We received a dead pigeon on September 20, 1938, from a breeder whose residence was adjacent to a farm on which two horses had died of the equine disease. The brain was removed, triturated in sterile sea sand, and suspended in 10.0 cc of infusion broth. After centrifugation, part of the supernatant liquid was filtered through a Berkefeld V filter. Young albino Swiss mice were injected intracerebrally with both filtered and unfiltered material (four animals in each group). All these mice died in 48 to 96 hours, having exhibited symptoms similar to those observed in mice infected with the equine virus of the Eastern variety. Bacteriological cultures of the pigeon brain suspension before injection and of the brains of the dead mice were sterile. Subsequent experiments confirmed the filterability of the infectious agent.

The identification of the pigeon virus was determined by intracerebral injection of guinea pigs previously immunized[3] against the Eastern and the Western varieties of virus, and in normal controls. Each animal received a dose of 0.15 cc of a 1:1000 dilution of mouse-brain passage virus. Of six guinea pigs immunized against the Eastern virus, two died, and from one of these we were unable to isolate the virus; the other four remained well and showed no symptoms. Two animals immunized against the Western virus and all six controls died. From one of the guinea pigs previously immunized with the Western virus, and from two of the controls, the virus was again recovered in mice. We conclude from this experiment that the virus isolated from the pigeon was the virus of the Eastern variety of equine encephalomyelitis.

Pigeons were shown to be susceptible to the virus obtained directly from the original pigeon, following intracerebral inoculation. In other experiments, pigeons were shown to be susceptible to the strain of the Eastern variety of equine virus isolated from the brain tissue of the human case elsewhere described.

Giltner and Shahan[4] first demonstrated the susceptibility of pigeons to experimental inoculation of equine encephalomyelitis virus (probably Western type). These authors and, later, Ten Broeck, Hurst and Traub,[5] suggested the possibility of pigeons as host reservoirs of the virus. The susceptibility of a number of species of birds to experimental inoculation of the virus was demonstrated by Remlinger and Bailly.[6] We have been unable to find any report of the isolation of this virus from pigeons dying from natural infection by it.

Summary: The virus of the Eastern variety of equine encephalomyelitis has been recovered from the brain of a pigeon[7] which had spontaneously contracted the disease in an area where equine encephalomyelitis was prevalent among horses.

LEROY D. FOTHERGILL
JOHN H. DINGLE,
James Jackson Cabot Fellow
DEPARTMENT OF BACTERIOLOGY AND IMMUNOLOGY AND DEPARTMENT OF PEDIATRICS, HARVARD MEDICAL SCHOOL

THE DEMONSTRATION OF PHAGE PRECURSOR IN THE BACTERIAL CELL[1]

IN their original study of the kinetics of the phage-bacterium reaction, Krueger and Northrop[2] found bacterial growth to be the essential conditioning factor for phage production. Under the conditions of their experiments any interference with bacterial growth was accompanied by a corresponding reduction in phage formation as stated in the equation:

$$\frac{P}{P_o} = \left(\frac{B}{B_o}\right)^n$$

where P = [phage] at any time, P_o = initial [phage], B = [bacteria] at any time and B_o = initial [bacteria].

More recently Krueger and Baldwin[3] have reported experimental evidence for the production of phage according to the reaction: phage precursor + phage→ phage. The precursor, apparently a normal cell metabolite, was obtained in ultrafiltrates of growing phage-susceptible cell suspensions. The essential reaction then is analogous to the autocatalytic transformation of inactive enzyme precursor into active enzyme upon the addition of small amounts of the active form to a solution of the precursor, *e.g.*, trypsinogen + trypsin→trypsin. The detection of phage precursor led to the conclusion that precursor formation, rather than bacterial growth as such, should condition phage production and that the apparent role of bacterial growth is due to the fact that both these expressions of cellular metabolism have nearly identical optimal milieus.

[3] We are indebted to Dr. R. W. G. Wyckoff and to Lt. Col. Raymond A. Kelser for providing some of the immune guinea pigs. Others were immunized by ourselves with chick-membrane vaccine kindly furnished by the Lederle Laboratories.

[4] L. T. Giltner and M. S. Shahan, SCIENCE, 78: 63, 1933.

[5] C. Ten Broeck, E. W. Hurst and E. Traub, *Jour. Exp. Med.*, 62: 677, 1935.

[6] P. Remlinger and J. Bailly, *Compt. rend. Soc. de Biol.*, 121: 146, 1936; *ibid.*, 122: 518; *ibid.*, 123: 562.

[7] Our findings in pigeons were briefly mentioned, with our permission, by Dr. Roy Feemster in an address before the American Public Health Association meeting in Kansas City, on October 26, and will be published in the *American Journal of Public Health*. They were likewise referred to by one of us (L.D.F.) in an address before a meeting of the the New England Veterinary Association in Boston, on October 26.

[1] The experimental work cited has been supported by grants-in-aid from the American Medical Association, the Research Corporation of New York and from various interested friends.

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

[3] A. P. Krueger and D. M. Baldwin, *Proc. Soc. Exp. Biol. and Med.*, 37: 393–395, 1937.

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.

ALBERT PAUL KRUEGER
JEAN H. MUNDELL
DEPARTMENT OF BACTERIOLOGY,
UNIVERSITY OF CALIFORNIA, BERKELEY

[4] A. P. Krueger and Jacob Fong, *Jour. Gen. Physiol.*, 21: 137–150, 1937.

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.

F. P. VEITCH, JR.
H. S. MILONE
E. L. EVERITT
DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY,
GEORGETOWN UNIVERSITY SCHOOL OF MEDICINE

[1] Odell and Marrian, *Biochem. Jour.*, 30: 9, 1936.
[2] Marker, *Jour. Amer. Chem. Soc.*, 60: 2442, 1938.
[3] Venning, *Proc. Soc. of Exp. Biol. and Med.*, 34: 792, 1936.
[4] Katzman and Doisy, *Jour. Biochem.*, 98: 739, 1932.