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:

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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] 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.