the medium containing C^{14}-glycine (0.02 μc/ml), benzylpenicillin (10 μg/ml), or cephalothin (200 μg/ml). This suspension was shaken at 37°C, and 2-ml portions were removed after 5 and 10 minutes, respectively, and added to 0.5 ml of 25 percent trichloroacetic acid. The assay methods for determining the degree of incorporation of C^{14}-lysine into mucopeptide and cell protein were, with slight modification, those described by Park and Hancock (*3*). Accumulation of *N*-acetyl glucosamine was determined after exposure of the staphylococci to 10 μg/ml of benzylpenicillin or 200 μg/ml of cephalothin in shake culture. The treated cells were extracted with 0.3*N* $HClO_4$ in the cold and then with hot 1*N* HCl. *N*-acetyl glucosamine was assayed by the borate method (*4*).

The results of studies of the effect of cephalothin and penicillin on the incorporation of C^{14}-lysine into the cell wall and cell protein fraction of *S. aureus* are given in Table 1. A striking degree of reduction in the quantity of labeled amino acid incorporated into the mucopeptide of the bacterial wall was observed. This was 89 percent after 5 minutes and 91 percent after 10 minutes, when the organisms were exposed to cephalothin and 82 percent and 89 percent, respectively, after the same duration of contact with penicillin. No appreciable diminution in the uptake into the protein of the "wall" amino acids was noted.

The data derived from investigations of the effect of cephalothin and penicillin on the accumulation of *N*-acetyl glucosamine in *S. aureus* show that a striking increase in the intracellular concentration of this material occurred. The quantities of *N*-acetyl glucosamine, detected when the organisms were exposed to cephalothin and penicillin, were 60 and 79 μg, respectively; only 0.02 μg was present in untreated bacterial suspension.

These studies indicate that the biochemical lesions produced in bacteria by both cephalothin and penicillin are results of selective inhibition of cell wall formation (*4*). The morphologic changes produced in bacterial cells by exposing them to both of these agents are also similar.

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Asynchronous Synthesis of RNA in Nucleoli of Root Meristem

Abstract. *During pulse incubations of onion roots with RNA precursors, incorporation into meristematic nucleoli is asynchronous for some, but not for all, cells. After a 30-minute labeling period, the "zero-class" is as large as 25 percent, and the asynchrony is intracellular rather than cellular. This suggests an individual specificity of nucleolar function in a population of differentiating cells.*

A number of recent reports and reviews are concerned with the possibility that the ultimate site of nucleolar RNA synthesis is the nucleolus-associated chromatin (*1*). While there is as yet insufficient evidence to permit a firm conclusion concerning the type or types of RNA elaborated in the nucleolus, the case for production therein of ribosomal precursor RNA has been greatly strengthened. This is particularly a result of experiments in which autoradiography was combined with sedimentation analysis of RNA synthesized during "pulse-and-chase" incubations (*2*). Also, the base composition of nucleolar RNA is like that of "cytoplasmic" RNA's (*3*).

It is of interest now to determine whether synthesis is synchronous in nuclei containing more than one nucleolus. True synchrony would be explicable on almost any hypothesis, but demonstrable asynchrony would weaken proposals requiring that the nucleoli act as nonspecific sinks or depots for RNA molecules assembled elsewhere.

Information on this point has emerged from studies in progress on nucleic acid synthesis in plant roots. The initial observation was that in roots given short pulses of tritiated RNA precursors, an unexpectedly large number of nucleoli remained unlabeled, even when the grain count over other nucleoli in the same cells was quite high. This suggested that nucleoli in the meristematic cells might belong to more than one population. An experiment was therefore designed to test this impression in a quantitative way.

Roots of *Allium cepa L.*, sprouted from bulbs, were treated with H^3-uridine (*4*) at 100 μc/ml (1 c/m mole), dissolved in Bonner's medium (*5*). Exposure to the labeled precursor was for 10 minutes in one series and for 30 minutes in another. After the pulse exposure, the excised root tips were washed thoroughly in fresh medium containing a 100-fold excess of unlabeled uridine, and then fixed in acetic acid–alcohol (1:3). Sections 3μ thick were obtained by routine histological methods. Prior to coating with emulsion, the sections, mounted on slides, were washed for 1 hour in 2 percent perchloric acid and for 18 hours in running tap water. This treatment removed unincorporated radioactivity. The slides were then dipped in diluted nuclear-track (Kodak NTB2) emulsion (1 part emulsion: 1 part H_2O) and stored in the dark for exposure periods of 5 days to 1 month.

One group of slides was treated with 20 μg of ribonuclease per milliliter (*6*) in tris-EDTA buffer (tris-hydroxymethylaminomethane-HCl, 0.05*M*, and ethylenediamine tetraacetic acid dipotassium salt, 0.025*M*) at *p*H 7.2. For this group, a control subset was incubated in the buffer without added enzyme. The digestions were done prior to dipping, and the resulting autoradiograms showed that for pulses of the durations employed all radioactivity incorporated into acid-insoluble material could be removed by ribonuclease. Hence, grains developed in the photographic emulsion, except for back-

Table 1. Distribution of grain counts over nucleoli in root meristem cells of *Allium*

Grain count class (n)	Number of nucleoli (c_n)	Frequency [$f(n)$]
0	136	.269
1	60	.118
2	94	.185
3	69	.136
4	61	.121
5	29	.057
6	36	.071
7	11	.021
8	9	.017
9	1	.002
	$\Sigma c_n = 506$	

Total counts: $\Sigma n \cdot c_n = 1218$;
$\mu = \Sigma n \cdot c_n / \Sigma c_n = 2.407$

ground, identified RNA alone. The autoradiograms were developed normally and stained through the emulsion with Azure B.

Median longitudinal sections of roots given a 10-minute exposure to H^3-uridine showed a gradient of incorporation through the four or five most peripheral cell layers, a central cylinder of the root remaining unlabeled. After a 30-minute pulse, radioactivity had been incorporated throughout the meristem. The grains were mainly over nuclei, and therein, mainly but not entirely over the nucleoli.

Figure 1 represents a situation very commonly encountered after the 30-minute pulse: two or more nucleoli within the same nuclear section, one of them either showing much less radioactivity than the others or being entirely unlabeled. It was necessary, therefore, to determine whether the observed distribution of silver grains was a result of the random decay process alone, or whether it indicated the presence of two or more populations of nucleoli, one of them effectively inactive in RNA synthesis during the period of the pulse incubation.

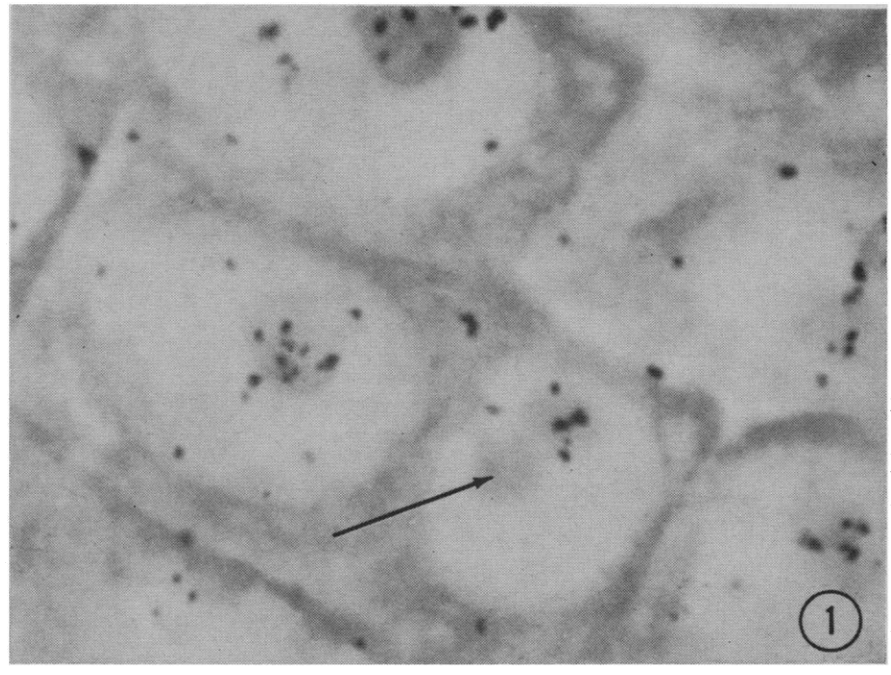

Fig. 1. Autoradiogram of *Allium* root meristem cells, showing incorporation of H^3-uridine into nuclei during a 30-minute pulse incubation. Most of the radioactivity is nucleolar, but some is extranucleolar. The arrow indicates an unlabeled nucleolus paired with a radioactive one in the same nucleus. Nuclei such as this, with two nucleolar bodies (but not necessarily with one unlabeled) were scored at random for the observations described in the text. Exposure time, 7 days; Kodak NTB2 liquid emulsion. Azure B stain. Approximately × 5000.

The normal number of nucleoli in *Allium* is four, but late in interphase coalescence takes place, resulting in a smaller number of distinct morphologic entities surviving to the beginning of mitosis. For the observations to be described, a large number of cells were selected at random in which two and only two distinct nucleoli were present. The emulsion films for this series had been exposed for 7 days, at which time the grain count per nucleolus could be scored with certainty, and the number of grains over labeled nucleoli was sufficiently high to suspect a very large zero class. Grains due to background radioactivity were negligible. Autoradiograms of a month's exposure also contained many zero-class nucleoli, but here the background had risen sufficiently to render scoring somewhat uncertain. Further analysis was therefore confined to the 7-day sets. A total of 506 nucleoli were scored, with grains varying from zero to nine per nucleolus.

Variation in grain count could arise from (i) unequal labeling of the nucleoli, (ii) unequal thickness of emulsion above the sections and unequal distances from nucleoli to the emulsion plane, (iii) randomness of background grains over the emulsion, (iv) local inhomogeneities in the film arising during photographic processing, and (v) the randomness of radioactive decay. We can estimate true unequal labeling most conveniently by determining whether the observed variation can be accounted for by the other sources. The emulsion is a liquid and is applied under conditions that minimize local inhomogeneities in thickness and composition. Also, the paired nucleoli were usually of about the same size and were in the same optical plane. Paired nucleoli were separated in their nuclei by only a few microns, often by less than one nucleolar diameter (Fig. 1). It is therefore unlikely that (ii) and (iv) contribute importantly to the variation in grain count. The background, as mentioned, was negligible everywhere in the film; this eliminates source (iii). Therefore, either observed variation in nucleolar grain counts is due solely to the randomness of radioactive decay or it represents true unequal labeling as well. For variation due only to random decay, it is possible to predict that the nucleoli will give grain-count frequencies which follow a Poisson distribution. If the observed frequency f, for a grain-count class n ($n = 0,1,2,3 \ldots 9$) depends primarily upon random decay, then the frequency should equal the Poisson probability $P(n)$,

$$f(n) = P(n) = \frac{\mu^n e^{-\mu}}{n!}$$

where μ is the arithmetic mean grain count.

The data obtained for a group of 506 nucleoli are given in Table 1.

The Poisson distribution function can be written in logarithmic form as:

$$ln\,[P(n)n!] = nln\mu - \mu$$

Therefore, if the observed frequencies are converted to terms $[f(n)n!]$, and the logarithms are plotted against the values of n $(0,1,2,3 \ldots 9)$, a straight line will be obtained if the data fit the theoretical distribution. The plotted points (Fig. 2) contain no assumption about the value of μ, the population mean, but the graph gives two estimates, one from the slope and the other from the intercept (7).

Figure 2 is a graph of the data from this experiment. The points fit a straight line reasonably well, except for the zero-class. The line drawn through them on the figure is calculated for a Poisson distribution with arithmetic mean of 3.21, obtained from the slope and intercept for the best-fitting func-

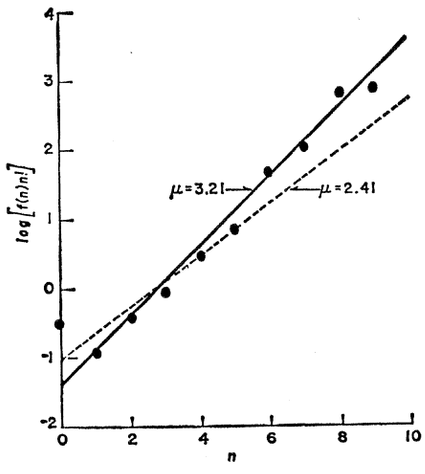

Fig. 2. Graph of observed grain-count frequencies from the data in Table 1. See text for method of plotting and interpretation.

tion. The zero class, represented by the point on the ordinate line, is very much too large. A population average calculated from all of the counts in Table 1 has the value 2.41. The line corresponding to a Poisson distribution having this mean value is also drawn on the graph. Even here, the observed zero class is too large, and in addition, this function does not fit the other data points.

It could be argued that a large zero class might indicate only the presence of some nuclei in which RNA synthesis was slower than in the general population. In such a case, the nuclei with zero-grain nucleoli should in general have only a small grain count over the second nucleolus. This was not observed. On the contrary, slightly more than half of the nuclei (52 percent) had one unlabeled nucleolus, and these nuclei accounted for 40 percent of all the silver grains. Since one of the two nucleoli in each had no grains, it is clear that zero grains over a nucleolus were not correlated with a low grain count over the partner. This is in itself evidence of asynchrony.

Thus, the nucleoli scored in this experiment are members of at least two classes; about 75 percent were synthesizing RNA during the pulse period and 25 percent were not. All the nucleoli engaged in synthesis were doing so, on the whole, at about the same rate, since their grain-counts are distributed as expected for a homogeneous population of radioactive units.

It is important to stress that the large zero class represents intracellular rather than cellular asynchrony, since all nonradioactive nucleoli included in the scoring were paired with normally labeled radioactive ones.

Root meristem is an asynchronous population of cells undergoing both mitosis and differentiation. Several important studies have been reported on variations in cell size, mitotic index, intermitotic time, and patterns of macromolecule synthesis within different zones (*8*). Asynchrony of RNA synthesis in the nucleoli of some, but not all cells of the meristem may be an early signal of cellular differentiation within the tissue. Our observation favors the idea that nucleoli have individual specificity of function within a single nucleus, since their rates of RNA synthesis may be very different at some point in the cell cycle.

Note added in proof. Since this manuscript was submitted for publication, it has been reported that the nucleoli of pea seedlings are centers for the methylation of transfer RNA (*9*). Nucleolar RNA from the same source can be hybridized with denatured homologous DNA (*10*), and the hybridizable stretches of DNA are complementary to ribosomal RNA. Further evidence is presented that some ribosomal RNA is manufactured on non-nucleolar chromatin and then transferred to the nucleolus. The authors cite evidence from their own and other laboratories which suggests that the ribosomal RNA's may be structurally diverse.

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Radiation-Induced Mouse Leukemia: Consistent Occurrence of an Extra and a Marker Chromosome

Abstract. *Granulocytic leukemia, induced in the primary mouse by x-irradiation, was serially transmitted to RF/Up mice. An extra chromosome, as well as a morphologicaly unusual chromosome, was found in the bone marrow cells of all the leukemic mice that had been injected previously either with leukemic spleen cells or with cell-free ultracentrifugates. This suggests that the changes in the chromosomes are caused by a virus.*

Granulocytic leukemia, induced in a mouse of the RF/Up strain by x-irradiation, was serially transmitted more than 25 times by inoculation of leukemic spleen cells, cell-free spleen ultracentrifugate, or plasma, to RF/Up recipient mice (*1*). The transmissability of the leukemia by cell-free filtrates containing virus particles was demonstrated by Parsons *et al.* (*2*), by means of electron microscopy. The similarity of the clinical and pathologic features of this disease (*2*) to those of chronic granulocytic leukemia of humans, and the regular occurrence of the Ph[1] chromosome in patients with the latter disease (*3*) prompted a search for a similarly characteristic abnormality of the chromosomes in RF/Up mice with this leukemia. In the marrow of all such mice examined to date we have found cells which show a consistent karyotypic abnormality.

The first mouse studied was a female who received an inoculum of plasma ultracentrifugate at birth. All the other recipient mice were 10-week-old males. The inoculum of leukemic spleen cells was prepared from tissue obtained from freshly killed leukemic donors. The spleens were minced with surgical scissors in chilled, sterile Tyrode's solution to give a suspension of 1.0 to 1.5 $\times$ 10^8 nucleated cells per milliliter. The re-