

This work was supported in part by National Institutes of Health grants AM 05812, AM 07583, and 5T1 AM 5335.

J. GRAHAM SMITH, JUN.
RICHARD D. CLARK

Division of Dermatology,
Department of Medicine, and Center for the Study of
Aging,
Duke University Medical Center,
Durham,
North Carolina.

¹ Lucas, F., Shaw, J. T. B., and Smith, S. G., *Adv. Protein Chem.*, **13**, 107 (1958).

² Smith, J. G., jun., Davidson, E. A., and Clark, P. D., *Fed. Proc.*, **21**, 172 (1962).

³ Boas, N. F., *J. Biol. Chem.*, **204**, 553 (1953).

⁴ Gardell, S., *Acta Chem. Scand.*, **7**, 207 (1953).

⁵ Stoffyn, P. J., and Jeanloz, R. W., *Arch. Biochem. Biophys.*, **52**, 373 (1954)

Solubility of Renal Stones

THE cause of renal stone formation is unknown. Of the many current theories, majority opinion probably favours a 'supersaturation' mechanism, in which the urine concentrations of the ions of a poorly soluble salt exceed the 'precipitation product' for that particular salt, thereby causing spontaneous nucleation and subsequent growth of salt crystals.

The chemical composition of most stones is complex, and even if one considers only the calcium phosphates found therein, it is not clear which particular stoichiometry is actually involved in the nucleation and crystallization processes leading to stone formation and growth. Although the presence of $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ (ref. 1), $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ (ref. 2) and $\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4$ (ref. 3) has been claimed from the use of X-ray crystallographic techniques, and the presence of apatites and other more basic calcium phosphates suspected, we feel that identification of these compounds in renal stones may not be relevant to the actual physicochemical relationship which exists between the ions of calcium and inorganic phosphate in the stone and their concentrations in the corresponding urine. The critical feature of 'solubility' investigations is the identification of the lattice structure of the crystal surface, since it is the surface stoichiometry which determines the solubility of the solid phase in a bathing fluid, and consequently whether a stone will dissolve or grow under a given set of conditions.

Recently, MacGregor and Brown⁴ have applied a non-prejudicial method of determining the stoichiometry of a solubility equilibrium to bone mineral, and the same technique has now been applied to renal stones.

An unselected series of renal stones obtained from a urological clinic were finely ground and equilibrated in 'Cellophane' dialysis tubing with buffers, at constant ion strength, over the pH range 7-8. The details of the equilibration technique have been published elsewhere⁵.

The pH and calcium and inorganic phosphate ion concentrations were determined at equilibrium, and the negative logarithm of the ion products $[\text{Ca}^{++}][\text{OH}^-]^2$ and $[\text{H}^+]^8[\text{PO}_4^{3-}]$ calculated. The fit of $\text{pCa}(\text{OH})_2$ (y) against pH_2PO_4 (x) was determined by linear regression analysis, where the regression coefficient is the negative reciprocal of the Ca : P ratio of the solubility equilibrium.

The equilibration investigations with renal stones yielded a regression coefficient of -0.7591 ($S.E. \pm 0.0201$) which represents a Ca : P ratio of 7.90/6. The confidence limits of this ratio, using the 't' distribution, are 7.44/6-8.42/6 (5 per cent) and 7.25/6-8.66/6 (1 per cent).

We therefore believe that these data indicate that the solid phase determining the 'solubility' of calcium phosphate stones is octo-calcium phosphate (OCP), even although it may only be present as a surface structure

and may indeed only be a small proportion of the total mineral present.

J. MACGREGOR
B. E. C. NORDIN
W. G. ROBERTSON

University Department of Medicine,
Gardiner Institute,
Western Infirmary, Glasgow.

¹ Smothers, W. J., and Siegel, L. H., *J. Urol.*, **71**, 647 (1954).

² Prien, E. L., and Frondel, C., *J. Urol.*, **57**, 949 (1947).

³ Lagergren, C., *J. Urol.*, **87**, 199 (1962).

⁴ MacGregor, J., and Brown, W. E., *Nature*, **205**, 359 (1965).

⁵ MacGregor, J., and Nordin, B. E. C., *J. Biol. Chem.*, **235**, 1215 (1960).

Stimulation of the Uptake of Water and Ions by Indolyl-3-acetic acid : its Dependence on Nucleic Acid and Protein Synthesis

THE plant growth hormone indolyl-3-acetic acid (IAA) affects almost all aspects of growth and development, and the very remarkable promotion of growth is associated with a simultaneous stimulation of water uptake, ion uptake and several metabolic processes. We have previously presented evidence to show that the plant growth substances, IAA, gibberellic acid and kinetin, all regulate the synthesis and release of nuclear RNA and that the fraction of RNA involved has a base composition complementary to that of DNA¹⁻⁴. The synthesis of nuclear protein, as well as some enzyme systems concerned with the metabolic pathways known to be affected by IAA, is also stimulated by growth promotive concentrations of IAA^{4,5}. In this communication we report that the IAA-induced stimulation of water uptake and ion uptake also involves a nucleic acid system, that this process is inhibited by actinomycin D and that the inhibition is removed by IAA.

The materials used were disks of potato tuber 5 mm in diameter and the epidermal cells of the lower surface of the leaves of *Rhoeo discolor*, which are rich in anthocyanin and thus easy to observe under the high-power lens of a light microscope. These tissues have been used successfully by several workers in previous investigations on the effects of IAA on water uptake⁶. Potato disks were treated with actinomycin D, chloramphenicol, puromycin and porphyromycin 2 h after they were removed from the tuber. After a suitable incubation period IAA was added depending on experimental requirements. Thin peels of the midrib regions of *Rhoeo* were first plasmolysed in 0.5 M mannitol and deplasmolysed in water or in appropriate concentrations of the antibiotic to facilitate their uptake. This was followed by re-plasmolysis in mannitol before they were placed in IAA solution of varying concentrations. The concentrations of actinomycin D, porphyromycin, chloramphenicol and puromycin were 100 $\mu\text{g}/\text{ml}$., 10 $\mu\text{g}/\text{ml}$., 3.2 mg/ml., and 100 $\mu\text{g}/\text{ml}$.. Water uptake by the potato disks was determined by weighing them 22 h after incubation. Water uptake by *Rhoeo* cells was measured by noting the time taken for the cells to deplasmolyse. ³²P uptake was estimated by measuring the radioactivity of the dried tissue in an end-window β -counter. Actinomycin D, porphyromycin and puromycin were obtained through the courtesy of Dr. V. Bryson, of Rutgers University, Dr. G. Savage, of the Upjohn Co., Kalamazoo, Mich., U.S.A., and Dr. Fritz Lipmann, of the Rockefeller Institute, New York. Orthophosphate-³²P was supplied by the Atomic Energy Establishment, Trombay, India.

Experiments concerning the effect of IAA on water uptake by potato disks yield a two-phase concentration curve characteristic of the effects of IAA on growth; low concentrations are promotive but higher concentrations are inhibitory. With 10^{-6} M IAA water uptake is practically trebled, but when actinomycin D is also included in the incubation mixture the IAA-induced stimulation is cut down by about 50 per cent. Higher concentrations