

Histological Changes in the Inner Ear of Sheep following a Round Window Ultrasonic Irradiation

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Introduction

Ultrasonic irradiation of the inner ear applied through the round window is used as treatment of Meniere's Disease, Kossoff et al (1967, 1968), Basek (1970). The aim of this project was to study histological changes that occur in the inner ear of sheep following an ultrasonic irradiation. The ultrasonic generator and round window applicator used were those designed by the Commonwealth Acoustic Laboratory and which are currently commercially available.

The 1.5 mm transducer operates at 3.5 MHz and has an output of up to 100 milliwatts. In this study the inner ears of three sheep were irradiated at intensities ranging from 40-100 milliwatts for a duration of 20 minutes. This is a typical dose used for human treatment.

Method

Five sheep were used in this experiment. Histological sections of the inner ears of two normal, non-irradiated sheep were prepared as standard anatomical controls. In two other sheep the inner ear of one side was irradiated while the opposite ear served as control. Both inner ears of another sheep were irradiated with the same intensity to study uniformity of the effect of ultrasound. Following the irradiation the animals were kept alive for five weeks when they were then sacrificed.

The temporal bones were quickly excised and fixed in 300 mls of Heidenhain's Susa solution at 5°C for 48 hours. Decalcification was with 5% trichloroacetic acid and the temporal bones were finally embedded in celloidin. Sections were cut at a thickness of 20 microns and were stained with haematoxylin and eosin. Orientation of the sectioning was in the vertical plane in all cases except the sheep irradiated

with 100 milliwatts where the sections were cut in the horizontal plane. Every fifth section was examined under the light microscope.

Results

DOSAGE: 40 Milliwatts, 20 Minutes

In the two ears irradiated at this dosage all the cristae of the semicircular canals exhibited changes in the neuroepithelium and cupula (Figure 1 top). The sensory hair cells had atrophied and in some sections could not be seen due to the cupula being somewhat shrunken and collapsed on to the upper surface of the neuroepithelium (Figure 1 lower). Vacuolation within the cells of the neuroepithelium was evident. The sensoneural elements were atrophied and displaced in the upper region of the neuroepithelium. The sensory hairs were atrophied appearing very short and stubby. In all the cristae this type of change was apparent involving vacuolation or slight lifting of the neuroepithelium from its basement membrane.

The maculae showed changes principally in the otolithic membrane. The utricular maculae showed minor changes to the sensory hair cells, although the endothelium of the utricle opposite the oval window was collapsed on to the macula (Figure 2) and the otolithic membrane was damaged. In the saccular macula the hair cells were atrophied but the otolithic membrane was intact. The sections here were cut slightly oblique making positive distinction at a cellular level rather difficult. Small changes occurred in the utricular macula in the form of stunted sensory hairs and vacuolation of the cells of the neuroepithelium (Figure 3 top). The saccular macula however exhibited slightly more widespread effects involving breaking of the neuroepithelium from its basement membrane, squashing and bending of the sensory hairs, a collapsed endothelium and a lesion extending through the otolithic membrane, neuroepithelium and the

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underlying matrix (Figure 3 lower). This lesion was located directly opposite the round window and received the full power of the ultrasonic beam entering the saccule.

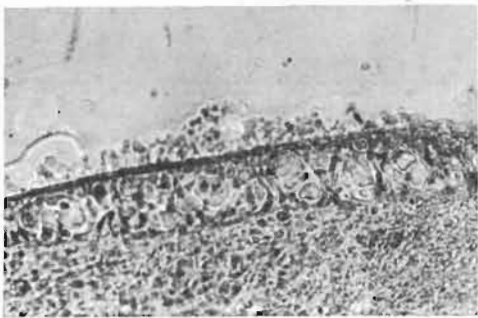


FIGURE 1

(Top): Posterior crista with atrophied cupula and sensory hairs. (Magnification x40).

(Lower): Posterior crista showing atrophied cupula and cellular disorganisation in the neuroepithelium. (Magnification x200.)

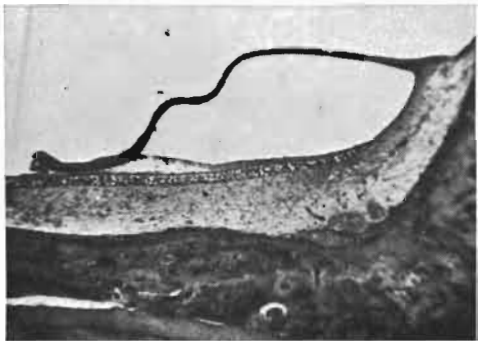


FIGURE 2

Macula of utricle with collapsed endothelial lining and damaged otolithic membrane. (Magnification x40.)

DOSAGE: 80 Milliwatts, 20 Minutes

In the left ear irradiated at this dosage there was extensive damage to the endothelial lining of the utricle. Associated with this there was disorganisation of the cells of the neuroepithelium, and the hair cells were atrophied

with the sensory hairs being very short and sparse. The cristae exhibited vacuolation, loss of sensory hair cells, and apparent enucleation of the cells of the neuroepithelium. Where present, the cupula appeared as a shrunken structureless mass. There was thickening of the membranous lining of the semicircular canals and obliteration of the perilymphatic space by increased fibroblastic activity (Figure 4).

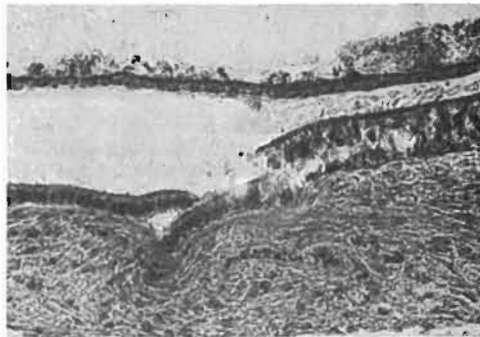
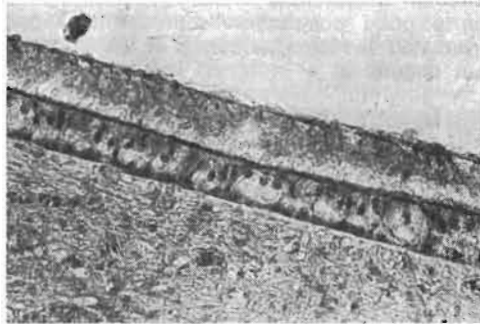


FIGURE 3

(Top): Macula of utricle with large vacuoles within the neuroepithelium. The otolithic membrane squashed the sensory hairs. (Magnification x200.)

(Lower): Macula of saccule showing a lesion in the neuroepithelium. Note the complete break in the epithelium and thickening of the underlying tissue. (Magnification x200.)

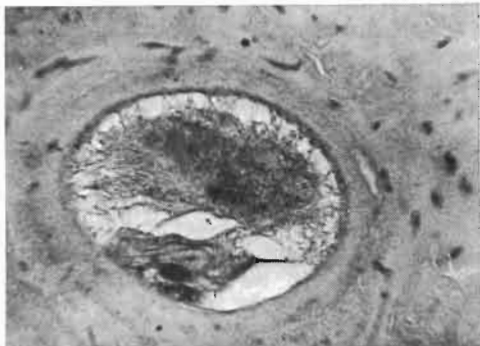


FIGURE 4

Superior semicircular canal with collapsed membranous labyrinth. Fibroblastic tissue obliterates the perilymphatic space. (Magnification x40).

The right ear served as a control. With the exception of artefacts in three sections involving the lateral semicircular canal ampulla, all three cristae appeared normal and undamaged in the neuroepithelium and secretory epithelium, (Figure 5). The cupula showed a tendency to become dislodged in a few sections, which can be attributed to forces inflicted in the preparation of the temporal bones prior to fixation and embedding. The semicircular canals were undamaged and served as a good comparison with the damaged irradiated semicircular canals of the opposite ear (Figure 6).

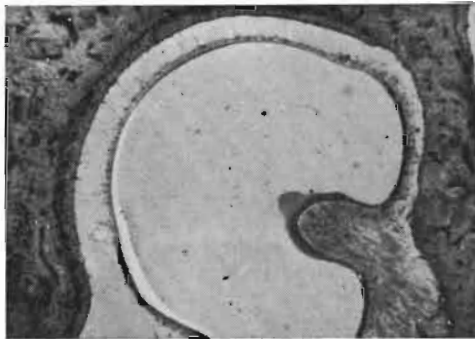


FIGURE 5
Ampulla of superior semicircular canal with a normal crista. (Magnification x20.)

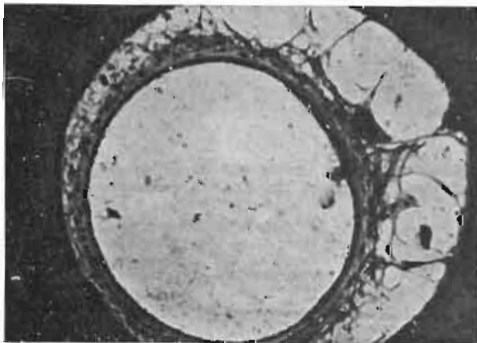


FIGURE 6
Superior semicircular canal with normal membranous labyrinth and perilymphatic trabeculae. (Magnification x100.)

The saccule was partially filled with fibrinous material resulting in some damage and obscuring of its macula, while the macula of the utricle was undamaged (Figure 7). Apparently the round window membrane was perforated during the operation, allowing fibrin, or blood from the wound to enter.

DOSAGE: 100 Milliwatts, 20 Minutes

At this dosage many of the vestibular sensorial structures were absent from the sections or were unable to be identified due to

the great amount of damage associated with the collapsed endothelial lining of the vestibule. The crista of the posterior semicircular canal appeared with complete disorganisation of its apical neuroepithelium and hair cells, also the endothelium from the opposite wall of the utricle has collapsed onto it obscuring the damaged cupula.

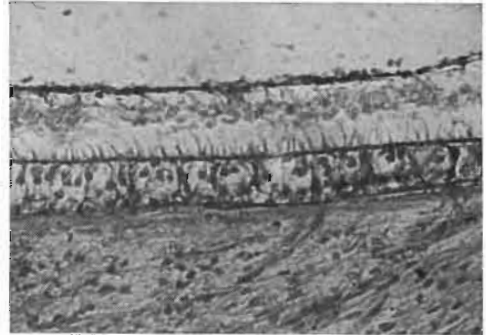


FIGURE 7
Macula of utricle showing sensory hair cells with the otolithic membrane suspended above the hairs. (Magnification x200.)

All three semicircular canals showed damage to the endothelial lining and perilymphatic trabeculae (Figure 8). The membranous labyrinth was collapsed throughout the length of all the semicircular canals and in the ampullae.

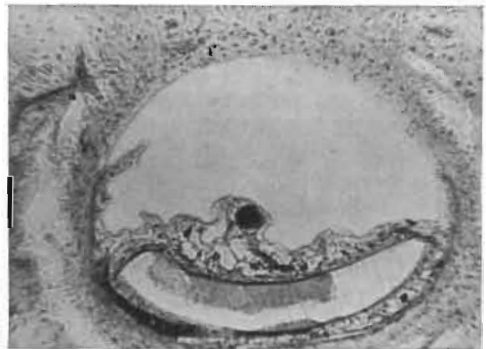


FIGURE 8
Superior semicircular canal. (Magnification x70.)

By comparison the control inner ear was completely free from damage with the exception of a very few artefacts formed due to shrinkage of the celloidin embedding material.

Discussion

The results indicate damage to the vestibular structures at all intensities of ultrasound used. The period of irradiation was constant while the intensity was different for each sheep. Where both the left and right inner

ears were irradiated with 40 milliwatts the vestibule in both cases exhibited discrete damage to the sensoneural elements. Cellular vacuolation existed within the neuroepithelium of the cristae and maculae. Sensory hair cells and their cilia were atrophied with the cilia appearing shortened and stubby. Severe damage to the crista has also been indicated by Basek (1970) after irradiating the inner ear of cats through the round window with 40 milliwatts.

Only type I sensory cells could be clearly distinguished, indicating degeneration of mainly type II sensory cells. Shrinkage of the cupula was common, with it often appearing closely withdrawn to the upper surface of the neuroepithelium. Cellular disorganisation of the secretory epithelium (Planum semilunatum) was common. This region has been cited in the production of acid-mucopolysaccharides and its destruction would have a marked effect on endolymph production. Degeneration of the planum semilunatum was also recorded by Lundquist et al (1971) when they irradiated the lateral semicircular canal of guinea pigs. They also noted vacuolation and shrinkage of the ampullar cristae similar to that described above.

A lesion in the macula of the saccule directly opposite the position of the round window consisted of damaged otolithic membrane, neuroepithelium and basement membrane, together with the formation of thickened layers of tissue within the matrix below the basement membrane. Being directly in the "line-of-fire" of the ultrasonic beam the tissue in this region was most extensively damaged. The utricular macula showed only signs of vacuolation by comparison, as the reflected ultrasonic energy would be less damaging. The endothelial lining of both saccule and utricle was collapsed, although only in the saccule was there a complete break in the membrane. Formby (1961), Sjöberg (1963), Dalton (1964), and Seda et al (1969) have described degeneration and disorganisation of the neuroepithelium of the utricular and saccular maculae.

At a dosage of 80 milliwatts sections of the irradiated temporal bone showed damage to similar vestibular structures but to greater extent than the inner ears irradiated with 40 milliwatts. Also the utricle and saccule exhibited thickening of the endothelial lining together with partial obliteration of the perilymphatic space, which may be attributed to ultrasonic irritation inducing increased fibroblastic activity. In a study of the effects of ultrasound on the domestic pigeon Stahle (1964) found some obliteration of the perilymphatic spaces due to fibroblastic tissue. He also noted an increase in local bone formation

in the bony labyrinth but this did not occur in the present investigation. Increased fibroblastic activity was also later recorded by Seda et al (1971) after irradiating the lateral wall of the otic capsule in cats with 0.7 watts for 20 minutes. In contrast, they observed no pathophysiological changes at 0.35 watts for 20 minutes, but they were irradiating through a bony wall and much of the ultrasound would have been attenuated. All the previous workers except Basek (1970) have irradiated the labyrinth through bone either at the semicircular canal or directly through the wall of the otic capsule. Therefore it is difficult to draw any meaningful correlation between their results and those achieved in this investigation where the ultrasonic energy was applied through the thin round window membrane.

An intensity of 100 milliwatts for 20 minutes resulted in complete disruption of all structures including total collapse of the membranous labyrinth and consequent obliteration of all the sensoneural elements. The lumen of the semicircular canals was completely obliterated by collapsed endothelium and increased fibroblastic activity, indicating the extent to which the ultrasonic energy is reflected through the vestibular labyrinth.

In human subjects with Meniere's Disease treatment with 100 milliwatts for 20 minutes does not appear to affect hearing. Therefore one would not expect cochlear damage to occur in the sheep whose inner ears are somewhat similar in size and spatial arrangement. However, the post-operative hearing tests given to Meniere's disease patients after ultrasound therapy tend to examine cochlear response to only the low to medium frequency range. In fact the basal turn of the cochlea which is concerned with the high frequency response is the area most likely to be damaged by direct ultrasonic energy. The low frequency responsive area in the apical turn of the cochlea is the least likely to be damaged by reflected energy.

Examination of the cochlea indicated damage was generally widespread and because of artefacts it was sometimes difficult to distinguish damage due to ultrasound from that due to mechanical forces exerted on the tissue during the operation, excision, sectioning and histological processing of the temporal bones. During tissue fixation and the histological preparation a certain amount of tissue shrinkage is almost inevitable resulting in damage to the finer structures. Incomplete decalcification is a common problem when sectioning specimens of inner ears of sheep where such a large amount of bone is encountered. The choice of a 50% solution of nitrocellulose in alcohol: ether as a final embedding material has resulted in artefacts due to the formation

of air bubbles within the tissue resulting from incomplete infiltration of this substance through the tissue of the specimen. For this reason it was considered that there were inadequate controls to allow an objective assessment of the histological effect of ultrasound on the cochlea. However Crysdale & Stahle (1972) irradiating the cochleas of guinea pigs have determined that in the Organ of Corti the outer hair cells are more sensitive to destruction by ultrasound than the inner hair cells.

Summary

The most common types of damage attributed to the effects of ultrasound which occurred in all the irradiated ears in chronological order of appearance were:— vacuolation of the neuroepithelium, atrophy of sensory hair cells, degeneration of the neuroepithelium in the crista and macula, collapse of the cupula, loss of cell nuclei, thickening of the membranous labyrinth wall, obliteration of the perilymphatic space by fibroblastic tissue, loosening of the neuroepithelium from its basement membrane, and collapse of endo-
thelium.

There is evidence of an irritant effect of ultrasound where an increased level of fibroblastic activity occurred in the perilymphatic space after irradiation. The results also indicate that a significant amount of ultrasonic energy is reflected throughout the entire vestibular labyrinth.

It would appear that a 40 milliwatts dosage is above the level required to initiate changes in the vestibular sensory structures in sheep. There appears to be a relation of vestibular damage to intensity, in such a way that an irradiation for 20 minutes at 40 milliwatts caused discrete and localised damage, an 80 milliwatt dose caused damage to the same structures but to a greater extent, while 100 milliwatts results in extreme widespread damage.

Acknowledgement

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