greater in the mutant (P < 0.001) and remained constant with age. The heart was not affected.

These results indicate that the myotonic mutation has a significant effect on the total glucose-6-phosphatase activities of skeletal muscle and kidney, but whereas the former is raised the latter is lowered. There is a small effect on the liver and no effect on the heart. Myotonic dystrophy is known to affect membrane structure [5] while the activity of glucose 6-phosphatase is known to be very sensitive to changes in the membrane environment [see [1]]. The activity changes reported here are therefore most probably secondary to changes in the membrane. The results do, however, implicate the kidney as well as skeletal muscle and may help explain the involvement of glucose metabolism [6, 7].

Analysis of urinary proteins in urolithiasis by isoelectric focusing using ultra-thin-layer gel

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Urinary proteins are generally analysed by SDS/PAGE whereby separation is achieved on the basis of molecular mass. An increased presence of low molecular mass urinary proteins in the range of M, 100,000–66,000 approximately, was detected in urinary samples from patients with urolithiasis [1] by ultra-thin-layer SDS/polyacrylamide-gradient-gel electrophoresis. Increased excretion of protein in renal disease has also been reported [2–4].

In the present communication, we present the results of isoelectric focusing on ultra-thin-layer gels, of urinary samples from patients with urolithiasis.

Twenty-four-hour urinary samples were obtained from 30 patients with urolithiasis. The total protein concentration of these samples ranged from 1.9 to 37.5 mg/dl as determined by the method of Bradford [5]. Although these samples contained much higher concentration of protein than those from normal individuals (>1.5 mg/dl), very faint protein bands were obtained in the ultra-thin-layer isoelectric focusing gel (0.4 mm × 120 mm × 180 mm). Hence, samples were concentrated five times by dialysis. For dialysis, samples contained Triton X-100 (0.005%, w/v), phenylmethanesulphonyl fluoride (1 mm) and aminoacetonitrile hydrogen sulphate (1 mm). The mixture was dialysed, in a membrane with an M, 6,000–8,000 cut-off range, against Tris/HCl buffer containing polyethylene glycol (20%, w/v) and the same concentrations of Triton X-100 and the proteolysis inhibitors for 1 h followed by dialysis against Tris/HCl buffer containing only the proteolysis inhibitors. The concentrated samples were subjected to ultra-thin-layer isoelectric focusing.

The isoelectric focusing results of the concentrated urinary samples from patients with urolithiasis revealed several protein bands in the pH range 3.5–6.5, but mainly between 4.5 and 5.5. The result obtained for a pathological sample is as shown in Fig. 1 and indicates the presence of acidic proteins. The result shows that the increased protein in urolithiasis are acidic in nature. Thus the procedure adopted was rapid and effective in showing increased presence of acidic proteins in urine from patients with urolithiasis.

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Fig. 1. Isoelectric focusing of urinary proteins in urolithiasis, using an ultra-thin-layer gel

A 5% (w/v) polyacrylamide gel (0.4 mm thick) containing 5% (v/v) amphotolys of 3.5–6.5 pH range was used for isoelectric focusing (IEF) on a horizontal system. Phosphoric acid (1 M) and sodium hydroxide (1 M) were used at the anode and cathode, respectively. After pre-electrophoresis of the gel at 500 V (constant voltage) for half an hour, sample (25 μl) was applied on an IEF sample applicator (10 mm × 5 mm) and the gel was run at 2000 V (constant voltage) for 4 h. The gel was then fixed followed by staining with Coomasie Blue R250 and scanning at 550 nm on a CAMAG densitometer using the transmittance mode. pH was measured using a surface electrode.


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