

creased sites for polysome attachment per unit membrane. The two changes under consideration occur at the membrane, progressively throughout the log phase of growth. Consequently, our findings suggest that membrane constituents may play a regulatory role during bacterial growth, possibly through alterations in the number of sites available for polysome attachment, and for the localization of enzymes responsible for messenger degradation.

The range of changes observed in membrane content, and in the extent of ribosomal attachment to membranes during culture aging, might explain some of the negative results obtained on electron microscopic examination of bacterial cultures from early log phase for membrane-bound polysomes (14).

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## Newberyite in Ancient and Modern Urinary Calculi: Identification and Space Group

Abstract. Relatively large amounts of newberyite,  $MgHPO_4 \cdot 3H_2O$ , are found in old or very large urinary calculi. Single crystals of struvite,  $MgNH_4PO_4 \cdot 6H_2O$ , sometimes show some decomposition to newberyite on aging. The fact that large amounts of struvite are also found in ancient stones implies, however, that special conditions, as yet unknown, are required for decay to occur.

In 1956 (1) newberyite was first identified as a crystalline component of a kidney stone, the other main component being hydroxyl apatite. In 1962 (2) it was recorded as a very minor constituent of 250 stones from Leeds,

England, and T. Rokkones has found it in one out of 50 Indian and in five out of 25 Indonesian bladder stones studied in Norway (3). On the other hand Lagergren (4) does not mention it in his studies of bladder and kidney stones from Sweden, nor does Prien (5), although the crystallographic studies he made extended over a period of 23 years and covered 25,000 urinary calculi obtained from the United States.

We have found newberyite in 17 percent of the urinary calculi so far analyzed by x-ray diffraction sampling techniques. For identification purposes, the spacings and intensities of the reflections for the naturally-occurring material (6) were determined, since different samples of commercial preparations gave slightly different x-ray powder patterns all of which contained strong lines not listed in the ASTM Index (7). In the natural deposit a small amount of  $Mg^{2+}$  is replaced by  $Fe^{2+}$  and  $Mn^{2+}$ . Analysis figures (8) give the total amount of FeO and MnO as 1 percent. The effect on the unit-cell dimensions is negligible.

Accurate unit-cell dimensions were obtained from single-crystal oscillation photographs taken with the Ievniš-Straumanis mounting. The space group

Table 1. Interplanar spacings and the relative intensities of strong and medium reflections in naturally occurring newberyite. S, strong; M, medium; V, very.

hkl	d(Å)	I
111	5.941	V S
020	5.340	S
200	5.109	M
021	4.711	V S
210	4.609	S
102	4.496	S
112	4.145	S
220	3.691	M S
022	3.652	M S
202	3.574	M S
221	3.465	S *
122	3.437	S †
131	3.187	M S
311	3.086	V S
113	3.043	V S
023	2.832	M S †
302	2.816	S †
231	2.805	S †
132	2.789	S †
312	2.723	S
213	2.703	M
040	2.672	M
041	2.578	S †
400	2.555	S †

\* Combine to give one V S line; † Combine to give one S line.

Table 2. Occurrence of newberyite,  $MgHPO_4 \cdot 3H_2O$ , in collections of bladder and kidney stones examined by x-ray diffraction methods, compared with occurrence of struvite,  $MgNH_4 \cdot PO_4 \cdot 6H_2O$ . (a) Number of stones examined; (b) number containing newberyite; (c) number containing struvite; (d) number containing both; (e) percentage of newberyite estimated by sampling; (f) the same for struvite. All analyses were carried out at University College, London.

	Site	Date	(a)	(b)	(c)	(d)	(e)	(f)
Norwich, England (10)								
Juvenile	Bladder	1773-1909	50	19	12	12	8.6	2.9
Adult	Bladder	1773-1909	33	7	2	2	6.1	1.2
London, England (11)								
	Bladder	1829-47	8	1	1	1		
Norwich, England (12)								
(Mostly adult)	Bladder	1932-61	57	2	33	2	0.4	32.7
Indonesia (13)								
	Bladder	Modern	40	23	24	18	13.4	17.9
Northeast Hhailand (14)								
Juvenile	Bladder	Modern	58	0	4	0	0	0.7
Adult	Bladder	Modern	19	0	3	0	0	9.9
Adult	Kidney	Modern	63	5	15	4	1.8	17.4
Turkey (15)								
Juvenile	Bladder	1963-66	50	0	11	0	0	8.1
Juvenile	Kidney	1963-66	91	0	16	0	0	10.5

is *Pbca* with  $a = 10.215 \text{ \AA}$ ,  $b = 10.681 \text{ \AA}$ ,  $c = 10.014 \text{ \AA}$  (all  $\pm 0.002 \text{ \AA}$ ). Weissenberg photographs were used for indexing the reflections and for intensity estimations. The interplanar spacings  $d$  and relative intensities  $I$  of the strong and medium lines (including some not listed in the ASTM Index) are given in Table 1. These were obtained from a well-resolved Nonius powder photograph of the Skipton material, taken with  $\text{CuK}\alpha$  radiation.

Table 2 shows the amounts of newberyite found in our crystallographic studies of various collections, the percentage compositions being determined on an arbitrary basis founded on the sampling technique used (9) and compared with those of struvite,  $\text{MgNH}_4 \cdot \text{PO}_4 \cdot 6\text{H}_2\text{O}$ .

In general, it may be stated that newberyite is more common in the ancient than in the modern stones, with the exception of the modern collection from Indonesia, about which, unfortunately, no information is available except that they are bladder stones. This fact, together with the prevalence of ammonium acid urate (which occurred in 18 out of 40 stones and in 16 nuclei) indicates that many of these very large stones were probably begun in childhood (9).

We have found that a single crystal of struvite became contaminated with newberyite on the surface after some months. It seems probable that

the newberyite in urinary calculi or some of it, is formed by decay of struvite; but special conditions may be necessary for the decomposition to occur.

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  10. Dated stones from the Norfolk and Norwich Hospital Museum, England, by courtesy of Mr. Ridley Thomas, F.R.C.S.
  11. From University College Hospital Museum, London.
  12. Courtesy R. Thomas.
  13. This larger collection included those examined in Norway by T. Rokkones (see 3). The stones were all very large (some 4 cm or more linear) but no details were available concerning the ages of patients, and so forth.
  14. From Ubol Hospital, Northeast Thailand, courtesy Dr. C. Chutikorn.
  15. From Hacettepe Medical Centre, Ankara, Turkey, courtesy Dr. Dogan Remzi. It is rare to obtain such a large collection of kidney stones from children.
  16. We thank Dr. D. A. Andersen, F.R.C.S., for initiating these studies and for obtaining collections for us; and the Medical Research Council for financial support.
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*Saccharomyces cerevisiae*) and *Escherichia coli* tRNA were obtained from General Biochemical Co. The liver tRNA was a commercial preparation from Nutritional Biochemicals Corp.; tRNA from yeast in the logarithmic phase of growth was prepared from *Saccharomyces lactis* (4). Ribosomal RNA (rRNA) from yeast was prepared from repeatedly washed 80S ribosomes isolated from *S. lactis* in the log phase of growth (5). The tRNA fractions, Nos. 36, 41, 50, and 63, were prepared by chromatography of the purified log-phase yeast tRNA on a diethylaminoethyl cellulose (DEAE) column at pH 7.5 with a urea gradient, in the presence of 0.35M NaCl (6). The amino-acid-acceptor activities of these fractions have been described (6, fig. 1). Preparation of the purified tRNA species tested has also been described (6).

The samples of unfractionated tRNA and rRNA that were obtained as dry powders were taken up in dilute NaCl solutions. Fractions from chromatography were already in 0.1M NaCl. The content of RNA in test samples was estimated on a diluted portion in a Beckman DU spectrophotometer on the basis that 1 mg of RNA gives an optical density (O.D.) of 22 at 260 m $\mu$ . Stock test solutions were prepared accordingly. One tenth the volume of 1.0N HCl was added, and the solutions were hydrolyzed at 100°C for 1 hour. This relatively mild treatment presumably results in the release of about 50 percent of the purines in RNA (7). The hydrolyzates, neutralized with NaOH, were incorporated in RM-1965 nutrient medium (8) and tested in fivefold serial concentrations from 10 to 6250  $\mu\text{g/liter}$ . The media were sterilized in an autoclave (15 minutes, 120°C) after incorporation of the test samples. The NaCl contributed to the medium by the samples was no more than 10 mmole/liter and had no influence on the yields of tissue. The bioassay was done essentially as described (3, 8). The stock tobacco tissue was maintained on RM-1965 medium with 2000  $\mu\text{g}$  of 3-indoleacetic acid and 200  $\mu\text{g}$  of kinetin per liter; but before it was used for bioassays, it was successively subcultured twice on medium with the kinetin concentration lowered to 30  $\mu\text{g/liter}$ . To conserve the limited supplies of RNA, four replicate 50-ml flasks with only 20 ml each of medium were used to test each concentration. Two pieces of tobacco cal-

## Cytokinin Activity: Localization in Transfer RNA Preparations

**Abstract.** *Transfer RNA from yeast, liver, and Escherichia coli has cytokinin activity in the tobacco callus bioassay, whereas ribosomal RNA from yeast is inactive. In contrast to fractions of yeast transfer RNA rich in serine acceptor and cytokinin activity, preparations (70 to 90 percent pure) of arginine transfer RNA<sub>2</sub>, glycine transfer RNA, phenylalanine transfer RNA, and valine transfer RNA<sub>1</sub> and of highly purified alanine transfer RNA from yeast were inactive at concentrations of 20 to 2500 micrograms per liter. One molecule of 6-( $\gamma,\gamma$ -dimethylallylamino)purine per 20 molecules of yeast tRNA would account for the observed cytokinin activity. The number of major molecular species contributing to cytokinin activity of transfer RNA, therefore, must be small.*

The isolation and localization of one of the minor bases in serine transfer RNA (tRNA) by Zachau *et al.* (1) and its identification by K. Biemann *et al.* (2) as 6-( $\gamma,\gamma$ -dimethylallylamino)purine together with the earlier finding that this substance has exceptionally high cytokinin activity—that is, it promotes cell division, growth, and organ formation in the tobacco callus test (3)—suggest that specific tRNA

fractions may exhibit cytokinin activity. Conversely, these findings suggest that the action of cytokinins in growth and morphogenesis may be due to their function as constituents of specific tRNA molecules. Therefore, various RNA preparations from yeast and other sources have been examined for cytokinin activity, as judged by the tobacco bioassay.

Commercial yeast (stationary-phase