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A Contribution to the Formation Mechanism of Calcium Oxalate Urinary Calculi

IV. Experimental Investigations of the Intrarenal Crystallisation of Calcium Oxalate in Rabbit

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Summary. Rabbits were given glyoxylic acid to induce intrarenal calcium oxalate crystal formation. The point of crystallisation in the renal tubule, the structure and the composition of the intrarenal crystals were studied. The initial crystallisation takes place in the proximal tubule. Calcium phosphate formation was excluded by microprobe examination. The comparison of the structures of the intrarenally formed crystals with those of Whewellite stones by scanning electron microscopy and the examination of isolated crystals by x-ray diffraction showed the intratubular crystals to consist of Whewellite.

Key words: Urolithiasis - Calcium oxalate - Intrarenal crystallisation - Scanning electron microscopy.

In previous studies (2, 8, 9) the formation of two calcium oxalate hydrates, Whewellite and Weddellite, were examined in vitro. The structure and conversion phases of the calcium oxalate hydrates in both the urine sediments and the stones were characterised by polarisation microscopy and scanning electron microscopic investigations (3, 6, 7).

We have studied now the in vivo formation of Whewellite and Weddellite. Because of our special interest in the primary stages of crystallisation we have used a method which results in reproducible intrarenal crystallisation of oxalate (12). The present study deals with the structure and the composition of the intrarenally formed crystals.

MATERIAL AND METHODS

The glyoxylic acid administration technique in rabbits served as an experimental model of the calcium oxalate crystallisation in animals. Male rabbits weighing 2-3 kg were used. The intrarenal crystallisation was induced by intraperitoneal administration of 120 mg of sodium glyoxylate per kg body weight, repeated three times (at 12 h intervals). Six hours after the last administration the kidneys of the anaesthetised animal were in situ irrigated vascularly with a perfusion solution (0.05% procaine and 25,000 I.U. heparin per litre of physiological saline) for 20 s and fixed with 2.5% glutaric aldehyde in physiological saline for another 20 min. Portions of the renal cortex and the renal medulla were dehydrated by the critical point method and coated with gold or carbon. The scanning electron microscope and microprobe investigations were carried out with a scanning electron microscope (IXA 50 A, JEOL, Tokyo).

In some cases sufficient quantities of intrarenally formed crystals were obtained for examintion by x-ray diffraction. For this, unfixed renal tissue was homogenized with an Ultra-Turrax TP 18-10 apparatus (Janke and Kunkel K. G.) and the crystals separated by repeated sedimentation. The occurrence of typical birefringent crystal shapes after sedimentation was checked by polarisation microscopy. There were no morphological changes. The air-dried crystals were examined by x-ray diffraction(x-ray diffraction unit, VEB Freiberger Präzisionsmechanik, Freiberg).

The experiments were carried out on 20 animals. Three untreated animals were examined for morphological comparison.

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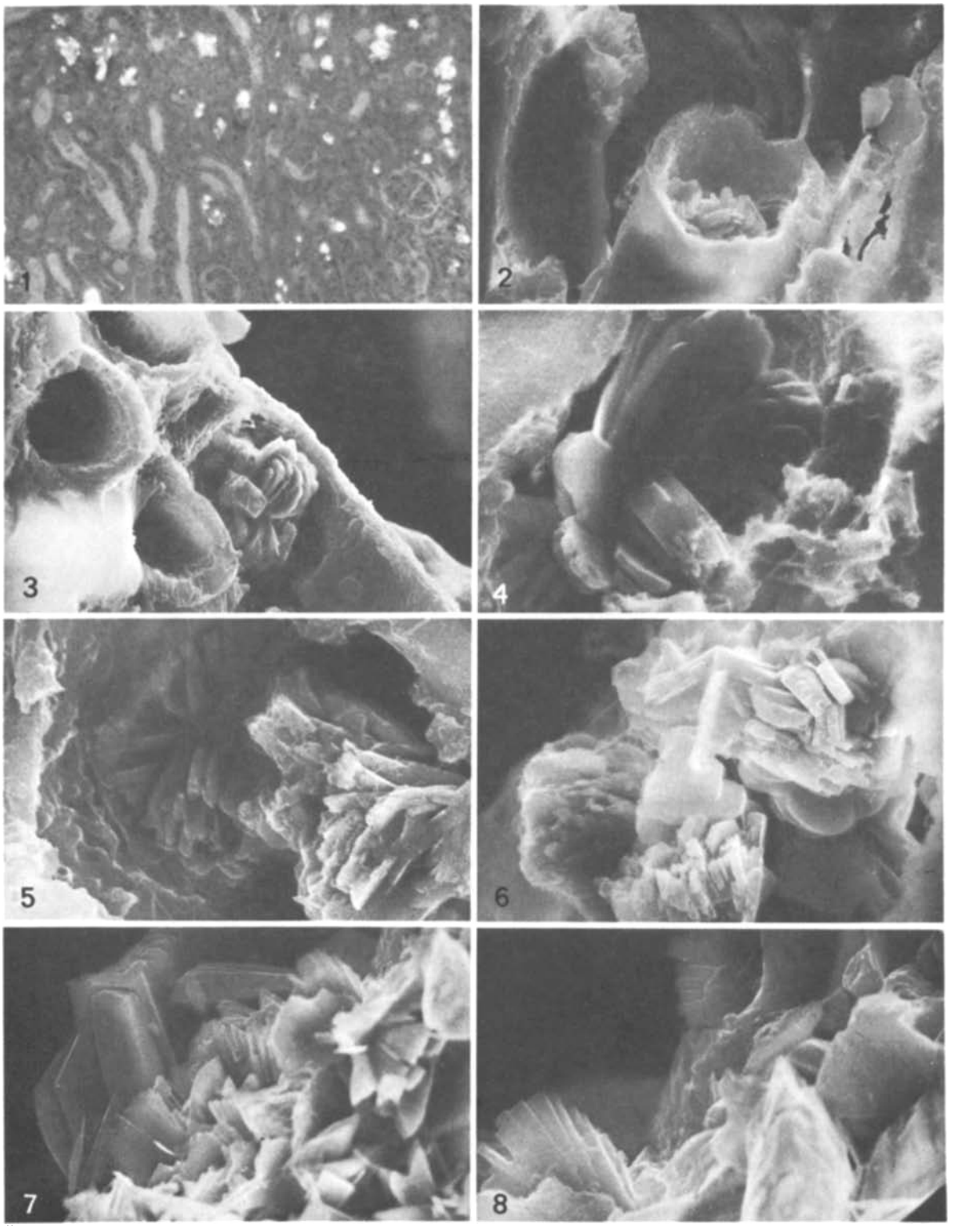

Fig. 1. Micrograph of the intrarenal crystal formation following glyoxylic acid administration; HE-staining, polarized light, crossed polarizers. x 50

Figs. 2 and 3. Proximal tubules with typical wafer-shaped crystal deposits, SEM. x 750

Figs. 4-6. Clod- and rosette-like structures of the intrarenal crystal formation, SEM. x 2, 250

Figs. 7 and 8. Scanning electron micrographs of Whewellite uroliths. x 750, x 1, 500

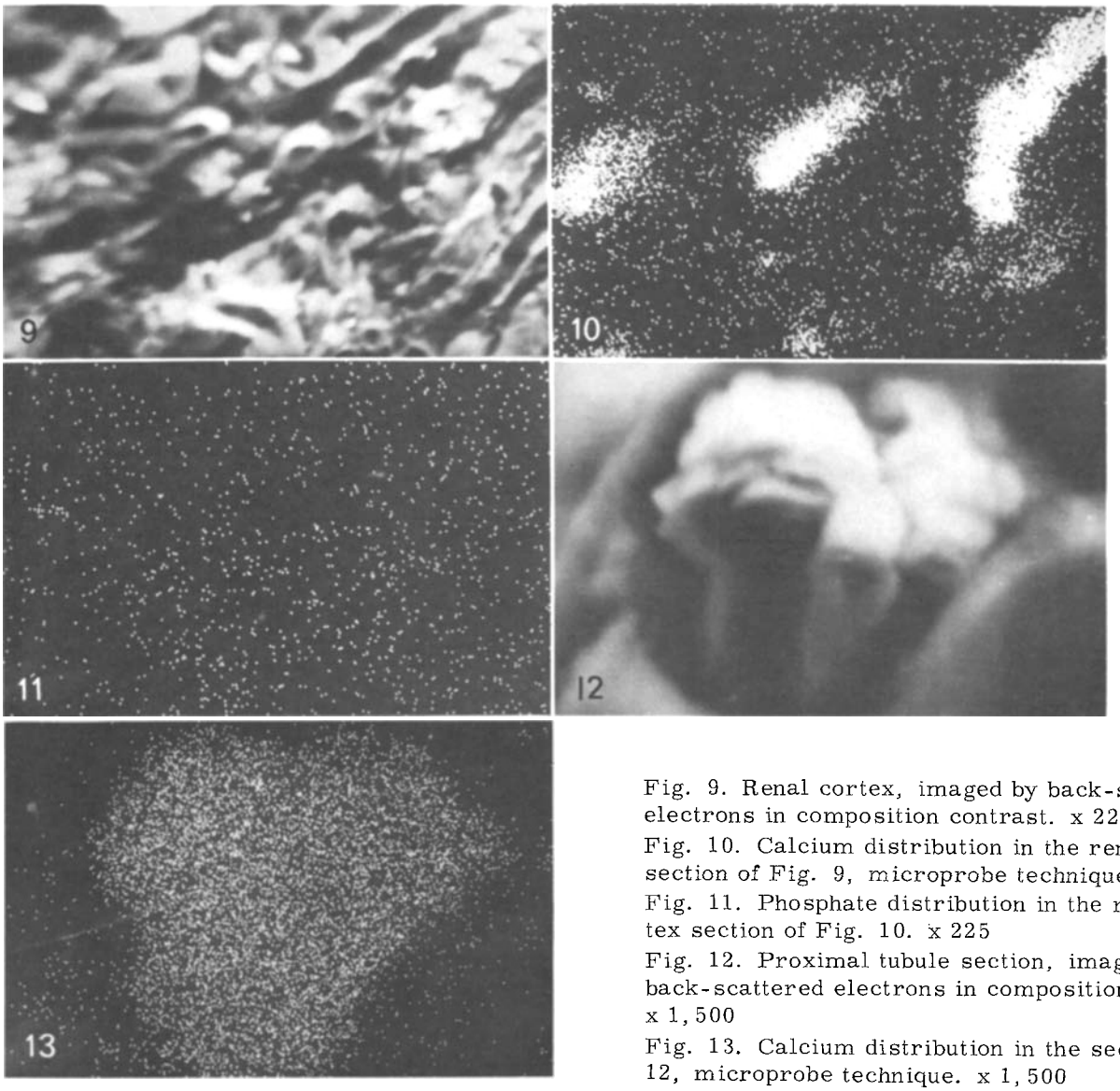

Fig. 9. Renal cortex, imaged by back-scattered electrons in composition contrast. x 225
Fig. 10. Calcium distribution in the renal cortex section of Fig. 9, microprobe technique. x 225
Fig. 11. Phosphate distribution in the renal cortex section of Fig. 10. x 225
Fig. 12. Proximal tubule section, imaged by back-scattered electrons in composition contrast. x 1,500
Fig. 13. Calcium distribution in the section of Fig. 12, microprobe technique. x 1,500

RESULTS

The glyoxylic acid intoxication method resulted in reproducible intrarenal crystallisation. The progress of the experiment was checked by optical microscopy (Fig. 1). The crystals were found exclusively in the renal cortex (see also Fig. 9), but never in the medulla. The transmitted light and scanning electron microscopic examinations of all specimens demonstrated that the initial crystallisation takes place in the region of the proximal tubule, seldom in more distal parts and never in Bowman's capsular space or the collecting tubule.

Compared with the control kidneys, the podocytes were swollen, Bowman's capsules were often enlarged and the brush border of proximal tubules had partially or completely disappeared or was deformed. More serious morphological changes were not observed. Sections of the renal cortex are shown in Figs. 2 and 3. The tubules were usually filled with crystal aggregates. Frequently the crystals were coated by tissue and intratubular albumin. The visible crystallites had various structures, clod-like and wafer-shaped aggregates being most common. Typical examples are seen in Figs. 2-6. In Fig. 5 the rosette-like microlith structure is clearly seen.

The calcium content of the crystals was affirmed by microprobe examination. Phosphorus had only a statistically normal distribution. The results of the microprobe examinations are shown in Figs. 9-13. The calcium enrichment in the renal cortex samples may be unambiguously attributed to intrarenal crystals and microliths.

Clod-like and radially symmetrical structures of the uroliths were similar to those found in Whewellite concrements (Figs. 7 and 8). The similarity of structure of the intrarenal crystallites and the Whewellite uroliths suggests similar compositions. X-ray diffraction analysis showed the experimental crystals to be composed of pure Whewellite.

DISCUSSION

Treatment of urolithiasis depends upon the examination of urinary sediment, urinalysis and determination of stone composition. The initial crystallisation point can only be determined in an animal model. By feeding 2.5% oxamide diet, Buss et al. (4) produced oxamide concrements in the rabbit kidney, which could be detected in the pelvi-calyceal system and in the distal urinary tract only. By oral application of 600 mg/kg of DL-3.a-dimethyltyrosine methylester hydrochloride, Datsis (5) succeeded in detecting the hydrolysed amino acid by scanning electron microscopy in the upper and lower urinary tract.

Bastian et al. (1) produced intrarenal calcium phosphate crystals by administering dihydrotachysterol and calcium and found calcium phosphate sediments in the Bowman's capsules.

These animal models described above cannot be fully applied to the conditions of human lithiasis.

Oxalic acid excretion can be raised by administration of ethylene glycol and glyoxylic acid, which was used for studying the response to treatment in animals (11, 12). As a consequence of recent investigations the glyoxylic acid administration was applied to the study of intrarenal crystal formation in the rabbit. The first microliths were formed in the proximal tubules. No crystals were detected in the glomerulus and are unlikely to be found there. Even with general oxalosis this part of nephron is usually not affected by the crystallisation process (10). Vainder and Kelly (13) demonstrated calcium oxalate crystallisation in the proximal tubules without oxalosis in a hyperoxaluric patient, secondary to jejunoileal bypass. Occasionally the pathologist may detect several intratubular calcium oxalate crystals even in routine examinations of human kidneys (14).

Microprobe investigation and x-ray diffraction analysis of the isolated crystals and comparison with the structure of calcium oxalate microliths in uroliths revealed the Whewellite structure of the intrarenal crystals. Our previous opinion (9), that Weddellite can also be formed by this method has not been substantiated.

According to the results presented here, in addition to the in vivo conversion of Weddellite into Whewellite (3, 7, 8, 9) the possibility of direct Whewellite formation must be considered.

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