Poliovirus strains may be differentiated by the influence which the presence of cystine in the nutrient medium exerts on their multiplication. Some of them multiply effectively only in the presence of cystine (1, 2); reproduction of others is inhibited by this amino acid (2, 3); still others multiply with approximately similar intensity regardless of the presence or absence of exogenous cystine (2, 3). So far there is no generally accepted designation for the corresponding mutants. By analogy with the designation of mutants with different sensitivity to guanidine we designated cystine mutants in the following way: cystine-dependent — cyd, cystine-resistant — cyr and cystine-sensitive (those that are inhibited by cystine) — cy$.  

In the present study the mechanism of phenotype expression of a cyd mutation of poliovirus was investigated. Attempts to elucidate such mechanisms had been made earlier too. The first step was made when it was found that the majority of cyd mutants were thermolabile (2, 3). It should be mentioned that in those studies thermosensitivity was evaluated by the rate of inactivation of virions at temperatures around 50°C. Shortly thereafter it was demonstrated (4) that dependence upon cystine was particularly effectively manifested at supraoptimal temperatures; at sufficiently low temperatures cystine exerts no influence on multiplication of cyd mutants at all. These facts led Tolbert and Dubes (4) to suggest that cystine-dependence was due to thermolability of the coat protein of mutant viruses on the one hand, and the capacity of cystine to increase thermal resistance of poliovirus (5), on the other.
There were some difficulties with this hypothesis at that time, however. First of all, previously described strains were sufficiently thermostable at temperatures below 40°C, that is at temperatures at which they show cystine-dependence. Secondly, in some cases no significant differences could be found in thermolability of cyd and cyr mutants. Thirdly, cystine was reported not to prevent inactivation of poliovirus which, even though slowly, occurred at temperatures below 37°C (6).

A significant handicap in elucidation of the nature of the cyd phenotype consisted in the lack of a system in which cystine-dependence could have been reproduced within one infectious cycle with total and simultaneous infection of all the cells in the population. After we had developed such a system we succeeded in showing that cystine did not influence the synthesis of the viral RNA of cyd mutant. Besides it was shown that in our case the above objections against the hypothesis of Tolbert and Dubes did not hold true. Accordingly, we were able with sufficient certainty to decipher the mechanism of the phenotypic expression of one cyd mutation of poliovirus. Results of this study are described in the present paper. A preliminary report on this subject was published elsewhere (7).

Materials and Methods

Viruses. A type 3 poliovirus strain, cyd — 40/1 (8), was used as a cystine-dependent variant. After 6 passages of this strain in a cystine-free medium and subsequent cloning, a mutant was isolated which had nearly completely lost its dependence upon cystine. This mutant was designated as cyr/I.

Tissue cultures. Primary cultures of kidney tissue from African green monkeys (Cercopithecus aethiops) were grown in Hanks' solution with 0.5% lactalbumin hydrolysate and 5% normal bovine serum. Continuous culture obtained from green monkey kidney (9) was grown in a mixture consisting of 60 ml Eagle's medium, 30 ml 0.5% lactalbumin hydrolysate in Earle's solution and 10 ml normal bovine serum. When it was appropriate the cells were transferred into Eagle's medium without cystine and serum at 18—20 hours before the experiment.

Virus titration was carried out in primary green monkey kidney cultures by the plaque method. The overlay consisted of a previously described mixture (10) with the following modifications: concentrations of agar, serum and sodium bicarbonate were 1%, 2% and 0.5%, respectively; the overlay contained additionally 0.5% lactalbumin hydrolysate. In experiments testing the effect of cystine on plaque formation the agar overlay contained no serum or lactalbumin hydrolysate but Eagle's medium (with or without cystine). The bottles were incubated at 34°C, if not otherwise indicated.

Study of synthesis of viral RNA. Infectious viral RNA was isolated from infected cells by phenol deproteinization and assayed in the presence of DEAE-dextran (11). In order to determine incorporation of labelled precursors into the viral RNA, cells of continuous monkey kidney culture were inoculated with virus, unadsorbed virus was washed off 30 minutes later and the cells were incubated at 34°C; 21⁄2 hours after the beginning of incubation actinomycin C or aurantaine (final concentration 5 µg/ml) and 30 minutes later uracil-