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ELECTRON-MICROSCOPIC EXAMINATION OF THE DISTRIBUTION OF SODIUM IN CORTI'S ORGAN OF ANIMALS, THAT ARE LOCATED IN THE STATE OF RELATIVE REST AND IN THE CONDITIONS OF SONIC ACTION

by

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А а	*А а*	A, a	Р р	*Р р*	R, r
Б б	*Б б*	B, b	С с	*С с*	S, s
В в	*В в*	V, v	Т т	*Т т*	T, t
Г г	*Г г*	G, g	У у	*У у*	U, u
Д д	*Д д*	D, d	Ф ф	*Ф ф*	F, f
Е е	*Е е*	Ye, ye; E, e*	Х х	*Х х*	Kh, kh
Ж ж	*Ж ж*	Zh, zh	Ц ц	*Ц ц*	Ts, ts
З з	*З з*	Z, z	Ч ч	*Ч ч*	Ch, ch
И и	*И и*	I, i	Ш ш	*Ш ш*	Sh, sh
Й й	*Й й*	Y, y	Щ щ	*Щ щ*	Shch, shch
К к	*К к*	K, k	Ъ ъ	*Ъ ъ*	"
Л л	*Л л*	L, l	Ы ы	*Ы ы*	Y, y
М м	*М м*	M, m	Ь ь	*Ь ь*	'
Н н	*Н н*	N, n	Э э	*Э э*	E, e
О о	*О о*	O, o	Ю ю	*Ю ю*	Yu, yu
П п	*П п*	P, p	Я я	*Я я*	Ya, ya

*ye initially, after vowels, and after ъ, ь; e elsewhere.
When written as ё in Russian, transliterate as yё or ё.

RUSSIAN AND ENGLISH TRIGONOMETRIC FUNCTIONS

Russian	English	Russian	English	Russian	English
sin	sin	sh	sinh	arc sh	$\sinh^{-1}$
cos	cos	ch	cosh	arc ch	$\cosh^{-1}$
tg	tan	th	tanh	arc th	$\tanh^{-1}$
ctg	cot	cth	coth	arc cth	$\coth^{-1}$
sec	sec	sch	sech	arc sch	sech^{-1}
cosec	csc	csch	csch	arc csch	csch^{-1}

Russian	English
rot	curl
lg	log

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CYTOLOGY.

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ELECTRON-MICROSCOPIC EXAMINATION OF THE DISTRIBUTION OF SODIUM IN CORTI'S ORGAN OF ANIMALS, THAT ARE LOCATED IN THE STATE OF RELATIVE REST AND IN THE CONDITIONS OF SONIC ACTION.

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Numerous work of physiologists, pharmacologists and biochemists established physiological role of ions of sodium as some other inorganic ions, in process of vital activity of cell. However, until recently it was impossible to morphological trace localization of sodium in the structures of cell because of the absence of the corresponding histochemical methods. Was only recently proposed the method of the detection of sodium, based on interaction of pyroantimonate of potassium - $KSb(OH)_6$ - and the cations of sodium with the formation of the insoluble residue of pyroantimonate of sodium - $NaSb(OH)_6$ and with the subsequent identification of the small

granules of reaction product in the structures of cloths under electron microscope (Komnick, 1962). As shown by the works of a number of authors (Komnick, 1962; Kaye et al; 1965, 1966; Hartmann, 1966, etc.), and also by the works of our laboratory (Ivanov and Leont'yev, 1968; Govardovskiy, 1969), this method possesses sufficiently high specificity and high sensitivity. Usually granular reaction products are timed to the cellular membrane, which, apparently, is connected with the active transport of the ions of sodium (Kaye et al, 1966). In the present investigation is posed the problem of the detection of localization of sodium in the cells of Corti's organ of mammals both in the state of their relative rest and under the conditions of sonic action. This study with respect to Corti's organ acquires special importance in connection with the fact that its receptor cells are located in the unique conditions, since their surface membranes are washed by liquids with different ionic composition. So, the apical surfaces of these cells contact with endolymph, which contains the high percentage of potassium and the very low percentage of sodium (Smith et al, 1964), while lateral surfaces are washed by the so-called Corti lymph, which is sufficiently rich in sodium (Engstrom, 1960, etc.). Histochemicals in the receptor cells Corti organ revealed a number of ferments and biologically important substances, activity and localization of which changes under the conditions of the action of sounds. These data allowed Vinnikov and Titova (1961) to formulate the "cytochemistry auditory theory", which focuses special attention on the role of cytochemistry reaction in the primary processes of the reception of

sounds. The comparison of these results with the data about the localization of sodium will enlarge our ideas about the cytochemistry processes in Corti organ.

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Material and procedure.

Was investigated Corti organ of guinea pigs and rats under conditions of relative rest and sonic action (for guinea pigs - 350, 1000, 2000 Hz, 95 dB for 30 min., for rats - 2000, 5000, 10000 Hz, 95 dB for 30 min.). Material was recorded according to the method of Komnick (Komnick, 1962), filled in by araldite Fluka, was cut into sections on ultratome LKB-8802A and was examined by electron microscope LEM-6s with voltage 50 and 80 kV. As a rule, the additional contrasting of preparations was not produced.

Results.

Products of histochemical reaction to sodium in elements of Corti organ are system of granules, whose diameter reaches 1500 e. These granules are localized, as a rule, near the diaphragm structures of cell. Granules are the crystals of pyroantimonate of sodium $NaSb(OH)_6$. Of course each granule presents the complex of those aggregated in the course of reacting the molecules, which usually occurs, also, in the presence of other histochemical reactions. It should be pointed out that while conducting of this reaction the ultrastructural organization of cells excellently remains.

Under conditions of relative rest both on external and in

internal receptor (hair) cells of Corti organ granular reaction products are revealed first of all on membranes, which cover stereocilia (Fig. 1 see. ins. 1). Separate granules are located also on the fibrils inside stereocilia. In this case it is possible to note that the granule of residue in stereocilia of internal hair cells are larger, but less numerous, that in the external hair cells. Small granules rarely are encountered on the external surface of the cuticle of receptor cells. The plasmatic membrane, which covers the lateral surfaces of receptor cells, is extremely poor in the granules of pyroantimonate of sodium. In the cytoplasm the granular deposits of reaction products are revealed in the cuticular plate, in the mitochondria. The insignificant number of small granules is located between the membranes of cristae, and also in the matrix of cytoplasm. For us with authenticity could not be revealed on the nuclear membranes granular reaction products to sodium. The at the same time distinct accumulations of small granules were observed in the chromatin and in the chromatospherite (Fig. 6; see ins. II).

Insignificant deposits of granules were found near presynaptic and postsynaptic membranes of receptor cells and nerve ends (Fig. 2; see ins. I). In the synaptic slot of the granules residue could not be detected. "Dark" - efferent nervous ends - are more rich in sodium, residue is distributed in them in the form of small granules. In the "bright" - afferent nervous ends - are encountered only single, but larger granules. In the nervous interlacements the reaction products distinctly are outlined along the plasmatic membrane of

nervous portages for entire their extent in the tunnel and further up to the level of the passage of the fibers through the basilar membrane and the appearance in them of myelin shells (Fig. 3; see ins. I).

In Deiters's cells, supporting elements of Corti organ, reaction products are revealed by entire elongation/extent of plasmatic membrane. In the cytoplasm and in the cristae of mitochondria separate small granules are encountered. The latter are located also in the chromatin of nucleus/kernel. Cell-posts are relatively poor in the deposits of pyroantimonate. Cells of Hensen are characterized by a comparatively rich content of sodium (Fig. 4; see ins. I). The small granules of residue in them are localized from the side of the internal surface of the plasmatic membrane, which covers microvills. Are especially significant the deposits of the granules of sediment/residue on the plasmatic membrane of these cells, inverted to the basilar membrane, and also over their lateral surface.

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In the cytoplasmic matrix of the cells of Hensen the reaction products to sodium practically are absent, although the sufficiently significant deposits are observed on the cristae of the mitochondria, which are located, as a rule, near the plasmatic membrane of these cells. In the nucleus/kernel a quantity of granules is insignificant. The abundant deposit of the granules of pyroantimonate of sodium is observed on the surface of the large and gigantic vacuoles of Hensen cells. Very intensively reaction products are accumulated over the

internal surface of the plasmatic membranes of the cells of internal and external spiral cut. In the tektorial [?] membrane the small granules of sediment/residue more or less evenly are distributed on the whole thicker (Fig. 5; see ins. II). In the basilar membrane granules are absent. Then intensive granular accumulation is revealed in the endothelial cells, which cover basilar membrane from the direction of basilar stairs. In the cells of reisner [?] membrane from the side of cochlear channel the granules are revealed very rarely. But in the cells of its surface, inverted to the vestibular stairs, the content of the granules of residue is very high.

Contents of tunnel, vestibular and drum stairs and cochlear channel, apparently, is washed out purely mechanically during fixation and installation through alcohols. Nevertheless sometimes in the drum stairs and in the space between the external hair cells is revealed flake-shaped residue, which always proves to be connected with the very large and abundant granules of reaction product. This indicates the high concentration of sodium ions in perilymph and Corti lymph.

After sonic actions is noted significant increase in deposit of products of histochemical reaction on plasmatic membrane, which covers stereocilia and cuticle of receptor cell (Fig. 6, 7, 8; see ins. II and III). Granules are considerably larger than in the control (to 1500 Å), they are very numerous and cover the surface of stereocilia and cuticle with almost continuous layer. The deposit of granules increases also in cuticle and cytoplasm of receptor cell and sometimes

along its lateral surface (Fig. 6). Furthermore, grows the number of granules in the mitochondria, the chromatin of nucleus/kernel and near the plasmatic membrane of the base of cell. At the same time are encountered the single cells, in which reaction products is contained even somewhat less than in the control. In the nerve ends the granular sizes noticeably rise. In this case, just as in the state of relative rest, granules are revealed predominantly in the efferent ("dark") nervous ends (Fig. 9; see ins. III).

It should be pointed out that increase of deposit of reaction products in receptor cells of Corti organ occurred in strict correspondence with gradient of perception of audio frequencies (Vinnikov and Titov, 1961). The application of high frequencies was accompanied by an increase of the deposits of reaction products in the receptor cells on the lower coils of snail, low - practically on all coils of snail. Under the influences used by us it was impossible to note explicit changes in the nature of reaction to sodium in the auxiliary structures and the supporting cells of Corti organ.

Consideration.

Results of conducted investigation show that with the aid of method in structures of Corti organ used can be revealed regular distribution of reaction product - pyroantimonate of sodium. Thus, for instance, in the receptor cells it is revealed mainly in stereocilia, in the cuticle and the cytoplasm and in the considerably

smaller measure in the plasmatic membrane, which covers lateral surface.

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In the supporting/reference cells the reaction product is located along the internal surfaces of plasmatic membranes, bordering intercellular spaces for entire their elongation/extent. It is thus far unclear, is revealed in the presence of the used reaction only free or also the part of connected sodium. In this case it is necessary to keep in mind the possibility of redistributing the free ions of sodium as a result of diffusion. However, even now it is possible to draw some conclusions from the obtained results.

First of all should be noted high concentration of sodium in Corti lymph, which will agree with literature data (Engstrom, 1960). The nonuniform distribution of reaction product along the plasmatic membrane of hair cell (surface of stereocilia, lateral surface) can be connected with the difference in the properties of these sections of the membrane. In favor of this assumption speak the results, obtained under the sonic influences. A significant increase in the deposit of the granules of pyroantimonate of sodium on the apical surface of hair cell can be explained by the accumulation of intracellular sodium. It is possible that under the effect of sound increases the permeability of the surface membrane of stereocilia, which can be considered as synaptic (Nakhmanzon, 1967). This increase in the permeability, possibly, connected with the action of the acetylcholine, adjusted by

acetylcholinesterase, which is contained in stereocilia (Vinnikov and Titov, 1961), leads to the entering of sodium into the hair cell from endolymph on the electrical gradient. As is known, a potential difference hair cell - endolymph reaches 150 mV (Bekesy, 1960; Davis 1960). By this, apparently, is explained the noted increase in the content of the products of histochemical reaction to sodium in the excited receptor cells.

It is thus far unclear, how it is possible to connect different content of reaction products in efferent and afferent nervous ends and preferred response of latter to sonic action.

Specific nature of described shifts is confirmed by their appropriate localization in different coils to snail depending on frequency of sonic action.

Resume.

Content and localization of sodium ions in Corti organ of guinea pigs and rats, that are located in conditions of relative rest and sonic action, was investigated according to method of Komnick (Komnick, 1962). The characteristic distribution of the granules of reaction product in the structures of receptor and supporting/reference cells is discovered. Under the sonic influence was observed an increase in these granules mainly on the membranes of stereocilia and in the cuticle of receptor cells, that, apparently, it

is possible to connect with a change in the permeability of the excited membranes. In the supporting/reference cells similar shifts are not discovered.

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