Animal Cell Strains

The Cell Culture Collection Committee has assembled and certified 23 strains of animal cells.

Over the past decade, the development of animal cell cultures in biological research has led to a profusion of cell lines, strains, clones, and derivatives. During their maintenance through continuous serial culture, substrains, even in one laboratory, have arisen which differ in characteristics. Many such differences result from significant biological events related to the natural history of cell populations, and these are proper subjects for analysis. On the other hand, technical artifacts also appear which may be related to handling and culturing of cells, to contamination with microorganisms (notably *Mycoplasma*) paradoxically occurring in the presence of antibiotics, and even to inadvertant admixture of animal cells of different lineage.

By 1959 it became clear that the scientific community needed standard or reference cell lines possessing constant and dependable characteristics. Several conferences and discussions led subsequently to the formation of a Cell Culture Collection Committee (*1*) in 1960 to initiate and coordinate a national program for characterizing and preserving animal cell strains and to establish a repository and distribution center for reference cultures. The committee's specific functions were to advise and aid in the development of a collection of animal cell strains; to formulate policies, procedures, and standards; to accept and certify cell lines which satisfy established standards; to facilitate cooperation among participating laboratories and other interested investigators and institutions; and to encourage research in the characterization and preservation of cell lines.

The feasibility of implementing the program depended in large part upon the accumulated evidence (i) that cells could be frozen and preserved at extremely low temperatures for long periods of time and (ii) that cells could then be reactivated without significant loss of viability or apparent change in properties. However, characterization procedures were mostly in the developmental rather than the implemental stage, and further research was required to provide a basis for classification of animal cell strains. Means were available or were sought to permit cells to be certified as to freedom from contamination, to be identified with regard to animal species of origin, and to be characterized on the basis of karyology, morphology, growth characteristics, and in some instances, by metabolic activities, nutritional requirements, genetic or biochemical markers, virus susceptibilities, tumorigenicity, or other special properties. Incomplete as these might be for classification or even identification of cell strains, they have at least provided an approach toward a systematic characterization.

The operation of the program, in large part, has been entrusted to grantees in participating laboratories (*2, 9*). These laboratories developed freeze-preservation methods suitable for preparation and storage of reference seed stocks wherein reasonable numbers of viable cells could be recovered unaltered in their properties and repropagated within a relatively short period of time. They have adapted known characterizing procedures or have developed new ones as the program has progressed (*3–7*).

Cell lines or strains for the collection are accepted by the committee on the basis of wide usage, special characteristics, or historical interest. Cultures with known histories are submitted by the original investigator or other authoritative sources. In the cooperating laboratories, each cell line undergoes an initial series of sterility, identification, and characterizing tests. The cells are processed in antibiotic-free media, and the cultures are expanded to provide sufficient numbers for a frozen reference seed stock. All essential tests are performed on samples of the latter, and critical tests are verified by a second cooperating laboratory or the submitting investigator. After review and certification by the committee, the frozen reference seed stocks are placed in the cell repository at the American Type Culture Collection for expansion and distribution as necessary.

The reference seed stocks are prepared essentially as follows. Cells are cultivated as monolayers or in suspension cultures in antibiotic-free media as specified by the submitting investigator (departures from this procedure being made only with approval of the submitting investigator). To obtain suitable numbers of cells (usually about 10^9) for preparation of reference seed stocks, cultures are expanded in flasks of large size, with minimum handling to reduce opportunities for contamination. When applicable, *p*H is controlled by incubation in a CO_2-air atmosphere. Cells are harvested with trypsin, trypsin-EDTA, or by mechanical dispersion, then centrifuged and usually resuspended in the specified culture medium to which a protective freezing additive such as glycerol or dimethylsulfoxide has been incorporated. Sufficient numbers of cells are frozen in each portion so that subsequent thawing and tenfold dilution with fresh medium produces a desirable inoculum without separate handling to remove the additive. Thus, portions of 2 to 5 $\times$ 10^6 cells are dispensed into 1-ml thick-walled glass ampules and sealed. Reference seed stocks in approximately 200 ampule batches are slow-frozen at controlled rates in liquid nitrogen vapor and stored at liquid nitrogen temperatures (−160°C to −190°C). The efficiency of the preservation procedure is assessed principally by three indicators of viability, trypan-blue exclusion, plating efficiencies, and growth response by replicate culture procedures; each test is performed both on the suspension to be frozen and immediately after the frozen samples are rapidly thawed. A portion of the reference ampules is subjected to various characterization tests by the

The present membership of the Cell Culture Collection Committee includes investigators from laboratories cooperating in the program, representatives from study sections of the NIH (Cell Biology, Pathology, Virology, and Rickettsiology), from the National Cancer Institute, and others as follows: W. F. Scherer (chairman), Cornell University Medical School, New York, L. L. Coriell, T. C. Hsu, D. W. King, S. H. Madin, H. T. Meryman, H. R. Morgan, H. M. Rose, K. K. Sanford, J. E. Shannon, R. E. Stevenson, C. S. Stulberg.

cooperating laboratories, and when indicated, to special characterizations by the submitting laboratory.

Reference cells prepared and preserved in this manner are accepted for the repository and certified by the committee when the following criteria and tests of the expansion process and of the reference seed stock have been judged satisfactory. Cell cultures are tested and shown to be free of contaminating microorganisms (including *Mycoplasma*) and cytopathic agents. The species of origin of the cell lines are verified with reference antiserums by one or more immunological procedures —the fluorescent antibody test (*4*), the mixed agglutination test (*5*), the cytotoxic dye exclusion test (*6*), the hemagglutination test (*7*), or their equivalent.

The media used to propagate and prepare the reference cultures are those employed or suggested by the submitting investigators. Growth characteristics are determined from data obtained on cell yields at each harvest and from cultures initiated from the frozen seed stocks. Growth rates, viabilities, or plating efficiency properties are established for each cell line. Morphologic characteristics both of living and stained cells (including colonial morphology, if feasible) under the particular conditions of growth are recorded by photomicrographs. Exact chromosome counts are made on 50 cells to determine the chromosome stemline number, distribution and presence of structural rearrangements, and chromosome breaks. Karyotypes are performed on ten cells in the stemline number to identify markers and to determine the stability of chromosome morphology within the stemline chromosome numbers. The reference cell lines are also characterized by their response to polioviruses of known cytopathic capacity in primate cells, and with other viruses when such tests are indicated. Other specific characterizations, such as tumorigenicity, biochemical markers, and so forth, are applied to the reference cultures when indicated, in order to verify their identities with respect to characteristics described in the literature. The methods of characterization and preservation adopted at present are similar to those described in (*3*).

Table 1 lists and summarizes some essential characteristics of 23 cell lines currently certified by the committee and deposited in the cell repository at the American Type Culture Collection. For cataloging purposes, cell strains have been designated as "certified cell lines" (CCL) and have been given accession numbers in the order in which they have been accepted by the committee. Variants, derivatives, and clones have been indicated by decimal annotation of the parent strain, for example, CCL 2 is HeLa, CCL 2.1 is HeLa 229, and so on. The committee has prepared a registry of certified cell lines (*8*) which contains full descriptions, histories, and pertinent references for each cell line, descriptions of the characteristics of the reference seed stocks, and information regarding the availability and procurement of certified cell strains from the repository. It is expected that strains originating from domestic ani-

Table 1. Cell lines certified by the Cell Culture Collection Committee (as of May 1964).

Accession No. (CCL)	Name of cell line	Species of origin	Tissue of origin	Characteristics of preserved reference cells								
				Serial passage		Morphology*	Karyology			Viability (%)	Plating efficiency (%)	Susceptibility to polio virus
				From origin	Of clone or derivative		Ploidy†	Modal No.	Markers			
1	NCTC, Clone 929	Mouse	Connective	648	553	FB–L	Aneu	66	Yes	81–96	‡	No
1.2	L–M	Mouse	Connective	U§	119	FB–L	Aneu	60	Yes	≈63‖	‡	No
2	HeLa	Human	Carcinoma, cervix	U	90–102	EP–L	Aneu	79		≈90	45	Yes
2.1	HeLa 229	Human	Carcinoma, cervix	U	78–88	EP–L	Aneu	82		≈92	44	Yes
3	Detroit-6	Human	Sternal bone marrow	160		EP–L	Aneu	64		≈85	42	Yes
3.1	Detroit-6, Cl.12	Human	Sternal bone marrow	214	54	EP–L	Aneu	60, 63		≈90	52	Yes
4	Minnesota-EE	Human	Esophageal epithelium	66		EP–L	Aneu	67	Yes	≈95	33	Yes
5	L–132	Human	Embryonic lung	U		EP–L	Aneu	71		≈90	37	Yes
6	Intestine 407	Human	Embryonic intestine	273		EP–L	Aneu	76		≈94	55	Yes
7.1	LLC–MK_2, Derivative	Monkey¶	Kidney	203	8	EP–L	Aneu	70		≈92	45	Yes
8	CCRF S–180 II	Mouse	Sarcoma 180	88		FB–L	Aneu	86	Yes	≈92	64	No
11	NCTC, Clone 2472	Mouse	Connective tissue	458	260	FB–L	Aneu	52	Yes	79–84	10	No
12	NCTC, Clone 2555	Mouse	Connective tissue	472	235	FB–L	Aneu	56	Yes	81–90	70	No
13	Chang liver	Human	Liver	255	5	EP–L	Aneu	70		≈88	21	Yes
14	B14–FAF–G3	Ch. Hamster#	Peritoneal cells	450	63	FB–L	Quasidi	22		≈93	47	No
15	HaK	Syr. Hamster**	Kidney	99		EP–L	Aneu	57		≈74	50	No
16	Don	Ch. Hamster#	Lung	29		FB–L	Eu	22		≈90	13	No
17	KB	Human	Carcinoma, oral	361		EP–L	Aneu	77		≈90	64	Yes
18	Detroit-98	Human	Sternal bone marrow	117		EP–L	Aneu	63		≈90	50	Yes
21	AV_3	Human	Amnion	270		EP–L	Aneu	74		≈95	20	Yes
23	HEp–2	Human	Carcinoma, larynx	350	110	EP–L	Aneu	76	Yes	≈85	44	Yes
24	J–111	Human	Peripheral blood††	314		EP–L	Aneu	111	Yes	≈89	54	Yes
25	WISH	Human	Amnion	167		EP–L	Aneu	74, 75		≈91	65	Yes

* FB–L, Fibroblast-like; EP–L, epithelium-like. † Aneu, di, and eu indicate aneuploid, diploid and euploid, respectively. ‡ Not applicable. § U, unknown. ‖ The symbol in this table indicates "approximately." ¶ *Macaca mulatta*. # *Cricetulus griseus*. ** *Mesocricetus auratus*. †† *Monocytic leukemia*.

mals, aquatic animals, and exotic animal species, as well as strains grown in chemically defined media, diploid strains from a variety of species, and strains with special chromosomal configurations, biochemical markers, and virus susceptibilities, will be placed in the repository within the next year. Investigators who wish to submit or suggest cell strains to the collection in these or other categories are requested to write to the chairman of the committee.

References and Notes

1. The committee was called together by the late J. T. Syverton under grant auspices and by authorization of the former Viruses and Cancer Panel, National Advisory Council, National Cancer Institute, after conferences held at the National Cancer Institute, 15 May 1959, and the Rockefeller Institute, 3 June 1959.
2. Laboratories currently participating in the program are: Child Research Center of Michigan, Detroit (C. S. Stulberg); South Jersey Medical Research Foundation, Camden (L. L. Coriell); Naval Biological Laboratory, University of California, Berkeley (S. H. Madin); and Cell Repository, American Type Culture Collection, Rockville, Md. (J. E. Shannon).
3. *Syverton Memorial Symposium on Analytic Cell Culture, National Cancer Institute Monograph 7*, R. E. Stevenson, Ed. (Washington, D.C., 1962).
4. C. S. Stulberg, W. F. Simpson, L. Berman. *Proc. Soc. Exptl. Biol. Med.* **108**, 434 (1961); W. F. Simpson and C. S. Stulberg, *Nature* **199**, 616 (1963).
5. R. R. A. Coombs, *Natl. Cancer Inst. Monograph 7*, 91 (1962).
6. A. E. Greene, L. L. Coriell, J. Charney, *J. Natl. Cancer Inst.* **32**, 779 (1964).
7. K. G. Brand, and J. T. Syverton, *ibid.* **24**, 1007 (1960).
8. *Registry of Animal Cell Strains Certified by the Cell Culture Collection Committee* (U.S. Govt. Printing Office, Washington, 1964). Copies of the Registry may be obtained from the Cell Repository, American Type Culture Collection, 12301 Parklawn Drive, Rockville, Md.

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A System for Studying Microbial Morphogenesis: Rapid Formation of Microcysts in Myxococcus xanthus

Abstract. *A method has been found for inducing the rapid, quantitative, and relatively synchronous conversion of vegetative rods of a fruiting myxobacterium to microcysts. The conversion is induced by the addition of 0.5*M *glycerol to a dispersed, growing, liquid culture of* Myxococcus xanthus. *The vegetative rods are converted to microcysts in about 120 minutes.*

Part of the life cycle of the fruiting myxobacterium, *Myxococcus xanthus*, involves the conversion of vegetative rods to round refractile cells called microcysts (*1*). Adye and Powelson (*2*) were the first workers to describe a method for inducing this conversion under relatively defined conditions in liquid medium. Under these conditions, however, the conversion is highly asynchronous and requires about 3 days.

As part of our interest in describing this conversion in biochemical terms, we have sought a method for carrying out the conversion in liquid medium rapidly, quantitatively, and synchronously. We need not elaborate on the value of such a system for examining microbial morphogenesis. This report describes such a technique.

Myxococcus xanthus, strain FB, was grown at 30°C with shaking in liquid CT medium (Bacto-Casitone, 2 percent; $MgSO_4$, 0.008*M*; K_2HPO_4–KH_2PO_4, 0.01*M*, *p*H 7.6). Cells from the exponential phase of growth were centrifuged at 3°C; washed once with cold 1 percent Casitone containing 0.008*M* $MgSO_4$; inoculated at a cell density of about 3×10^8 cells per milliliter (200 Klett units with a No. 54 filter) into a liquid medium containing 1 percent Casitone, 0.008*M* $MgSO_4$, and 0.5*M* glycerol; incubated at 30°C. The presence of glycerol caused the conversion of the vegetative rods to microcysts.

Figure 1 illustrates the sequence of events during a typical experiment. There is invariably a sharp increase in turbidity immediately after addition of glycerol. Within 1 minute the turbidity returns to the original value or goes below it. [Mager *et al.* (*3*) have described similar changes in optical density resulting from the addition of solutes to cultures of a variety of gram-negative bacteria.] For the first 35 minutes there is no visible morphological change. Then, as indicated in Fig. 1, the cells convert to short rods, ovoids, nonrefractile spheres, and refractile spheres in sequence. The entire conversion requires about 120 minutes and is relatively synchronous. Total cell counts during this period indicate no decrease in cell numbers.

Factors which affect the conversion are as follows: (i) Aeration is required (20 ml of medium in a 250-ml erlenmeyer flask subjected to about 250 strokes per minute on a shaker is sufficient). (ii) $MgSO_4$ is required, while 0.01*M* potassium partially inhibits the conversion. (iii) The conversion will take place when Casitone is replaced by the amino acid mixture which supports growth of *M. xanthus* (*4*). Under these conditions, however, the process requires about 5 hours and is highly asynchronous. (iv) Exponentially growing cells convert much more satisfactorily than lag or stationary phase cells.

Microcyst formation may also be induced by the addition of 0.5*M* glycerol directly to the growth medium during

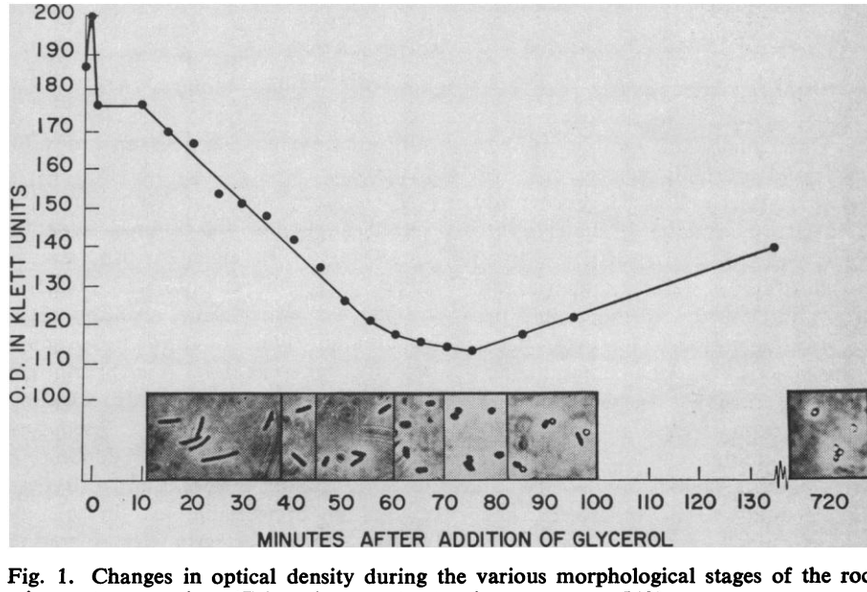

Fig. 1. Changes in optical density during the various morphological stages of the rod-microcyst conversion (Zeiss phase-contrast microscope, × 540).