Carbohydrate Content of Transitional Epithelium of the Urinary System

Histochemical data and electron microscopic studies indicated the presence of complex carbohydrates in the cell surface of transitional epithelium of the urinary tract which could be a source of the Tamm Horsfall glycoprotein[1,2]. It was reasonable to predict that this tissue would contain large concentrations of carbohydrates. In order to obtain relatively pure samples of transitional epithelium, a simple procedure derived from cell dissociation techniques was developed[3]. Hexoses, hexosamines, methyl pentoses and sialic acid was assayed on pellets of transitional epithelium obtained by that procedure.

Material and methods. Renal pelvis, ureters and bladders of sheep were collected from the slaughter-house. The organs were cut open and placed in petri dishes containing 0.25% ethylenediamine tetraacetic acid (EDTA) in 0.1 *M* phosphate buffer pH 7.0, for 12 h at 4 °C, shaking frequently. The fluid, containing the exfoliated cells, was centrifuged at 3000 *g* for 20 min, at 4 °C (pellet I). This pellet was resuspended in 0.001 *M* sodium bicarbonate to induce the lysis of red cells which were often found mixed with the epithelial cells. The suspension was centrifuged again at 3000 *g* for 20 min (pellet II). For controls, sections of the organs, prior to incubation in the EDTA-buffer solution and after this treatment, were embedded in paraffin for microscopic observations. Tissue sections and ether-ethanol-fixed smears were stained with H–E or a PAS-alcian blue sequence. For chemical studies, pellets of washed exfoliated cells of urinary bladder and slices of kidney and liver were homogenized with 5 volumes of an aqueous solution of 0.1% Triton X-100. Proteins[4,5], hexoses[6,7], hexosamines[8,9], methyl pentoses[10] and sialic acid[11] were assayed.

Results and discussion. Transitional epithelium is the stratified cell lining covering the mucous membrane, the innermost layer, of the organs of the urinary system (renal pelvis, ureters and bladder) (Figure 1). The procedure herein described leads to a complete exfoliation of the epithelium from the underlying connective tissue (Figures 2 and 3). The contamination of the sample by red cells in the process of exfoliation was overcome by a dilute sodium bicarbonate solution.

Levels of hexoses, hexosamines, methyl pentoses and sialic acid in sheep transitional epithelium are significantly higher than in kidney and liver (Table).

Data on the sialic acid content of various organs seem to indicate that transitional epithelium of urinary tract is remarkably rich in sialic acid. Values are similar to those reported for tissues which have very high amounts of sialic acid, such as rat epididymis[12]. There is little knowledge of the biological significance of the various carbohydrates herein reported. Information available pertains mostly to sialic acid. In certain biologically active glycoproteins such as gonadotrophins, erythropoyetin, etc. the activity is largely dependent on their content of sialic acid. Enzymatical removal of sialic acid from FSH

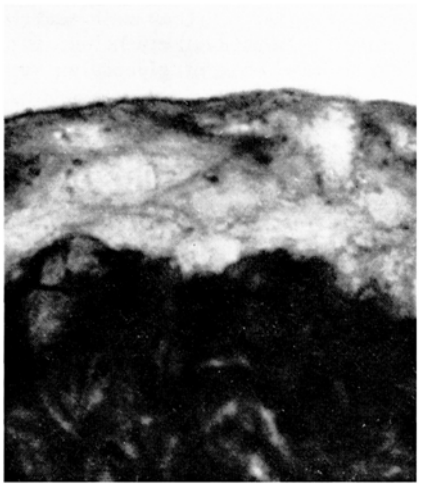

Fig. 1. Urinary bladder (sheep). Epithelial layers and the underlying connective tissue of the lamina propia. The free border of the epithelium correspond to the glycocalyx. AB-PAS. ×1600.

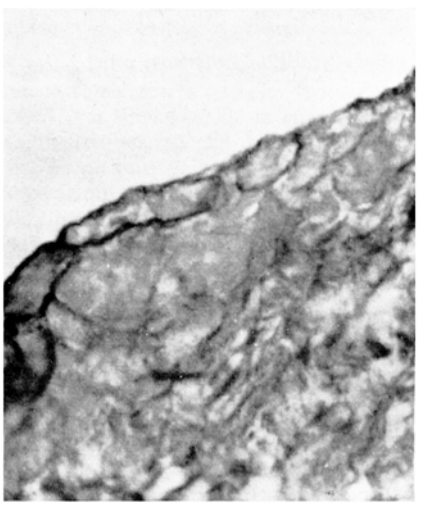

Fig. 2. Mucosa of bladder (sheep) after incubation in the EDTA-buffer phosphate solution, for 12 h at 4 °C. The epithelium is no longer seen. The free border corresponds now to the basement membrane. PAS. ×1600.

Carbohydrates in transitional epithelium

Tissue	Neutral carbohydrates	Hexosamines	Methyl pentoses	Sialic acid
Transitional epithelium	162.86[a] ±13.91[c]	18.13[a] ±0.90	16.93[a] ±0.34	20.33[b] ±0.24
Liver	91.10 ±3.71	4.76 ±0.23	16.28 ±0.41	3.70 ±0.15
Kidney	62.50 ±1.80	8.31 ±0.16	10.66 ±0.27	7.96 ±1.06

[a] mg/g protein. [b] μM/g protein. [c] Standard error.

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reduces its biological activity by 97% or more[13]. It has been suggested that sialic acid could be involved in the transport of the hormone or have some action on activating the receptor sites at the target organs[14]. There are indications that sialic acid may have a role in the spatial configuration of glycoproteins which is drastically changed by releasing sialic acid residues[15]. Furthermore, the ability of certain glycoproteins to inhibit viral hemagglutination is related to their sialic acid content[16,17]. The cell surface of transitional epithelium is covered by a thick carbohydrate coat or glycocalyx rich in sialic acid[2]. This led to postulate that the glycocalyx of transitional epithelium is a source of certain urinary complex carbohydrates, such as the mucoprotein of TAMM and HORSFALL[2]. The present results, indicating the high carbohydrate content, particularly of sialic acid, in sheep transitional epithelium are followed by fractionation studies and comparison of its electrophoretic behaviour with the TAMM-HORSFALL mucoprotein[18].

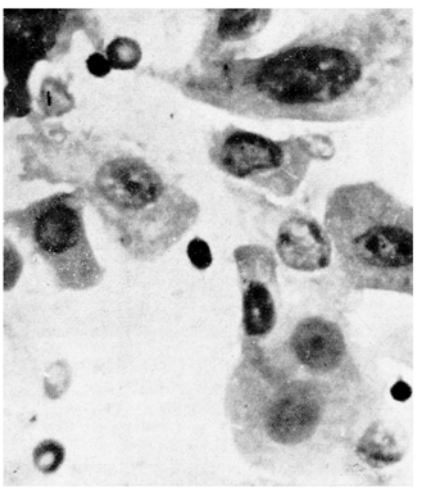

Fig. 3. Transitional epithelial cells from pellet I (see text). H + E. ×1600.

Resumen. El epitelio de transición tiene alto contenido de carbohidratos. Este tejido posee una de las concentraciones más elevadas de ácido siálico en el organismo. Se describe un procedimiento simple para exfoliar epitelio de transición.

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Irreversible Depigmentation of Hair by N-Methyl-N-Nitrosourethane

Recently it has been reported that skin application of a solution of 8-hydroxyquinoline 0.5% in acetone causes striking, but reversible depigmentation of the subsequent hair growth in several strains of pigmented mice[1]. Several other copper reagents, however, failed to inhibit hair pigmentation in female C57BL mice. It has been suggested that possibly a complex of 8-hydroxyquinoline with the metal is involved.

In the course of testing the carcinogen, N-methyl-N-nitrosourethane (MNU) by various routes in several animal species, single s.c. injections of MNU were observed to cause sometimes irreversible depilation at the site of injection, or irreversible depigmentation of the hair when the dark CBA mice were used (Figure 1). The depigmentation effects on the hair were particularly striking after a single dose, 0.5 ml of a 2.5% solution of MNU in 30% aqueous ethanol was injected into the marginal ear-vein of pigmented 'Dutch' rabbits ears. The hair overlaying the veins through which MNU had pressumably passed (before being diluted in the blood stream), became depigmented and showed the pattern of the underlaying veins (Figure 2). The depigmentation was noticed a few weeks after the injection and remained unchanged for more than 1.5 years.

Melanine is known to occur in melanocytes, specialized cells in the basal layer of the epidermis, probably derived from the neural crest. Its formation involves several steps, the first of which is the oxidation of tyrosine to L-Dopa (3,4-dihydroxyphenyl alanine) by tyrosinase, a copper protein complex present in melanocytes. This enzyme is believed to catalyse also some of the subsequent reactions leading from Dopa to melanine. These involve the oxidation of L-Dopa to the respective *ortho*-quinone, cyclization to 5,6-dihydroxy-dihydro-indole-carboxylic acid, oxidation of the latter to the respective red *ortho*quinone, hallachrome, its decarboxylation and eventually the polymerization of indole-5,6-quinone, to melanins, which probably form complexes with proteins[2].

The structures of melanins and the effects of various agents on their formation have been intensely studied. The melanins give ESR signals for free radicals; they appear to contain besides indole-5,6-quinone, small numbers of units of some of its precursors[3].

Irradiation, whether with ultraviolet rays, or small doses of X- and γ-rays as well as local application of carcinogenic polycyclic aromatic hydrocarbons are known to induce increased skin pigmentation[4]. These agents have been shown to activate the usually non-pigmented melanocytes and also to cause their proliferation. This

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