Experimentally Krueger and Fong[4] found it possible, by adjusting the pH and temperature of phage-bacteria mixtures, to completely inhibit bacterial reproduction, while phage formation continued. There is some question as to the theoretical significance of these results, however, inasmuch as the method employed for detecting Δ [bacteria] was not highly sensitive. Because of this and also because the yields of phage precursor have been both limited and irregular, we have sought a means of studying the precursor-phage reaction in the cell. The accumulated data offer excellent evidence for the existence of phage precursor within the bacterium and likewise for the dissociation of phage production and cell growth.

To demonstrate intracellular phage precursor we prepare "activated" suspensions of staphylococci by growing the organisms in a highly oxygenated medium. The activation process requires some two hours, after which the cells are separated from the medium, resuspended in Locke's solution and maintained at 5° C. for two hours in order to rule out the possibility of bacterial growth during subsequent reactions with phage. To demonstrate the precursor, 4 ml of activated cell suspension containing 5×10^8 bacteria/ml is added to 1 ml of phage diluted with Locke's solution to contain 1×10^9 activity units/ml. The initial phage concentration of the mixture, therefore, is 2×10^8 activity units/ml. The mixture is kept for five minutes at 5° C. and is then promptly titrated for total phage content. The end titre is found to be 2×10^9 phage units/ml, whereas that of a control (non-activated bacteria prepared from a 16-hour agar culture) is 2×10^8 units/ml.

A detailed study of the reaction occurring when phage is added to activated bacteria will be reported elsewhere. The data indicate that:

(*a*) Phage precursor is produced by the actively metabolizing bacterium and apparently is present on the cell surface.

(*b*) Precursor production is enhanced by conditions which favor general cell metabolism. Cell growth and phage formation as a consequence present a pseudo-relationship, for both are dependent upon similar environmental factors. The activation process mentioned above merely serves to induce the production of relatively large amounts of precursor before the cells are put into a resting state by storage in Locke's solution at 5° C.

(*c*) Precursor is transformed into phage within two to four minutes after the addition of phage to activated cell suspensions. The precursor content of activated cells is maintained for a period of 6 to 24 hours.

(*d*) Phage precursor is more thermolabile than the bacterial cell. It is possible to destroy the precursor content of the cell by heat and still keep the cell alive.

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THE HYDROLYSIS OF STEROL GLYCOSIDES BY EMULSION

IT has been shown by Odell and Marrian[1] that pregnanediol, as well as other sterol sex hormones, occurs in the urine conjugated with glycuronic acid.

A majority of the methods so far employed for the isolation or estimation of sex hormones depend upon acid or alkali hydrolysis of the glycosidal linkage.[1, 2] While most investigators feel that the sterol sex hormones are not destroyed to any appreciable extent by such treatment, proof that such is the case is inconclusive.

Venning[3] has proposed a method for the isolation of pregnanediol (as sodium pregnanediol glycuronate) by which she has determined the pregnanediol content of human pregnancy urine (eighth month) to be from 20 to 25 milligrams per liter of urine (equivalent to from 40 to 60 milligrams of pregnanediol glycuronate). Other investigators have reported findings of the same order of magnitude.

Since it has been suggested that pregnanediol glycuronate might be a beta glycoside,[3] it occurred to the authors that hydrolysis of the glycuronate might be effected rapidly, and without destruction of pregnanediol, by the enzyme emulsin.

Preliminary experiments have shown that pregnanediol glycuronate is hydrolyzed rapidly by low concentrations of emulsin at a pH of approximately 6.0, the hydrolysis being carried out directly in the urine. This may be considered proof that pregnanediol glycuronate is a beta glycoside. Isolation of the pregnanediol freed by this method and extracted from the urine by a modification of the original process of Katzman and Doisy[4] yields a product of high purity without additional purification (m.p. 235° C. uncorr.). Treated in this manner, second to eighth month human pregnancy urine yields from 40 to 50 milligrams of pure crystalline pregnanediol per liter; or approximately twice the amount previously reported by other investigators.

Full details of this investigation will appear soon in the literature.

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