Discussion of Genetics of Somatic Cells (Bar Harbor, Maine, Sept. 1960)
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its frequency and its relation to extrinsic and intrinsic factors. Critical experiments are yet to be done with cells derived from homozygous animals and with close passage-to-passage coordination of *in-vitro* cultivation and retransplantation to animals isogenic with the original donor. Isogenicity can be best insured by using highly inbred mouse strains, critically tested with skin grafts, and restricting the time elapsing between the origin of the line and its actual testing to the possible minimum, or, alternatively, by using some such technique as frozen storage of spermatozoa and artificial insemination to reproduce the histocompatibility factor equipment of the original donor or animals isogenic with it.

The possibilities for working out methods for transferring genetic information between somatic cells such as DNA-transformation or virus-mediated transduction have been discussed. *A priori* the feasibility of some such procedure is no less probable than it had been with bacteria.

DISCUSSION

DR. BURDETTE: Dr. Leonard A. Herzenberg will open the discussion of Dr. Klein's paper.

DR. HERZENBERG: The exciting reports of Drs. George and Eva Klein and their collaborators on isoantigenic variations in tumors stimulated us to explore the possibility of extending their genetic analysis *in vivo* to the cell-culture situation *in vitro*. In this symposium on methodology, let me indicate some of the methodologic advantages for detailed genetic analysis that cell-culture systems afford us.

First and foremost, since the demonstrations of Puck,[1029] cloning is a rapid and reproducible procedure in work utilizing cultures of cells. Populations derived from one cell are routinely available, and all the tricks of the microbiologist are applicable to the mammalian-cell system.

Cells have been cultivated from various tissues of a number of mammals in semi-defined media;[313] that is, in synthetic media to which are added a small percentage of dialyzed serum proteins. These media may contain traces of unknown materials, but the macroconstituents—amino acids, carbohydrates, purines, pyrimidines, most vitamins, and salts—are present in known concentrations. Thus, variations in nutritional needs or susceptibility to metabolic poisons can be searched for, and, if suitable ones are found, these can be employed as cellular genetic markers. One or two nutritional variants have been described.[244] However, these have not yet been too useful in genetic studies due to the difficulty in effectively selecting for these variants.

A number of drug-resistant variants of mammalian cells in culture have now been found. With some drugs, for example, 6-mercaptopurine, 8-azaguanine, and amethopterin, a resistant cell can easily be selected from a large population of sensitive cells,[366, 787] and it has been possible to answer some basic questions about the genetics of resistance to these compounds. With other drugs, for example the fluorinated pyrimidines, efficient selection of individual resistant cells from a background of sensitive cells has proved to be more difficult. Nevertheless, workers in a number of

laboratories, including our own, are continuing to characterize variations in resistance to antimetabolites, with the hope that some of these may become useful genetic markers.

The main emphasis in our laboratory is an exploration of the isoantigens of cells in culture. The *H*-2 antigens of the mouse seem particularly promising from a geneticist's point of view. These antigens are controlled by a complex locus on the ninth chromosome of the mouse and have been the subject of extensive and detailed immunogenetic analysis in several laboratories for a number of years.[455, 1238] At least 20 alleles have been described at this locus, and many antigenic components have been associated with most of these. Thus if the *H*-2 antigens can be detected on cultured cells with reasonable facility, a large number of markers become immediately available for genetic studies *in vitro* by simply preparing cultures from different strains of mice. Moreover, one can have confidence that the phenotypes (serotypes) of these cells bear a direct relationship with the genotype. One considerable advantage *a priori* is the possibility of using cultures derived from IR lines of Snell, or F_1 hybrids of these. Then one can have cultures which are genetically identical except for one gene, or at most a short region of one chromosome.

Several methods of scoring the *H*-2 antigens of cultured cells suggest themselves as possibilities. These all involve the use of isoantisera and are potentially as specific as the sera which can be obtained with all the skills of the immunogeneticist. We are now adapting the cytotoxic method to cultured cells.

Cells of a DBA/2 lymphoma *P*-388, whose nutritional needs *in vitro* have recently been determined,[552] are lysed when incubated with an anti-*H*-2*d* antiserum and guinea-pig complement. This lysis has been conveniently followed with an electronic cell counter. The viability of unlysed cells then can unambiguously be determined by plating the culture in growth medium and counting the number of clones which develop. With these procedures, we have found that these cells in long-term cultures retain the *H*-2*d* phenotype of the strain from which they were derived. Continued cycles of cellular killing and regrowth have selected no stable variants which have lost the *H*-2*d* antigens. No tests have been performed for individual antigenic components.

Now that the basic selection procedure has been tried, future work will be directed toward attempts to select variants from an F_1 hybrid-derived line of cells, where loss of only one dose rather than two doses are needed and to selection of variants for some of the individual antigenic components.

Means which do not result in the death of cells exhibiting an *H*-2 phenotype must be explored. We have demonstrated that, in the absence of complement, the isoantisera are completely without effect on cellular viability. Thus labeled antibodies, either fluorescent or radioactive, should be tried to score cells. The technique of mixed agglutination, where red cells which share an antigen with a cultured cell are coupled to such cells with antiserum, is another potentially useful method.

To conclude, the facility with which cells can be cloned, and the complete control of environment obtainable with methods of cellular culture is broadening the scope and increasing the analytic power of mammalian somatic-cell genetics.