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Calcium oxalate crystallizing properties of polyanions elaborated by cultured renal proximal tubular cells

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Abstract To study the influence of renal polyanions on crystallization of urinary calcium oxalate, we recovered polyanionic macromolecules from media conditioned by primary cultures of renal proximal tubular epithelial cells of rats in serum-free, hormonally defined medium. Cells cultured on microporous supports showed a higher degree of morphological and functional proximal differentiation into a polarized monolayer than those on plastic impervious substrata. Papainization of the polyanions yielded the glycosaminoglycans chondroitin/dermatan sulphate and heparan sulphate. These accounted respectively for 60% and 80% of the crystal nucleation-promoting activities of polyanions recovered from the apical and basal media conditioned by polarized cultures on microporous supports. Similar relative activities were observed among the urinary glycosaminoglycans and polyanions similarly tested. Primary cultures of polarized proximal tubular epithelial cells are useful then as an *in vitro* model to study the crystallizing activities of polyanionic macromolecules produced by renal cells.

Key words Calcium oxalate · Crystallization · Glycosaminoglycans · Renal polyanions · Renal cell culture

Crystallization of calcium oxalate in the renal tubular fluid constitutes a major predisposition to renal stone disease. In contrast to urinary microions such as citrate, pyrophosphate and magnesium which are endowed with crystallization-inhibitory activity [11], polyanionic urinary macromolecules assume a variety of roles in the crystallization of calcium oxalate. One factor which contributes to the varied role is the physical state or form in which the macromolecules can exist in the crystallizing environment – in vitro studies have shown that both glycosaminoglycans (GAGs) and Tamm-Horsfall glycoprotein exhibit crystallization-inhibitory activity due to the dissolved state of the macromolecules but promoting activity due to the immobilized state or aggregate form of the macromolecules [2, 6, 22, 24]. Structural microheterogeneity within any one class of GAG may additionally contribute to the varied role observed. This is evidenced by the unique crystal-active properties of the urinary chondroitin sulphate (CS) (a major urinary GAG class) of idiopathic renal stone formers, contrasting the non-active urinary CS of normal individuals or cartilage-derived commercial CS [28]. Similarly, urinary heparan sulphate (HS) (a minor urinary GAG class) of both stone formers and normal individuals was found to be distinctly more crystal-active than heparin [28], the commonly used commercial product taken to represent HS. These observations prompt a need to identify the major source of individual urinary GAG classes with a view to regulating urinary GAG output as a possible means of controlling urinary calcium oxalate crystallization.

In normal individuals, the output of urinary GAG classes was found to follow a circadian rhythm that was interpreted as a reflection of the metabolic turnover of proteoglycans of both extrarenal and renal origins [18]. Renal clearance and micropuncture studies, however, showed restricted filtration of the GAGs at the glomerular barrier and further uptake of filtered GAGs at the renal tubules [29]. We hypothesized then that in normal individuals and idiopathic calcium stone formers who do not suffer from disorders of connective tissue metabolism or diseases affecting the glomeruli, GAGs that end up in the urine reflect more closely the turnover of renal proteoglycans than that of extrarenal

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sources. As the proximal tubule is the major site of renal oxalate handling [25], cells of this nephron segment are the first target in our study of the output of renal proteoglycans and GAGs and their involvements in the crystallization of calcium oxalate.

In this paper, we report the establishment of primary cultures of polarized proximal tubular epithelial cells from the renal cortex of rats as an in vitro model to study the range of crystallizing activities of the polyanionic macromolecules produced by the renal cells.

Material and methods

Preparation of renal cell suspension

The method of Wong [33] was used with modifications. Minced cortices of decapsulated kidneys of 14-day-old Sprague-Dawley rats were digested (37 °C, 30 min) with 0.25% trypsin in Hanks' balanced salt solution and then centrifuged (800 *g*, 5 min). The pellet was further digested (37 °C, 60 min) with collagenase (1 mg/ml) and then centrifuged. The pellet was resuspended in a complete medium of basic medium supplemented with 10% fetal calf serum (Globe Pharm, UK). The basic medium was a 1:1 (v/v) mixture of Eagle's minimum essential medium (MEM, D-valine modified) and Ham's F12 medium (F12) containing hydrocortisone (5×10^{-8} M), insulin (5 µg/ml), transferrin (5 mg/ml), 3′,3′,5-triiodo-L-thyronine (5×10^{-12} M), prostaglandin E_1 (25 ng/ml), penicillin (100 IU/ml) and streptomycin (100 µg/ml). The suspension was transferred to a flask and incubated at 37 °C in 5% CO_2 for 3 h to allow attachment of non-epithelial cells such as fibroblasts to the plastic substratum. Measured aliquots of the unattached cells were sampled for counting in a haemocytometer as well as for enzymatic and protein assays. These primary isolates were then cultured.

Cell culture

Primary isolates were plated at 5×10^5 cells/cm^2 onto 100 × 20-mm dishes (Falcon) or 12-mm collagen-coated filters (Millicell inserts, Millipore, Mass., USA) in complete medium and incubated in humidified 5% CO_2/95% air at 37 °C. After 16 h, the monolayers were maintained in the serum-free (SF) basic medium. Conditioned media were collected 3 days after SF media had been introduced and every 3–4 days thereafter for 7 days for cultures on plastic. For cultures on filters, media were collected separately from the apical and basal compartments at similar intervals for 21 days. Each collection was centrifuged to remove cell debris. The supematants were mixed with protease inhibitors – phenylmethylsulphonylfluoride (1 mM), *N*-ethylmaleimide (5 mM), aminohexanoic acid (50 mM) and benzamidine (5 mM) and then stored at −20 °C until use.

As a diagnostic for cells of epithelial origin, cytokeratin expression in the filter cultures was evaluated using monoclonal antibodies (Sigma) against cytokeratin 18 and Pan-cytokeratin followed by staining with the horseradish peroxidase-labelled ABC complex (Vectastain, Vector Laboratories) and diaminobenzidine-hydrogen peroxidase as chromogen. Negative controls were processed using similar steps but without application of the primary antibody.

The cell layers were assayed biochemically for the activities of proximal tubular enzyme markers alkaline phosphatase [14] and leucine aminopeptidase [12], as well as for the activity of the distal cytosolic enzyme marker hexokinase [9]. Protein contents were estimated [15] with the use of bovine serum albumin as standard. As the primary source of the proximal enzyme markers is the brush border [12], the media collections were also assayed for these markers to provide an indication for differentiation of the cells into a polarized epithelium. Cell viability was assessed using the trypan blue exclusion method (Sigma Technical Information).

For morphological examination at the ultrastructural level, monolayers were fixed at 4 °C with 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Samples were post-fixed in 1% (w/v) osmium tetroxide, dehydrated by sequential immersion in ethanol and then embedded in Epon. Ultrathin sections were stained with lead citrate and uranyl acetate for examination with a Jeol SX100 transmission electron microscope (Japan) operated at 650 kV.

Recovery of macromolecules, polyanions and glycosaminoglycans from the conditioned media

Conditioned media from cultures on (a) plastic, (b) filters, apical compartment, and (c) filters, basal compartment, were pooled separately and treated with 4 volumes ethanol to precipitate the macromolecular components. Alternatively, the polyanionic components of the media were recovered by sequential precipitation, first as the cetylpyridinium (CP) salt and subsequently as the sodium salt as described [6]. Protein components in the polyanionic extract were digested by papain at 65 °C and the GAG components were recovered from the digest by the sequential precipitation procedure [28]. Urine samples from normal individuals were similarly treated to provide extracts for comparison.

Analytical electrophoresis of the recovered GAGs was performed on cellulose acetate in 0.05 M barium acetate buffer, pH 5.8, with the use of the Microzone apparatus (Beckman). The membranes were stained with 0.2% alcian blue [19]. Chondroitin/dermatan sulphate (CS/DS) and HS in the GAGs were identified by the disappearance of stained bands in the electrophoretograms of materials treated respectively with chondroitinase ABC [23] and nitrous acid [27]. Hexuronate contents of the extracts were estimated by the carbazole reaction [1] with the use of D-glucurolactone as standard.

Assay of crystallization activity

The assay [28] was performed on test samples dissolved in ultrafiltrates (pH 5.3, 1250 mOsm) at a nominal cutoff of 10 kDa (YM-10 membranes, Amicon) from urine of normal individuals. These solutions had been checked to be crystal free by microscopic examination before they were frozen (−20 °C, 16 h) to induce crystallization. The samples were then thawed at 37 °C and the population densities and sizes of envelope crystals of calcium oxalate dihydrate therein were determined with a haemocytometer. The neat urine ultrafiltrate was similarly treated. The data from the test sample were expressed as ratios to those observed in the neat urine ultrafiltrate to minimize effects due to microions in the ultrafiltrate and thus reveal the effects due to the test sample: a value of >1 indicates promotion and a value of <1 indicates inhibition.

Statistical analysis

All statistical analyses were performed using the Mann-Whitney U-test.

Results

Renal cortical tubular cells in culture

Cells cultured on plastic substratum exhibited partly a spindle-shaped and partly a cobblestone appearance at confluence (5 days after seeding). Collapsible domes

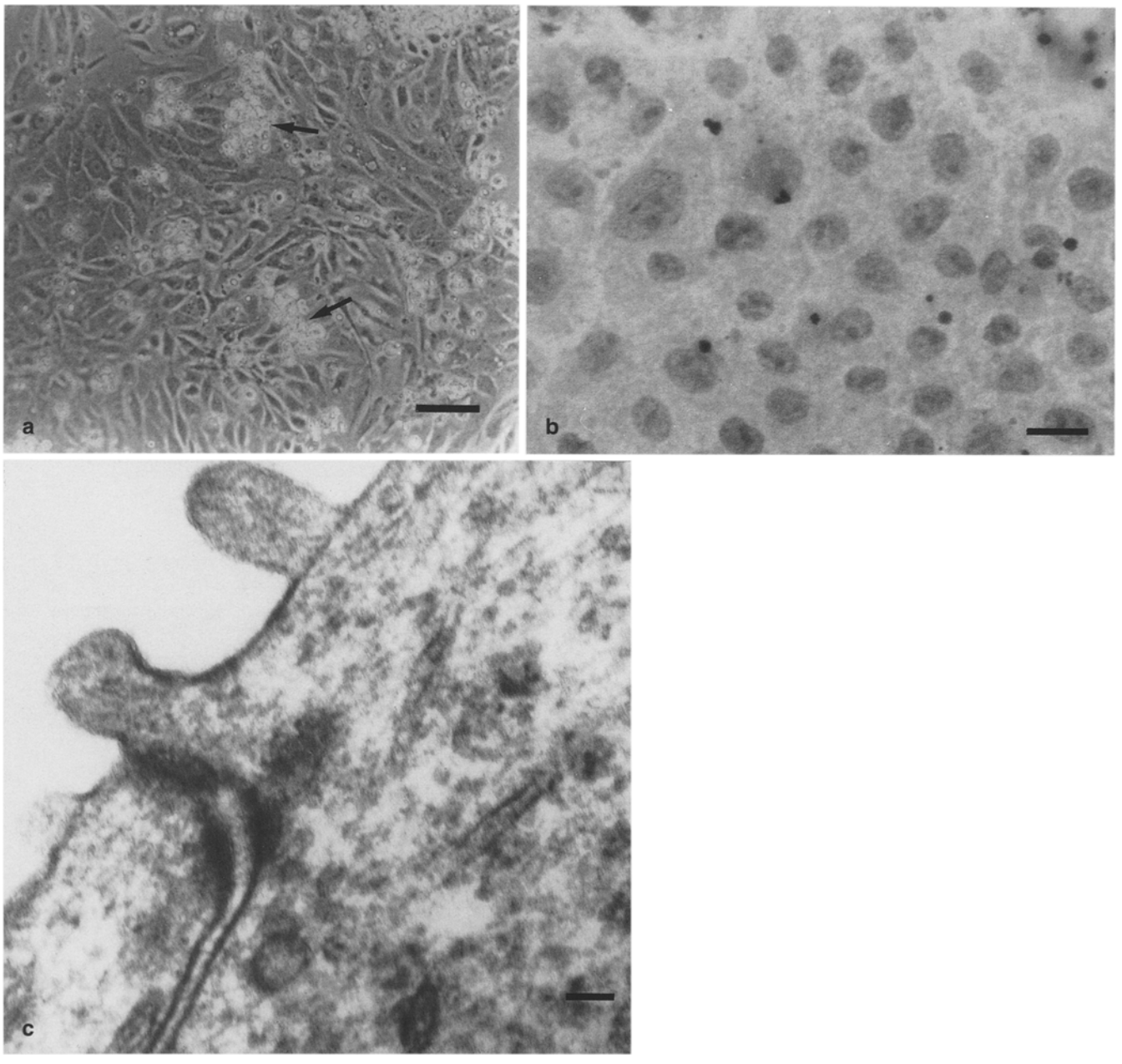

Fig. 1a–c Primary cultured proximal tubular epithelial cells grown in basic medium, at confluence. **a** Phase-contrast micrograph of cultures on plastic (4×10^6 cells/dish). Domes in the monolayer are indicated with *arrows* ($\times 400$). *Bar* 100 μm. **b** Light micrograph of cultures on filter, stained with H&E (3.5×10^6 cells/well). No domes are apparent ($\times 640$). *Bar* 15 μm. **c** Electron micrograph of the apical region of cells grown on microporous support, at confluence, showing microvilli, tight junctions and intercellular spaces ($\times 100\,000$). *Bar* 6.2 μm

were evident (Fig. 1a), indicating fluid transport from the medium on the apical side to the basolateral side facing the plastic substratum. Beyond confluence, this functional differentiation into a polarized monolayer on plastic could, however, not be maintained and viability was at 80%. In contrast, cells cultured on filters reached confluence 7 days after seeding and could be maintained for another week beyond confluence with viability maintained at 90%, the cells assumed the cobblestone appearance typical of epithelial cells in culture and no domes were observable (Fig. 1b). Electron micrographs of these latter showed single layers of polarized cells typical of simple epithelia-apical microvilli and tight junctions between cells at the apical end of the intercellular spaces (Fig. 1c). The elaboration of alkaline phosphatase exclusively into the apical media

Table 1 Enzyme characteristics of cultured proximal tubular epithelial cells

	Specific activities (units/mg protein)			Ratio of specific activities	
	AP	LAP	HK	AP:HK	LAP:HK
Cultures on plastic (up to 5 days in culture)					
Cultured cells	0.073 ± 0.004 (43%)	0.044 ± 0.009 (48%)	0.0014 ± 0.0003 (140%)	52	31
Conditioned media	$2.8 \times 10^{-4} \pm 0.60 \times 10^{-4}$	0.060 ± 0.005	ND		
Cultures on filter (up to 15 days in culture)					
Cultured cells	0.125 ± 0.005 (65%)	0.068 ± 0.018 (113%)	0.0013 ± 0.0002 (125%)	96	52
Conditioned media (a)	0.003 → 0.017	0.107 ± 0.008	ND		
(b)	0	0.092 ± 0.002			

Cells and media were collected at regular intervals from at least duplicate cell culture chambers of a representative experiment over 5 days for cultures on plastic and 15 days for cultures on filter. With the exception of alkaline phosphatase in the apical media of filter cultures, specific activities determined for the collections over the period in culture each varied about a mean and are therefore presented as means ± SEM. The specific activity of the indicated enzyme determined in the cultured cells relative to that in the primary isolate is represented as a percentage (parenthesis) in the column of that enzyme (*AP* alkaline phosphatase; *LAP* leucine aminopeptidase, *HK* hexokinase, (*a*), (*b*) media collected from the apical and basal compartments respectively of cultures on filter; *ND* not determined)

and leucine aminopeptidase, higher in the apical than in the basal media in the course of the 2 weeks in culture (Table 1), also provides evidence for functional differentiation into polarized cells. Beyond 2 weeks in culture, ultrastructural examination revealed increasing numbers of electron-dense bodies (possibly lysosomes) and readily detached senescent cells.

Epithelial cells can be distinguished from other cell types by the expression of proteins from the cytokeratin intermediate filament family. Cytokeratin 18 is present in simple epithelial cells and Pan-cytokeratin is expressed in both simple and stratified epithelial cells [17]. Confluent monolayers showed positive staining for cytokeratin 18 and Pan-cytokeratin in 76% and 78% of cells respectively; 19% and 21% respectively of these immunostained cells were identifiable as fibroblasts while the remaining 5.0% and 0.3% were negatively stained but showing epithelial morphology. The majority (80%) of the cultured cells are thus simple epithelial in origin.

The enzymatic characteristics of the cultured epithelial cells are compared in Table 1. Over the period in which the majority of cells remained viable, the filter cultures showed higher specific activities of the proximal marker enzymes, alkaline phosphatase and leucine aminopeptidase than the cultures on plastic. Similarly, higher percentages of cellular specific activities of the proximal markers relative to the corresponding primary isolates were observed in the filter cultures. In contrast, the cultured cells showed similar specific activities of hexokinase, the distal marker enzyme, and these were slightly higher than the corresponding primary isolates. It is apparent then that the filter cultures were more differentiated in proximal tubular characteristics than cultures on plastic and contamination of these cultures by distal tubular epithelial cells was generally low.

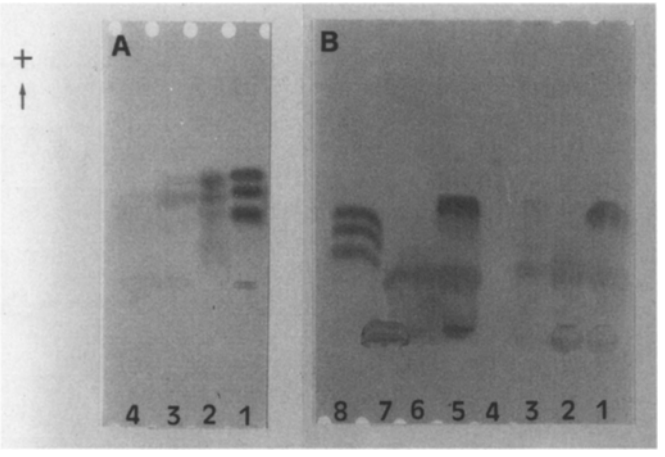

Fig. 2A,B Cellulose acetate electrophoretograms (alcian-blue stained) of glycosaminoglycans from conditioned media of renal proximal cells grown on (**A**) plastic or (**B**) microporous supports (*lanes B1–3* the apical compartment, *B5–7* the basal compartment). *Lanes A1 and B8* contain the reference standard mixture: heparin, DS and CS, in increasing order of mobility. *Lanes A2, B1 and B5* contain the untreated GAG mixtures of the conditioned media. CS/DS in the samples was digestible by chondroitinase ABC (*lanes A4, B2 and B6*) and HS in the samples was removed by nitrous acid treatment (*lanes A3, B3 and B7*). Electrophoresis was performed at 5 mA (constant current) for 30 min

GAG composition of media conditioned by renal cortical tubular cells

Macromolecules recovered from the conditioned media by ethanol precipitation contained both proteins and GAGs. After digestion of the proteins, the GAGs that remained were separable electrophoretically into alcian blue-stained bands of mobilities similar to reference CS/DS and heparin (Fig. 2). The bands were confirmed

as CS/DS and HS by their respective removal subsequent to treatments with chondroitinase ABC and nitrous acid (pH 4). In accordance with these criteria, GAGs recovered from cultures on plastic were identified as CS/DS and HS. In the case of GAGs recovered from filter cultures, the electrophoretograms showed not only the presence of CS/DS and HS, but also alcian blue-stained material resistant to both chondroitinase ABC and nitrous acid (Fig. 2). The resistant material recovered from the apical compartment showed a band of mobility intermediate between reference DS and heparin and that from the basal compartment remained immobile at the origin. These were also resistant to hyaluronidase, keratanase, ribonuclease and deoxyribonuclease, thus ruling out the possible occurrences of hyaluronate, keratan sulphate and the nucleic acids in the preparation. The relative intensity of alcian blue-stained bands suggests that in all media studied, CS/DS and HS were predominant and the unidentified material resistant to the test treatments was a minor component, if present.

Crystallization study

As reported earlier [6], urine ultrafiltrates showed minimal crystallization ($0.78 \pm 0.56 \times 10^4$ crystals/ml, 2.30 ± 0.44 μm) of calcium oxalate from the ions endogenous in urine. Introduction of macromolecular materials recovered from either normal human urine or the culture media into the urine ultrafiltrate resulted in enhanced crystallization over that observed in the neat urine ultrafiltrate. Ratios of population density of crystals were, to varying extents, greater than unity, indicating crystal nucleation-enhancing activities of the test macromolecules; ratios of size of crystals were invariably slightly less than unity, indicating crystal growth-inhibitory activities of the test macromolecules. A comparison of the nucleation-promoting and growth-inhibitory effects of the different macromolecular extracts (on the basis of the same hexuronate concentration) on crystallization of calcium oxalate from ions endogenous in urine ultrafiltrate is shown in Fig. 3. Since the crystal growth-inhibitory effects are similar

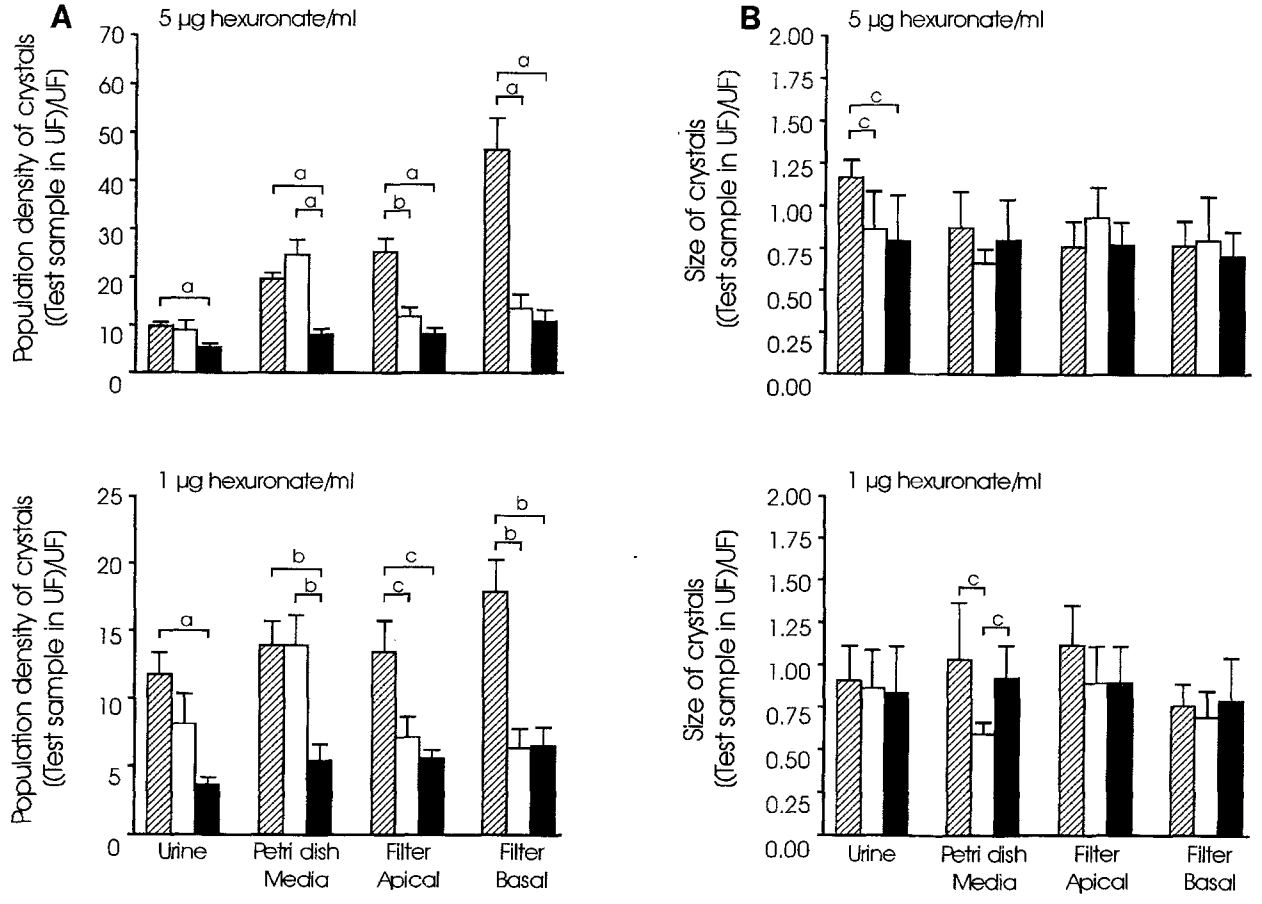

Fig. 3A, B Calcium oxalate crystal nucleation-promoting (**A**) and growth-inhibitory (**B**) activities of ethanol-precipitated macromolecules (*striped*), CP-precipitated polyanions (*blank*) and GAGs (*solid*) recovered from human urine, media of cultures on plastic and media of apical and basal compartments of cultures on microporous supports. Assays were performed at hexuronate concentrations of 5 μg/ml and 1 μg/ml. All values are expressed as mean ± SEM ($n = 12$). *UF* urine ultrafiltrate, *a*, *b*, *c* statistically different at $P < 0.001$, $P < 0.005$ and $P < 0.050$, respectively

among the macromolecular extracts, difference in crystallization activities of the macromolecular extracts is due to their different crystal nucleation-promoting effects.

Extracts from urine

Both the ethanol-precipitated macromolecules and CP-precipitated polyanionic component showed similar nucleation-promoting activities at a hexuronate concentration of 5 μg/ml. This indicates that the activity demonstrated by urinary macromolecules is largely due to the polyanions therein. That the activities due to both the macromolecular and polyanionic extracts were not reduced when the extracts were studied at the lower concentration of 1 μg hexuronate/ml suggests that the effect of the protein components predominates over that of the GAG components when both are present in the test mixture. After papain digestion, the recovered GAGs still exhibited 60% of the level of activity shown by the polyanions at the hexuronate concentration of 5 μg/ml and 37% at 1 μg/ml. Thus the crude urinary GAG mixture, considered apart from the urinary glycoproteins, does exhibit concentration-dependent nucleation-promoting activity on the crystallization of urinary calcium oxalate.

Extracts from culture media

The ethanol-precipitated macromolecules from the medium conditioned by cells cultured on plastic showed nucleation-promoting activity similar to the CP-precipitated polyanionic component from the same medium at hexuronate concentrations of either 5 μg/ml or 1 μg/ml. The activity shown by the macromolecules is therefore largely due to the polyanions therein, similar to the observations made of the urinary counterparts. These activities were, however, sensitive to the GAG content as significantly lower ($P < 0.05$) nucleation-promoting activities were observed at the lower hexuronate concentration. The papainized product, consisting essentially of GAGs, showed activities half that of the polyanionic extract. It is therefore possible for the GAGs elaborated by renal cells in culture to exert their nucleation-promoting influence when they are present in a mixture with the glycoproteins.

The materials recovered from media conditioned by cells cultured on filters also showed gradation of nucleation-promoting activity with decrease in hexuronate content. When comparisons are made at the same hexuronate concentrations, the macromolecules recovered from the apical compartment showed activity similar to those recovered from media of cells cultured on plastic but those recovered from the basal compartment showed significantly higher activity. The difference became non-apparent when the activities shown by extracts of polyanions were compared. The difference was therefore attributable to macromolecules that are not polyanionic. Of the activity due to the polyanionic component, 60% was observable in the GAG mixture of the apical media (similar to that observed in the case of urine) and 80% in that of the basal media. Thus, the activities shown by these renal polyanions are largely due to the GAG components.

Discussion

In the present study, monolayer cultures of renal cortical cells of the rat kidney were established either on tissue culture plastic or on filter supports. These assumed morphological polarity with sparse apical microvilli and well-defined tight junctions. Functional polarity was evidenced by the ability to form multicellular collapsible domes [31] in the case of cells cultured on plastic and the preferred apical release of brush border marker enzymes in the case of cells cultured on filters. Eighty per cent of the cells at confluence were positively immunostained for both cytokeratin 18 and Pan-cyokeratin [17], thus indicating that the majority of the cultured cells were of the simple epithelial type. One index to determine whether the epithelial cells are of proximal or distal tubular origin is the ratio of activities of marker enzymes for the respective segments – the alkaline phosphatase/hexokinase activity ratio of proximal:distal nephron segment preparations was 63 for nearly pure preparations of the segments from rat kidney [4]; this was derived from ratios of 285 determined for proximal segments and 4.5 for the distal segments. Our cultured cells showed ratios (Table 1) lower than that of the proximal segment preparation but higher than that of the distal segment preparation, suggesting that the cultured cells were differentiated with more proximal than distal enzymatic characteristics. Our ratios are also higher than those calculated from the data [16] of 12- to 30-day cultures of epithelial cells of the S1 segments obtained by microdissection of renal proximal tubules of the rabbit.

We also followed the activities of the marker enzymes to monitor the characteristics of the cells during the period of culture. Specific activities of alkaline phosphatase and leucine aminopeptidase in our cultures were lower than in the fresh isolates. Similar reduced activities were reported in primary cultures of proximal tubular cells on impervious supports [3, 16, 32] and attributed to the reduced development of the brush border system of cells in vitro. Our cultures on filters showed less reduction than cultures on plastic. It follows that the microporous support is preferred to plastic as a substratum that sustains differentiation of proximal tubular characteristics.

In order that polyanionic proteins and proteoglycans endogenous in serum do not interfere with the

characterization of crystallizing properties of polyanions arising from the cultured cells, a SF, hormone-supplemented medium was adopted [31] for the maintenance of the cells in vitro. D-Valine was included in our SF medium to select for growth of epithelial cells and suppress growth of fibroblasts [5]. Attempts to use MEM [D-Val] in the SF medium resulted in poorly attached "dangling" cells; the problem was overcome in part by the use of F12 in combination. L-Valine, a component of F12, is therefore present but at deficient levels in our SF medium of a 1:1 mixture of MEM [D-Val]/F12. An initial incubation in serum-containing medium was also necessary to facilitate attachment of the cells to the substratum. Subsequent change to the SF medium affected neither attachment nor survival of the cells, whether they were on plastic or on filters. Immunohistochemical examination of cells at confluence showed a distribution of 80% simple epithelial cells and 20% fibroblasts. Thus, the SF, D-valine- and hormone-supplemented medium was competent in maintaining proximal tubular epithelial cells of the rat in vitro but contamination by fibroblasts, though reduced, cannot yet be eliminated.

This is the first report of the primary culture of proximal tubular epithelial cells for the study of the polyanions elaborated by these cells in the crystallization of urinary calcium oxalate. Thus far, renal epithelial cell lines have been raised to allow the study of segment-specific transport functions and neurohormonal regulation of these functions [7]. Epithelial cells of the collecting duct have also been cultured to study the crystal-binding property of the polarized epithelial cells [20, 21]. Until the present study, no attempt has been made to assess the crystallizing activity of polyanionic metabolites elaborated from renal epithelial cells, separate from those which result from renal handling of metabolites from other tissues. The polyanionic materials recovered from the papainized extract of medium conditioned by the cultured cells were found to contain CS/DS and HS. In SF cultures, the expression of proteoglycan families by cultured renal epithelial cells appears to depend on the glucocorticoid supplement. In cultures supplemented with the glucocorticoid analogue dexamethasone, alone or in combination with insulin, only HS proteoglycan was detected and it was immunolocalized to the basal layer of the extracellular matrix [10, 13]. Hydrocortisone, the glucocorticoid included in our culture medium, appears to differ in that it allows both CS and HS proteoglycans to be synthesized and elaborated by the cultured cells into the medium. This composition is similarly observed in serum-containing cultures of renal epithelial cells [13] as well as in the polyanionic population recovered from urine [28]. That the cultured renal epithelial cells are able to elaborate into the medium the same GAG classes as those found in urine provides support for renal cell culture as a model to study the renal cellular contribution to "crystal-active" proteoglycans and related GAGs.

Despite assessment at the same hexuronate concentration, the calcium oxalate crystal nucleation-promoting activity of the GAGs differed with the source from which they were extracted (Fig. 3). This would be the case if the GAG classes in the different extracts differed in crystal nucleation-promoting activity. Among the urinary GAGs, HS from renal stone formers or normal individuals were found to be active; isomers of CS recovered from urine of stone formers were also active, contrasting the basal activity of urinary CS of normal individuals [28]. Among the tissue-derived reference GAGs, HS was found to be active, but neither CS, DS nor heparin showed basal, if any, activity in promoting crystal nucleation [28]. In the present study, the urinary GAGs were from normal individuals – given that urinary CS is preponderant over urinary HS, activity of the urinary GAGs is expected to be low and in agreement with the result. On the other hand, HS is the preponderant GAG in the basement membrane of renal epithelial cells [10, 13, 30] as well as in basal secretions of our cultured cells. If HS can be considered to be generally nucleation-promoting, irrespective of source, it is then not unexpected that GAGs recovered from the basal media of our cultured cells should show higher activity than those of urine. Only when the structural features that confer crystallization activities in the GAGs are identified can assays targeted at these features provide meaningful estimates of concentration of crystal-active species in the different extracts.

Assessment at the same hexuronate concentration also showed significantly higher crystallization activity due to macromolecules recovered from the basal than from the apical compartment of filter cultures. As activities due to the polyanionic components of the apical and basal compartments do not differ significantly (Fig. 3), the difference shown by macromolecules of the two compartments is due to glycoproteins low in anionic charge. Urinary glycoproteins reported to affect calcium oxalate crystallization include Tamm-Horsfall mucoprotein [24], nephrocalcin [8] and uropontin [26]. They are found on the apical surface of cells lining the lumen of subsets of nephrons and would be shed into the apical medium of polarized epithelial cells in culture. Of these glycoproteins, only nephrocalcin is of proximal tubular origin and would likely contribute to activities that we observed in macromolecules recovered from the apical compartment of our filter cultures. The other two are products of the distal tubules and the ascending limbs of the loops of Henle; their contribution, if any, would be minor when the majority of our cultured cells are proximal in characteristics. Apart from the study of crystal-retention activity due to exposure of basal lamina of injured nephrons [21], little attention has been paid to possible crystallization activities arising from basal secretions. Our culture model has therefore made it possible for future studies in this direction.

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