On the Content of Hexosamines in Cell Nuclei of Calf Thymus and Calf Liver

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In 1951 Bychkov *et al.*[1] claimed that mucopolysaccharides and mucoproteins are regular constituents of the cell nuclei. They based this conclusion on hexosamine determinations made on dried tissues and on nuclear preparations.

However, the method of Elson and Morgan [2] used by them is known to give too high values in the presence of basic amino acids and neutral sugars [3]. This combination is present in the cell nuclei, where basic proteins and pentoses from the nucleic acids make up a considerable part of the dry matter.

The application of a chromatographic step [4] before applying the Elson and Morgan reaction offers a possibility for the determination of hexosamines without any interference of foreign substances.

Bychkov *et al.*[1] used dilute citric acid for the isolation of the cell nuclei [5]. Data presented by Mirsky *et al.*[6] indicate that dilute citric acid may remove from 18 to 55 % of the proteins of the nucleus as compared with the results obtained in using anhydrous lipid solvents for the isolation of the nuclei. It thus seemed necessary to reinvestigate the possible content of hexosamines in cell nuclei.

We used the method of Mirsky *et al.*[6] for the isolation of cell nuclei from calf thymus and calf liver. The final purity of the preparations was judged by staining with crystal violet.

The hexosamine content was determined according to Gardell [4] on hydrochloric acid hydrolysates of the preparations.

From calf thymus we obtained 4.38 g of nuclei from 100 g of dried tissue. Mirsky *et al.*[6] obtained 20—25 g/100 g. Our preparation looked very pure on microscopical examination, and seemed to be practically free from cytoplasmic contamination.

The liver preparation, on the contrary, was not so pure. Our yield of 7.9 g of nuclei out of 200 g of dried tissue is even more than double the yield of Mirsky *et al.*[6]

The analytical results are shown in Table 1.

The figures for the hexosamine content in whole thymus tissue are derived from six chromatographic runs on three different hydrolysates. In five of them two peaks were obtained, one for glucosamine, and one for galactosamine. The galactosamine peak contained some 10—15 % of the total hexosamine content, the glucosamine peak the remaining 85—90 %. The sixth chromatogram showed only a glucosamine peak.

The figures for the hexosamine content in thymus cell nuclei are also derived from six chromatographic runs on three different hydrolysates. Only in three of them there was a trace of galactosamine.

The same results were obtained in analyzing another preparation of cell nuclei from calf thymus.

As the purity test of the nuclear preparations is entirely subjective, one cannot draw extensive conclusions from our results. We cannot exclude the possibility that the amount of hexosamines in our nuclear preparations is due to cytoplasmic contamination. One can state anyhow, that the amount of hexosamine found by Bychkov *et al.*[1] is at least five times too large. If the cell nuclei really contain hexosamines, the amount is of the order of

Table 1.

Percentage of	Thymus		Liver	
	Whole tissue	Nuclei	Whole tissue	Nuclei
Nitrogen (micro-Kjeldahl)	15.65	16.60	10.40	10.87
Ash	12.20	12.2	4.98	5.06
Moisture	5.1	7.1	7.1	6.8
Hexosamine	0.24	0.09	0.23	0.13

magnitude of 0.1 % of the dry substance or less. Mucopolysaccharides containing galactosamine are not present in the cell nuclei of calf thymus.

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Uptake of ^{35}S-labelled Sulfate in the Heparin of a Dog Mastocytoma

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Oliver, Bloom and Mangieri [1] in 1947 found dog mast cell tumors to be very rich in heparin. The heparin content of a highly differentiated tumor was some fifty times that of the dog liver. This finding has been confirmed by Riley and co-workers [2] in 1954. In 1953 Jorpes, Odeblad and Boström [3], by means of an autoradiographic technique, observed that ^{35}S-labelled sulfate is taken up by the mast cells in the subcutaneous tissue of the rat. They could not, however, determine whether the exchange observed occurred in heparin, in any of its precursors or in chondroitin sulfuric acid.

It was therefore considered of interest to know if the sulfate groups of heparin can be labelled with ^{35}S. The large content of heparin in mast cell tumors makes them a suitable material for the study of this question. At the suggestion of Dr. B. Åberg the following experiment was therefore performed.

Experimental. To a dog suffering from a rather highly differentiated mastocytoma was given 1 mC of carrier free sodium sulfate intravenously. After 24 hours the tumor, weighing 16.7 g, was removed and frozen to —20° C. After mincing in the Latapie mill, the material was digested for three weeks with proteolytic enzymes according to the technique of Gardell [4]. After digestion the suspension was treated with several volumes of 96 % ethanol at pH 8.8. The precipitate formed was suspended in water at pH 8—9 and after centrifugation the solution was passed through an anion exchange column, Dowex 2, (Boström and Månsson [5]) in order to remove inorganic sulfate. In a control test with a mixture of heparin and radioactive sulfate it was found that the radioactivity was retained, while the heparin passed through.

The sulfate free solution was evaporated *in vacuo* to dryness and dissolved in water to a concentration of 5 %. The pH was adjusted to 7—8. Then 0.2 volumes of 20 % barium chloride were added. The precipitate was separated by centrifugation. The supernatant was precipitated by adding a third of its volume of glacial acetic acid. The precipitate weighed 3.060 mg. To the mother liquor three volumes of 96 % ethanol were added. This precipitate weighed 9.795 mg. The former precipitate should contain heparin and the latter chondroitin sulfuric acid or the heparin monosulfuric acid found by Jorpes, Werner and Åberg [6] to occur in the normal mast cells. These compounds can easily be separated by

Table 1.

	Weight in mg	Counts/min./cm² of 3 mg	I. U. of heparin / mg	
			Whole blood method	Thrombin method
Barium chloride-acetic acid precipitate	3.060	6 690	61	96
Ethanol precipitate from the mother liquor	9.795	1 750	8.5	12