5. Moore, A. E., Friend, C., *Proc. Am. Assn. Cancer Res.*, 1958, v2, 328.
6. Manaker, R. A., Strother, P. C., Miller, A. A., Piezak, C. V., *J. Nat. Cancer Inst.*, 1960, v25, 1411.
7. Fischer, G. A., Welch, A. D., *Science*, 1957, v126, 1018.
8. ———, *Ann. N. Y. Acad. Sci.*, 1958, v76, 673.
9. Bethell, F. H., Swenseid, M. E., *J. Clin. Invest.*, 1946, v25, 918.
10. Swenseid, M. E., Bethell, F. H., Bird, O. H., *Cancer Res.*, 1951, v11, 864.
11. Lajhta, L. G., Oliver, R., *Lab. Invest.*, 1959, v8, 219.
12. Friend, C., *J. Exp. Med.*, 1957, v105, 307.
13. Gross, L., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 767.
14. Moloney, J. B., *Nat. Cancer Inst. Monograph No. 4*, 1960, p7.
15. Eagle, H., *Science*, 1955, v122, 501.
16. ———, *J. Exp. Med.*, 1955, v102, 595.
17. Fitzgerald, P. J., Simmel, E., Weinstein, J., Martin, C., *Lab. Invest.*, 1953, v2, 181.
18. Craddock, C., in *Ciba Symposia on Hematopoiesis*. Little, Brown and Co., Boston, 1960.
19. Gavosto, F., Maraini, B., Pileri, A., *Blood*, 1960, v16, 1555.
20. Scherer, W., *Am. J. Path.*, 1953, v29, 113.
21. Snedecor, G. W., *Statistical Methods*. Iowa State College Press, 1946.
22. Huennekens, F. M., Osborn, M. J., Whiteley, H. R., *Science*, 1958, v128, 120.
23. Schindler, R., Day, M., Fischer, G. A., *Cancer Res.*, 1959, v19, 47.

Received July 24, 1961. P.S.E.B.M., 1961, v108.

Efficiency of Plaquing Certain Poliovirus on Tissue Cultures at Higher Temperatures as a Function of L-Cystine Concentration.* (26954)

OTIS TOLBERT AND GEORGE R. DUBES (Introduced by H. A. Wenner)
Department of Pediatrics and Hixon Memorial Lab., University of Kansas School of Medicine, Kansas City, Kansas

In 1956 3 wild-type polioviruses were shown to require exogenously supplied cystine for efficient plaque production on monkey kidney tissue cultures at 36° (1). Subsequently, it was shown that their efficiency of plaque production on cultures not supplied with cystine is dependent on the temperature during plaque development: lowering the temperature below 37° increased this efficiency (2).

That the temperature of the host cell has a marked influence on growth of poliovirus has recently been well recognized [see reviews by Lwoff and Lwoff (3) and by Sabin (4)]. However, the relation of cystine to this influence of temperature has not been adequately explored; the results of such investigation are described here.

Materials and methods. Polioviruses. The wild-type virus, the 30°-adapted (ca^{30}) mutant (5), and the 23°-adapted (ca^{23}) mutant (6) of the Akron strain of antigenic type 1 poliovirus were used. *Growth of monkey kidney tissue cultures (MKTC's).* Kidney cells from cynomolgus monkeys (*Macaca irus*) or rhesus monkeys (*M. mulatta*) were dispersed with trypsin (7), suspended in lactalbumin growth medium (8), delivered to Petri plates, and then kept at 37°-38° in an atmosphere of 3% CO_2 and 97% air for 5-9 days, after which time suitable sheets of cells had developed. *Inoculations and plaque development.* The sheets were washed twice, each time with 4 ml phosphate-buffered saline (PBS) (9), then were inoculated with the appropriate virus concentrations, 0.3 ml inoculum per plate. Inoculated sheets were put at 37° for 30 minutes. Then a sheet received 6 ml altered Eagle's maintenance medium (AE_m) (2), adjusted with HCl and/or NaOH usually to pH 6.9-7.1, containing 0.9% washed (9) agar and 0, 0.025, 0.050, 0.20, or 1.0 mM

* Aided by grant from National Fn.

L-cystine: during plaque development the pH was somewhat higher, ranging from about 7.2 to about 7.7. In some experiments the effect of purposely varying the pH during plaque development was assessed; for these the initial pH of the agar-AE_m was adjusted with HCl and/or NaOH to various values between 5.7 and 7.8. The pH of the agar-overlay during plaque development was measured periodically either colorimetrically, using phenol red as pH-indicator and standard plates containing phosphate buffers of known pH's, or with a pH meter. For some tests (Table II) compounds not present in this medium were added; or concentration of DL-methionine was increased. The plates were then put at various temperatures (29°-41°) in an atmosphere of 3% CO_2 and 97% air. Plates were kept under these conditions 4 days (wild-type and *ca*^{30} mutant) or 5 days (*ca*^{23} mutant); the temperature was measured periodically and the mean calculated. The surviving cells were stained with neutral red. *Calculation of efficiency of plating (eop.).* Within certain temperature and L-cystine concentration ranges the titer of a given virus population was high and independent of the temperature and L-cystine concentration. Such high titers, which are maximal for the given virus populations in this MKTC system, were used as standards. The titer of a population under other conditions, e.g. at a higher temperature, was divided by its maximal titer to give its eop under these other conditions; an eop so calculated is of course a relative one.

Results. Akron plaque production as a function of temperature and the supply of L-cystine. In Table I are presented data from a representative experiment showing the promotion by L-cystine of plaque development by the Akron *ca*^{30} mutant on MKTC cells at a relatively high temperature. Curves which show growth of poliovirus on cells *in vitro* as a function of temperature are typically steep at the warmer end;

TABLE I. Promotion by L-cystine of Development of Plaques of the *ca*^{30} Mutant at 37.3°.

Conc. (mM) of L-cystine	No. plaques/plate when inoc. virus conc. is 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
0	ov	16	0	*	*
.025	*	80	3	1	*
.050	*	*	46	11	0
1.0	*	*	*	ov	30†

ov = extensive overlap of plaques.
* Not done. † eop = 1.

i.e., when the temperature exceeds a certain value, growth of the virus decreases rapidly with increasing temperature (10, 11, 12). By graphing the data from Table I and other experiments we see (Fig. 1) that the position of this steep portion is displaced toward higher temperatures by increasing the concentration of L-cystine in the medium over the cells. This displacement was observed with all 3 Akron viruses, which represent 3 degrees of genetic ability to grow at relatively high temperatures: with the initial concentration of L-cystine in the medium at the rather low value of 0.025 mM, the steep portion commences at about 30.7°, 36.2°, and 37.4° for the *ca*^{23}, *ca*^{30}, and wild-type viruses, respectively (see curves for the 2 mutants in Fig. 1). During

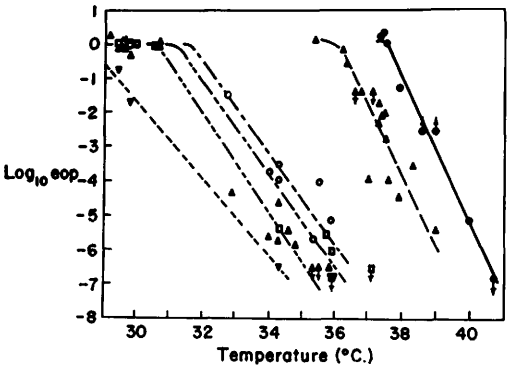

FIG. 1. Plaque production by *ca*^{30} and *ca*^{23} mutants as a function of temperature and supply of L-cystine. The 4 curves at left (open symbols) are for the *ca*^{23} mutant and the 2 at right (closed symbols) for the *ca*^{30} mutant. Results with media containing 0, 0.025, 0.20, and 1.0 mM L-cystine are indicated by triangles with horizontal side up, triangles with horizontal side down, rectangles, and circles, respectively. Points with arrows pointing up and down indicate minimal and maximal values, respectively.

is in a thermolabile state: its *in vitro* stability is low at 45°-53°. Such thermolabile particles can be greatly stabilized against thermal inactivation by *in vitro* reaction with L-cystine (14, 15): apparently the protein coat of a thermolabile particle reacts with several molecules of L-cystine, resulting in a coat much less easily denatured by heat (14). Applying this idea to the effect of L-cystine on viral growth in cells at different temperatures, we hypothesize that L-cystine stabilizes precursor viral protein against thermal denaturation at 31°-41°. Such an hypothesis appears compatible with (a) the finding of Lwoff and Lwoff (16) that a relatively late stage of intracellular viral development is inhibited by high temperature, (b) the report by Darnell and Levintow (17) that poliovirus protein synthesis proceeds rapidly at a similarly late stage of intracellular viral development, and (c) the very high dependence of rate of protein denaturation on temperature.

Summary. L-Cystine enables viruses of the Akron strain of antigenic type 1 poliovirus to grow on *in vitro* monkey kidney cells at comparatively high temperatures; at lower temperatures little or no exogenously supplied L-cystine is needed by these viruses. These results were shown for Akron viruses of 3 different degrees of genetic ability to grow at higher temperatures. The activity of L-cystine is not due to its slight lowering of the pH during plaque development. L-Cysteine and glutathione are active. DL-cystathionine is slightly active, and a sample of D-cystine showed slight activity. Rare plaques produced at temperatures too high for the L-cystine supply contained hot-adapted mutants.

The authors thank Dr. Herbert A. Wenner for critical reading of the manuscript and Dr. W. Daniel Kundin for the sample of S-ethyl L-cysteine.

1. Dubes, G. R., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 129.

2. Dubes, G. R., Chapin, M., *J. Gen. Microbiol.*, 1958, v18, 320.

3. Lwoff, A., Lwoff, M., *Ann. de l'Inst. Pasteur*, 1960, v98, 173.

4. Sabin, A. B., *Perspectives in Virology*, Wiley, New York, in press.

5. Dubes, G. R., Chapin, M., *Science*, 1956, v124, 586.

6. Dubes, G. R., Wenner, H. A., *Virology*, 1957, v4, 275.

7. Youngner, J. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 202.

8. Melnick, J. L., *Ann. N. Y. Acad. Sci.*, 1955, v61, 754.

9. Dulbecco, R., Vogt, M., *J. Exp. Med.*, 1954, v99, 167.

10. Lwoff, A., in *Recent Progress in Microbiology*, Symposia held at VII Internat. Cong. for Microbiol., 1958.

11. Likar, M., Wilson, D. C., *Brit. J. Exp. Path.*, 1958, v39, 674.

12. Lwoff, A., Lwoff, M., *Compt. rend. séances l'Acad. Sci.*, 1959, v248, 1725.

13. Vogt, M., Dulbecco, R., Wenner, H. A., *Virology*, 1957, v4, 141.

14. Pohjanpelto, P., *ibid.*, 1958, v6, 472.

15. Dubes, G. R., Chapin, M., Tolbert, O., *Arch. für Gesamte Virusforsch.*, 1960, v10, 315.

16. Lwoff, A., Lwoff, M., *Compt. rend séances l'Acad. Sci.*, 1958, v246, 190.

17. Darnell, J. E., Jr., Levintow, L., *J. Biol. Chem.*, 1960, v235, 74.

18. Lwoff, A., Lwoff, M., *Compt. rend. séances l'Acad. Sci.*, 1959 v248, 154.

Received July 24, 1961. P.S.E.B.M., 1961, v108.