Genetic Heterogeneity of Cloned Animal Virus Preparations

THE procedure used to purify genetically a virus preparation does not guarantee the homogeneity of the derived population[1]. Genetic purity can be defined by markers characteristic of a given virus. During studies of the selective effects of cloned and uncloned lines of human epithelial cells on a relatively non-cytopathic strain of type 2 poliovirus, MEF_1[2,3], efforts to obtain genetically pure virus stocks failed. We describe here a study undertaken to explain this failure.

A seed stock of virus was prepared in cultures of cynomolgus monkey kidney cells. This virus produced a trimodal distribution of plaque size[3] when plated on HeLa–Gey cells, subline GHJ[4]. After staining with Wright's stain[5], plaques were classified as large, $5{\cdot}0 \pm 0{\cdot}8$ mm; small, $1{\cdot}3 \pm 0{\cdot}4$ mm; or intermediate, $2{\cdot}6 \pm 0{\cdot}5$ mm. To confirm the visual identification, plaque sizes were measured and analysed by the F ratio and Student t tests. Differences in plaque size were highly significant ($P \ll 0{\cdot}001$). Genetic purification of the large and small plaque types was attempted using the terminal dilution and plaque techniques.

Monolayers of uncloned HeLa–GHJ cells were used for plaque assays. The media and methods of cell culture were reported[4]. Virus was diluted (between none and five plaques a bottle) and plated as described before[2]. After attachment, the inoculum was discarded. Cells were washed twice and covered with 1 ml. of diluent and 4 ml. of overlay medium. After incubation (4 days at 37° C), an agar plug was removed from an isolated plaque with a bent-tip capillary pipette. The virus was dispersed, diluted and replated. Each plaque type was purified four or more times. Working stocks of plaque isolates were also prepared in HeLa–GHJ cells. Virus titres and the distributions of variants were calculated from total counts of 150–250 plaques on three replicate cultures in each of two experiments. This method detected 0·4–0·7 per cent of contaminant plaques.

Table 1. DISTRIBUTION OF PLAQUE TYPES DURING GENETIC PURIFICATION FOR THE LARGE VARIANT

Source of virus	PFU/ml.*	Per cent distribution of plaque variants		
		Small	Inter-mediate	Large
Seed stock†	39×10^4	70	10	20
Purification 1	6×10^5	33	0	67
Purification 2	8×10^5	75	12·5	12·5
Purification 3	$4{\cdot}8 \times 10^5$	21	10	69
Purification 4	$5{\cdot}3 \times 10^5$	59	19	22
Purified working stock‡	$10{\cdot}2 \times 10^6$	88	10	2

* Titrated in HeLa–GHJ cells.
† Prepared in cynomolgus monkey renal cells.
‡ Prepared from virus at the third level of genetic purification.

Table 2. DISTRIBUTION OF PLAQUE TYPES DURING GENETIC PURIFICATION FOR THE SMALL VARIANT

Source of virus	PFU/ml.*	Per cent distribution of plaque variants		
		Small	Intermediate	Large
Seed stock†	39×10^4	70	10	20
Purification 4	11×10^3	68	0	32
Purification 5	35×10^3	61	21	18

* Titrated in HeLa–GHJ cells.
† Prepared in cynomolgus monkey renal cells.

No consistent purification was achieved for the large plaque type (Table 1). Working stocks, prepared from cloned virus enriched for the large variant (plaque passage 3), were as impure as the seed stock. Results were similar with the small plaque type (Table 2). Isolates from bottles containing only one plaque were also impure. Other studies have indicated that the cell types in established human cell lines influence the genetic composition of virus populations grown in these lines[3]. Such findings suggest that the difficulties in isolating pure lines of virus result from the use of uncloned cells for plaque purification. Accordingly, cloned lines of human epithelial cells were used to purify the variants; that is, Chang–liver–CS (CH–CS); Chang–liver–CL (CH–CL); HeLa S3–CS (S3–CS); HeLa–Gey–CS (HG–CS); and HeLa–Gey–CL (HG–CL)[6,7].

Seed virus was purified once for the small plaque type in S3–CS cells. The isolate was plated on HeLa–GHJ cells (uncloned) to test its purity. Only the small plaque type was detected (Table 3). A working stock of this small variant, prepared in HeLa–GHJ cells, also was pure. This step eliminated the possibility of phenotypic modification through cloning of virus in S3–CS cells. The large variant, however, could not be purified in S3–CS, HG–CS or HG–CL cells. Clones CH–CS and CH–CL were too insensitive to use for isolation of either plaque type.

Table 3. GENETIC PURIFICATION OF THE SMALL VARIANT IN HELA S3 CELLS, CLONE–CS

Source of virus	PFU/ml.*	Per cent distribution of variants		
		Small	Intermediate	Large
Seed stock	39×10^4	70	10	20
S1†	18×10^2	100	0	0
Working stock of S1 prepared in HeLa–GHJ cells	25×10^4	100	0	0

* Titrated in HeLa–GHJ cells.
† First purification of the small variant in HeLa–S3–CS cells.

The results illustrate an additional limitation in cloning procedures used for the genetic purification of viruses. Besides the mechanical problems involved[1], the use of uncloned cell populations, or "cloned" cell lines of unspecified purity, may also contribute to the genetic impurity of the derived virus. Our results suggest that the types of cells within a plaque influence the composition of a cloned virus population.

The failure to obtain pure large virus preparations may be related to the genetic instability of this variant. Genetic instability of a cloned virus[8,9] must be distinguished, however, from genetic heterogeneity related to other factors[10].

This work was supported by grants from the US National Institutes of Health and the US Army.

KENNETH J. MCCORMICK*
WILLIAM H. MURPHY
Department of Microbiology,
University of Michigan.

Received June 17; revised September 9, 1968.

* Present address: Division of Experimental Biology, Baylor University College of Medicine, Houston, Texas.

[1] Mosley, J. W., and Enders, J. F., *Proc. Soc. Exp. Biol. and Med.*, **108**, 406 (1961).
[2] Murphy, jun., W. H., and Armstrong, R., *J. Exp. Med.*, **110**, 629 (1959).
[3] McCormick, K. J., and Murphy, W. H., *Bact. Proc.*, 105 (1965).
[4] Murphy, W. H., and Wisner, C., *J. Bact.*, **83**, 649 (1962).
[5] Holland, J. J., and McLaren, L. C., *J. Bact.*, **78**, 596 (1959).
[6] Murphy, W. H., Bullis, C., Landau, B. J., and Acosta, R., *Cancer Res.*, **22**, 906 (1962).
[7] Murphy, W. H., and Landau, B. J., *J. Nat. Cancer Inst. Monog.*, **7**, 249 (1962).
[8] Fenner, F., and Sambrook, J. F., *Ann. Rev. Microbiol.*, **18**, 47 (1964).
[9] Cooper, P. D., *Brit. Med. Bull.*, **23**, 155 (1967).
[10] Walen, K. H., *Virology*, **20**, 230 (1963).

Inhibition of Rhinovirus by Isatin Thiosemicarbazone Analogues

ISATIN 3-thiosemicarbazone is known to have antiviral activity against certain poxviruses[1,2]. The antiviral spectrum of 1-methylisatin 3-thiosemicarbazone, which reportedly extends to adenoviruses, may be rather broader but is still limited to several groups of DNA viruses[2,3]. It was therefore an unexpected finding that these compounds are also active against certain rhinoviruses. As a result, related heterocyclic compounds were tested of which several examples are shown here; two (D and E)