scribed by Mussen,[3] though details are yet to be worked out.

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CRYSTALLINE PAPAIN[1]

CRYSTALS showing proteolytic activity have been ioslated from the undried latex of green papaya fruit. The crystals show the usual properties of proteins. The substance contains nitrogen precipitated from aqueous solution by trichloracetic acid and has been isolated by methods commonly employed in the purification of protein.

The crystalline material clots milk, digests casein and splits hippurylamide in the presence of added cysteine under the conditions usually employed for demonstrating the activity of papain. The activity of the crystalline preparation per mg of protein nitrogen as measured by milk clotting or by casein digestion is from 25 to 50 per cent. higher than that of any of the amorphous preparations made in this laboratory, and is about twice as great as that of the best commercial preparations.

No essential difference in activity was observed between thrice and five times crystallized material, and the ratio of the milk-clotting, casein-digesting and hippurylamide-splitting properties is approximately the same as found in dried latex and in amorphous precipitates prepared from fresh latex.

Without added activator, the activity of the crystals varies, apparently depending upon the treatment during preparation. Determinations made without added activator are obviously not as accurate as those run in the presence of cysteine, because of oxidation during the time of digestion. Accordingly the values obtained in short-time intervals (milk clotting data) are probably the most accurate. On this basis some of the crystals were nearly inactive, others showed originally about half the maximum activity. All these preparations reached the same level of activity when pre-treated with cysteine. A small sample of thrice crystallized material which was half active was incubated with dilute hydrogen peroxide and then crystallized three times more to remove the reagent. The final crystals were between 94 to 97 per cent. inactive.

Due to lack of raw materials, the quantity of crystals available thus far has been extremely small, and many desirable experiments have had to be postponed until more material is available. Recently crystals have also been obtained from commercial papain but have not yet been freed from amorphous material.

Improvements in the method for preparing the crystals will also be studied. Those used presently were prepared in outline as follows:

Coagulated papaya latex, preserved with toluene, was suspended in some cases in about four times its weight of water, in other cases in about two volumes of 0.25 saturated ammonium sulfate. After about an hour the material was filtered and the clear filtrate was made 0.6 to 0.7 saturated with ammonium sulfate and filtered. The semi-dry filter cake was suspended in about an equal weight of water. The pH was adjusted to light green to brom thymol blue and the solution was cooled slowly from 20° to 5° C. (24 hours). The solution containing about 15 mg protein nitrogen per cc became turbid on cooling and in a few days developed a sheen due to the formation of small needle crystals. Particularly after crystal formation, slow addition of a saturated solution of ammonium sulfate may increase the yield. Recrystallization was carried out by essentially the same technique.

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THE MECHANISM OF BACTERIOPHAGE PRODUCTION[1]

WHEN bacteriophage is added to a culture of susceptible bacteria growing in an appropriate medium two phenomena occur. First, during the period of contact there is produced a considerable additional amount of phage and, second, as a terminal event the bacteria quite suddenly break up, leaving the medium clear.

d'Herelle[2] and Burnet[3] have stressed the importance of cellular lysis in the production of phage. According to them phage particles penetrate into the bacterium, multiply, but remain under spatial constraint until set free when the cell undergoes dissolution. Sufficient experimental evidence has accumulated to prove that bacterial lysis is not causally related to the phage-producing mechanism.[4] In the place of lysis, bacterial growth has come to be considered a *sine qua non* for phage production. Krueger and Northrop[5] found that factors such as reduced temperature or limitation of nutrients, which interfere with bacterial growth, likewise reduce phage formation. They de-

[1] Food Research Division contribution No. 343.

[1] The experimental work cited in this paper was supported by grants-in-aid from the National Research Council, the American Medical Association and the Board of Research, University of California.

[2] F. d'Herelle, "The Bacteriophage and Its Behavior." Williams and Wilkins Co., 1926.

[3] F. M. Burnet, *Brit. Jour. Exp. Path.*, 10: 109, 1929.

[4] A. P. Krueger, *Physiol. Reviews*, 16: 1, 1936.

[5] A. P. Krueger and J. H. Northrop, *Jour. Gen. Physiol.*, 14: 223, 1930.

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.

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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.
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[5] A. F. Ross (Abstract), *Phytopath.*, 25: 33, 1935.