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Biochemical Parameters of Guinea Pig Perilymph Sampled According to Scala and Following Sound Presentation

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Guinea pigs were exposed to sound varying from 2 to 8 kHz in frequency and 80-100 dB (SPL) in intensity for periods of 1 hr. The biochemical parameters, glucose, sodium, total protein, and the glycolytic enzymes, aldolase, phosphohexose isomerase, and total LDH as well as isozymes of the latter were ascertained for blood serum, perilymph, and, in some instances, cerebrospinal fluid. The three enzymes occurred at lower levels in perilymph as compared to blood serum. Except for a small difference in serum total protein, sound presentation incurred no significant effect on any of the above parameters. Definite differences in several metabolites were discerned for perilymph sampled according to scala and which were independent of the respective acoustical treatments. Thus, as compared to the scale tympani, the scala vestibuli perilymph displayed a higher glucose content and a diminished total LDH level and of the latter isozymes, LDH_1 ranged lower and LDH_2, higher. As further evidence pointing to cerebrospinal fluid as the possible origin of perilymph, similarities in glucose contents and LDH isozyme patterns were noted for both fluids.

Introduction

The biochemical investigation of the cochlear fluid so important from the standpoint of noise pollution, has been beset by several difficulties. One problem lies in the minute quantities of sample for analysis, amounts of 10–20 μl of perilymph and about 1–2 μl endolymph being collected from both ears of an animal such as the cat or guinea pig. Accordingly, workers have been forced to resort to microtechniques. A persistent handicap encountered in all labyrinthine fluid studies is the contamination with blood, cerebrospinal fluid (CSF), and tissue fluids during removal from the cochlea. Despite extensive precautions, most samples are found to contain some erythrocytes; such contamination, however slight, must be considered in the interpretation of certain biochemical findings. Such difficulties notwithstanding, data have been accumulated on the electrolyte, protein and glucose contents of labyrinthine fluids. However, little work has been carried out on

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various enzyme levels, the published accounts relating almost exclusively to ambient conditions. The enzymes screened have included phosphohexose isomerase (PHI), total lactic dehydrogenase (LDH), and the component isozymes and malic dehydrogenase (1–6).

The present study was undertaken to ascertain changes in electrolyte, glucose, total protein and the glycolytic enzymes, PHI, total LDH and isozymes and aldolase (ALD) in perilymph after acute exposure of guinea pigs to auditory stimulation. Particular attention was directed to comparisons with the mean values for blood serum and in several cases, for CSF and especially, to any differences in the distribution of such parameters between the scalae vestibuli and tympani of the perilymph.

Materials and Methods

Guinea pigs displaying a Preyer's reflex, namely, involuntary movements of the ears produced by auditory stimulation, were employed. The animals were organized into blocks on the basis of age, sex, weight, and pigmentation and the members of any particular block were assigned at random to the experimental auditory treatments.

Acoustical Treatments

A Knight sine/square wave generator, Model KG–688, was used to produce tones of a sine waveform and of specific frequency. A Knight stereoamplifier, Model KG–60, served to amplify the output of the sound source to the desired intensity level. The tone was transmitted to two 8-in. two-way speakers, Model EDI–810, from Electronic Distributors Incorporated, which were placed at right angle with respect to one another. A General Radio Company sound-level meter, Type 1551–C, allowed for the measurement of the intensity of the sound field. The sound-pressure level (SPL) at the microphone of the meter is expressed throughout in decibels with respect to a standard reference intensity of 0.0002 dyne/cm^2. The guinea pig was introduced into a wire cage (28 x 23 x 16 cm) which was set in the right angle formed by the speakers. The center of the cage was perpendicularly 17 cm from the front of the speakers. The cage and speakers were housed in a separate small room.

The auditory stimulus was adjusted to the desired frequency and intensity before introduction of the animal into the sound field for one hour. Six experimental treatments were applied: ambient; 2kHz, 80 dB; 2 kHz, 90 dB; 2 kHz, 100 dB; 4 kHz, 100 dB; and 8 kHz, 100 dB. The intensity level of the ambient stimulation measured about 30 dB.

Collection of Fluids

The animals were sacrificed by swift decapitation immediately at the end of their acoustical treatment. Auditory stimulation was continued during the decapitation. Blood was collected at the time of sacrifice and the serum centrifuged at 3000 rpm. CSF was collected from the cisterna magna.

For removal of perilymph, the ventral side of the head was cleared of muscle and connective tissue so that the acoustic bullae were exposed. The ventral surface of each bulla was cracked and the opening enlarged by means of tissue forceps until the cochlea was exposed. Some outer and middle ear structures and portions of the semicircular canals were removed in order to facilitate access to the oval and round windows of the cochlea. The perilymph was obtained from both ears under a dissecting microscope. The areas surrounding the oval and round windows were dried by application of slivers of filter paper so as to minimize blood and tissue fluid contamination of the perilymph during its removal. Samples were withdrawn from the cochlea by means of glass capillary tubes drawn to very narrow tips (less than 100 μ in diameter). The tip of a capillary was used to push aside the footplate of the stapes so as to enter the scala vestibuli. A second capillary was inserted through the round window membrane into the scala tympani. Perilymph was drawn up into the tubes by capillary force and gentle suction and the samples transferred to calibrated micropipets which were sealed and centrifuged at 3000 rpm for 10 min to determine the extent of contamination by erythrocytes. Any sample which was macroscopically contaminated in this

way was not included in the statistical analyses. Samples were maintained at −5°C until analyzed.

Biochemical Analyses

Sodium was determined in an Instrumentation Laboratory Model 143 flame photometer, samples of 1−2 ul being treated with lithium diluent. Total protein was analyzed by the ultraviolet spectrophotometric technique of Waddell *(7)*. Serum was diluted either 1:1000 or 1:2000 with saline and CSF and perilymph, 1:100 or 1:200, the absorbance of the solutions being measured at 215 and 225 nm. For the analysis of glucose, the method of Stein et al. *(8)* was used. The procedure is based on the hexokinase reaction followed by conversion of glucose-6-phosphate to 6-phosphogluconate. The rate of reduction of nicotinamide adenine dinucleotide phosphate (NADP), an indirect measure of glucose concentration, was followed spectrophotometrically at 340 nm.

Kinetic assays were applied to the evaluation of the enzymes. The determinations were facilitated by the use of Worthington and Calbiochem enzymatic kits. For PHI, the glucose-6-phosphate formed from fructose-6-phosphate was converted into 6-phosphogluconate *(9)*. The rate of reduction of NAD affords an indirect index of PHI activity, the increase in absorbance at 30°C being followed for 1 min at 340 nm. Activity is expressed as international units (U/100 ml) at 30°C. The ALD assay was also based on a series of coupled reactions resulting in the oxidation of NADH *(10)*, the activity being given as international milliunits (mU/ml) at 37°C. For total LDH, oxidation of NADH attending the conversion of pyruvate into lactate was ascertained, activity being expressed as mU/ml fluid at 30°C *(11)*.

LDH isozyme distribution was determined by the polyacrylamide disc electrophoretic technique as detailed by Dietz and Lubrano *(12)*. The patterns were quantitated by scanning the gels in a densitometer.

Results

Glucose, sodium, total protein and enzyme levels of fluids from guinea pigs subjected to the specified acoustical treatments for 1 hr appear in Table 1, and the distribution of LDH isozymes is presented in Table 2. The data were examined initially by the F_{max} test *(13)* for homogeneity of error variance. Analyses of variance were subsequently performed in order to determine the significance of the biochemical findings. Factorial experiments, either 1× 6 or 2 × 6 with a randomized group design *(14)* were employed. If a significant F ratio was obtained, the multiple comparison method of Newman and Keuls *(13)* was applied to ascertain the significant pairs among the treatment means.

Sodium

Sodium levels were similar for both blood serum and perilymph and no significant differences were encountered in the acoustical treatments. Sodium concentrations were in the same range for both perilymph scalae.

Glucose

The glucose content of perilymph ranged lower than that of serum but simulated the level of CSF. Analyses of the means for serum, CSF, and perilymph revealed no significant effect due to auditory treatment. However, the mean values of the perilymph scalae obtained over all auditory conditions were significantly different. The fluid from the scala vestibuli contained a higher glucose content than that from the scala tympani.

Total Protein

Perilymph total protein was less than that of serum. With the acoustical treatments, a significant effect on serum total protein could be ascertained between the means for 2 kHz, 90 dB and 4 kHz, 100 dB. However, no effect of sound on perilymph could be deduced and the two scalae were comparable in their protein contents.

PHI, ALD, and Total LDH

The PHI, ALD, and total LDH levels of perilymph were lower than those of serum. Analyses of the mean values showed no

Table 1. Mean glucose, sodium, protein, and enzyme levels of blood serum and perilymph from guinea pigs subjected to auditory treatments for 1 hr [a]

Fluid	Auditory conditions Frequency, kHz	Intensity, dB SPL	Glucose, mg/100 ml [b]	Sodium meq/l.	Protein, wt per 100 ml	LDH, mU/ml [c]	ALD, mU/ml	PHI, U/100 ml
Blood serum	Ambient		148 ± 4.8	138 ± 5.8	4.2 ± 0.2	709 ± 88	102 ± 12.9	206 ± 15.0
	2	80	142 ± 6.2	144 ± 4.8	4.1 ± 0.3	712 ± 94	119 ± 15.5	170 ± 16.2
	2	90	151 ± 3.9	133 ± 7.1	5.0 ± 0.3	883 ± 106	146 ± 18.6	218 ± 30.5
	2	100	148 ± 9.7	140 ± 3.4	4.6 ± 0.3	660 ± 65	98 ± 11.0	215 ± 21.3
	4	100	129 ± 5.5	140 ± 4.7	3.8 ± 0.2	728 ± 118	137 ± 29.1	261 ± 33.4
	8	100	156 ± 5.5	128 ± 11.4	4.2 ± 0.2	698 ± 89	134 ± 32.9	233 ± 22.4
Perilymph								
Scala vestibuli	Ambient		51.3 ± 10.6	141 ± 16.3	169 ± 28.5	227 ± 73	20.0 ± 3.5	18.6 ± 3.1
	2	80	52.1 ± 4.4	162 ± 16.2	167 ± 25.9	119 ± 12	13.9 ± 4.1	22.7 ± 4.9
	2	90	44.2 ± 6.1	162 ± 17.6	253 ± 34.2	136 ± 25	17.0 ± 2.6	25.2 ± 4.0
	2	100	56.2 ± 6.5	149 ± 7.4	218 ± 33.2	184 ± 31	15.3 ± 2.1	23.4 ± 4.9
	4	100	52.6 ± 6.3	141 ± 16.6	197 ± 41.8	119 ± 18	31.2 ± 13.8	24.4 ± 3.0
	8	100	58.2 ± 4.1	145 ± 5.1	187 ± 13.2	241 ± 71	16.1 ± 3.0	25.7 ± 4.6
Scala tympani	Ambient		34.3 ± 4.7	177 ± 17.0	181 ± 34.1	317 ± 97	21.4 ± 3.2	29.5 ± 7.0
	2	80	31.4 ± 3.7	159 ± 9.8	186 ± 35.9	141 ± 35	18.3 ± 4.2	26.9 ± 6.4
	2	90	30.0 ± 6.7	155 ± 12.8	231 ± 41.0	276 ± 48	23.7 ± 2.9	29.9 ± 6.1
	2	100	41.7 ± 12.1	166 ± 14.4	215 ± 28.7	273 ± 88	29.2 ± 6.8	28.2 ± 7.5
	4	100	32.2 ± 4.9	143 ± 11.2	186 ± 48.7	181 ± 21	32.2 ± 4.8	27.4 ± 5.5
	8	100	40.0 ± 3.7	164 ± 15.0	235 ± 48.1	319 ± 104	22.2 ± 4.1	20.1 ± 2.6

[a] Each value ± standard error is based on 10 animals.

[b] For CSF, the glucose values (mg/100 ml) based on six animals each amounted to 51.8 ± 8.2, 43.1 ± 6.5, 43.5 ± 2.4, 40.3 ± 8.0, 54.2 ± 5.6 and 61.2 ± 11.3 for ambient, 2 kHz, 80 dB; 2 kHz, 90 dB; 2 kHz, 100 dB; 4 kHz, 100 dB; and 8 kHz, 100 dB conditions respectively.

[c] Protein is expressed as g and mg for serum and perilymph, respectively.

remarkable differences attributable to stimulation by sound. Also, the perilymph samples from the two scalae displayed similar PHI and ALD activities. However, a significant difference in perilymph LDH was discerned for the scalae. The scala tympani mean based on all auditory conditions was significantly higher than that of the scala vestibuli. Whether this difference is related to the perilymph scala as such or to some other variable associated with the scala cannot be discerned from the data.

LDH Isozymes

For statistical analysis of the LDH isozyme data, the values of Table 2 were transformed from percentages to angles by the inverse sine function *(15)*. An examination of the isozymes from serum, CSF, and perilymph disclosed no significant effect of sound stimulation. However, a definite difference between the perilymph scalae in LDH_1 and LDH_2 could be demonstrated. The scala vestibuli value averaged over all conditions showed a lower LDH_1 but a higher LDH_2 level than that of the scala tympani. Whereas serum presented the usual pattern of five isozymes, perilymph and CSF exhibited primarily three bands, those of LDH_4 and LDH_5 being either faint or absent.

Discussion

The biochemical nature of the labyrinthine fluids and their possible modification by auditory stimulation were investigated in the present program. The electrolytes, sodium and potassium, have been implicated in the mechanism of hearing *(16, 17)* but in the current

Table 2. Mean LDH isozyme percentages of blood serum, CSF, and perilymph of guinea pigs following auditory treatment for 1 hr[a]

Fluid	Auditory conditions Frequency, kHz	Intensity, dB SPL	LDH_1, %	LDH_2, %	LDH_3, %	LDH_4, %	LDH_5, %
Blood serum	Ambient		27.38 ± 5.82	21.76 ± 3.91	34.60 ± 3.25	15.56 ± 3.25	0.70 ± 0.37
	2	80	40.53 ± 4.73	15.23 ± 3.07	27.22 ± 2.25	15.48 ± 2.05	1.54 ± 0.66
	2	90	38.74 ± 5.51	14.79 ± 2.49	27.45 ± 2.45	16.86 ± 4.06	2.16 ± 0.87
	2	100	27.83 ± 4.03	15.61 ± 2.25	33.15 ± 2.60	23.41 ± 2.44	—
	4	100	31.43 ± 4.75	17.17 ± 2.32	32.99 ± 2.31	17.31 ± 3.71	1.10 ± 0.53
	8	100	22.56 ± 3.70	17.35 ± 2.64	36.34 ± 2.26	22.69 ± 3.45	1.07 ± 0.56
CSF[b]	Ambient		57.86 ± 3.03	27.31 ± 3.35	13.08 ± 4.05	1.76 ± 0.50	—
	2	80	57.45 ± 2.65	30.30 ± 0.57	10.87 ± 1.55	1.38 ± 0.86	—
	2	90	57.00 ± 2.17	30.28 ± 2.14	11.20 ± 0.54	1.52 ± 0.64	—
	2	100	61.26 ± 1.82	25.28 ± 1.38	12.12 ± 0.92	1.33 ± 0.62	—
	4	100	55.41 ± 2.44	25.50 ± 1.18	15.68 ± 1.83	3.41 ± 1.60	—
	8	100	55.01 ± 2.19	29.91 ± 1.13	12.30 ± 1.47	2.78 ± 1.36	—
Perilymph							
Scala vestibuli	Ambient		62.62 ± 3.06	23.97 ± 0.98	12.04 ± 1.69	1.37 ± 0.82	—
	2	80	60.52 ± 2.66	26.47 ± 2.24	12.00 ± 1.51	1.02 ± 0.85	—
	2	90	67.24 ± 4.19	20.52 ± 2.08	10.22 ± 1.84	2.02 ± 1.49	—
	2	100	62.13 ± 3.04	23.72 ± 3.27	13.97 ± 3.24	0.19 ± 0.19	—
	4	100	69.83 ± 4.51	22.63 ± 3.00	7.54 ± 1.87	—	—
	8	100	62.00 ± 3.18	25.09 ± 2.25	10.86 ± 1.81	2.05 ± 1.18	—
Scala tympani	Ambient		72.02 ± 2.82	15.65 ± 0.74	11.82 ± 2.52	0.46 ± 0.46	—
	2	80	74.51 ± 3.40	18.36 ± 2.19	7.13 ± 1.52	—	—
	2	90	64.83 ± 3.76	25.20 ± 4.01	8.83 ± 1.29	1.14 ± 0.81	—
	2	100	70.61 ± 3.31	20.92 ± 1.94	7.76 ± 1.33	0.71 ± 0.50	—
	4	100	72.81 ± 3.47	18.69 ± 1.92	8.24 ± 1.71	0.25 ± 0.25	—
	8	100	68.81 ± 4.26	21.31 ± 2.51	8.72 ± 1.86	1.16 ± 0.57	—

[a] All means are based on ten animals except for those of the CSF values employing five guinea pigs.

[b] Because of technical difficulties in obtaining sufficient uncontaminated amounts, few CSF samples could be screened for total LDH activity.

study, no remarkable alterations in sodium could be observed following the various treatments. In this conjunction, Rauch *(18)* noted an increase in potassium content of the second cochlear turn after presentation of a 2 kHz tone at 140 dB for 4–6 hr. Furthermore, electrophysiological experiments have demonstrated that changes in electrolyte concentrations do occur during sound presentation but immediately return to the initial values when the stimulus is terminated *(19–23)*. The discrepancies may be related to the difference in technique of this study where the perilymph may not have been removed rapidly enough, about twenty minutes elapsing before all fluid samples were collected.

Glucose is of primary interest as it constitutes the main hexose *(24)* and is involved in the functions of the inner ear *(25, 26)*. Silverstein *(2)* reported a lower glucose level in the endolymph than in the perilymph and attributed the decrease to the need of the hair cells for this source of energy. It was thought that the degree of metabolic activity within the cochlear fluid might also be reflected by changes in perilymph glucose contents. However, the current results do not support such a hypothesis, since perilymph glucose was not altered under auditory stimulation.

With regard to fluid total protein, the present results showed no significant change in the perilymph level in spite of application of high

frequency, up to 8 kHz and intensities as great as 100 dB. These findings are in marked contrast to the decrease in perilymph total protein content reported by Komarovich and Plouzhnikov *(27)* as occurring 30 min after intense stimulation followed by an increase 4 hr later and to the diminution in protein level 3 days after administration of ultrasound according to Lawler et al. *(28)*. Again, the parameters utilized in the current investigation might not have been adequate to incur a measurable change, especially, allowance of a longer time interval after stimulation for an effect. The results of this study likewise showed no statistically significant differences between perilymph scalae in total protein content. This finding does not corroborate the contention of Scheibe et al. *(29)* that the protein level of the scala vestibuli ranges higher than that of the scala tympani. However, the conclusion of the latter workers is merely subjective and not based on any statistical analysis. Scheibe et al. reported that the presentation of a tone of 135 dB for 1 hr. did not alter the protein composition, even when measurements were made at intervals of up to 4 days after stimulation. On the other hand, Beck *(30)* showed that immunoelectrophoresis of perilymph after sound presentation revealed the appearance of new submolecular protein fractions in the fluid. Unfortunately, this investigator did not specify parameters of the acoustical stimulation nor did he identify the additional protein components.

Biochemical pathways occurring in the inner ear fluids and which may participate in hearing were investigated by screening select enzymes of the glycolytic metabolic scheme. In confirmation of earlier work, perilymph contains PHI and LDH in addition to ALD which was studied for the first time. It will be noted that PHI, like LDH and ALD, occurred at a lower activity in perilymph as compared to serum in the guinea pig. Antonini et al. *(1)* reported the reverse relationship, namely, a higher PHI level in perilymph; the conflict may be due to species differences, the last group employing equine material. As with the other biochemical indices, no effect of sound on the three glycolytic enzymes nor on the LDH isozyme patterns was evident in the current study. Stimulation employing higher intensities and the manipulation of temporal parameters might predispose to more definitive effects. Such changes might not necessarily lead to destruction of the hearing mechanism but rather to a functional defect which, as speculated by Elredge *(31)*, might involve necessary metabolite depletion or the accumulation of toxic components on a temporary basis.

Further insight into biochemical pathways operating in the labyrinthine fluids can be gained from the LDH isozyme patterns. LDH_1 is more prominent in tissues where aerobiasis occurs whereas LDH_5 figures notably in those undergoing glycolysis. In this and other studies *(3–6, 32)*, patterns for perilymph showed the presence of high LDH_1 levels, thereby implicating aerobic pathways as a significant source of metabolic energy. The tricarboxylic acid cycle as an important pathway for the fluid is also suggested from the findings of Silverstein *(2)*. Lotz and Kuhl *(4)* observed that whereas LDH_1 and LDH_2 predominated in mammals below man, all five isozymes occurred in human perilymph, thus indicating the presence of both aerobic and anaerobic pathways.

Although no effect was noted in several parameters of perilymph with sound stimulation, a few findings are of interest. One of these results entails the effect of sound presentation on serum total protein, namely, that the mean values for 2 kHz, 90 dB and 4 kHz, 100 dB were significantly different. This apparent lack of a dose–response relationship suggests that the increase at 90 dB may not represent a true biological effect but rather, a chance occurrence. When the number of current statistical comparisons is considered, an instance of significance on the basis of chance might be expected. In this conjunction, Jozkiewicz et al. *(33)* observed rises in serum total protein in men exposed to intense noise of 110–130 dB in their working environment, and Gregorczyk et al. *(34)* reported increases in serum ALD and LDH of workers following exposure to a frequency of about 100 dB. Other analyses of the present study did not point to any noteworthy changes in sodium, glucose, or the enzymes, LDH, PHI, or ALD in the serum of animals exposed to sound stimulation. In these experiments, the guinea pig is an ideal species since its ears are especial-

ly vulnerable to high frequency and intensity *(31)*. It is possible that tones which are not damaging to the human labyrinths may injure those of the guinea pig.

The present data also have a bearing on the possible origin of perilymph. Some workers believe the latter to be derived from CSF via the cochlear aqueduct and other routes like the internal auditory meatus, while yet several feel that the fluid originates from blood as an ultrafiltrate from the vascular network of the perilymphatic space *(35)*. To support their particular hypothesis, investigators have drawn upon results from two different approaches, the injection of isotopically labeled material or dyes into the circulation following passage into the perilymph *(36–39)* or by comparison of biochemical parameters of blood, CSF and perilymph for similarities and differences. The current results indicate that perilymph and CSF share similar glucose levels and LDH isozyme patterns; blood serum displayed higher total protein and glycolytic enzyme levels than perilymph. Although further comparisons were complicated by the inavailability of CSF from all animals, the data indicate that perilymph may originate from CSF. Additional findings by others have shown that CSF and perilymph possess similar inorganic phosphate content *(40)*, albumin mobility *(41)*, and malic dehydrogenase patterns *(6)* but differ in protein fractions *(42, 43)*. Furthermore, perilymph was reported to differ from both serum and CSF in free amino acid content *(44)* and, according to Antonini et al. *(1)*, in LDH and PHI levels.

It should be pointed out that significant differences in some parameters occurred between the perilymph scalae. Compared to the scala tympani, the scala vestibuli displayed a greater glucose content and a diminished total LDH activity. The change in the latter is further amplified in the isozyme pattern, LDH_1 ranging lower and LDH_2 being greater in the fluid of the scala vestibuli. The relative distribution of these constituents was not affected by sound presentation. In the absence of additional findings, it can only be speculated that the biochemical variations between the two scalae reveal an underlying functional difference. However, the possibility of other factors should be assessed. For example, the partial destruction of the semicircular canals prior to the removal of the cochlear fluid may have caused contamination of the samples. Since the membranous semicircular canals are almost entirely surrounded by perilymph which is continuous with that of the inner ear scalae, blood and tissue fluids may have been carried into the latter perilymph. A comparison of perilymph obtained from animals with and without damage to the vestibular system would determine the validity of the current results.

Summary

Biochemical parameters, glucose, sodium, total protein, the glycolytic enzymes, aldolase, phosphohexose isomerase, and total LDH as well as the component isozymes were determined for blood serum, perilymph, and, in some instances, cerebrospinal fluid from guinea pigs exposed to sound of frequencies up to 8 kHz and of intensities to 100 dB for periods of 1 hr. The activities of the three enzymes in perilymph ranged lower than those of serum. Except for a difference in serum protein of animals exposed to the various tones, sound presentation had no significant effect on any of the biochemical parameters. As one of the facets of the current study, differences were deduced for several metabolites according to perilymph scalae. Thus, as compared to the scala tympani, the scala vestibuli displayed a greater glucose content and a diminished total LDH level and of the latter, the isozyme, LDH_1 ranged lower and LDH_2 higher.

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