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COLLOQUIUM ON 'BIOCHEMISTRY OF URINARY CALCULUS FORMATION'

Physical Chemistry of Calcium Stone Formation

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The simplest explanation of calcium stone disease is that it is due to precipitation of calcium oxalate and/or calcium phosphate from an oversaturated solution. The degree of saturation of urine with respect to the stone-forming salts depends on the products of the relevant ion concentrations (or, more correctly, ion activities). Until recently it was not possible to measure activity products accurately because of the difficulty in measuring the concentrations of calcium, magnesium, phosphate and oxalate ions, as distinct from their total concentrations, but methods are now available for the measurement of Ca^{2+} by using tetramethyl murexide (Raaflaub, 1962), and liquid or solid-state electrodes (Ross, 1967; Schultz, Petersen, Mask & Buck, 1968). A few limited methods are also available for measuring Mg^{2+} concentrations (Walser, 1960; Heaton, 1967) but at present it is not possible to measure concentrations of phosphate and oxalate ions and these must be calculated from a knowledge of urine pH, ionic strength and the stability constants of the relevant complexes (Robertson, Peacock & Nordin, 1968; Pak, 1969; Finlayson & Miller, 1969).

Using these techniques Robertson *et al.* (1968) distinguished two states of supersaturation, (1) 'metastable supersaturation' in which urine may exist for long periods without precipitation occurring and (2) 'oversaturation' in which spontaneous homogeneous nucleation can occur. Most urine samples, whether from controls or stone-formers, were in a state of metastable supersaturation with respect to calcium oxalate and calcium phosphate

but the degree of supersaturation tended to be higher in the patients, owing to mainly higher concentrations of Ca^{2+} . Similar results and conclusions were reached by Pak (1969), who confined his studies to calcium phosphate stones.

Although these observations account for the growth of calculi, they do not explain the initiation of lithiasis, since nucleation requires either oversaturation or the presence of a suitable nidus (heterogeneous nucleation). Robertson *et al.* (1968) and Pak (1969) confined their studies to 24h urine collections, whereas it is clear that oversaturation need be only temporary for nucleation to occur. We have therefore examined the hour-to-hour variations in the activity products and have found that temporary oversaturation with respect to calcium oxalate or calcium phosphate occurs in both controls and stone-formers but is generally greater in patients than in controls. These periods of oversaturation generally occur after meals and are due mainly to an increased Ca^{2+} concentration, combined with a lower H^+ concentration and/or an increased oxalate ion concentration. The activity products at any given time of the day or night are generally higher in the patients than in the controls, suggesting that any crystals formed during periods of oversaturation will grow more rapidly (or dissolve more slowly) in the patients than in the controls.

These results indicate that homogeneous nucleation of either calcium oxalate or calcium phosphate may occur at some time of the day or night. The calcium crystalluria in recurrent stone-formers (Robertson, Peacock & Nordin, 1969b) and the chemical composition of small urinary-tract calculi (Hodgkinson, Peacock & Nicholson, 1969) suggested that crystallization of calcium oxalate was the first step in the formation of most calcium stones. Subsequent studies with the electron-probe microanalyser have confirmed that most small calculi consist predominantly of calcium oxalate but the

majority also contain small areas of calcium phosphate, which are not readily detectable by existing techniques such as X-ray crystallography. The morphological appearance of these deposits suggests that deposition of calcium phosphate may often precede the deposition of calcium oxalate.

These data permit a rational hypothesis for the cause of calcium stones. The first prerequisite is almost certainly hypercalciuria, which is generally due to an increased intestinal absorption of calcium (Peacock, Hodgkinson & Nordin, 1967). In the presence of a high calcium concentration, oversaturation of the urine and precipitation of calcium salts is readily produced by quite small variations in urine oxalate (for oxalate stones) or urine pH (for phosphate stones).

Although the above outline may suffice to explain renal stone disease, other more subtle mechanisms have been invoked at various times. Thus it is frequently suggested that normal urine contains factors that help to keep the calcium salts in solution. These include protective colloids (Butt, 1952), polyphosphates (Fleisch & Bisaz, 1962) and small peptides (Howard, Thomas, Barker, Smith & Wadkins, 1967). Although there is good evidence that these compounds will inhibit the precipitation of calcium salts *in vitro*, their role in stone formation remains unproven (MacLagan & Anderson, 1958; Russell & Hodgkinson, 1966; Lewis, Thomas & Tomita, 1966; Robertson, Hambleton & Hodgkinson, 1969a). Indeed, the recognition of a metastable state in urine (Robertson *et al.* 1968) appears to obviate the need to invoke nucleating inhibitors, although the possibility remains that these compounds inhibit crystal growth, rather than nucleation (Francis, 1969). Unfortunately, most studies of inhibition have been made without precise control of the degree of saturation of urine with respect to an appropriate solid phase and there is a need to re-examine this problem in terms of the state of saturation of urine.

Crystallization frequently involves, not only nucleation and crystal growth, but also crystal aggregation (Van Hook, 1961; Doremus, 1970), and this phenomenon may play an important part in stone formation. For example, large octahedral crystals of calcium oxalate dihydrate and aggregates of crystals up to 500 μ m in diameter were observed in the urine of stone-formers after an oral dose of sodium oxalate (Robertson *et al.* 1969b). In contrast, control subjects given the same dose of sodium oxalate showed little or no crystalluria. Whether these differences were due only to the higher Ca^{2+} and oxalate concentrations or to a deficiency of crystallization inhibitors in the stone-forming urines is under investigation.

Finally there is the 'matrix hypothesis', which postulates that certain macromolecular substances

in urine may actively bind Ca^{2+} to form a nidus (Boyce, Garvey & Norfleet, 1955). However, there is little experimental evidence to support this hypothesis (Keutel, King & Boyce, 1964), and it seems likely that the organic matrix of calculi is adventitious, its presence being due to the coprecipitation of protein with calcium salts (Vermeulen, Lyon & Fried, 1965).

We thank Dr R. Marshall for the data on diurnal variations in urine activity products, Mr A. Chambers for the electron micro-probe analyses and Dr. June Sutor for the X-ray-diffraction analyses of small urinary-tract calculi.

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Crystallographic Studies on the Formation of Renal Calculi

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At University College London we are studying urinary calculus disease by crystallographic tech-