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OFFICE OF THE DIRECTOR

ADVANCED RESEARCH PROJECTS

Report Prepared by - Dr. Samuel Graff

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For Period - July 1, 1961 to June 30, 1964

Contract - Nonr 266 (76)

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Contractor - Columbia University

Principal Investigator - Dr. Samuel Graff

Assistants - Dr. Hermann Adolf Moser and Mrs. Ada M. Graff

Title of Project - The Development of Functional Cell Strains

Summary of Results

a) During the period of this Nonr contract we have developed, and employed in related research problems, three different cell culture devices which enable us to simulate in vivo conditions under rigid control.

1. The cytogenerated is a perfused suspension system in which the cell population is retained wholly within the growth chamber. The population increases logarithmically to a ceiling. As high as 5×10^6 cells per ml have been reached in such systems, the generation times varying, under control, from 14 to 40 hours. Our apparatus has a capacity of 500 ml.

2. The chemostat is a device in which the population of logarithmically replicating cells is held constant by harvesting the cells at precisely their replication rates. Exquisitely sensitive steady-states are achieved in this device. Moreover, these steady-states are extremely sensitive to alterations of the

1.6

environment. Thus, this instrument is a valuable tool for studying the effects of drugs, hormones, etc., on a cellular level.

3. The pachycytogenerator is a device for growing solid masses of cells in artificial stromas in vitro to more nearly simulate their in vivo status, where cells rarely, if ever, grow in suspension. While our experience with this particular system is not yet uniformly good, we have obtained as high as 20 grams of cells in some instances. This system has been used by McCoy even more successfully.

We have employed the several systems in a number of different researches, some fundamental, but others in the direction of providing systems capable of truly massive production of selected types of cells and cell products. We can now grow a number of cell types in mass, but increasing the scope of cultivation to include cell types not hitherto grown, for burn replacement tissue banks or for post-radiation therapy, to cite only two possible applications, requires a more complete knowledge of the factors influencing replication and differentiation. This knowledge we are acquiring.

b) During the current report period

1. We have completed an extensive analysis of the population dynamics and transition states of cells cultivated in vitro. Such cells are heterogeneous not only in the randomness of their distribution through the cycle, but also with respect to their functional and replicative capacities. These transitions form fully replicative cells capable of synthesizing both DNA and RNA, to functional, but non-replicative cells capable of synthesizing RNA but not DNA, and senescent cells in which the capacity to

synthesize either RNA or DNA is able to, constitute a primitive type of cell differentiation. Not only do these findings have pathological diagnostic implications, they also provide a part of the fundamental information necessary for expanded application of the culture systems.

2. We have solved the forty year enigma of glycolysis by animal cells. All cells exhibit inordinate glycolysis in culture, whereas ordinarily only cancer cells do so in vivo. We have demonstrated for the first time that glycolysis is in fact a transitory defense mechanism against excessive influx of glucose. Normal cells in vivo do not have to avail themselves of this protection device because they have no glucose influx problem; the hormonal milieu, to which they are responsive, controls the transfer of glucose across the normal cell membrane. Cancer cells, on the other hand, are refractory to hormonal control, and, therefore, do have a glucose influx problem. This flux is dissipated by glycolysis. These findings not only suggest therapeutic implications, they also provide the basis for the investigation of hormonal reactions at a cellular level.

3. Animal cells react to virus infection by synthesizing a substance which inhibits viral replication. This is the basis of the interference phenomenon. The substance, either peptide or protein in nature, is called interferon. It is our avowed intention to develop methods of production and purification of interferon, to produce sufficient amounts of the material for clinical trial, and to characterize the substance chemically. We find that this laboratory is uniquely fitted for this project. Only by our mass cultivation systems can the enormous numbers of cells, both for assay and production be produced. We have reconstructed the

laboratory, engaged another professional worker, and have already made good progress. We have produced respectable quantities of interferon (respectable in comparison with what other laboratories have done) and are now improving our unit yields.

4. We have continually improved our cell culture systems, not only to increase their applicability for research, but also to arrive at fool-proof fully automatic controlled systems for general use in any laboratory. We have already constructed chemostats of novel design in which a much higher precision in temperature control is assured, and in which homogeneity of the culture is assured by means of a vibratory stirrer of novel design. We have learned how to fabricate elastic seals out of silicone, and our controls over gas atmospheres are now more economical and efficient.

5. The major source of energy for our cell cultures is obviously not carbohydrate (see paragraph 2 of this report). The energy source must be one or more of the amino acids in the medium. Accordingly, we have worked out most of the operations involved in the gas chromatographic quantitative microanalysis of the amino acids. We have worked out the quantitative volatilization of very small amounts of the acids by conversion to their acetyl propyl ester. We expect not only to measure the utilization of amino acids by the cells but also the effects of hormones on the character and distribution of the intra-cellular free amino acids.

6. We have continued work on the pachyocytogenerator in an effort to produce large masses of cells by tissue-like adhesion to artificial stromas. We have had only indifferent success with this device, but on occasion, we have been able to produce 20

to 30 grams of cells in this way. We believe that the forthcoming amino acid utilization data (see paragraph 5 of this report) will prove helpful in this case. We believe that successful solid mass cultures will find their greatest usefulness in interferon production (see paragraph 3 of this report).

Plans for the Future

Immediate plans - 1) We plan to improve our apparatus and its control devices. We have already designed pH and O₂ tension monitors and feed-back devices. We have designed new growth chambers which can be upscaled readily as desired.

2) We plan to study amino acid metabolism in great detail. We have already begun analysis of amino acid consumption and of free intracellular amino acid composition. The technical aspects have all been worked out.

3) We plan to study the mechanism of hormone reactions on a cellular level by measuring the effects of these hormones on carbohydrate metabolism, amino acid consumption and intracellular free amino acid distribution.

4) We plan to pursue the interferon problem vigorously.

5) We plan to study the influence of O₂ tension.

Long range plans - We plan to pursue the several problems cited with the eventual goals of devising reliable general purpose devices for large-scale production of cells of any type desired, of establishing cell banks for therapeutic use, and of producing valuable cellular products not only for experimentation but for broader application.

Graff, S., Moser, H.A., Kastner, O., Tanenbaum, M., and Graff, A.M. The Significance of Aerobic Glycolysis. Abstract, VIth International Congress of Biochemistry, New York, 1964. Full paper submitted to the Journal of the National Cancer Institute.