EFFECT OF CELL CULTURE ON CELL ANTIGENS[1]

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FOREWORD

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The publication of this paper in the Academy Journal represents a unique trend in reporting some of the youth science activities of the Tennessee Academy of Science. Here for the first time, a scientific investigation performed by a high-school student is presented in recognition of the excellence of his work and for his contributions in promoting the objectives of the Tennessee Academy of Science. This report was submitted by the author in December 1964 for entry in the Westinghouse National Science Talent Search of 1965 and in the Tennessee Science Talent Search of 1965. This paper won State and National Honors. It is hoped that the publication of this paper by John William Cross, Jr., a senior of White Station High School, Memphis, Tennessee, will inspire other junior scientists to follow his wonderful example.

INTRODUCTION

Cell culture, the growing or maintaining of the cells of higher organisms in vitro, (Parker 1961, White 1963) has uncovered many new phenomena. One of these is that cells explanted and grown in vitro for a minimum of five weeks through several monolayer subcultures frequently take on a different appearance from that of cells growing directly from the primary explant, (Barsky and Cassingena 1963, Faulkner 1964, Shelton *et al* 1963).

In some cases these "changed" cells have been shown to be malignant, and in others, normal (Barsky and Cassingena 1963). These facts lend importance to the study of such changes.

Frequently changes in morphology are accompanied by differences in protein structure (Landsteiner 1963). Such differences are often detectable by immunological reactions. These methods were applied in this project. The aim was to grow mouse kidney cells in culture for over six weeks with repeated subculture, prepare antisera to them and to isologous uncultured cells, and compare the antigens of the two types.

PROCEDURE

Four C3H predominately inbred mice were killed by etherization, and the following procedures were carried out using sterile technique: The kidneys were removed, rinsed in Hanks' balanced salt solution (HBSS) (Parker 1961) and chopped finely. The tissue fragments were pipetted into a tube and centrifuged at 500 rpm for ten minues (as were all other centrifugations unless otherwise noted). The supernatant fluid was removed, and the tissues were resuspended in 10 ml. cell-free chick embryo extract.

[1] These experiments were performed by the author, a senior in the White Station High School of Memphis, at the Kennedy V.A. Hospital in Memphis in the Medical Research Laboratory under Dr. Wolcott B. Dunham and Mrs. Frances Ewing.

Five milliliters of the suspension were inoculated into a 200 ml. flat-sided bottle and 1 ml. into each of the four 5 ml. Falcon plastic flasks. To the bottle was added 5 ml. of chicken plasma, and to each flask 1 ml. of the plasma. Clots were allowed to form as the bottles were incubated overnight at 35.5°C. The next day 1 ml. of growth medium (calf serum 20%, 199 medium 80%) (Parker 1961) was added to each culture, and the cultures were then placed in an incubator at 35.5° C. in an atmosphere with 5% CO_2. The remainder of the tissue suspension (to be called UK) was frozen at −70° C. for later use in serological reactions.

The cultures (to be called MKII) were each supplied 0.5 ml. growth medium two or three times per week, and examined microscopically at those times. When possible, subcultures were made to 200 ml. bottles without clots for monolayer growth.

The plastic flask cultures suffered contamination on the 21st day of culture when a subculture was attempted, and they had to be disposed of. The bottle culture, however, remained sterile and was serially subcultured five times by the end of the project.

Five or six weeks after the beginning of culture, many cells began to have a different appearance from cells growing directly from the original explants. In the beginning, epithelial and fibroblastic cells predominated, but later a new, round cell appeared, and was in the majority by the end of the culture period (87 days) (see Fig. 1).

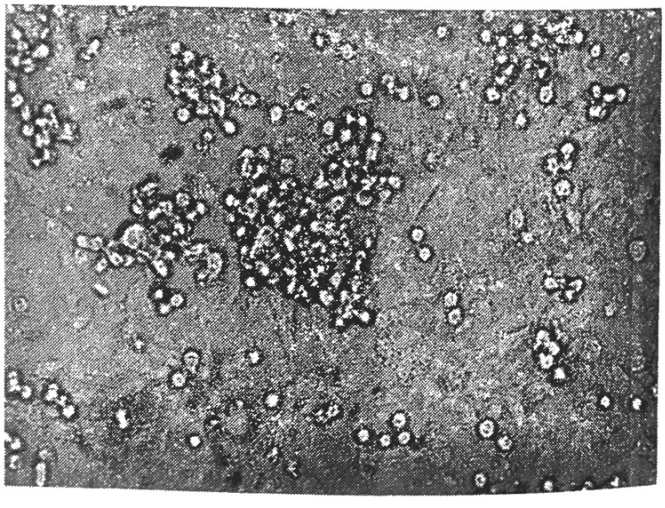

Fig. 1. MKII Cell Strain after 80 days in culture. Note the rounded appearance of many of the cells (taken at 100x).

The rounded appearance was not due to trypsinization (no trypsin was used), and the growth rate, though good, was insufficient for all the rounded cells to have been in mitosis. Since the trait lasted through repeated transfer, it can be ventured that there was a change in basic morphology.

While the cells were in culture, two female albino rabbits were bled for normal serum. Bleeding was by cardiac puncture. The serum obtained was frozen for future use in testing to insure that the rabbits were not previously immune to mouse kidney.

After six weeks of culture, immunization was begun. Rabbit "A" was injected with MKII cells scraped of the glass of monolayer subcultures. The cells were centrifuged, the supernatant fluid discarded, and the cells were resuspended in HBSS for injection.

Rabbit "B" was injected with an uncultured kidney tissue suspension (K). This had been prepared from the kidneys of five mice of the same stock as supplied the kidneys for MKII. They had been removed, ground in a Ten Broeck tissue grinder, and suspended in HBSS. The three subcutaneous injections, each of 0.5 ml. of the respective suspensions, were each made one week apart. The rabbits were bled 10 ml. one week after the last injection to obtain sera for testing.

This being completed, the immunological testing was begun. Aggulutination and immunodiffusion reactions were used.

For agglutination the procedures of the Kahn test were followed, but the mouse kidney antigen suspensions and the "A" and "B" antisera were substituted in the procedures. No agglutinations could be noted, even with microscopic examination. After two repetitions failed to produce noticeable results, it was decided to rely entirely on the immuno-diffusion test.

An agar diffusion plate test was used. Six nine-centimeter plates of Difco Noble Agar with merthiolate and methyl orange stain added were poured 1 cm. deep. Near the center of each plate three 1 cm. diameter holes were cut 1 cm. apart with a cork borer. A drop of liquid agar was placed in each well to give it a bottom.

Sera were placed in well #1, and antigen suspensions were placed in wells #2 and #3. The plates were incubated at approximately 37°C. in a metal pan covered with plastic film to maintain moisture. The reagents were replenished once a day.

The antigens and the antibodies in the antisera diffuse in all directions from the wells in the moist agar. Where a nomologous antigen-antibody pair meet, a precipitin reaction takes place. The diffusion of the reagents is halted at those points, and a visible precipitate line is formed when the reagents are in sufficient concentration. The lines can be of several types indicating different relationships between the antigens.

TABLE I

RESULTS OF THE AGAR IMMUNODIFFUSION

Plate	Well 1. (antisera)	Reaction	Well 2. (antigens)	Reaction	Well 3, (antigens)
1.	Anti-MKII	+	MKII	+	K
2.	Anti-MKII	+	MKII	+	UK
3.	Anti-K	+	MKII	+	K
4.	Anti-K	+	UK	+	K
5.	normal serum	—	MKII	—	UK
6.	normal serum	—	UK	—	K

The results of the reactions are shown in Table I. The reactions were all of the U-curve type indicative of similar antigens. The antigens MKII, K and UK may be identical, or merely similar; the reactions were not specific enough to say. The quantitative nature of the Kahn test might have clarified this situation if it had been successful. An immunoelectrophoretic study to determine if differences do exist would be extremely helpful.

In conclusion, in this case it was not possible to differentiate antigens of mouse kidney cells from those of similar cells in culture for over six weeks using the immunodiffusion plate test of Ouchterlony.

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