A Cytological and Histochemical Study of the Stria Vascularis of the Guinea-pig's Ear

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With one plate (fig. 1)

Summary

The cells of the stria vascularis of the guinea-pig have been studied morphologically and histochemically. The stria vascularis consists of three layers of cells with blood-capillaries. Apart from some yellowish pigments, the only cytoplasmic inclusions seen in the living cells and in the fixed materials are numerous rod-shaped mitochondria and spherical lipid globules. The mitochondria are about 1·5 μ in length with a diameter of 0·8 μ; they contain phospholipid. The lipid globules are about 1·0 μ to 1·5 μ in diameter; they contain phospholipid and a small amount of cerebroside.

The cytoplasm of these cells contains a small amount of diffused phospholipid and cerebroside.

Introduction

RECENT results of the analysis of the labyrinthine fluids suggest that the endolymph is formed and maintained by certain cells of the endolymphatic system of the inner ear. The present investigation is concerned with the histochemistry of the stria vascularis, which has been supposed to be involved in the formation of endolymph (Citron, Exley, & Hallpike, 1956).

The epithelial cells of the stria vascularis of the cochlea of the guinea-pig are attached to the spiral ligament as a ribbon (fig. 1, A) consisting of three layers of cells (fig. 1, B).

The extensive network of blood-capillaries in the stria vascularis and the position of the stria vascularis in the cochlear duct have been regarded as evidence of a secretory mechanism for supplying the endolymph. Shambaugh's histological study (1908) of the stria vascularis showed a true secretory, gland-like structure. Held (1926) confirmed this finding and expanded his view in support of Shambaugh's contention that the stria vascularis secretes the endolymph of the scala media. Von Fieandt and Saxén (1936) described the stria vascularis as epithelial in origin, and they considered that the endolymph is probably formed in the basal part of the cells. Saxén (1951), in his reinvestigation of the stria vascularis, stated that the columnar cells of the stria vascularis have broad, branched protoplasmic feet which are often closely connected with capillaries. From this feature and the well-developed 'Golgi net', he concluded that the cells of stria vascularis have a secretory function.

[Quarterly Journal of Microscopical Science, Vol. 102, part 1, pp. 75-82, 1961.]

In biochemical studies Smith, Lowry, and Wu (1954) showed that the utricular endolymph of the guinea-pig has a high potassium and low sodium content and it is in this respect comparable to intracellular fluid. Citron, Exley, and Hallpike (1956) confirmed their finding and suggested that the endolymph is probably produced by the secretory activity of specialized cells. Whether these are to be found in the stria vascularis or in the succus endolymphaticus or in both, these structures nevertheless remains unsolved (compare Naftalin & Harrison, 1958).

The present study gives an account of the cytological and histological characteristics of the cytoplasmic inclusions of the stria vascularis. It is based upon the results of the study of the living cells by phase-contrast microscopy and vital staining.

Methods

A number of coloured guinea-pigs and a few white ones were used in this investigation. For the study of the living cells of the stria vascularis, 0·5 ml of 25% urethane per 100 g of body-weight of the guinea-pig was injected into the peritoneum to produce anaesthesia. The animal's head was firmly immobilized and the tympanic bulla opened by a posterior ventral approach to expose the cochlea. A ribbon of spiral ligament together with the stria vascularis was removed from the cochlea and transferred to 0·9% sodium chloride solution containing 0·1% of 10% anhydrous calcium chloride. In the coloured guinea-pig the stria vascularis could be clearly seen as a grey band through the bone of the cochlea. The spiral ligament was separated from the stria vascularis by teasing under a binocular. The stria vascularis was transferred to a slide with saline and examined by direct and phase-contrast microscopy.

For supra-vital staining, neutral red, Nile blue, brilliant cresyl blue, Janus green, and Janus black were used. The dye was dissolved at 0·5% in distilled water and then diluted with saline to 0·05%. The cells of stria vascularis were stained in the diluted dye for 5 to 10 min, flattened under a coverslip, and examined by direct microscopy.

For the study of fixed material, the cochleae were decalcified in Kristensen's buffered formic acid decalcifying solution (Lillie, 1954) for a week after fixation.

Baker's standard Sudan black method (1944, 1949, 1956*b*) and Sudan black after fixation in Lewitsky-saline solution were used for general cytological

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FIG. 1 (plate). Photomicrographs of vertical sections of the stria vascularis of the guinea-pig.

A, a basal coil of the cochlea, showing Reissner's membrane (*r*) separating the scale media (*sm*) from the scala vestibuli (*v*); the basilar membrane (*bm*) separates the scala media from the scala tympani (*t*). The cells of the stria vascularis (*s*) lie on the spiral ligament (*sl.*).

B, haematoxylin and eosin preparation to show the three layers of cells [basal (*ba*), middle (*mi*) and surface (*su*) layers], and two transversely-cut capillaries (*c*).

C, standard Sudan black method to show the distribution of the lipid globules (*l*).

D, Altmann–Metzner's method to show the short, rod-shaped mitochondria (*m*).

E, acid haematein method, to show the distribution of phospholipid in the mitochondria (*m*) and lipid globules (*l*).

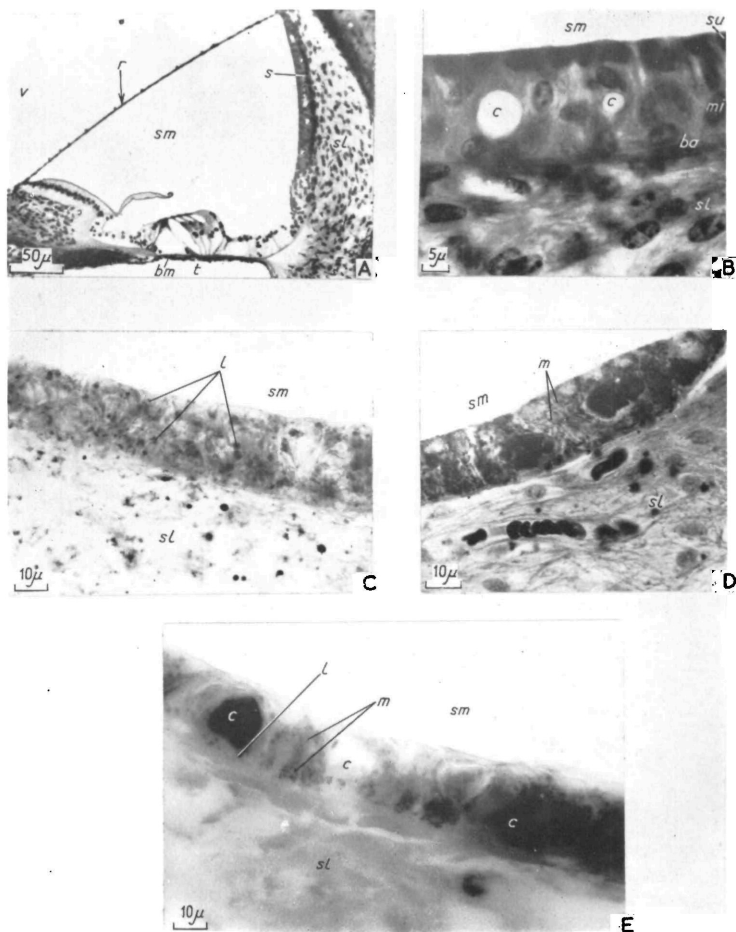

FIG. 1

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study. Aoyama's (1929) and Weigl's techniques (1910) were also used in this investigation. Routine histological preparations (Masson's tricolor stain and Mallory's phosphotungstic acid / haematoxylin) were also made.

To show mitochondria, cochleae were fixed in Helly's, Altmann's, and Mann's fixatives. Sections were cut at 2 μ and were stained by Metzner's (1928) and Hirschler's (1927) methods.

The details of the histochemical tests are set out in a summary form in the Appendix (p. 81).

Results

Unstained living cells

The stria vascularis consists of several layers of cells with a rich capillary system. By direct microscopy, small granules naturally devoid of colour are seen to be present in all the cells. These granules are mostly scattered near the periphery of the cell, but some are near the nucleus. They vary in diameter from 1·0 μ to 1·5 μ. The larger ones are slightly irregular in shape. In addition to granules there are light yellowish bodies in some of the cells. They are about 0·5 μ in diameter.

When examined by phase-contrast microscopy, the most striking feature of the cells of the stria vascularis is the presence of numerous short rods, which nearly fill the entire cytoplasm. These short rods are about 1·5 μ long and about 0·8 μ in diameter. The colourless granules appear darker than the cytoplasm and homogeneous; they are rather refractile. The pigmented bodies are also seen clearly.

Vitally stained cells

The granules are stained faintly reddish by neutral red, and blue by Nile blue and brilliant cresyl blue. Janus black and Janus green were also used. Occasionally the short rods were stained grey by Janus black, while the cytoplasm of the cells was not stained. These short rods are probably mitochondria.

Fixed preparations

In preparations stained with haematoxylin and eosin, Masson's tricolor stain, or Mallory's phosphotungstic acid haematoxylin, the stria vascularis appears to be stratified columnar epithelium (fig. 1, B). It consists of three layers of cells but the cell boundaries are extremely ill-defined.

Basal layer. Owing to the absence of a distinct basement membrane, the cells of the basal layer of the stria vascularis are not sharply separated from the underlying spiral ligament. They are about 16 μ to 18 μ long with elongated nuclei about 7 μ in length.

Middle layer. The cells of the middle layer are fusiform or columnar, each cell being about 16 μ to 20 μ long and having a large, oblong nucleus about 8 μ to 9 μ in length with 2 or 3 nucleoli. Abundant capillaries form loops in this layer.

Surface layer. The surface layer of the stria vascularis, that is the marginal cells which come in contact with the endolymph, are rectangular. They

contain very few pigmented bodies. The cells are about 10 μ long, have oval or round nuclei about 4·5 μ in diameter, and are always situated near the surface of the cell. The surface of the stria vascularis is smooth.

Baker's standard Sudan black and Sudan black after Lewitsky-saline fixation (Baker, 1949) coloured the granules of all the cells of the stria vascularis blue-black (fig. 1, C), except the pigmented bodies. The cytoplasm is also slightly blue. When Weigl's technique was applied to these cells the

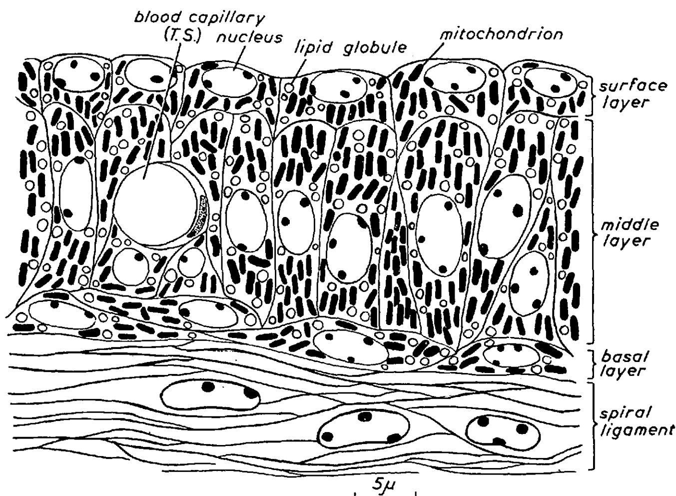

FIG. 2. Diagram to show the distribution of the mitochondria and lipid globules in the middle region of the stria vascularis of the guinea-pig.

cytoplasm became grey. Silver was deposited in the cytoplasm by the method of Aoyama without definite arrangement. No classical appearance of 'Golgi apparatus' was shown by either of these methods.

The mitochondria of the cells of the stria vascularis are shown by Metzner's method and Hirschler's method as short rods. They are present in all the cells. In the middle region of the stria vascularis the mitochondria are mostly short rods, but a few of granular type are also present (fig. 1, D). The rods are arranged with their long axes parallel to the length of the cells. The mitochondria are mostly granular in the cells of the upper and lower regions of the stria vascularis. The number of the mitochondria in the stria vascularis is very high. An impression of the characteristic arrangement of the mitochondria in the middle region of the stria vascularis is shown in the form of a diagram (fig. 2).

Histochemistry of the cells of stria vascularis

Two inclusions formed the main subject at the histochemical investigation, namely, the granules and the mitochondria. A study of the pigmented bodies is not included in this paper.

The granules in the cells of the stria vascularis react positively to Sudan colouring agents (fig. 1, C), and this suggests that they consist of lipid. They give a positive reaction to Baker's acid haematein test for phospholipids, but negative after pyridine extraction. When a piece of stria vascularis is fixed in cold acetone and coloured with Sudan black, the lipid granules become pale grey; they cannot be coloured by Sudan black or any other Sudan colouring reagents if the tissue is extracted with hot acetone in a Soxhlet extraction apparatus. This fact suggests the presence of cerebroside. The pale colour indicates the presence of a very small amount of cerebroside. Cain's Nile blue test (1947) shows that the lipid in the granules is not neutral lipid. The lipid granules are negative to Windaus's and to Liebermann's tests and also to protein tests. The lipid seems to be evenly distributed in the granules. No crescents or ring appearances are seen.

By the use of Baker's acid haematein test it can be demonstrated that the mitochondria contain a great amount of phospholipid (fig. 1, E).

In general, the cytoplasm of the cells of stria vascularis gives a weakly positive reaction to the coupled tetrazonium test. Positive results were also given by Baker's Hg/nitrite test (1956*a*) and the Sakaguchi test (Baker, 1947). The PAS reaction is very weakly positive, and it is still so if the section is treated with saliva before the test. Since the cytoplasm of these cells is not chromotropic and is weakly positive to the Sudan colouring agents after cold acetone fixation, the PAS-positive reaction is perhaps due to a very small amount of diffused cerebroside. Since the cytoplasm is also weakly positive to the acid haematein test, it presumably contains diffused phospholipids.

The cytoplasm of the cells of stria vascularis is basiphil, colouring pink with pyronine in the pyronine / methyl green technique (Jordan & Baker, 1955). The basiphilia does not appear when the section is previously heated with salivary ribonuclease (Bradbury, 1956), and must therefore be presumed to be due to the presence of ribonucleic acid.

Usually two nucleoli are present in each nucleus. The chromatin of all the nuclei of stria vascularis reacts strongly to Feulgen's test.

Citron and Exley (1957) found that in contrast to the endolymph in fishes (Vilstrup & Jensen, 1954), that of guinea-pig has a low viscosity and low amino-sugar content. This finding is of interest in connexion with the apparent absence of both mucopolysaccharide and metachromasy in the stria vascularis.

Discussion

The major finding of the present study is that the cytoplasmic inclusions of the stria vascularis of the guinea-pig consist of lipid granules and a large number of mitochondria; some yellowish bodies are also present in a few cells.

The lipid granules contain phospholipid and a small amount of cerebroside.

As would be expected the mitochondria contain phospholipid. The basiphilia of the cytoplasm of these cells is due to the presence of ribonucleic acid. Evidence was also obtained that the cells are rich in arginine and tyrosine.

Chromotropic granules, reported by Plotz and Perlman (1955) in the cells of stria vascularis of the bat, are not found in the epithelial cells of the stria vascularis of the guinea-pig.

Smith (1957) studied the cells of the stria vascularis of the guinea-pig with the electron microscope. She reported that small vesicles and short rod-shaped mitochondria were numerous in the cells of the stria vascularis. The present investigation has not only confirmed this, and has also provided some chemical information about the cytoplasmic inclusions of these cells. Smith also identified several groups of membranous in her electron micrographs as 'Golgi apparatus'. She gave no evidence of the presence of any 'Golgi apparatus' in the living cells. Chou and Meek (1958) showed that phospholipid globules in other tissues tended to break into crescentic membranous structures when buffered osmium tetroxide was used; calcium ions tend to preserve their spherical shape (compare Baker, 1958). It is possible that the objects which Smith referred to as 'Golgi apparatus' were either distorted lipid globules or the diffused phospholipid.

Saxén (1951) studied the cells of the stria vascularis and the epithelium of the prominentis spiralis of man and the dog. He considered the well-developed 'Golgi nets' in his preparations as evidence of a secretory function. These structures appeared in his photomicrographs as aggregations of spheres. He did not study the living tissue.

Nogi (1958) noticed the numerous mitochondria in the cells of the stria vascularis of the rabbit and also reported that there were structural changes of the mitochondria after electric stimulation of hypothalamus and intravenous injection of adrenalin or pilocarpine.

The present cytological and histochemical evidence, suggests that the epithelial cells of the stria vascularis are active cells and may well be related to the function of secretion or resorption of the endolymph.

The author wishes to express his gratitude to Professor Sir Hans Krebs, F.R.S., and Dr. D. E. Hughes for reading the manuscript, and to Dr. Hallpike, F.R.S., for his criticism and encouragement. Part of the work was done while the author was a member of staff of the Neuropsychiatric Research Unit of the Medical Research Council. Thanks are also due to the Rockefeller Foundation and to the National Institutes of Health, United States Public Health Service (Grant No. A–3369).

APPENDIX

A summary of the histochemistry of the stria vascularis of the guinea-pig

Test	Reference	Results		
		Lipid globules	*Mito-chondria*	*Cyto-plasm*
Standard Sudan black	Baker, 1944, 1956*b*	+++	—	+
Sudan IV	Herxheimer, 1901	+++	—	O
Windaus	Lison, 1953	O	—	O
Liebermann	Lison, 1953	O	—	O
Fischler	Pearse, 1954	+	—	O
Acid haematein	Baker, 1946	++	+++	+
Acid haematein control	Baker, 1946	O	O	O
Sudan black after hot acetone	Casselman & Baker, 1955	O	—	O
Sudan black after cold acetone	Casselman & Baker, 1955	+	—	+
Nile blue	Cain, 1947	+ (blue)	—	O
PAS	Pearse, 1954	O	O	+
PAS control	Pearse, 1954	O	O	O
PAS after saliva digestion	Pearse, 1954	O	O	+
Pyronin / methyl green	Jordan & Baker, 1955	O	O	+
Pyronin / methyl green control	Bradbury, 1956	O	O	O
Feulgen	Feulgen & Rossenbeck, 1924	O	O	O
Feulgen control	Feulgen & Rossenbeck, 1924	O	O	O
Coupled tetrazonium	Pearse, 1954	—	—	+++
Sakaguchi	Baker, 1947	—	—	+
Hg/nitrite	Baker, 1956*a*	—	—	+++
Alkaline phosphatase	Gomori, 1952	—	—	O
Acid phosphatase	Gomori, 1952	—	—	O
Basiphilia	—	—	—	++
Metachromasy		O	O	O
Acid mucopolysaccharide	Steedman, 1950	O	—	O

KEY. +++, strong reaction; ++, moderate reaction; +, weak reaction; O, negative; —, no observation.

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