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Protein Studies of Endolymph and Perilymph of the Inner Ear

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The amount of total protein in the endo- and perilymph of sharks has been estimated at 1.8 and 1.2 % respectively. It is found that the endolymph contains α, β and γ globulins, but no albumin, and that the perilymph contains both α, β and γ globulins and albumin.

A study of the literature shows that our knowledge concerning the chemical composition of the liquids of the inner ear (the labyrinth) is very small and rather fragmentary. As a part of a general investigation on these fluids we report here the determination of total protein and the ratios of the various protein fractions. The reason why sharks (*Acanthias vulgaris*) are employed is mentioned in a recent paper by one of the authors [1].

DETERMINATION OF PROTEIN

The amount of total protein was determined by precipitation partly with 1 volume of 20 % trichloroacetic acid, partly with 1 volume of absolute alcohol at —10° C just after withdrawal of the fluids. The trichloroacetic acid was removed from the precipitates by extraction with ether. All protein precipitates were dried to constant weight *in vacuo* over silica gel and phosphorus pentoxide. The precipitate obtained by addition of alcohol was soluble in water. The results are in Table 1.

Table 1. The protein content of endolymph and perilymph and the nitrogen content of these proteins.

Fluid	Precipitating agent	% Protein	% N in the precipitates	
Endolymph	CCl_3COOH	1.76	15.27 *	15.30 *
	C_2H_5OH	1.71	15.02 *	
Perilymph	CCl_3COOH	1.18	13.98 *	13.35 **
	C_2H_5OH	1.11	13.27 **	

* Determined by C. E. J. according to Kjeldahl modified by Blom [3].
** Determined at the University Institute of Chemistry, Copenhagen.

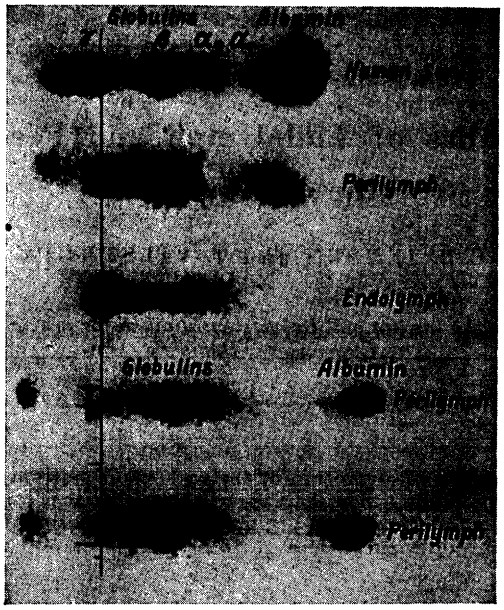

Fig. 1. The electrophorogrammes of endolymph and perilymph. The vertical line indicates the spots where the applications of the various samples have taken place. The separation of albumin from the globulins is evident in all perilymph electrophorogrammes. Only in lowest diagram (perilymph) a fair separation of the α, β and γ globulins is observed.

In the use of these and other reagents precipitating proteins it must be borne in mind that certain of the reagents are better precipitating agents in some instances than in others depending upon the particular type of protein that is to be precipitated.

In order to verify the protein nature of the precipitates the nitrogen content was determined. The results are in Table 1. The precipitates also give the ordinary reactions for protein *e. g.* the biuret test, the test for sulphur and Millons test.

PAPER ELECTROPHORESIS OF THE ENDOLYMPH AND THE PERILYMPH

On account of the scantiness of the fluids we had to make use of a micro-method to separate the proteins in the endolymph and the perilymph. We chose the procedure of paper electrophoresis which is a typical micro-method, requiring only very small quantities for analysis (5—50 μl).

Immediately prior to the electrophoresis the endolymph was liquified by a trace of hyaluronidase, as described in a recent paper by the present authors [2]. Thus the sample for analysis acquired a consistency suitable for the procedure of electrophoresis. The perilymph was used in its natural state. A sample of 25 μl is applicated as a spot on a 15 × 35 cm buffered filter paper (Whatman

No. 3). The buffer is a barbituric buffer pH 8.6: 0.05 *M* sodium diethylbarbiturate (10.3 g per litre) 0.01 *M* diethylbarbituric acid (1.84 g per litre); ionic strength 0.05. A potential gradient of 3 V/cm is applied across the paper for 24 hours in a closed chamber.

The strip is dried, and the various protein fractions stained by immersing the strip for 5 minutes in 1 % bromophenol blue in saturated alcoholic mercuric chloride solution. The mercuric chloride fixes the proteins in the paper strip. For further fixation the strip is then placed in a 1 % solution of mercuric chloride for 15 minutes. After washing for 10 minutes in methanol, 10 minutes in ethanol and again in methanol for 3 minutes, the strip is dried. Fig. 1 shows the electrophorogrammes with the electrophorogram of human serum as reference. At our selected pH (8.6) all the protein fractions are negatively charged, and so it could be expected that all the protein particles would migrate in the same direction (towards the positive pole). The apparently anomalous behaviour of the γ globulin in moving towards the negative pole is caused by a displacement of the entire protein pattern towards the negative pole as a result of an electroendosmotic flow in this direction.

The intensity of the staining together with the area covered by the dye may be used for exact estimation of the relative ratios of protein components in the sample examined, provided care is taken of the fact that albumin binds relatively more dye than do the globulins. However, if the extinctions for the globulin-bound amounts of dye are multiplied by a factor 1.6 we have a relatively good measure of the ratio of albumin/globulin.

This ratio was found to be zero for the endolymph and roughly 21/79 for the perilymph (for further details see Köiw [3]) The average figure for normal human serum is 70/30 (we found 75/25).

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