veloped a differential equation expressing the rate of phage production in terms of the rate of bacterial growth and from it derived integral forms satisfactorily predicting the time of lysis, concentrations of bacteria at the moment lysis begins, etc.

More recently Scribner and Krueger,[6] investigating the kinetics of the phage-bacterium reaction in the presence of 0.25 N. NaCl, demonstrated that just before lysis there is a period of some 0.7 hour during which phage production continues, although the bacterial population remains stationary. Additional experiments reported by Krueger and Fong[7] indicate that such dissociation of bacterial growth and phage formation can be accomplished under different circumstances even in the absence of increased salt concentrations. By adjusting the pH and temperature, the bacterial substrate can be maintained in the resting phage, *i.e.*, without growth, while phage formation continues at the rate of a tenfold increase per hour.

The selection of bacterial growth as the essential conditioning factor for phage production and the use of bacterial growth data in deriving the equation for the kinetics of the phage-bacterium reaction were then merely fortuitous and, as shown here, without significance in defining the mechanism. The expression for bacterial growth should be replaced by the terms of some other reaction proceeding logarithmically with time as the growth does, and paralleling growth quite closely in the conditions requisite for its operation.

There is good reason to believe that phage is a protein with the properties of an enzyme,[4, 8] and the experiments cited above show that the mechanism of phage production can be studied like any other cellular mechanism of enzyme formation under conditions which set it apart from the complexities of cellular growth.

A. P. KRUEGER

UNIVERSITY OF CALIFORNIA

PURIFICATION OF TOBACCO MOSAIC VIRUS AND PRODUCTION OF MESOMORPHIC FIBERS BY TREATMENT WITH TRYPSIN

TREATMENT of impure tobacco mosaic and other virus solutions with trypsin has been stated[1] to facilitate purification of the virus proteins. The proteins were later isolated as liquid crystalline concentrates, but it was not indicated whether trypsin aided isolation in the usual crystalline forms. Pure virus protein is not digested at a measurable rate by any proteolytic enzyme yet tried.[1, 2] Preparations of crude virus protein of tobacco common mosaic, which had received very little preliminary treatment and could not be crystallized by any of the usual methods, yielded pure protein readily after incubation with trypsin. The purified protein separated first in long mesomorphic fibers at pH 7.5, and crystallized in the typical needle form immediately upon acidification to pH 4.5.

In a typical experiment, the impure virus protein, in approximately 1 per cent. solution, was incubated for 3 to 5 hours with 3.3 mg/cc of Fairchild's trypsin. The protein precipitable by trichloroacetic acid decreased in a few minutes from 11.4 mg to 8.3 mg/cc, and in a few hours the solution assumed an opaque appearance. Apparently pure virus protein had separated at pH 7.5, in a form readily identified microscopically as that described recently by Best[3] as mesomorphic fibers of virus. Shaking the solution disintegrated the fibers. The liquid was thereafter opalescent, but quite clear. On standing an hour or two the satin-like opaque appearance of the solution returned and the fibers had reformed. Acidifying the solution to pH 5 precipitated excellent crystals of the typical needle form of the protein, which were readily recrystallized.

Lojkin and Vinson[4] and Ross[5] have reported that purified solutions of virus incubated with Fairchild's trypsin are not infectious, but become so after heating to 70°. Assays were made of the virus protein purified by incubation with trypsin and subsequently crystallized, and also of virus crystals obtained in the usual way (referred to as the controls). All samples were brought to an equivalent protein content, 5.8 mg/cc, in 0.1 M phosphate buffer at pH 7; they were then diluted at suitable steps in the same buffer and used to inoculate the first leaves of 8-day old bean plants, *Phaseolus vulgaris* L., variety Scotia.

TABLE 1

	Dilutions		
	10^{-3}	10^{-4}	10^{-5}
Control*	300.2‡	19.3	5.40
Trypsin treated	215.4‡	32.3	8.26
Control*	....	46.1§	...
Trypsin treated	....	54.3§	...
Control†	....	97.3¶	...
Trypsin treated	....	164.6¶	...

* Crystalline virus stored in pH 7 buffer.
† Crystals stored under 0.5 saturated ammonium sulfate.
‡ Average number of lesions per plant on 15 plants.
§ " " " " " " " 10 "
¶ " " " " " " " 100 "

[6] J. Scribner and A. P. Krueger, *Jour. Gen. Physiol.*, 21: 1, 1937.
[7] A. P. Krueger and J. Fong, *Jour. Gen. Physiol.*, 21: 2, 1937.
[8] J. H. Northrop, SCIENCE, 84: 90, 1936.
[1] F. C. Bawden, *et al.*, *Nature*, 138: 1051, December 19, 1936.
[2] F. C. Bawden and Pirie, *Nature*, 139: 546, March 27, 1937.
[3] R. J. Best, *Nature*, 139: 628, April 10, 1937.
[4] M. Lojkin and C. G. Vinson, *Cont. Boyce Thomp. Inst.*, 3: 147, 1931.
[5] A. F. Ross (Abstract), *Phytopath.*, 25: 33, 1935.

Although there is a high degree of variability in virus assay methods, it appears that the infectivity of the trypsin purified protein is as great or greater than that of the controls. The virus protein after treatment with trypsin had been precipitated by ammonium sulfate at pH 4.5–5.0, filtered on Celite, and recrystallized three times by Stanley's procedure,[6] using CaO for elution, and acetic acid in ammonium sulfate for crystallization. It is probable that this subsequent treatment freed the preparation of trypsin, which either has only a temporary inactivating effect,[5] or acts on cells of inoculated plants so as to prevent entry of virus into living cells, when it is still present in the inoculum.[7]

LAWRENCE F. MARTIN
FOOD RESEARCH DIVISION
BUREAU OF CHEMISTRY AND SOILS
H. H. MCKINNEY
L. W. BOYLE
DIVISION OF CEREAL CROPS AND DISEASES
BUREAU OF PLANT INDUSTRY

CATALYTIC REDUCTION OF THE METHYL ESTER OF 2:3:4-TRIACETYL α-METHYL-GALACTURONIDE TO METHYL-GALACTOSIDE

IN a recent note[1] there was described the reduction of methyl ester of 2:3:4-trimethyl α-methyl-d-galacturonide to 2:3:4-trimethyl α-methyl-d-galactoside. We now wish to report on the reduction of the methyl ester of 2:3:4-triacetyl α-methyl-galacturonide to methyl-d-galactoside. Thus, in one step, reduction of the $-COOCH_3$ and deacetylation are accomplished.

The composition of the methylgalactoside was as follows: C 43.51, H 7.4, OCH_3 16.91 (for $C_7H_{14}O_6$ Calc. C 43.30, H 7.3, OCH_3 15.98).

The application of this method to the study of aldobionic acids and other uronic acid derivatives is in progress.

P. A. LEVENE
C. C. CHRISTMAN
THE ROCKEFELLER INSTITUTE
FOR MEDICAL RESEARCH
NEW YORK, N. Y.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A METHOD FOR THE SECTIONING OF PROTOZOA EN MASSE

OF the methods commonly used for sectioning protozoa *en masse,* those of Sharp[1] (imbedding in a hard paraffin mold), Calkins[2] (entanglement in zooglea) and Belar[3] (glass tubing closed at one end by bolting cloth) are no doubt the most practicable. In an attempt to reduce the loss of specimens, the present method of imbedding in a bag made of mammalian mesentery was devised. I have used the method successfully in sectioning several lots of large endocommensal ciliates, and an account of the procedure may be useful to others.

To make a form over which the bag may be shaped, roll a bit of warmed hard paraffin of such size that a sphere 2 mm in diameter results; while still warm impale it on the end of a round wooden toothpick or on a wooden pin, pointed at both ends, about 5 cm long and 0.6 mm in diameter near the ends.

Excise a transparent piece of fresh mesentery (cat or rabbit) 1.5 cm in diameter, transfer it to a dish filled with warm salt solution to a depth of 7 cm and, after rinsing, dip it out with the paraffin head of the pin so that it hangs symmetrically over the head. Stick the pin head-upright into a piece of sheet cork and tie with fine, black, cotton thread (No. 80) an overhand knot securely around both mesentery and pin just below the head. Handling the pin by its cork base, plunge it head first into a deep dish of Bouin's fluid and shake it about head-downward in the fixative; the free edge of the bag can thus be made to stand out nearly at right angles to the pin and to harden in this position. Leave it immersed head-downward for 24 hours, allowing the cork to serve as a float.

Remove the pin from the cork, wash in 80 per cent. alcohol as usual, dehydrate and transfer to xylene for four hours to dissolve out the paraffin interior of the bag. Reimmerse in absolute alcohol, transfer to 80 per cent. for a day and with forceps push the bag off the pin. There results a tough, fairly rigid, thin-walled bag for receiving the protozoa; it has an opening equal to the diameter of the pin and has a draw string in place; it may be stored in alcohol until needed.

To get the fixed protozoa into the bag, transfer them to a watch glass of 80 per cent. alcohol. Make a small basket or low cylinder of fine-mesh wire gauze just large enough to receive the bag, immerse the basket completely in a watch glass of 95 per cent. alcohol and set the bag into the basket with its opening uppermost. With a pipette having a straight, slender tip about 2 cm long, transfer the protozoa to the bag under the dissecting binocular. They need merely to be released from the pipette directly above and near to the opening in the bag; they will drop or stream into it because of the greater specific gravity of the 80 per cent. alcohol. The process should not be

[6] W. M. Stanley, *Jour. Biol. Chem.*, 115: 673, 1936.
[7] W. M. Stanley, *Phytopath.*, 24: 1055, 1934.
[1] R. G. Sharp, *Univ. Calif. Publ. Zool.*, 13: 43, 1914.
[2] G. N. Calkins, *Jour. Exp. Zool.*, 27: 293, 1919.
[3] K. Belar, *Methodik der Wiss. Biol.*, 1: 735, 1928.
[1] SCIENCE, 86: 2232, 332, October 8, 1937.