early bactericidal effects of penicillin on enterococci, i.e., had some antagonistic action to that of penicillin. This lowering of the rate of bactericidal action occurred in combinations of Chloromycetin (10 μg/ml) with a wide range of concentrations of penicillin (6 to 60 μg/ml). This interference phenomenon was observed with all nine strains of enterococci but not always to the same degree.

Another observation should be noted: Whereas penicillin (6 μg/ml) alone permitted a great increase in the numbers of viable bacteria after the original sharp decline (Fig. 1), Chloromycetin-penicillin mixtures resulted in a slow but steady decrease of the population. Thus the late effects of this antibiotic mixture resembled those of high concentrations of penicillin (e.g., 300 μg/ml) alone, far outside the optimal zone. In both instances the number of viable bacteria diminished more slowly than at the optimal concentration of penicillin alone (6 μg/ml) but after long periods of incubation occasionally all enterococci succumbed.

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Cytologic Demonstration of Nucleic Acids in Tissue Culture[1]

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The tissue culture technique offers a means of investigating a great variety of biochemical and physiologic processes such as the nutritional requirements of the various cell types (*6, 15*), the action of drugs and other chemicals on growing tissue (*12*), the nature of various metabolic reactions and other biochemical changes in the medium (*7, 8*), and many other vital manifestations of growth phenomena (*9*).

The authors' interest in nucleic acid metabolism and the effect of nucleic acid inhibitors on growth and their relation to protein synthesis suggested the possibility of using tissue culture techniques in these investigations. It has been shown recently (*5*) that cells grown *in vitro* may be analyzed biochemically for desoxyribonucleic (DNA) and ribonucleic (RNA) acids, and that cytochemical methods are available for the demonstration of the presence of these nucleic acids in fixed sections of tissue culture. The following procedures have proved successful in demonstrating nucleic acids in cells grown *in vitro*.

The classical double cover slip technique of Maximow was used. Tissue (chick embryo muscle) was cut into small fragments (1 mm^3), embedded in a medium composed of equal parts of 50% chick embryo extract and rooster plasma,[3] incubated at 37° C for 72 hr, and fixed.

The following three fixatives were used: *Cowdry's*—equal parts saturated aqueous solution of mercuric chloride and 95% ethyl alcohol for 3 hr. *Carnoy's*—acetic acid-alcohol (1: 3) for 1 hr. *Serra's*—alcohol-formol-acetic acid (6: 3: 0.5) for 3 hr. Although Carnoy's and Serra's are not ideal fixatives, nevertheless they preserve nucleic acids well and permit excellent staining (*2*).

The cover slip preparations were washed thoroughly in running water after fixation in Cowdry's and Serra's fluids, and washed in descending strengths of alcohol after Carnoy's. They were rinsed in distilled water and dried slowly in air (*4*). This drying technique has proved very successful in eliminating the opacity produced in the plasma coagulum by fixation.

The basic dyes, methyl green and pyronin, have been shown to be specific for the two nucleic acids DNA and RNA (*1, 10*). By employing these stains in conjunction with specific enzyme digestion (desoxyribonuclease and ribonuclease), it is possible further to identify these acids. The Feulgen reaction was also used to demonstrate the presence of DNA.

Staining solutions used were:

Methyl green[4] (Grübler or National Aniline)	0.15 g
Pyronin (Grübler or Eastman)	0.25 g
Ethyl alcohol, 95%	2.5 ml
Glycerine, C. P.	20.0 ml
Carbolic acid-water (0.5%)	77.5 ml

The method of staining used was as follows: The cover slip preparations were stained in dye solutions for 20–30 min. They were rinsed in distilled water, blotted with filter paper, differentiated and dehydrated in tertiary butyl alcohol for 1–3 hr, then cleared in xylol and mounted in clarite. By this method, chromatin is stained green, nucleoli and cytoplasm red.

In studying enzyme digestion of desoxyribonuclease,[5]

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[3] The lyophilized embryo extract and plasma were kindly furnished by Dr. C. W. Christenson of Difco Laboratories, Inc., Detroit, Michigan.

[4] This dye contains a small amount of a violet compound which stains nonspecifically and must be removed. To achieve this, the chloroform purification method described by Pollister (*11*) was used.

[5] The desoxyribonuclease and ribonuclease were obtained commercially from Worthington Biochemical Laboratory, Freehold, New Jersey.

the crystalline enzyme was dissolved in triple-distilled water to give a concentration of 0.2 mg/ml. Magnesium sulfate was added to a final concentration of M/100, and gelatin, as a protective colloid, to a concentration of 0.01%. Ribonuclease the crystalline enzyme, was dissolved in triple-distilled water to a concentration of 0.2 mg/ml.

Three cover slip preparations were used. One specimen was placed in the desoxyribonuclease solution for 1 hr at 37° C, the second in the ribonuclease solution for 1 hr at 37° C, and the third, the control, in distilled water for the same period of time and at the same temperature. The specimens were then rinsed in distilled water and stained with methyl-green-pyronin as described. The Feulgen reaction may also be used to demonstrate DNA.

The results of desoxyribonuclease digestion are: Nucleolus and cytoplasm stain red, chromatin does not stain. The Feulgen test is negative, showing the absence of DNA. In ribonuclease digestion: chromatin stains green, nucleolus and cytoplasm are unstained.

It has been shown that nucleic acids can be extracted quantitatively by heating in 0.3 M trichloracetic acid (*10*). The application of this technique to cytologic preparations without destroying the cellular architecture has been reported (*10*). This method proved successful in tissue culture preparations by using the following procedure:

1. Place cover slip preparations in 0.3 M trichloracetic acid at 90° C for 5–10 min.
2. Rinse in three changes of distilled water.
3. Stain with methyl-green-pyronin or Feulgen reagents.

Since the nucleic acids are removed, the cells remain unstained and are Feulgen-negative.

The methods described provide techniques whereby RNA and DNA can be demonstrated in cells grown *in vitro*. These methods can be put on a semiquantitative basis by measuring the absorption at 2600 A with the ultraviolet microspectrophotometer (*3, 13*), or extinction with the photomultiplier tube photometer (*14*).

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Synthesis of 1-C^{14}-L-Ascorbic Acid

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To make possible an extension of research under way, dealing with the biological functions of L-ascorbic acid in the tissues of guinea pigs and albino rats, it was necessary to have available 1-C^{14}-L-ascorbic acid. The ascorbic acid thus labeled has been synthesized by a modification of the methods of Ault *et al.* (*1*) and Reichstein *et al.* (*3, 4*).

The steps involved cyanide addition to L-xylosone,[2] carried out under conditions to insure the maximum yield based on the labeled sodium cyanide. Forty-nine mg of sodium cyanide (1.0 millimole) which contained approximately 1 millicurie (mc) of C^{14} was added to 155 mg of L-xylosone (1.05 millimole) in 19 ml of water adjusted to pH 6.5. The addition of cyanide as shown by titration of the imino-L-ascorbic acid was 95% of the theoretical value. An additional 18 mg of sodium cyanide (0.37 millimole) was added to react with excess L-xylosone which, if not removed, interferes with final crystallization of the L-ascorbic acid.

The resulting imino-L-ascorbic acid was hydrolyzed to L-ascorbic acid in 2.6*N* HCl at 50° C for 22 hr. The radioactive carbon dioxide arising in part from decarboxylation of L-ascorbic acid was collected in saturated barium hydroxide solution. In pilot runs, the amount of barium carbonate formed was equivalent to the 15%–20% of ascorbic acid lost during hydrolysis.

The L-ascorbic acid was purified from mineral salts and other impurities by employing an ion-exchange column technique similar to that used in isolating the 2,4-dinitrophenyl-osazone derivative of L-ascorbic acid from the urine of the rat (*2*). An Amberlite IR-100 column was used to remove all cations which would prevent the adsorption of ascorbic acid on the Amberlite IR-4-B column. Impurities less acidic than ascorbic acid were preferentially eluted by 4% acetic acid prior to elution of ascorbic acid with 1*N* HCl.

The eluate from the anion column was evaporated to a syrup *in vacuo* under nitrogen, taken up in 15 ml of absolute alcohol, and further purified through an alcohol-ether fractionation. The final product was obtained in good yield as colorless crystals from methanol-ether-ligroin solvent mixture at −15° C.

In the radioactive synthesis, 180 mg of L-ascorbic acid was obtained which contained approximately 75% of carrier ascorbic acid added to facilitate crystallization in the course of the synthesis. This represented a final yield of approximately 25% based on the labeled sodium cyanide. The L-ascorbic acid had an activity of 2.10×10^6 cpm/mg.

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[2] Prepared from L-xylose kindly supplied by Dr. Robert Hockett of the Sugar Research Foundation.