empty conchostracan valves in mudcracks of the few dry ponds encountered.

Black, silty, micaceous shales bore impressions of valves assignable to *Cyzicus* (*Lioestheria*) sp. Prominent hachure-like markings ornament growth bands; number of growth lines, 15; valve length, 2.25 to 2.40 mm; width, 1.65 to 2.10 mm. Three complete right and left valves and dozens of valve fragments, all with characteristic ornamentation.

Plant and molluscan fossils from this locality have recently been described [see bed A-33 (*9*)]. Conchostracans were not reported. Fragments of plant debris and a probable pelecypod (*Monotis*) occur on the black shale bearing fossil conchostracans. A brackish water environment is indicated.

A few comparisons can show the significance of the new data. Fossil flora at Santa Clara suggest an age equivalent to that of the Newark Group (Carnian) of Virginia (*9*, pt. 3, p. 7). *Lioestheria inornata* Raymond [=*Cyzicus* (*Lioestheria*)] is known from the Virginia beds (*10*). Other *Lioestheria* species have been found in equivalent beds in Pennsylvania and New Jersey (*11*), the Maritime Provinces of Canada (*12*), and the Shinarump formation of southern Utah (*13*). Thus, lioestheriid distribution in Carnian time ranged from Canada to Mexico and was parallel to the widespread North American distribution of living *Cyzicus*.

Since *Lioestheria* is found in the upper Navajo sandstone (Lower Jurassic) exposed near Shonto, Arizona (*14, 15*), there is indication of lioestheriid persistence beyond Carnian time at least in the Arizona-Mexico area. In this regard, the absence of living Cyzicidae from collections at the Chihuahua sites is of interest. Today *Cyzicus* occurs in all of our states bordering Mexico. As noted, fossil *Cyzicus* occurs to the west of Chihuahua. Farther west, in Lower California, *Eocyzicus digueti* (Richard) is known (*3, 16*). In Baja, California, Shaffer explored several large, dry, flat lake basins exceeding 8 km in diameter but no conchostracan valves were found. One certain locality for *Cyzicus mexicanus* (Claus) is Zimapan, State of Hidalgo (southeast of the Chihuahua collecting sites) (*7*).

The known species of *Leptestheria* and *Eulimnadia* are all Recent. The absence of a Mesozoic record of these genera (*17, 18*) therefore, indicates that the only form in the Upper Triassic pond sites in Chihuahua was *Cyzicus*. The occurrence of both *Leptestheria compleximanus* and *Cyzicus mexicanus* in a single Kansas pond or pool (*7*, p. 306) and the known distribution of *Leptestheria* and *Eulimnadia* in Texas today mean that these genera which have come into existence in late Mesozoic-Tertiary time have become widely dispersed in the Southwest.

Note added in proof. Eulimnadia geayi Daday 1926 and *Leptestheria vanhoffeni* Dad. 1923 are known from the State of Coahuila, which borders Chihuahua on the east.

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20 December 1963

Inhibition of Synthesis of the Cell Wall of Staphylococcus aureus by Cephalothin

Abstract. *Cephalothin, 7-(thiophene-2-acetamido)-cephalosporanic acid, suppresses synthesis of the cell of* Staphylococcus aureus. *Exposure to this agent led to a reduction in the degree of incorporation of carbon-14-lysine into the mucopeptide of the cell wall material and to an accumulation of* N*-acetyl glucosamine in the cell. The intensity of the lesions was comparable to that produced by penicillin.*

Studies in this laboratory have indicated that organisms exposed to cephalothin, 7-(thiophene-2-acetamido)-cephalosporanic acid, a derivative of cephalosporin C, undergo a series of morphologic changes which resemble closely those produced by penicillin and which were thought to result from a defective formation of the cell wall. Since cephalosporin C and penicillin are chemically related and both induce the same type of morphologic alterations (*1*), the mechanism of action of these antibiotics might be similar. This report presents evidence that this is indeed the case and that exposure to both cephalothin and penicillin leads to the accumulation of *N*-acetyl glucosamine in the cell, and to the reduction of mucopeptide synthesis in the bacterial wall.

Staphylococcus aureus SH (*2*) was inoculated into a medium consisting of yeast extract, peptone, and glucose. The inoculated medium was incubated at 37°C, with constant shaking, until the optical density of the culture reached 1.5 at 582 mμ. The culture was washed once with 0.03*M* phosphate buffer (*p*H 6.8), and the cells were suspended in

Table 1. The effect of cephalothin and penicillin on the incorporation of C^{14}-lysine into the cell wall or into cell protein. The signs in parentheses indicate: (+), no inhibition; (−), inhibition.

Time of exposure (min)	Specific activity (count/min per mg)		
	Control	Cephalothin	Penicillin
	Mucopeptide		
5	6,605	766 (−)	1176 (−)
10	13,327	1230 (−)	1496 (−)
	Protein		
5	2,808	2854 (+)	2750 (−)
10	4,868	5227 (+)	4515 (−)

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