associated with diabetes is neuropathy, or impairment of nerve conduction. As to whether this complication is secondary to the vascular insufficiency from capillary basement membrane thickening, or whether neuropathy results directly from the metabolic influences, is still a controversial matter. Unfortunately neuropathy is the least understood and least studied complication of diabetes. Neuropathy was not noted in the present study, but its importance in human diabetic auditory and vestibular systems seems to be significant.

Some studies show a moderate amount of atrophy of the spiral ganglia in the cochlear modiolus. In addition demyelination of the eighth cranial (vestibular) nerve has been noted. Vascular insufficiency has been thought to be one of the most important causative factors leading to neuronal degeneration. In the present study no loss of spiral ganglia neurons was found.

An important consideration is to what degree does the genetic component of diabetes affect these long range complications. There are several known genetic syndromes which link genetic deafness to diabetes mellitus, such as Alström syndrome (Konigsmark and Gorlin, 1976).

The Chinese hamsters used in this study demonstrated significant differences in capillary basement membrane thickening between all of the genetic lines, and between the middle ear pathology. The pathological conditions of the middle ear were due to frequency and/or severity of the inflammatory state. Both of these conditions if similar to humans would be expected to lead to auditory and/or vestibular loss.

CHAPTER IV

CONCLUSIONS AND RECOMMENDATIONS

Capillary basement membrane thickening associated with diabetes appears to be a complication of the insulin deficient state. The microvascular disease is a long range complication of diabetes mellitus and has been shown in the capillary networks of the Chinese hamsters evaluated in the present study. The thickening narrows lumens of the capillaries and leads to a decrease in vascularization to the tissues supplied. A correlation was found in the present study between the animals with capillary changes and inflammatory reaction. Significance was shown between diabetics and non-diabetics for capillary basement membrane thickening and middle ear pathology. All otopathological conditions assessed in this study were related to the inflammatory state and its chronic complications. Evidence suggests a correlation between the thickening of the basement membrane observed in the stria vascularis and other inner ear capillaries, and the inflammatory reaction observed in the ear.

The "M" hamsters demonstrated normal non-diabetic capillary basement membranes and there was a very limited amount of inflammatory reaction observed in this line. The "XA" individuals were moderate in their otopathological involvement, even though their capillary basement membrane thickening was not very different from the severe "AC" line of the Chinese hamster (59.7% of maximum capillary basement membrane thickening for "XA" verses 76.6% for "AC" animals). The

"AC" hamsters did have extreme otopathological involvement even though these animals have been shown to have elevated levels of the acid glycohydrolases (Chang, 1978). In the "AC" animals capillary basement membranes would be postulated to be normal even though the present study demonstrated severe thickening of auditory, vestibular, and central nervous system capillary basement membranes in the "AC" line. As of this date no other studies of the capillary basement membranes of the "AC" line in the central nervous system or elsewhere in the body have been reported (Gerritsen, March 30, 1983). A study at the Upjohn Company is presently in progress to look at this aspect of diabetes in "AC" animals. Until further evidence suggests differently the present study is the only known report of the possible affects of capillary basement membrane thickening in these "AC" animals.

The age of onset of the diabetic condition was similar to known characteristics listed in table two (ie the "AC" hamsters take longer to arrive at the diabetic condition than the "XA" animals). However, the time interval was longer for both diabetic lines of animals used in the present study.

The otopathological assessment demonstrated generally more severe chronic inflammatory reaction in the "AC" line with characteristic cholesterol crystal granulomas, areas of new bone formation, and ossicular erosion.

These conclusions support the original hypothesis of greater otopathological involvement of diabetic over non-diabetic Chinese hamsters.

Although there are difficulties involved in obtaining and

preparing human temporal bones, hopefully otopathological studies of diabetics will continue and be expanded. There is a great deal of room for further work and the Chinese hamster appears to be a very useful animal model.

In consideration of future research using the diabetic Chinese hamster and assessing the auditory/vestibular systems it will be advantageous to have a comparison microscope available so that direct side by side comparisons and photography of the capillary basement membranes of normal and diabetics can be made. Additional data could be obtained from fractionated basement membranes, a procedure which has been carried out for several areas of the body, but not in the auditory/vestibular systems.

Autoradiography studies to follow the amount of glycoprotein material incorporated into the basement membrane could also be used. This procedure will encounter preparation difficulties since the temporal bones are embedded in celloidin, and autoradiography techniques will need to be altered to account for this.

It would be of value to assess a ketotic "AC" animal. Both ketotic individuals in the present study were "XA" hamsters, and did not demonstrate any greater otopathological involvement greater than the average "XA" animal. Other lines of the Chinese hamster colony could also be evaluated.

Since many of the "M" animals were among the oldest animals at the time of sacrifice, this seems to support the conclusion that the changes observed were not age related changes, but only changes due to duration of the diabetic condition. This particular age control

is worth noting it is commonly known that aging changes in the capillary basement membrane appear to be very similar to capillary basement membrane thickening resulting from diabetes.

Although no cochlear neuronal atrophy was exhibited in the present study, further studies examining this aspect, using various neurological stains and electron microscopic techniques may demonstrate some changes, which are beyond the scope of the present investigation methods.

APPENDIX A

Hematoxylin and Eosin Procedure

1.	Tap water-	rinse
2.	Tap water-	rinse
3.	Lugol's Iodine-	10 minutes
4.	2.5% Sodium Thiosulfate-	5 minutes
5.	Tap water-	rinse
6.	Tap water-	rinse
7.	Harris Hematoxylin	8 minutes
8.	Tap water-	rinse
9.	Tap water-	rinse
10.	70% Acid Alcohol	15 seconds
11.	Running Tap water-	30 seconds
12.	0.5% Ammonium Hydroxide-	2 minutes
13.	0.5% Ammonium Hydroxide-	2 minutes
14.	0.5% Ammonium Hydroxide-	2 minutes
15.	Tap water-	2 minutes
16.	Tap water-	2 minutes
17.	Eosin Y-	30 seconds
18.	80% Ethanol-	1 minute
19.	80% Ethanol-	1 minute
20.	80% Ethanol-	1 minute
21.	95% Ethanol-	30 seconds
22.	Chloroform & Absolute Ethanol 1:1-	1 minute
23.	Xylene & Terpeneol 1:1	may leave
24.	Coverslip with permount	
25.	Weight slides-	2 weeks

Solutions

1. Lugol's Iodine: distilled water - - - - - 400 ml
potassium iodide- - - - - 8 ml
iodine crystals - - - - - 4 ml
Mix potassium iodide and distilled water. Add iodine crystals with heat.

2. Harris's Hematoxylin:
hematoxylin crystals- - - - - 1 gm
alcohol, 95%- - - - - 5 cc
Ammonium or potassium alum- - - - 20 gm
distilled water - - - - - 200 cc
Mercuric oxide - - - - - 0.5 gm
Dissolve the hematoxylin in the alcohol, the alum in the water by the aid of heat. Mix the two solutions. Bring the mixture to a boil as rapidly as possible and then remove from the heat, and add the mercuric oxide. Reheat the solution until it becomes a dark purple, about 1 minute, and promptly remove the container from

the flame and plunge it into a basin of cold water.
The solution is ready to use when cool. Add 2-4cc of
glacial acetic acid to 100cc of solution if desired.

3. 70% Acid Alcohol:

70% alcohol-	100 cc
hydrochloric acid, concentrated-	1 cc

4. Eosin Y:

Eosin Y, water soluble -	0.5 gm
distilled water-	40 cc
alcohol, 95% -	160 cc

Dissolve Eosin Y in distilled water. Add 95% alcohol.
One drop of acetic acid/100cc of solution may be added
to deepen shade.

APPENDIX B

Periodic Acid Schiff's - Allochrome Procedure

- | | | |
|-----|-------------------------------------|-------------|
| 1. | Tap water- | rinse |
| 2. | Tap water- | rinse |
| 3. | Lugols Iodine- | 10 minutes |
| 4. | 2.5% Sodium Thiosulfate- | 5 minutes |
| 5. | Tap water- | rinse |
| 6. | Tap water- | rinse |
| 7. | Periodic acid- | 10 minutes |
| 8. | Running tap water- | 5 minutes |
| 9. | Schiff's reagent- | 7 minutes |
| 10. | 0.5% Sodium Metabisulfite- | 30 seconds |
| 11. | 0.5% Sodium Metabisulfite- | 2 minutes |
| 12. | 0.5% Sodium Metabisulfite- | 2 minutes |
| 13. | 0.5% Sodium Metabisulfite- | 2 minutes |
| 14. | Running tap water- | 5 minutes |
| 15. | Weigerts Iron Hematoxylin- | 3 minutes |
| 16. | Running tap water- | 4 minutes |
| 17. | Picric Acid- | 1.5 minutes |
| 18. | 95% Ethanol- | 1 dip |
| 19. | 95% Ethanol- | 1 dip |
| 20. | 100% Ethanol- | 1 dip |
| 21. | 100% Ethanol- | 1 dip |
| 22. | Choloroform & Absoulte Ethanol 1:1- | 1 dip |
| 23. | Xylene & Terpeneol- | may leave |
| 24. | Coverslip with permount | |
| 25. | Weight slides- | 2 weeks |

Solutions

1. Periodic Acid Solution 0.5%

Periodic Acid-	0.5 gm
Distilled water-	100 cc

2. Schiff's Solution

Weigh 1 gm of basic fuchin and 1.9 gm of sodium metabisulfite. Dissolve these in 100ml of 0.15N hydrochloric acid. Shake the above at intervals until clear or yellow to tan color. If the solution is not the proper color, allow it to stand overnight in a dark place. Add 500mg of fresh activated charcoal and shake well for 1 to 2 minutes. Using a double layer of No. 1 filter paper, filter solution into a bottle. The filtrate should be clear. Wash the residue with distilled water to restore the original 100ml volume. Store in refrigerator.

3. Weigert's Iron Hematoxylin:

Solution A:

Hematoxylin- - - - - 1 gm
Alcohol, 95% ethyl - - - - - 100 ml

Solution B:

Ferric Chloride, 29% aqueous soln. 4 ml
Distilled water- - - - - 95 ml
Hydrochloric acid, concentrated- - 1 ml

Stock solutions of Solution A & B may be prepared and stored separately at room temperature. Just before use combine equal parts of Solution A and Solution B. Ferric salts oxidize hematoxylin immediately and the mixture will turn a deep black. Mixed solution is good for approximately 30 minutes.

APPENDIX C

Sample, Qualitative Assessment of Serial Sections (H&E)

J. Oostveen

Animal #9

7-9-82

Slide #36 Approximate mid-modiolar section. Right and left are not in the same plane. Mid-modiolar in the right only. The bone is well preserved and a nice section with no air bubbles. Looking at the cochlea and nuclei of all cells in the organ of Corti all stand out well. The organ of Corti itself had hair and support cells normal in number. The spiral ligament and stria vascularis also look grossly normal. Reissner's membrane does not appear to be markedly thickened. The spiral ganglia also stand out well with no decrease in cell numbers. At the helicotrema level there does appear to be a circle of cells present, which is probably Reissner's membrane. The bone connecting the middle turn and the helicotrema level is bent and distorted. The organ of Corti at the apical end is also distorted as well as the bone. Scarpa's ganglia is well seen with no gaps. Fiber tracts are present and appear to be grossly normal. There is very good preservation of the bone including all cell types throughout the cochlea. The cerebellum and pons area does contain some gaps between many of the cells due to loss of cerebral spinal fluid during preparation. The glial cells themselves look grossly normal. Basilar membrane is normal except for the distorted configuration of the entire cochlea, including all membranes and bone. The stapes is present with footplate, one crura and head, and does show a significant amount of otitis media surrounding the entire cavity with lymphocytes, plasma

cells and polymorphs. There are fibrous attachments to the stapedia artery which is somewhat thickened. There are some larger white blood cells within the artery itself and attached to the wall of the artery as well as hemolyzed red blood cells with invading macrophages. The footplate of the stapes is thickened in one area and definitely eroded in the other. The annular ligament on the more superior side does appear to be normal, but on the inferior side is not normal and appears to be continuous with the bone. This could be due to orientation of the section. The ligament appears to be replaced by bone. The fibrosis is attached to the footplate as well as head. Both macule of the utricle and saccule are present with hair cells normal in number. Otoliths are present. The beginning of the crista of the lateral canal is present. The middle ear cavity does not appear to have a significant amount of otitis media, but at the more inferior end there is amorphous hemolyzed red blood cells, with some invading macrophages. Also is evidence of fibrosis covering the medial aspects of the cavity, more toward the anterior end. The tympanic membrane is complete and is somewhat thickened along the pars tensa. Basically the middle ear condition is clear, except for some cells along the inferior and medial sides.

Left - shows two turns of the cochlea. The organ of Corti appears to be normal in the most apical portion. The middle ear condition shows a massive amount of inflammatory condition throughout the entire cavity dissimilar to the contralateral side. Cell types include lymphocytes, some plasma cells and polymorphs as well as other round cells. The malleus is present with several eroded cavities

in the center of the ossicle which are filled with the same type of fibrosis. The massive erosion is present and there is no incudo-malleolar joint. The sides of the malleus in some places show fibrous attachment directly to the malleus itself with erosion in some areas. The tympanic membrane is complete and somewhat thickened with fibrous connective tissue attachment. Just anterior to the lateral process of the malleus is an area of bone, probably an ossicle sectioned in a different orientation. There is fibrous attachment to this area and is also closely attached to the tympanic membrane itself. There is no cholesterol needle formation on the left.

Slide # 1 Right side shows the beginning of the cochlea with two turns and the malleus. The malleus itself does have some otitis media and fibrous attachment surrounding the bone itself and its lateral process. No incudo-malleolar joint is present. In the middle of the malleus is a cavity filled with the same type of fibrous cells making up the middle ear cavity. It does not have the large amount of inflammatory reaction as the contralateral side, but does have more of the hemolyzed red blood cells with invading macrophages. Tympanic membrane still appears to be somewhat thickened in its course. The vestibular systems have not been sectioned yet.

The left side has no structures available for assessment.

Slide # 6 Right does show the beginning of the ampullated and non-ampullated end of the superior canal. The cochlea is continued with two turns. The middle ear condition is the same as previously described. The Y-shaped incudo-malleolar joint is easier to see, but

still has areas of erosion within it. Nothing left side.

Slide # 11 Right is a continuation of all previously described structures as well as the beginning of the crista of the superior canal. Hair and support cells appear normal. The most apical portion of the cochlea has one organ of Corti which is bulged out with some attachment in the endolymphatic space below it. This could be due to preparation. Nothing left side.

Slide # 16 Right - looking at the same organ of Corti as previously described still shows the Reissner's membrane with the attachment to it in the scala media. It is a better section through the superior crista. The hair cells are somewhat swollen, although normal in number. Looking at the middle ear cavity, shows the malleus. The area previously described as new bone formation still seems to be full of loose connective tissue cells surrounding the ossicles. There is still fibrous attachment to the tympanic membrane which is somewhat thickened. Good section showing the neural connections directly to the crista of the superior canal.

Left - shows the middle ear cavity is completely full with inflammation with all types of cells. Also there is attachment to the tympanic membrane and does appear to be markedly thickened in some areas.

Slide # 21 Right - looking at the apical turn of the cochlea shows Reissner's membrane. The structure just below the membrane is well seen and appears to be the tectorial membrane which has been broken

off and lying just medial to Reissner's membrane. The organ of Corti in the middle turn does show a good organ of Corti with all structures appearing to be normal, including Reissner's membrane. The only question of any problem is that the bone itself surrounding the different turns of the cochlea is distorted. There is continuation of the superior canal crista and the middle ear condition is much the same with the incus present.

Slide # 26 Right shows a nice section through the vestibule with the macule of the utricle and saccule present. Condition is the same as previously described.

Left shows the beginning of the organ of Corti within the cochlea.

Slide # 31 Right - Continuation of the macule of the utricle and saccule and the lateral canal crista is seen in tangential. Large section of the cochlea with all turns. All sensory epithelium are normal, except for the structures previously noted.

Left - is a continuation and enlargement of the cochlea. The middle ear condition is the same as previously described with a massive amount of otitis media throughout. No vestibular system shown.

Slide # 41 On the right the Reissner's membrane shows some question as to whether it is thickened in this animal over previous animals. The stapes is just beginning to come into view with the footplate, one crura, and head. The head shows the same condition as previously described.

Left does show a large malleus with a condition similar to the contralateral ear. There is a massive amount of cellular material with polymorphs, plasma cells, and some lymphocytes, completely surrounding the ossicles. Also the section shows the beginning of the most superior portion of the superior canal.

Slide # 46 Left looking at the malleus shows the incudo-malleolar joint. The demarcation is not very distinct and well defined due to the chronic inflammatory condition.

Slide # 51 On the left, the cochlea and the organ of Corti appears to be normal. The hair and support cells are normal in number. The basilar membrane and Reissner's membrane are also grossly normal. Reissner's is not as thickened as in the contralateral side. Stria vascularis appears to be normal under these H & E preparations. The distortion of the cochlear bone is not as evident as in the contralateral ear. Also the crista of the superior canal is first seen. Hair and support cells look good in terms of number, but the hair cells do appear to be more swollen than the contralateral ear. The neural connections are nicely shown as well as the ganglia supporting the superior crista. Middle ear and ossicle condition is unchanged. The tympanic membrane is somewhat thickened.

Slide # 56 Right is the end of the cochlear system, but it is a good section showing neural connections to the cochlea, through the habena perforata. Otherwise middle ear conditions remain the same. Best section on the left showing a complete organ of Corti and crista

of superior canal with neural connections and condition of the middle ear.

Slide # 61 Left - shows the macule of both the utricle and saccule with otoliths. Hair and support cells appear to be normal in terms of number and don't appear to be as edematous as in previous sections.

Slide # 71 Right shows what appears to be the end of the cochlea and the beginning of the round window membrane which does appear to be somewhat thickened and has a large amount of otitis media in the round window niche, but not directly attached to the membrane itself. The cochlear side appears to be mostly clear, except for possible hemolyzed red blood cells or protein coagulate. The crista of the posterior canal is cut in tangential, but there is some histological artifact which distorts the picture.

Left shows the crista of the lateral canal. Other conditions remain the same. Parts of the stapes are shown with the head, and the stapedial artery. The artery does contain hemolyzed red blood cells with a few macrophages. There is a significant amount of fibrous attachment to the footplate and surrounding the stapedial artery. In the middle ear cavity, just superior to the head is a very rounded swirl of otitis media, tightly packed into a spherical shape. It is different than the otitis media above the swirl of cells.

Slide # 76 Left shows the crista of the lateral canal cut in tangential as well as the macula of both the utricle and saccule. All sensory epithelia appear to be normal. The swirl of cells present in the middle ear cavity just anterior to what now appears to be a crura.

Slide # 81 Right shows a better section through the posterior canal crista and the round window membrane. There is otitis media in the round window niche with no fibrous attachments to the somewhat thickened membrane.

Left still shows the swirl of cells in the middle ear with much the same condition for the stapes, etc. There is some erosion of the footplate near the annular ligament. The tympanic membrane is significantly thickened.

Slide # 86 Right shows a full section through the crista of the posterior canal. Hair and support cells appear to be normal with none missing except that the hair cells seem to be somewhat more thickened than in previous cristae.

Slide # 91 Right - better section through the crista of the posterior canal. The stain is darker and it is easier to see the edema of the hair cells and the condition of the support cells.

Left side appears to be nothing changed except that the swirl of cells in the middle ear cavity is gone.

Slide # 106 Left shows the end of the cochlea and the beginning of the round window membrane which is somewhat thickened. There is some otitis media in the niche. Appears to be more in strands than seen on the contralateral side.

Slide # 111 Left - following the round window niche and crista of the posterior canal shows their condition. The otitis media is seen in cords of cells.

Slide # 116 Left - nice section through the crista of the posterior canal and its condition as well as the end of the round window membrane and niche.

Slide # 121 Left - entire rest of the middle ear completely filled with otitis media and the end of the posterior canal.'

APPENDIX D

Sample, Quantitative Otopathological Form (H&E)

Animal #9 ("XA" line)

CONDITION-MIDDLE EAR

SLIGHT MODERATE SEVERE

Round Cell Infiltration			X
Fibroblasts & Cholesterol crystals	X		
Tympanic Membrane Thickening			X
Fibrous Attachment to T. Membrane			X
Fibrosis Around Ossicles		X	
Polyp Formations in M.E.			X
Cholesterol granuloma			
New Bone Formation		X	
New Gland Formation	X		
Hemorrhage		X	
Stapedial Cavity Inflammatory Cells			X
Fibrous Attachment within S. Cavity			X
Thickened Footplate or Crura			X
Fibrous Attachment to S. Artery			X
Thickened Stapedial Artery			X
Fibroblasts in S. Cavity		X	
Fibrous Attachment to the R.W. Memb.	X		
Inflammatory Cells in the R.W.Niche		X	
Fibroblasts in the R.W. Niche	X		
Polyp Formations in the R.W. Niche	X		
Total	<u>5</u> (5)	<u>5</u> (10)	<u>9</u> (27)

CONDITION-COCHLEAR VESTIBULAR

Round Window Membrane Thickening		X	
Strial Atrophy			
Precipitate	X		
Hemorrhage	X		
Reissner's Membrane Thickened	X		
Sensory Epithelium Changes	X		
Total	<u>4</u> (4)	<u>1</u> (2)	<u>0</u>

Total	Middle Ear	42
	C & V	<u>6</u>
		48

APPENDIX E

Sample, Qualitative Assessment of Serial Sections (PAS)

J. Oostveen

Animal # 18 PAS

10-8-82

General overview: Excellent stain quality, including PAS, hematoxylin, and allochrome. The left side has massive destruction due to air bubbles in the vestibular and cochlear systems. Right has air bubbles, but not to the same degree as the left ear.

Slide # 51 Right - Cochlea does present several turns. The most superior left hand stria has several capillaries in cross section and longitudinal section. The capillary basement membrane seems to be somewhat thickened over the normal condition. Other stria also present a similar picture. The far right stria has approximately 7 cross sections of capillaries. The stapedial artery shows a definite thickening of the tunica intima. The fibrous material is continuous with the tunica adventicia.

Left - Although there is little left of the cochlea, one far left stria does have several cross sections. They present a similar picture as the contralateral side with a thickened tunica intima.

Slide # 6 Left - Crista of the superior canal with attendant blood supply. There are some examples of capillary blood supply to the cochlea, and they are somewhat more difficult to distinguish.

Slide # 11 Right - beginning of the crista of the superior canal.

Left - Better picture of the crista of the superior canal with several capillaries supplying it.

Slide # 16 Right - Better section through the crista of the superior canal with several capillaries. The stain is somewhat darker than ideal.

Slide # 21 Left - Macule of the utricle and the cristae of the lateral canal. Very good examples of slight thickening to the basement membrane material in the blood supply to both the vestibular and cochlear systems.

Slide # 26 Left - Crista of the lateral canal and the macule of the utricle and saccule with a better example of the capillary network. The pons and cerebellum also show several good examples for capillary basement membrane thickening in the central nervous system. The capillary basement membrane is greater than in the normal state. No occluded vessels are seen. (Photo example).

Slide # 41 Right - Extreme superior turn of the cochlea has one stria with an excellent example of capillary basement membrane thickening along a longitudinal course. The capillaries in other stria also show the thickening over the normal state.

Left - Presents an identical picture for the contralateral side.

Slide # 46 Right - Again superior turn of the cochlea contains a stria with a cross section showing significant thickening of the capillary basement membrane.

Left - Presents identical picture for the stria in the superior turn. There is little left to the interior of the cochlea

and vestibular system to assess.

Slide # 56 Right - The modiolas capillary system lends credence to the idea of possible thickened capillary basement membrane material of this animal. The stria vascularis in this particular slide points to a thickened capillary basement membrane.

Left - The most superior stria is a good section of a longitudinal cross section through a capillary with a thickness to the capillary basement material. (Photo example).

Slide # 71 Right - Superior aspects of the cochlea show one particular capillary which can be followed from one side of the field of view to the other and does show significant thickening throughout its course. It is not completely occluded, but is thickened over the normal condition.

Slide # 76 Left - Crista of the posterior canal. Good picture through several capillaries which can be followed throughout their course within the interior of the crista.

Slide # 81 Right - Crista of the posterior canal with much the same picture as described in the contralateral cristae. Better for possible photo of capillaries to the posterior crista.

Slide # 91 Top of both the cochlea, have many good examples of capillary basement membrane thickening.

APPENDIX F

Sample, Quantitative Capillary Basement Membrane Form

Animal #13 ("M" line)				
	<u>Normal</u>	<u>Moderate</u>	<u>Severe</u>	<u>Slide #</u>
<u>Stria vascularis</u> 6 per side	4L	2L		36L
mid-modiolar 12 total	5R	1R		21R
<u>Modiolas</u> 1 per side	1L			36L
mid-modiolar 2 total				
<u>Superior Cristae</u> 1 per bone	1			16L
1 total				
<u>Lateral Cristae</u> 1 per bone	1			36L
1 total				
<u>Macule of the Utricle</u>	1S			36L
<u>or Sacculle</u> 1 per bone				
1 total				
<u>Posterior Cristae</u> 1 per bone	1			61L
1 total				
<u>Pons - Cerebellum</u> 2 per bone	1P	1C		21
2 total				
Total	15 (0)	4 (1)	0 (2)	

Total assessment 4

Total Capillaries assessed 19/20

L = left side
R = right side
S = sacculle
U = utricle
P = pons
C = cerebellum

GLOSSARY OF OTOPATHOLOGICAL TERMS

Annular ligament: a circular ligament which attaches the footplate of the stapes to the inner surface of the round window

Basal Turn of the Cochlea: the largest turn of the cochlea

Basilar Membrane: forms the boundary between the scala media and the roof of the scala tympani, and also supports the organ of Corti

Bony labyrinth: houses all of the inner ear structures

Canalicular System: three osseous and membranous semicircular canals (superior, lateral, and posterior). They are oriented at right angles to each other and respond to angular acceleration and deceleration.

Crista ampullaris: an elevation projecting into the lumen of the ampulla of a semicircular canal, containing the sensory end organs. It is stimulated by movements of the endolymph.

Cochlea: A cavity of the inner ear resembling a snail shell; it contains the end organ of hearing (acoustic analyzer)

Endolymph: the fluid that fills the membranous labyrinth of the ear low in sodium and high in potassium as compared to perilymph

Helicotrema: opening at apex of cochlea which allows for communication between the scala vestibuli and the scala tympani

Incudomalleal articulation: diarthrodial joint binding the incus and malleus at their articular surfaces

Incus: anvil shaped ossicle of the middle ear

Labyrinth: the internal ear, consisting of the osseous labyrinth (semi-circular canals, vestibule, cochlea) within which lies the membranous labyrinth (semicircular ducts, utricle, saccule, and cochlear duct)

Macule: termination of the auditory nerve in the spoon shaped sensory epithelium containing hair cells which are stimulated by the movement of otoliths in the overlying otolithic membrane (sensory end organ of the utricle and saccule)

Malleus: the hammer shaped ossicle of the middle ear

Membranous labyrinth: follows the contours of the bony labyrinth and includes the semicircular ducts, utricle and saccule and cochlear duct

Mid-modiolar: the midsection of the cochlea giving mirror halves

Modiolus: the central pillar or axis of the cochlea, around which the spiral canal makes two and one half turns

Organ of Corti: the end organ of hearing or the sensory part of the cochlear duct

Otitis media: inflammation of the middle ear

Otolith: one of the calcareous concretions within the membranous labyrinth of the ear

Otolithic Membrane: a gelatinous substance on the surface of the macula into which the hair cells project. Contains the otoliths which when moved stimulate the hair cells

Otology: the science of the ear, its anatomy, function, and diseases

Otologist: one versed in otology

Otopathology: study of the diseases of the ear

Oval window: leading to the vestibule of the inner ear, and is located between the recess of the facial nerve canal and the cochlear promontory

Perilymph: fluid in the exterior of the membranous labyrinth with chemical properties similar to cerebral-spinal fluid, high in sodium and low in potassium, and separates the membranous from the osseous labyrinth of the ear

Reissner's membrane: separates the scala media from the scala vestibuli

Round Window Membrane: membrane leading to the scala tympani of the cochlea, lies in a plane which is posterior and inferior to the cochlear promontory

Round Window Niche: cup shaped depression in the medial wall of the middle ear which forms a canal that ends at the round window membrane

Saccul: the smaller of the two vestibular sacs of the membranous labyrinth of the ear and also houses a macule or sensory organ

Scala: a subdivision of the cavity of the cochlea, especially one of the perilymphatic spaces

Scala media: another term for the cochlear duct which contains the endolymph

Scala tympani: the perilymphatic space below the osseous spiral lamina and the basilar membrane

Scala vestibuli: the perilymphatic space above the osseus spiral lamina and the Reissner's membrane (vestibular membrane)

Scarpa's ganglia: ganglion of the vestibular nerve

Spiral ligament: thickened outer attachment of the basilar membrane which is covered with the secreting epithelium of the stria vascularis

Stapes: the stirrup shaped bone of the middle ear articulating with the incus and the fenestra ovalis (oval window). It is composed of the capitulum (head), crura (legs) and the footplate.

Stria vascularis: vascular epithelium lying on the internal surface of the spiral ligament

Tectorial membrane: a gelatinous structure extending from the vestibular lip of the limbus over the organ of Corti and which has contact with the hair cells of the organ of Corti

Temporal bone: bone forming a part of the side and base of the cranium housing the auditory and vestibular systems

Tinnitus: a ringing noise in the ear

Tympanic membrane: ear drum which closes the external auditory meatus

Utricle: a delicate membranous sac communicating with the semicircular canals; a part of the vestibular system

Vertigo: a profound form of dizziness characterized by a perceived circular motion of the environment

Vestibule: the oval cavity of the inner ear which forms the entrance to the cochlea

The Glossary was compiled from the following:

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