STRUCTURAL COCHLEAR CHANGES AFTER NOISE EXPOSURE

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INTRODUCTION

Excessive noise stimulation is known to cause either temporary or permanent hearing losses by damaging the sensory hair cells and associated structures in the cochlea of the inner ear. The nature of this damage, its extent and location, are determined by the intensity, frequency and duration parameters of the noise exposure. During the initial, dynamic phase of the damaging process, changes occur at the cellular level; they may be temporary and recover, or they may later become permanent. Both degenerative and repair processes need time for completion and this will vary from a few hours to weeks or even months. When these processes have finished, an apparently static phase is reached. But in reality even during this phase, small changes may still be proceeding. Thus a study of permanent hair cell loss in relation to noise dose can be a valuable measure in trying to prevent noise induced hearing loss in humans.

Basic experimental studies are important also in elucidating the development and progression of noise damage. Although animal studies cannot be directly applied to humans, nevertheless basic principles must apply. All mammalian cochleae possess the same structural arrangements and can be presumed to function in the same way. Various parameters may differ, for example the length of the basilar membrane, how tightly the cochlea is coiled and the different frequency responses. Man and higher primates have a poorer high frequency range than most other mammals. Our experimental animal, the guinea pig, has a hearing range from 250 Hz to 45 kHz. It also appears to be more susceptible to noise damage than man.

Numerous studies have been performed by exposing guinea pigs to industrial and impulse noise, for example Hamernik et al [3], Nilsson et al [4], Pye et al [6] and Spoendlin and Brun [11]. But the damage obtained is variable and widespread and the results are more difficult to interpret. Other experimenters, Spoendlin [10] and Robertson and Johnstone [8] have used continuous pure tones of different frequencies, intensities and durations. However, variability is still present, even after strictly controlled exposures [2]. High frequencies, to which the guinea pig is still very sensitive, have been less well researched [8,5]. The post-exposure intervals have also varied considerably. Thorne et al [12] looked at damage immediately after exposures of 2-30 min in duration and found stereocilia abnormalities already present after the shortest time. Slepecky [9] gives a comprehensive overview of mechanical damage to the cochlea.

Thus the present study aims to follow and quantify the progression of surface changes to sensory hair cells after a short, intense pure tone exposure. The changes seen immediately after an exposure are thought to be the first, primary structural alterations [12]. A preliminary report of the early changes has been submitted for publication [7].

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MATERIAL AND METHODS

Groups of 6-7 young, pigmented guinea pigs were used. Only the left ears of unanaesthetised animals were exposed to 15 kHz for 7.5 min at an intensity of 133 dB SPL measured 1 cm from the pinna. Spoendlin [10] states that only above 130 dB SPL is one likely to obtain mainly primary structural alterations, below this intensity metabolic changes will predominate. Very high intensities (above 140-145 dB SPL), however, cause excessive displacements of cells and tissues and this was not the aim here.
The present exposure duration was found from preliminary experiments to be the minimum to cause damage to most ears. Thus these sound parameters were chosen to give a discrete area of damage at a specific location.
The contralateral, plugged ears were used as experimental controls, but non-exposed controls were also included in each group. The animals were killed after the following post-exposure intervals: immediately (0 min), 10 and 30 min, 4 h, 3 weeks and 6 months.

Scanning electron microscopy (SEM) was used to assess the structural modifications to the surface of the reticular lamina of the spiral organ. The study concentrated on the condition and changes to the stereocilia, the cuticular plates of the sensory cells and supporting cells. Thus surface damage was quantified as follows: the pattern, extent, location, type and severity. The cochleae were first fixed in 2.5% cacodylate buffered glutaraldehyde, then in 1% buffered osmium tetroxide, followed by the TOTO method (thiocarbohydrazide and osmium tetroxide) and finally critical-point dried. The dissected basal turn was left *in situ* and damage assessment of all hair cell rows was carried out field by field, each consisting of 17 first row outer hair cells (OHC1). However, it was impossible to view the whole of the round window region and thus the assessment started at 2 mm from the very base.

RESULTS

a) Usually one exposed cochlea in each group was found to be undamaged, amounting to 17% of the total. No damage was also observed in any of the contralateral or the control ears, the normal pattern being shown in fig. 1.
b) Stereociliary disturbances were already present immediately after the exposure (in the 0 min group), as can be seen in fig.2. If these changes are irreversible and the hair cells start to degenerate, then the phalanges of the supporting cells fill the spaces, thus ensuring that the integrity of the reticular lamina is kept as intact as possible. The replacements are known as phalangeal scars.

Pattern

The first row of outer hair cells (OHC1) was always the most susceptible row, followed usually by inner hair cells (IHC). Damage was restricted to these rows at the periphery of an area, while in the middle of the damaged zone, all four rows were affected. But at times OHC2 would show changes before the IHC row. The tallest stereocilia on any row would usually show the first signs of disturbances. At the 6-month stage, many IHC were still in the process of degeneration, while correspondingly fewer OHC showed this.

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Figure 1. The normal pattern, showing three rows of outer hair cells (1,2,3) and a single inner hair cell row (I). The rows of stereocilia on cuticular plates are clearly seen, forming an M shape on outer hair cells. The phalanges of supporting cells are covered with microvillae, illustrated in the first row.

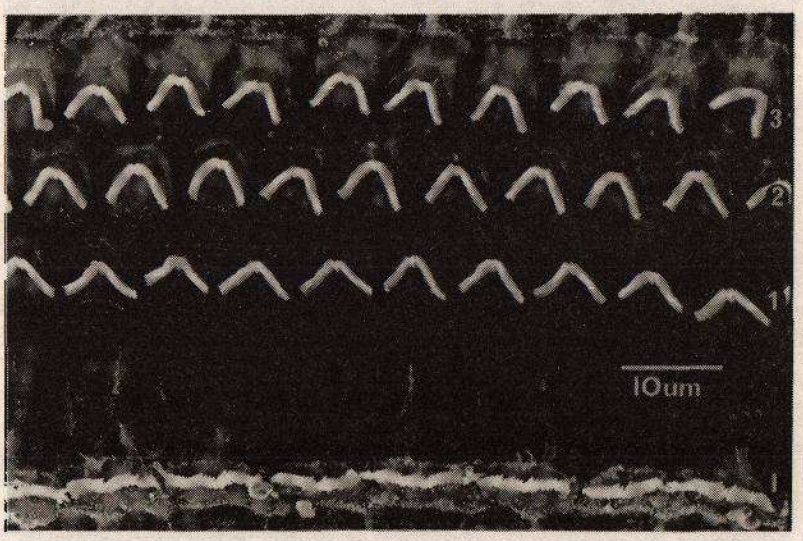
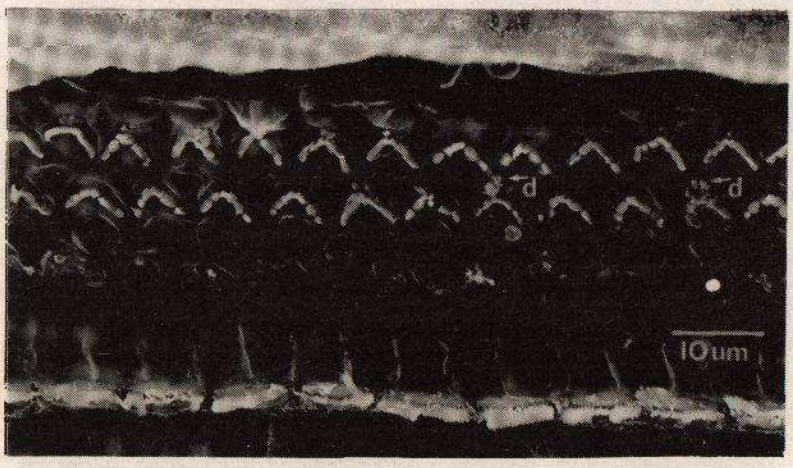


Figure 2. Types of stereociliary disturbances seen immediately after the exposure. Note that the first row of OHC is most affected. Debris (d) of cellular contents can be seen on a number of outer hair cells.



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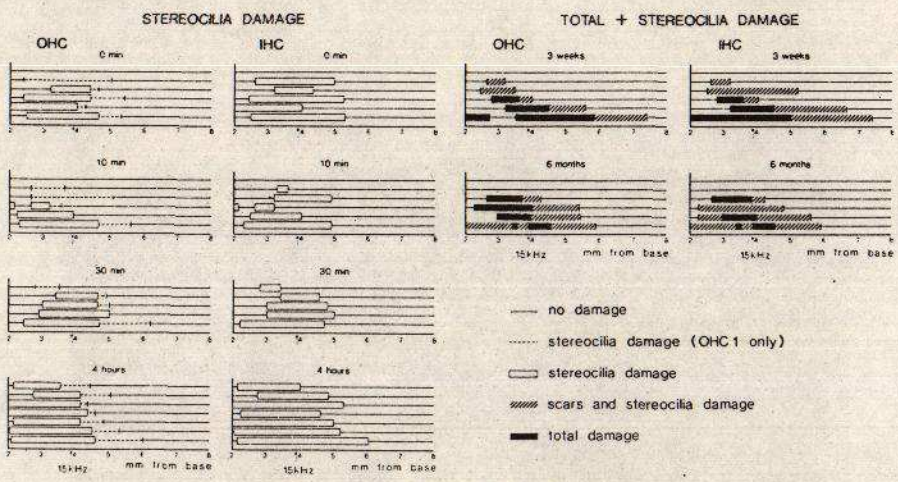
Extent

Table 1 illustrates the extents of the early stereociliary disturbances in the 0 min - 4 h groups on the left and total damage (phalangeal scars) and scars + stereociliary damage in the 3 weeks and 6 months groups on the right. When the extents of damage in all the experimental groups were compared, no statistically significant differences were found. However, individual variations were found to be great between animals in any one group. For the early changes, the areas of damage ranged from 0.6 - 4 mm. Two ears in the 3-week group were especially sensitive, with damage up to 5.4 mm in extent. Overall, in four cases, the stereociliary disturbances to OHC were confined to only the first row, with concomitant IHC damage. In many cases, OHC1 damage extended beyond the area which involved all OHC rows.

Location

Table 1 also shows that the main damage was found between 2 mm and 5 - 6 mm from the base and did not extend apically beyond one half of the basal turn. The maximum point of stimulation for the exposure frequency of 15 kHz is marked by an arrow and damage was usually spread on either side of this point, at times more basally. Individual variations were great in some groups eg. OHC for the 3-week group, but much less for others eg. IHC for the 4-h group.

Table 1 shows the extents and the locations of damage for all the experimental groups. OHC = combined outer hair cell rows, IHC = inner hair cells.



15kHz FOR 7.5 MIN AT 133dB SPL

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Type

Table 2 summarises the main damage categories for stereocilia, which have been minimised for clarity. Thus membrane changes are mainly responsible for causing "fusion", changes in actin filaments cause "floppy" stereocilia and "collapsed" stereocilia are mainly due to rootlet changes. Combinations of these categories could be seen immediately after this short sound exposure (fig. 2). They were also present at the 3-week stage (fig. 3) and to a lesser extent at 6 months, where at times only small stumps could be seen, especially on OHC1 (fig. 4). Total loss of stereocilia and formation of elongated forms were not immediate processes, with the latter being most abundant at 6 months on IHC (fig. 4).

Some damage to cuticular plates could be seen immediately after exposure (Table 2), their loss becoming evident at 4 h. Also immediately, cellular contents was seen oozing out of some hair cells (see fig. 2). This became more severe after 10 min and the process appeared to have finished by 6 months. The first signs of hair cell loss appeared at 30 min, but clearer indications were present at 4 h. Here debris from the degenerating cuticular plate and cells could be seen (fig. 5). In a number of specimens some holes were seen in the spaces earlier occupied by hair cells, but this was seen only at the 4-h stage (fig. 6). It was also at this stage that the first signs of repair processes to the damaged areas became evident (fig. 5). Scars or replacements of the hair cells were not seen until the 3-week stage (fig 3), where they existed adjacent to cells with degenerating stereocilia.

Table 2. Surface damage after exposure to 15 kHz for 7.5 min at 133 dB SPL-SEM

Post-exposure groups:	0 min		10 min		30 min		4 h		3 weeks		6 months	
Stereocilia:	IHC	OHC	IHC	OHC	IHC	OHC	IHC	OHC	IHC	OHC	IHC	OHC
total loss	-	-	+	-	+	+	+	+	+	+	-	+
fusion	+	+	+	+	+	+	+	+	+	+	+	+
floppy	+	+	+	+	+	+	+	+	+	+	+	+
collapsed	+	+	+	+	+	+	+	+	+	+	+	+
giant/elongated	-	-	-	-	+	+	+	+	+	+	+	+
Cut.plate: damage	+	-/+	+	+	+	+	+	+	+	+	+	+
: loss	-	-	-	-	-/+	-/+	+	+	+	+	-	-
Cellular contents	-/+	-/+	+	+	+	+	+	+	+	-	-	-
Hair cell: loss	-	-	-	-	-/+	-/+	+	+	+	+	-	-
: repair	-	-	-	-	-	-	-/+	-/+	+	+	-/+	-/+
scars/replacement	-	-	-	-	-	-	-	-	+	+	+	+

SEM= Scanning electron microscopy, IHC= Inner hair cells, OHC= Outer hair cells
+ = damage present, - = damage absent, cut. = cuticular

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Figure 3. 3 weeks post-exposure, showing phalangeal scars (ps) adjacent to cells where stereocilia are still degenerating.

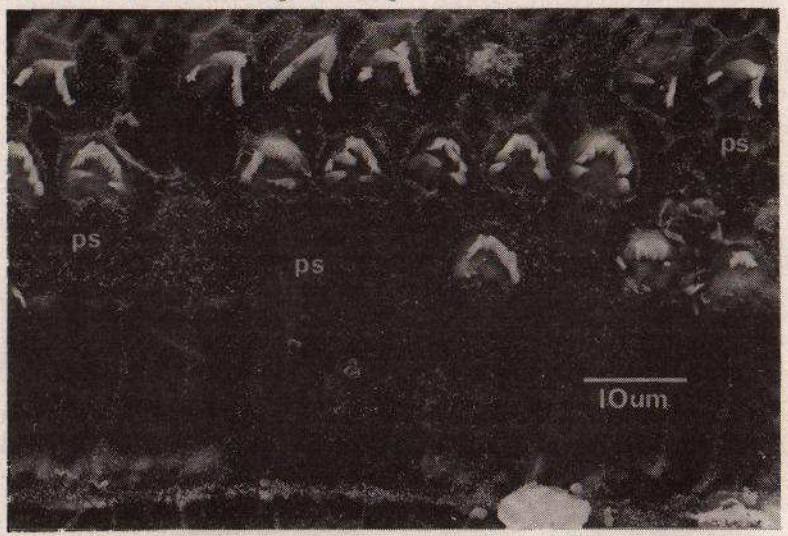

Figure 4. 6 months post-exposure, with some stereocilia remaining as stumps (s) on OHC1, adjacent to phalangeal scars (ps). Giant stereocilia are seen on IHC.

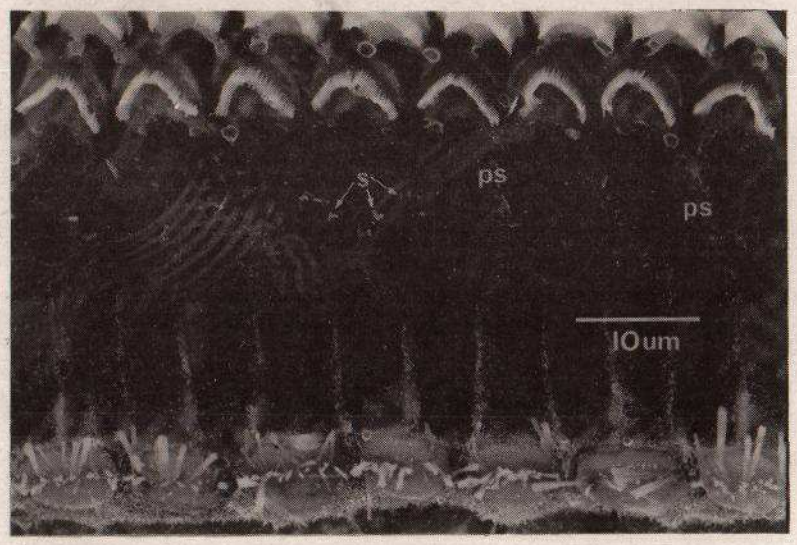

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Figure 5. At 4-h post-exposure many cells are degenerating, showing debris (d) and expelled cuticular plates (cp). Some inner and outer hair cells have already been replaced by the phalanges of supporting cells (p).

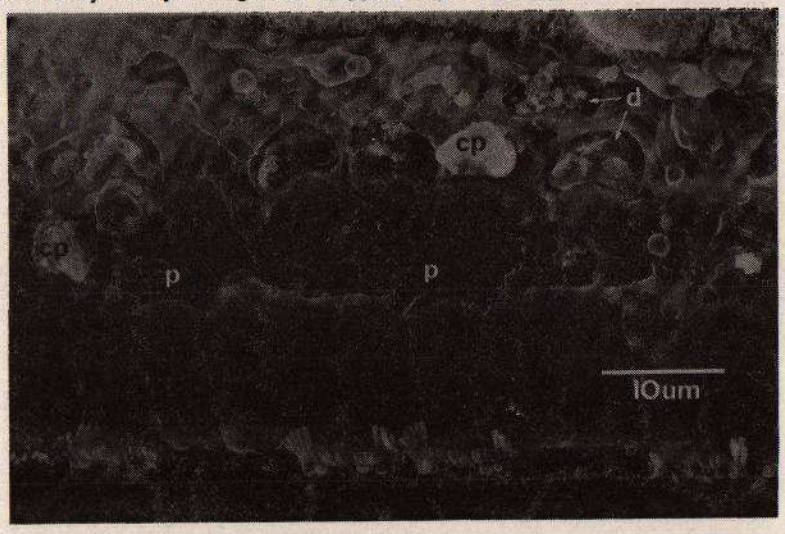
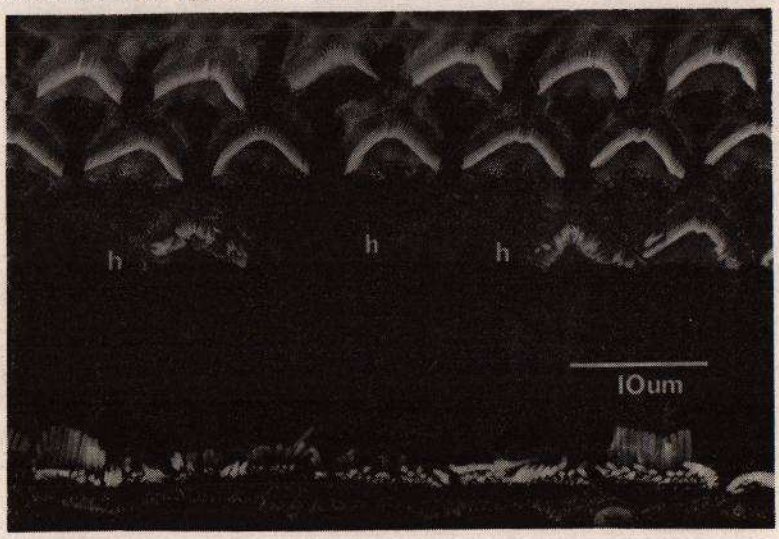


Figure 6. At 4-h post-exposure, 3 holes (h) can be seen in the spaces previously occupied by OHC1. Note disarray of stereocilia on adjacent cells and on IHC, although OHC2 and OHC3 appear normal.



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Supporting cells

The phalanges of outer pillar cells and of Deiters' cells, which fill the spaces between the OHC, are usually covered with microvillae. However, these are not always visible even in normal specimens (see fig. 1) and therefore any quantitative assessment in damaged specimens was thought to be inappropriate. In summary, examples were seen where, in areas with stereociliary damage, fewer microvillae were seen on phalanges, but this was not always so. At the early observation times the phalanges had enlarged enormously in areas where hair cell recovery was not anticipated (see fig. 2). If the hair cells had degenerated and the phalanges had filled the gaps, they were usually covered with an organised array of microvillae (see also fig. 4).

Severity

This was categorised in each field for every hair cell row from no damage through minimum, medium to maximum damage, then calculated for each cochlea and finally summarised for the different post-exposure groups. Generally, there was a fairly even distribution between the various categories for the groups, except for the 4-h group, where 5 specimens out of 7 (71%) were severely damaged. This was also the only group to show no undamaged cochleae (refer also to Table 1).

DISCUSSION

The results showed that stereocilia are affected immediately after this short noise exposure and that this process can be assumed to be the direct result of the noise. Thus after a fairly intense pure tone stimulation, stereocilia appear the most vulnerable structures. Some workers have reported also seeing swelling of the afferent nerve endings at the same time [10], while others have failed to demonstrate it [2]. Different types of alterations to stereocilia were described, their susceptibility to noise damage differing greatly, with a number of cochleae being unaffected. This variability has been found in many animal and human studies, even after carefully controlled experimental exposures as shown by Cody and Robertson [2]. Elongated/giant cilia as seen here most prominently at the 6-month stage, have been previously reported both in old human and old animal ears.

The progression of damage can be very rapid, changes taking place in a few minutes, rather than many hours. On the other hand, some cells are undergoing changes much more slowly, for example the degenerating cilia on IHC after 6 months. This could possibly be due to a secondary and later effect, maybe caused by the intermixing of cochlear fluids, when the integrity of the reticular lamina is broken, as suggested by Bohne and Rabbitt [1]. They reported seeing chinchilla hair cells missing within a few hours after exposure. In the present study, holes were seen in the reticular lamina in place of hair cells in a number of specimens at the 4-h stage. But could this account for all the cells still degenerating at 6 months? This is also contrary to our findings that no statistically significant differences in the extents of damage were seen when the early and later stages were compared. The same results were obtained in another series [7], when all damaged hair cells were counted 3 weeks post-exposure and compared with numbers observed soon after exposure.

However, Cody and Robertson [2] reported N1 threshold losses to be greater immediately after exposure than 21 days later, but their structural data on this point are not clear. There was no evidence in the present study of any stereociliary recovery to have taken place, although this aspect needs further investigation.

The speed of some repair processes evident in this study was quite amazing, where at the 4-h stage the phalanges of the supporting cells had completely filled the spaces left by the hair cells, at least at the surface of the reticular lamina. The one drawback to using only SEM for this study is that no assessment is possible of what is happening to cell bodies beneath the reticular lamina. The replacement of the damaged hair cells by the supporting elements needs a more thorough study by different procedures. This is being carried out at this Institute by Drs. McDowell and Forge after gentamicin intoxication and the results should be of great value to all researchers engaged in elucidating phenomena concerned with hearing damage.

As reported by other workers [8,2,5 and 7], OHC1 was the most susceptible row in every cochlea examined. This phenomenon, however, is thought by Slepecky in her review [9] to be the case for only pure tone stimulation, impulse noise, for example, causing most damage to OHC3.

Not much evidence was found for the half-octave shift in the location of damage, although this was also very variable in different specimens. Robertson and Jonstone [8] and Cody and Robertson [2] found that for less intense sounds (112-115 dB SPL) the main area of damage was inclined towards a higher frequency rather than the exposure frequency, but for intensities above 118-120dB SPL, the damage was mostly centred at the exposure frequency. This factor could also depend on the actual frequency and the duration of the exposure. Short, high intensity pure tones produce a more discrete area of damage near to the point of maximum stimulation. The severity of damage was also a variable feature within experimental groups.

An experimental study of this nature is helpful in trying to understand the sequences of structural cochlear damage in humans exposed to industrial noise. Although pure tones are seldom found in the environment, nevertheless at times the main component may be close to them, for example the ultrasonic cleaning bath study reported by us [6]. The present study, although only involved with structural alterations, extends the range of previous ones. It is hoped in the future to follow the structural changes by non-invasive brainstem audiometry (BSER), which has now been developed at this Institute.

ACKNOWLEDGEMENTS

I would like to thank Dr. Libuše Úlehlová for her assistance at the beginning of this project. Thanks are also due to the Electron Microscopy Unit of this Institute and to the Department of Clinical Photography for the illustrations.

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