Short Communications

Paper chromatographic behaviour of low molecular weight hexosamine-containing compounds from human urine

Approximately the same amounts of bound hexosamine are excreted in the urine either in the form of non-dialysable mucoproteins and/or polysaccharides, or in the form of low molecular weight compounds[1-3]. The great attention of scientists focussed on urine colloids in the past years has led us to investigate these low molecular weight compounds which may be in close relationship with mucoproteins.

The presence of these compounds in urine was mentioned by several authors, but only BOAS[1] made an attempt to investigate them in detail. In his preliminary report he could distinguish at least three distinct fractions, separated chromatographically on cellulose columns from lyophilized dialysate of urine.

The concentration of dialysable hexosamine in urine is about 50 μg/ml[1,3] and, therefore, low molecular hexosamine-containing compounds have to be concentrated before chromatographic analysis on paper. Our purification procedure is as follows. Pooled normal male urine was precipitated with 6 volumes of ethanol and filtered. The filtrate was concentrated *in vacuo* to one tenth of its volume and the greater part of the salts removed on ion-exchange columns (Zerolit 225-H^+, X 4.5, 50–100 mesh, 5 × 62 cm, and Zerolit FF-$HCOO^-$, 16–50 mesh, 5 × 31 cm). The water eluate from the latter column contained substances not adsorbable on ion exchangers under our experimental conditions. It was again concentrated *in vacuo* and then fractionated by chromatography on a charcoal–Celite column[4] (the charcoal was purchased from Messrs. Spolek pro chemickou a hutní výrobu, ČSSR; before use it was mixed in the ratio of 1:1, w/w, with Celite 535, Light & Co.). The column was step-wise eluted with water and water–ethanol mixtures (up to 30 % ethanol, v/v) and, finally, with aqueous acetone (30 %). The ten fractions thus obtained were examined by chromatography on paper. At least fourteen Elson-Morgan[5] positive spots yielding the purple colour, characteristic of N-acetylamino sugars, were obtained, as well as two other spots, which gave a red coloration. The R_F values for the most intense spots are listed in Table I. The substances designated as IIb and IVb were present in relatively greater amounts. The former was eluted with water, the latter with ethanol (10 %).

The substances described above contain, with one exception, neither glucuronic acid, nor sialic acid. The fraction X, eluted with aqueous acetone, contained 5 % sialic acid, as estimated by Bial's method[6]. The substance X is ninhydrin-positive, while the others are ninhydrin-negative and consequently, in character most similar to the hexosamine-containing oligosaccharides found in human colostrum by KUHN *et al.*[7]. They are also accompanied by several other neutral sugars. They might be present in fractions of urine ultrafiltrate separated on Sephadex[8]. The total yield of these substances accounts for 10–25 % of the dialysable hexosamine, so that it may be

presumed that other types of low molecular weight hexosamine-containing substances such as glycopeptides, are also excreted in urine, as shown by recent observations[9,10].

The details of our purification procedure will be published elsewhere. Further

TABLE I

R_F VALUES AND COLOUR REACTIONS OF LOW MOLECULAR WEIGHT HEXOSAMINE-CONTAINING SUBSTANCES

Solvent systems: S_1 = pyridine–ethyl acetate–water, 1:2:2 (v/v/v)[7], circular development; S_2 = *n*-butyl alcohol–acetic acid–water, 4:1:5[6], descending; S_3 = *n*-butyl alcohol–pyridine–water, 6:4:3[5], circular. No. 1 Whatman paper; solutions containing 1 mg of each fraction were spotted on the paper. For comparison, the R_F values of glucosamine (GN) and N-acetylglucosamine (AcGN) are given.

Substance	*Solvent systems*			*Colour reactions*[a]		
	S_1	S_2	S_3	*Elson-Morgan*[b]	*Aniline phthalate*	*Aniline-diphenylamine*
IIa_2		0.14	0.18	red[c]		no reaction
IIb	0.28	0.27	0.58	purple[c]	brown	yellow-green
IIc	0.42	0.33	0.65	blue-gray		
IVa	0.10	0.04	0.10	gray	brown-red	
IVb	0.30	0.10	0.40	purple[c]	brown-red	blue-green
VIa	0.03	0.00	0.10	purple	brown	
VIIa	0.02	0.00	0.05	gray	no reaction	no reaction
VIIb	0.60	0.22	0.55	gray	no reaction	no reaction
X			0.12	purple		
GN	0.15	0.09	0.30	red		
AcGN	0.30	0.30	0.50	purple[c]		

[a] The reactions were performed according to HAIS AND MACEK[5], where they are designated D47, D34, and D35.

[b] In solvent systems S_1 and S_2 the colours are not quite clear and have some gray or brown coloration.

[c] Reacts in the cold especially on chromatograms from S_3.

experiments are in progress to elucidate the composition and structure of the substances described in this communication.

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