is *Pbca* with $a = 10.215$ Å, $b = 10.681$ Å, $c = 10.014$ Å (all $\pm$ 0.002 Å). 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 CuK_α 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, $MgNH_4 \cdot PO_4 \cdot 6H_2O$.

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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6. From Skipton Caves, Victoria, Australia, provided by the Keeper of Minerals, British Museum.
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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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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_2, glycine transfer RNA, phenylalanine transfer RNA, and valine transfer RNA_1 and of highly purified alanine transfer RNA from yeast were inactive at concentrations of 20 to 2500 micrograms per liter. One molecule of 6-(γ,γ-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-(γ,γ-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 *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 80*S* 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 *p*H 7.5 with a urea gradient, in the presence of 0.35*M* 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 0.1*M* 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 mμ. Stock test solutions were prepared accordingly. One tenth the volume of 1.0*N* 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 μg/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 μg of 3-indoleacetic acid and 200 μg 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 μg/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-

lus, about 50 mg each, were planted in each flask. The fresh and dry weights of the tissue were determined after a 5-week growth period. All experiments were done twice.

Comparative tests of "stationary-phase" tRNA, "log-phase" tRNA, and rRNA from yeast (Table 1) show the former two to be active and the latter to be inactive at the concentrations tested. The fact that rRNA was inactive was not due to growth inhibitors, because kinetin (5 μg/liter) added to the three highest concentrations of the rRNA increased the yield almost to the same extent as when the kinetin was added to the basal medium. Certainly increasing the concentration of RNA did not result in a corresponding reduction in the yield of tissue in the assay even if there was a slight overall depressing action of the rRNA samples on the effectiveness of kinetin.

tRNA's from *E. coli* and from liver also were active. The *E. coli* preparation, in which 70 percent of the total RNA was tRNA, was somewhat less active than the log-phase tRNA from yeast. The commercial preparation of liver tRNA was considerably less active.

Selected chromatographic fractions of yeast tRNA (*4*, *6*) were tested for cytokinin activity (Table 2). Fractions numbered 36, 41, and 43, which are enriched in serine tRNA, have relatively higher cytokinin activity than the unfractionated log-phase tRNA has; fraction number 50 with low serine tRNA content has less cytokinin activity, and fraction number 64 with little or no amino acid acceptor function has much lower cytokinin activity than the other fractions. However, isoleucine- and tyrosine-acceptor activity were also enriched in parallel to the cytokinin activity.

Arginine $tRNA_2$, glycine tRNA, phenylalanine tRNA, and valine $tRNA_1$, obtained in 70 to 90 percent purity by further chromatography of the yeast tRNA fractions (*6*) and bioassayed individually at concentrations of 20 to 2500 μg/per liter, were completely devoid of cytokinin activity.

Purified yeast alanine tRNA for which the complete base sequence is known (*9*) was also inactive.

The finding of cytokinin activity in tRNA prepared from yeast and liver confirms the report by Hall *et al.* (*10*), and was expected in view of the presence of 6-(γ,γ-dimethylallylamino)purine. Whether the assay procedure provides it as free base or in combined form (*3*, *10*), and to what extent the bioassay may be sensitive to intact nucleotides is unknown. In the tobacco bioassay we use, 6-(γ,γ-dimethylallylamino)purine is several-fold more active than its 9-substituted riboside (*11*), but both are highly active. In comparisons of hydrolyzed and unhydrolyzed samples of log-phase tRNA and of fraction number 43, the unhydrolyzed samples in each case were only about one-quarter as active. (But all samples were exposed to autoclaving for 15 minutes in the testing procedure.)

If 50 percent of the potential cytokinin activity is measured and it is all due to 6-(γ,γ-dimethylallylamino)purine, which has ten times higher activity than kinetin, the presence of one such molecule in 20 tRNA molecules would account for the observed activity. From this value and on the basis of negative results with the five purified tRNA species, we conclude that the number of major molecular species contributing to the high cytokinin activity of tRNA must be small. Hall *et al.* (*10*) have estimated the 6-(γ,γ-dimethylallylamino)purine content as 0.1 mole percent of the total nucleotides in yeast tRNA and 0.05 mole percent in calf-liver tRNA.

As 6-(γ,γ-dimethylallylamino)purine has been found in *Corynebacterium fascians* (*12*), it is also likely to be present and responsible for the cytokinin activity in *E. coli* RNA.

However, a variety of substituents, on the N^6-position of the purine ring confer high cytokinin activity on adenine derivatives and may be present or incorporated in tRNA. Thus, Fox (*13*) has reported that ^{14}C-labeled 6-benzylaminopurine is preferentially incorporated and is recoverable as such from tRNA in tobacco and soy bean tissues. 6-Methylaminopurine is a widely distributed minor base in tRNA and has

Table 1. Cytokinin activities of RNA preparations from yeast. Results represent the average weight of new tissue (grams per flask) obtained.

RNA fraction	Kinetin added (μg/liter)	Yield of fresh tissue (g/flask) *RNA concentrations* 10 μg/liter	50 μg/liter	250 μg/liter	1250 μg/liter	6250 μg/liter	Relative cytokinin activities [KE* (μg/mg)]
tRNA	0	0.17	0.22	0.24	1.12	2.26	1.4
tRNA†	0	0.21	0.21	0.22	0.55	2.82	1.6
rRNA	0	0.17	0.20	0.24	0.18	0.18	0
tRNA	5			1.19	1.93	3.12	1.1
tRNA†	5			1.63	2.20	3.40	1.6
rRNA	5			1.28	1.38	1.41	0
Kinetin controls							
Kinetin conc. (μg/liter)		0		1		5	25
Fresh weight (g/flask)		0.17		0.44		1.91	5.07

* KE (kinetin equivalents), micrograms of kinetin required to produce the yield of tissue obtained with a specified weight of test sample. † Prepared from *S. cerevisiae* in stationary phase. All other RNA samples were prepared from yeast *S. lactis* harvested during log-phase of growth.

Table 2. Distribution of cytokinin activity in yeast (*Saccharomyces lactis*) tRNA fractions from DEAE-cellulose column. Results are the average of two experiments (grams per flask).

Log-phase tRNA fraction	Yield of fresh tissue (g/flask) *RNA concentrations* 10 μg/liter	50 μg/liter	250 μg/liter	1250 μg/liter	6250 μg/liter	Relative cytokinin activities [KE (μg/mg)]
36	0.17	0.20	0.38	1.80	5.20	4
41	0.25	0.20	0.38	0.90	4.42	2
43	0.28	0.31	0.38	1.11	4.90	3
50	0.23	0.22	0.26	0.48	1.92	1
64	0.22	0.24	0.18	0.43	1.04	0.5
Unfractionated	0.16	0.17	0.18	0.86	2.83	1.4
Kinetin controls						
Kinetin conc. (μg/liter)	0		1		5	25
Fresh weight (g/flask)	0.16		0.40		1.71	5.29

weak cytokinin activity; concentrations of 50 μmole/liter or more are required for its detection in the tobacco bioassay. Such a high concentration could not have been attained in the present tests, and thus any contribution by 6-methylaminopurine to the observed activity was negligible. While bases other than 6-(γ,γ-dimethylallylamino) purine could have contributed to the total observed activity, the several purified tRNA species which were devoid of cytokinin activity have normal amounts of dihydrouridine, pseudouridine, and methylated purine bases. Also the ribosomal RNA was inactive.

Whether there is a functional relation between cytokinin molecules localized in tRNA and the growth and morphogenesis of tissue in response to administered free bases remains to be determined.

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Protoperithecia in Neurospora crassa: Technique for Studying Their Development

Abstract. *A technique is described which facilitates analysis of the development of individual protoperithecia in* Neurospora crassa. *The formation of this organelle proceeds in several clearly discernible steps beginning with the looping of a single hyphal filament and ending with a heavily pigmented, densely packed structure that is the mature protoperithecial structure. The effects of a number of environmental conditions on the development of protoperithecia in two wild types and in a female-sterile mutant strain, ty-1, are presented.*

Genetic and environmental factors affect the development of protoperithecia in *Neurospora crassa* (*1*, *2*). However, molecular genetic studies of this relatively simple, differentiating system have been hampered in part by the difficulty of obtaining a clearcut picture of the morphological sequence of events leading to the formation of these organelles. Descriptions of the development of protoperithecia in *Neurospora* have been limited to *N. sitophila* and *N. tetrasperma* (*3*, *4*), and these descriptions were not accompanied by photographs.

We report a technique permitting a more extensive study of the development of individual protoperithecia in *N. crassa*. We have extended previous observations on the effects of temperature on protoperithecial formation and shown that a female-sterile mutant (*4*,

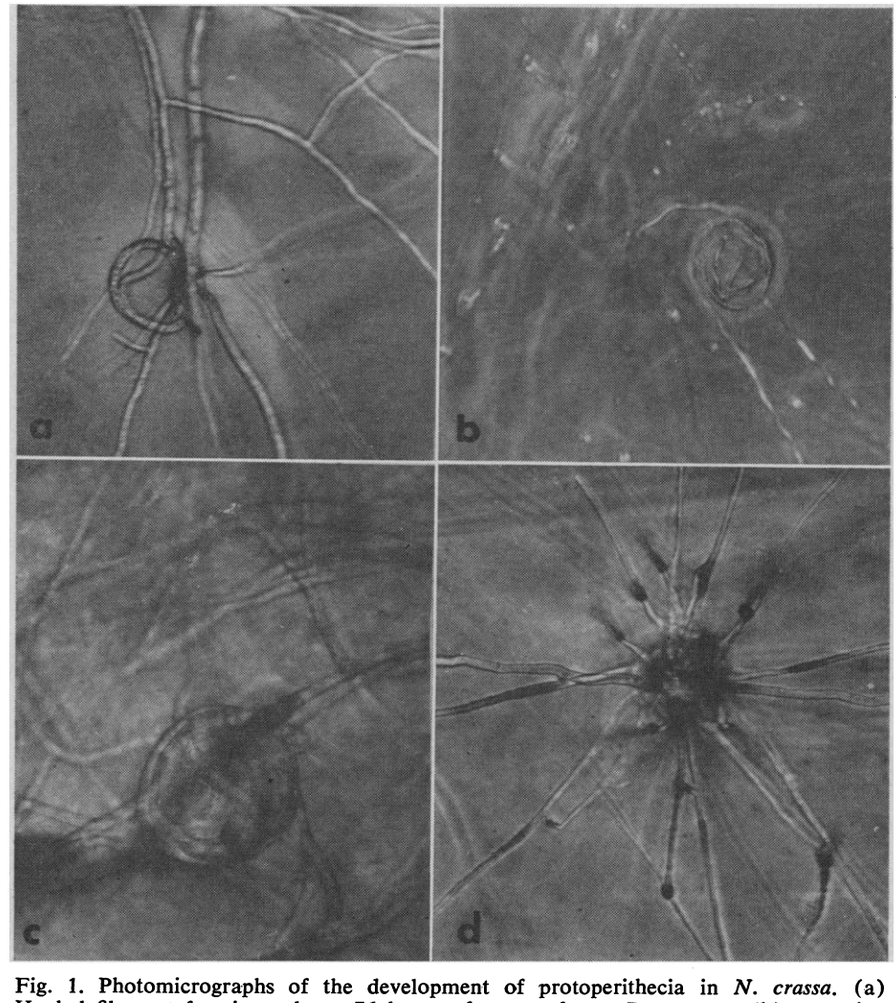

Fig. 1. Photomicrographs of the development of protoperithecia in *N. crassa*. (a) Hyphal filament forming a loop, 76 hours after transfer to Bacto-agar; (b) extension of hyphal loop to form coils, 78 hours; (c) further coiling, 84 hours; (d) filaments growing from an early protoperithecium, 88 hours (× 256). Photographs were taken on a Zeiss standard phase contrast microscope with 35-mm Zeiss attachment camera.