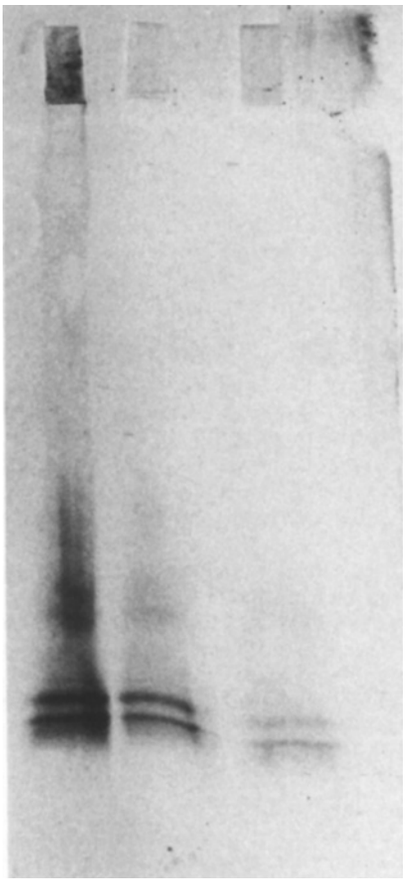

Fig. 1. *SDS/PAGE of renal stone proteins*

Samples were isolated and prepared according to conditions described in the text.

affected individuals. So far a number of biopolymers of quite diverse nature, including RNA, glycosaminoglycans and Tamm–Horsfall mucoproteins (THM) have been suspected to be involved in the inhibition or promotion of agglomeration of calcium oxalate and/or urate crystals, hence aiding renal stone genesis [3, 4]. More recently it has been shown that THM, albumin and transferrin, along with some low molecular mass proteins, exist as components of the organic matrix of calcium oxalate and urate stones [5].

In a previous communication [6], we reported the presence of at least three low molecular mass proteins in the urine of patients with nephrolithiasis, which might be specifically associated with this condition.

In order to investigate the possible involvement of proteins in stone formation we decided to analyse renal stones for the presence of such proteins which might be actively participating in this process. Identification of such proteins as essential constituents of renal stones would have an important bearing in determining a more specific promoter of renal stone formation. For this study we selected ten renal stones of various sizes and five gall stones. The outer surface of each stone was washed once with 0.1 M-HNO_3 and once with 2% (w/v) SDS. These washings were carried out to make all stones free of any contaminating mucus. After breaking the stone into two halves, the nidus was removed and crushed into fine powder. For the extraction of proteins, powdered nidus was mixed with a ratio of 1:4 (w/v) in 0.065 M-Tris–HCl buffer containing 2% (w/v) SDS, 5% (v/v) β-mercaptoethanol and 10% (v/v) glycerol. This mixture was then heated to 100°C for 10 min. After centrifugation, supernatant was removed and analysed by thin-layer SDS/PAGE [7]. Fig. 1 shows the electrophoretic profiles of proteins extracted from renal stones. Renal stone proteins extracted by this procedure were shown to be composed of three protein species as detected by SDS/PAGE. It appears that these proteins exist as very tightly attached components to other constituents of stone matrix, since these were totally unextractable by any other method using non-denaturing conditions. When β-mercaptoethanol and SDS were excluded from the extraction buffer no protein band was detected on SDS/polyacrylamide gels. These proteins also seemed to be specifically associated with renal stones as gall stone sample extracts did not show the presence of such proteins when analysed by the same procedure.

The presence of these low molecular mass proteins in the core of renal stones and their firm attachment with other matrix substances strongly suggests that these proteins might have some influence on stone formation. Further studies are underway to fully characterize these proteins and to carry out quantitative measurements of these in urine and serum of stone formers.

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Evaluation of creatine kinase in the cerebrospinal fluid of patients with various neurological diseases

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Abbreviations used: CK, creatine kinase; CSF, cerebrospinal fluid.

The evaluation of creatine kinase (CK) in the cerebrospinal fluid (CSF) is of potential interest for determining the extent of injury in cerebrovascular accident and hypoxic–ischaemic brain damage [1, 2]. While assessing the qualitative or quantitative changes in CSF most investigators have used techniques like immunoassays and/or agarose-gel electrophoresis. Although CK-BB is the predominant species present in CSF, other isozymes and subforms of iso-

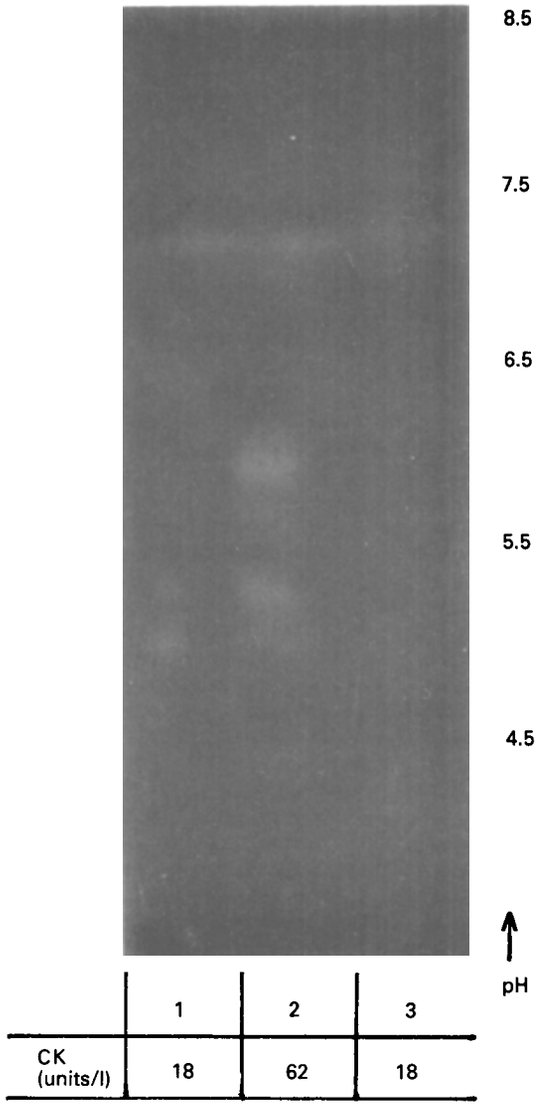

Fig. 1. *Isoelectric focusing of CSF CK*

Total CK activity is shown under each channel. 40 μl of CSF was applied in the centre of each lane. Lane 1 represents a sample from a myelitis patient, lane 2 a sample from a stroke patient and lane 3 a sample from an encephalitis patient.

zymes could also exist [3] which might prove useful in the diagnosis or monitoring of certain neurological disorders.

Recently, isoelectric focusing has been used to determine the CK-MM subforms in the sera of patients with myocardial infarction [4, 5]. This has instigated us to analyse the pattern of CSF CK by isoelectric focusing in disorders affecting the central nervous system. This technique, in combination with densitometric tracing, could provide information which might prove valuable in estimating the extent of brain damage. CSF samples from patients with various neurological disorders including stroke, myelitis, brain tumour, encephalitis and meningitis were examined for the presence of total CK activity using a spectrophotometric method. Detectable CK activity was present in two out of three samples of individuals suffering from myelitis and three out of four samples from encephalitis patients. Seven out of eleven CSF samples from patients with stroke showed a mean CK activity (S.D.) of 28.4 ± 15.8 units/l. No CK activity was detected in CSF samples from patients with brain tumours (2 samples) and meningitis (38 samples).

For the detection of the CK band(s) pattern, the ultra-thin-layer polyacrylamide isoelectric focusing technique was used. For this purpose the method of Williams & Marshall [6] was followed with some modifications. We used 160 mm × 120 mm × 0.3 mm polyacrylamide gels containing 2% (w/v) pharmalyte of pH 3–10. The samples (40 μl of CSF), in 5 mm × 10 mm strips of Whatman 1 filter paper, were loaded in the centre of focusing direction. The gels were focused at 2000 V for 4 h after a 30 min pre-run period at 1000 V. After isoelectric focusing a strip of cellulose acetate paper soaked in 2.5 ml of commercial CK reagent was evenly applied to the gel surface. Fluorescent bands of CK were visualized on a cellular acetate strip after 20 min under u.v. light.

Fig. 1 demonstrates the CK pattern of CSF samples. A major CK-BB band was present in the CSF samples from patients with three disorders, namely stroke, encephalitis and myelitis. The CK pattern in all three disease conditions was not identical as is evident from Fig. 1. In myelitis only one CK-BB was detectable while in stroke and encephalitis at least three other sub-bands in the region corresponding to MB (pI 5.0–7.0) were also present.

This study was directed to determine the effectiveness of isoelectric focusing in measuring the qualitative changes occurring in CK of CSF after brain damage. Our results indicate that CSF samples containing total CK activity as low as 18 units/l can be easily evaluated by this technique, since isoenzymic forms of CK such as BB, MB and MM, if present, can be separately analysed. This may prove beneficial in assessing the extent of brain damage in disorders like stroke. This technique has some definite advantages over other commonly used methods like immunoassays and agarose-gel electrophoresis currently in use to measure CK-BB activity in CSF [1, 3, 7]. The immunoassay procedures are sensitive but expensive. Agarose-gel electrophoresis is simple and convenient but less sensitive. Better resolving power and sensitivity puts isoelectric focusing ahead of electrophoresis, and its capacity to detect all isozymes and subtypes in a single application of sample gives it an edge over immunoassay. These observations suggest that isoelectric focusing could possibly serve as a useful tool in estimating the extent of brain damage in conditions like encephalitis, stroke and myelitis.

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