KB cells, easy to obtain and inexpensive to grow in tissue cultures, were used as successfully as monkey kidney cells for the isolation and identification of polioviruses.

Tissue Cultures of KB Epithelial Cells for Poliomyelitis Virus Tests

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PUBLIC HEALTH laboratories are often called upon to test various human specimens for polioviruses. A variety of human and monkey cell cultures are employed (*1*) for this purpose, but their use presents certain limitations for small laboratories operating on limited budgets or those at some distance from biologic supply firms. The use of such cells requires ready access to a source of materials and may prove expensive. The cultivation of certain cells, such as the HeLa cell, by serial passage, has proved troublesome at the Rocky Mountain Laboratory and other laboratories. Utilization of a cell line that can be more easily grown by serial passage, such as the epidermoid carcinoma, strain KB (*2*), which is susceptible to poliovirus (*3*), offers advantages of availability, economy, and convenience.

This paper describes a method for growing KB cells serially in tissue cultures and the results obtained in tests for the isolation and identification of polioviruses.

Material and Methods

KB cells grown and maintained as stock cultures in square 16-ounce bottles were transplanted to smaller containers for test purposes. Stock bottles seeded with 3 ml. of a cell suspension (approximately 3,600,000 cells) and 14 ml. of growth medium yielded confluent sheets of closely packed cells suitable for transplanting after 7 days' incubation at 36° C. These cells were trypsinized, centrifuged at 600 rpm for 10 minutes, and resuspended in a volume of medium 8 times that of the original cell inoculum. Thus, each stock bottle yielded 24 ml. (8 x 3 ml.) of transferable cell suspension. For use in tests, 2-ounce prescription bottles were inoculated with 0.5 ml. (600,000 cells) of the cell suspension and 10 ml. of medium. When test tube cultures were prepared, each tube received 1 ml. of the suspension diluted 1:20 in medium (60,000 cells).

Growth medium, 90L, for the KB cells consisted of Scherer's maintenance solution (*4*) containing 0.5 percent lactalbumin hydrolysate, 10 percent horse serum, and 125 units each of penicillin and streptomycin per milliliter. Cultures were ready for use after 3 days of incubation when bottles contained scattered islands of proliferating cells and tubes contained nearly confluent sheets of cells. Prior to inoculation with test specimens, the growth medium was replaced with medium 95M which consisted of 90L diluted with an equal volume of maintenance solution. To avoid heavy growth and

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consequent overcrowding of cells, test cultures were used within a few days. Stock cultures of KB cells could be stored for several weeks at 28° C.

Cultures also were prepared from trypsinized rhesus monkey kidneys (*5*). MK cells were planted in 2-ounce or 16-ounce bottles, or in test tubes, in Hanks' solution (*6*) containing 0.5 percent lactalbumin and 5 percent horse serum. This medium was replaced after 5 days incubation with a similar one containing 5 percent calf serum instead of horse serum, and an additional 20 ml. 4.4 percent $NaHCO_3$ per liter of medium. Before using the cultures, when grown to yield confluent sheets of cells after 7 to 10 days of incubation, the latter medium was replaced with a similar one containing only 2 percent calf serum but an additional 20 ml. $NaHCO_3$.

Prototype and types 1, 2, and 3 polioviruses were titrated in tube cultures of KB and of monkey kidney cells. One-tenth milliliter aliquots of tenfold dilutions of virus were inoculated into 6 tubes per dilution and the cultures were observed for 7 days for virus-induced cytopathogenic changes. Titers of virus obtained with the respective cells were calculated (*7*) from the highest dilutions which produced cellular changes.

Neutralization tests for identification and typing of polioviruses were performed (*5*) in both cell cultures with poliomyelitis antiserums from hyperimmunized monkeys. Neutralization of virus activity by pooled or by type-specific antiserum within the 4-day observation period was the basis upon which agents were identified as poliovirus or a type thereof.

Isolation of polioviruses from human specimens was attempted with both KB and monkey kidney cell cultures. Human fecal specimens were prepared as 20 percent suspensions in saline and centrifuged at 8,000 rpm for 30 minutes. Supernates, to which 125 units each of penicillin and streptomycin per milliliter had been added, were inoculated in 0.4-ml. amounts onto cultures. Throat washings, collected in 20 ml. of 1 percent bovine albumin in Hanks' solution with antibiotics, were inoculated in 1 ml. amounts. Daily for 7 days, cultures were examined microscopically for cytopathogenic changes; changes observed in second passage cultures were considered specific. Viruses in such culture fluids were then differentiated in poliomyelitis-neutralization tests. If a discrepancy occurred in the test results between the MK and KB cultures, the test specimens were retested in cultures of both cells. Data on original tests of most specimens on monkey kidney cells were obtained through Dr. Carl L. Larson and William Wicht, Rocky Mountain Laboratory.

Results

Results of titrations of 9 suspensions, representative of 3 types of virus, indicated that both tissue-culture systems were of approximately equal sensitivity for the detection of polioviruses (see table). In tests of virus dilutions near the end point, changes were sometimes noted earlier in monkey kidney cells than in KB cells, possibly because the irregular shape of the former cell permitted easier detection of changes. Differences noted were not significant, however, since cellular changes were essentially identical within several days. Alterations induced in KB cultures were similar to the progressive changes observed in monkey kidney cultures. Individual cells rounded into refrac-

Titers of polioviruses obtained in cultures of MK cells and KB cells

Poliovirus	Virus titer [1]	
	MK cells	KB cells
Stock strains		
Type 1:		
Mahoney	$10^{-6.6}$	$10^{-6.6}$
Brunhilde	$10^{-6.4}$	$10^{-6.7}$
Type 2:		
MEF	$10^{-6.5}$	$10^{-6.0}$
Lansing	$10^{-6.3}$	$10^{-6.4}$
Type 3:		
Saukett	$10^{-7.0}$	$10^{-6.4}$
Leon	$10^{-6.8}$	$10^{-6.7}$
Recent isolates		
Type 1: No. 3420	$10^{-6.8}$	$10^{-7.0}$
Type 2: No. 2682	$10^{-6.7}$	$10^{-6.8}$
Type 3: No. 2647	$10^{-6.7}$	$10^{-5.6}$

[1] Log of $TCID_{50}$/ml.

tile spheres which detached from the glass surface until the entire culture was destroyed.

Eighteen known polioviruses, 3 prototype and 15 recent isolates, previously identified in monkey kidney cultures as types 1, 2, or 3, were retested in KB cells with type-specific and pooled antiserums. In every instance type-specific and pooled antiserums caused clear-cut neutralization of virus. Results of these tests were identical with those previously obtained with monkey kidney cultures.

Identification of 80 recently isolated viruses of mixed species was attempted by neutralization tests in which only pooled poliomyelitis antiserums were used. Thirteen agents were identified as polioviruses with both MK and KB cells. The remaining 67 viruses were not inactivated by the antiserums when tested in either cell type. Apparently KB cells are as effective as monkey kidney cells for identification of polioviruses inasmuch as all 13 known polioviruses and 67 nonpolioviruses, subjected to tests in both cells, were accurately differentiated with KB cultures.

Approximately 300 suspensions of feces or throat washings were tested for the presence of cytopathogenic agents. Viruses were detected in 29 specimens when monkey kidney cells were used and in 62 specimens when KB cells were employed. Of the agents isolated, 22 in monkey kidney cultures and 24 in KB cells were polioviruses. These isolations were made from the same specimens except in the two instances where cytopathogenic effects appeared only in KB cells.

Agents other than polioviruses were detected in 38 specimens tested with KB cultures and in 7 of the same specimens with monkey kidney cells. Thus, the KB cells detected all polioviruses or other viruses that were isolated from human specimens with MK cultures.

Discussion

KB cell cultures used for the isolation or propagation of viruses should consist of a dispersed cell pattern so that cellular changes caused by virus can be detected readily. Cultures containing heavier growth or confluent cells can be used with polioviruses, which produce rapid and complete degeneration of cells. When viruses that are slow growing or that produce minimal cytopathogenic changes are cultured, the use of confluent cells may result in overcrowding and loss of cell detail, interfering with the detection of changes if tests are to be held longer than a week. Suitable cultures are easily prepared and quickly available if containers are inoculated with appropriate numbers of cells.

The sensitivity of KB cells to polioviruses, as demonstrated in titrations, in neutralization tests, and in isolation of the virus from naturally infected specimens, indicates that this cell may be substituted for or used to supplement MK cultures now used for these purposes. Also, KB cells appear more susceptible than monkey kidney cells to many nonpolioviruses. This observation will be considered fully in another paper.

Summary

Cultures of KB cells were as sensitive as cultures of monkey kidney cells in titrations of 9 suspensions of polioviruses representative of the 3 antigenic types. Eighteen known polioviruses were accurately identified by neutralization tests with KB cells. When 80 unidentified viruses were subjected to neutralization tests with poliomyelitis antiserums, 13 polioviruses detected by monkey kidney cells were also identified by KB cells. In tests of 300 human-fecal suspensions or throat washings, 22 polioviruses detected in cultures of MK cells were also isolated in KB cells. Since cultures of KB cells can be propagated serially, they are less expensive and more readily available than monkey kidney cells commonly used for the isolation and identification of polioviruses.

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State Mental Health Legislation in 1957

Broad expansion of community mental health services took place during 1957 as a result of the large volume of State legislation aimed at stimulating mental health programs in the communities and at improving conditions for the mentally ill.

During that year, according to reports received by the National Institute of Mental Health, Public Health Service, California, Minnesota, New Jersey, and Vermont passed laws providing grants-in-aid for community mental health services. Such legislation had been passed by Connecticut, New York, Pennsylvania, Indiana, Tennessee, and Florida in previous years.

Laws authorizing counties to levy taxes or to appropriate funds to support local mental health centers were passed by Iowa, Kansas, and South Dakota in 1957.

States and Territories which took steps to modernize laws governing commitment, detention, care, and treatment of the mentally ill were California, Colorado, Kansas, Minnesota, Montana, North Dakota, Texas, and Alaska.

Legislative action in Connecticut, Maine, Minnesota, New Hampshire, Oregon, Rhode Island, and West Virginia ratified the Interstate Compact on Mental Health, already subscribed to by Massachusetts, New York, and New Jersey. For care and treatment of the mentally retarded, Arkansas, Nebraska, and Texas authorized the construction of new institutions, the State of Washington set up a diagnostic and training center, and New York is planning a research institution. Idaho and Minnesota made it mandatory for local school districts to provide instruction for such handicapped children.

In Washington, a resident treatment center for emotionally disturbed children is being set up for research and treatment and a center for such children has also been authorized in Minnesota.

For juvenile delinquents, a special program of intensive treatment in California is being initiated in two institutions of that State. Among the States taking action to expand research and training were Texas, North Dakota, Iowa, and Ohio, where a research program in alcoholism was set up at the College of Medicine of the Ohio State University.