Table 2. Growth of *Sh. dysenteriae* 1 in Mixtures of Amino-acids With and Without Cystine. Cystine, when Present, was at 0·0005 *M*; All other Amino-acid Concentrations were 0·01 *M*

	Medium consisted of basal medium and the following amino-acid mixtures:	Growth
1	Glut. + meth. + tryp. + asp. + cys.	+
2	Glut. + meth + tryp. + asp.	−
3	Glut. + meth. + tryp. + cys.	+
4	Glut. + meth. + tryp.	−
5	Glut. + asp. + tryp. + cys.	+
6	Glut. + asp. + tryp.	−
7	Glut. + asp. + meth. + cys.	+
8	Glut. + asp. + meth.	−
9	Asp. + meth. + tryp. + cys.	+
10	Asp. + meth. + tryp.	−
11	Glut. + tryp. + cys.	+
12	Glut. + meth. + cys.	+
13	Glut. + asp. + cys.	+
14	Meth. + tryp. + cys.	+
15	Glut. + tryp.	−
16	Glut. + meth.	−
17	Glut. + asp.	−
18	Glut. + cys.	+
19	Meth. + tryp.	−
20	Tryp. + asp.	−
21	Tryp. + cys.	+

Glut., glutamic acid; meth., methionine; tryp., tryptophan; asp., aspartic acid; cys., cystine.

showing that growth of the strain occurred only when cystine was present in mixtures of amino-acids, as tabulated in Table 2, would also favour an essential nutritional role for the amino-acid for this particular strain of *Sh. dysenteriae* 1.

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VIROLOGY

Persistence of Herpes Simplex Virus in HeLa Cells

Persistence of herpes virus in cells cultured *in vitro* might provide a suitable model for investigating the phenomenon of latent herpes infection in humans. During a study on the interaction between herpes virus and HeLa cells, it was observed that an extensive cytopathic effect eventually resulted from inoculation of a monolayer culture with the *HFEM* strain of herpes virus[1,2]. A few isolated cells sometimes survived, however, and if growth medium containing antiviral serum was added, colonies grew from these surviving cells, leading eventually to a large population. These recovered cells were apparently healthy and grew as well as the original HeLa cells: there were no gross morphological differences such as those described by Vogt and Dulbecco[3], who studied cells recovered after poliomyelitis infection.

Despite continued growth in the presence of high-titre antiserum, however, the recovered cells were found to carry herpes virus for at least nine months, involving 29 subcultures. The presence of the virus was indicated by the appearance of typical microscopic foci of infected cells or plaques, from which virus could be isolated. These plaques appeared whether or not there was antiserum in the medium. When antiserum was present, however, the plaques remained localized and did not increase in number. It was therefore possible to deduce that there was one plaque-forming unit (presumably an infected cell) in approximately 10^5 cells. If antiserum was removed from the medium, the foci became larger and eventually confluent, with release of infective virus into the medium. No infective virus has been isolated from medium or cells at times when no plaques were visible.

By taking a small population of recovered cells it was possible to grow a sub-line which did not give rise to plaques spontaneously, even in medium free from antiserum. This sub-line was compared with the original HeLa cells for susceptibility to virus recovered from a single plaque in the recovered cells and then grown in chick cells to form a virus stock. With a large dose of virus, the virus-free recovered cells underwent a confluent cytopathic effect with release of virus. Comparative infectivity titrations in the recovered cells and the HeLa cell stock indicated, however, that 10–100 times as much virus had to be present to infect a recovered cell as that required to infect a stock HeLa cell.

The mechanism of persistence of herpes virus in recovered cells has yet to be elucidated; but the susceptibility of these cells to virus, although reduced, does not suggest a situation resembling temperature bacteriophage, and it is also unlikely that auto-interference of the type studied by Henle *et al.*[4] could be the explanation. It is possible that persistence is due simply to the very slow spread of virus in the selected population of less-susceptible cells in the presence of inhibitory medium. This would resemble persistence of poliomyelitis virus in partially resistant HeLa cells, studied by Ackermann and Kurtz[5] and Vogt and Dulbecco[3]. Unlike the poliomyelitis-infected cultures, however, the recovered cells continued to carry herpes virus in the presence of strong antiviral serum. This is in keeping with the known ability of this virus and the related *B* virus of monkeys to spread between contiguous cells in antiserum[6,7].

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