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Polioviruses in Tissue Cultures of Human Conjunctiva and Kidney Cells.* (22377)

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In the search for a stable strain of tissue culture cells which could be propagated serially on glass, and which could be substituted for monkey kidney cells in mass production of polioviruses, the presumably normal human cell lines established by Chang(1,2) were studied.

To determine if 2 of Chang's cell lines derived from conjunctiva and kidney could support the multiplication of the polioviruses, growth curve experiments were carried out in bottle cultures of these cells and compared to control growth curves in similar cultures of first generation rhesus monkey kidney cells.

Materials and methods. Technics used in this laboratory for the serial subcultivation of conjunctiva and kidney cells have been reported(3). The Mahoney, MEF-1 and Saukett strains of poliovirus were received from the Connaught Laboratories, Toronto, Canada, and were the 3 monkey kidney tissue culture adapted strains, Types 1, 2 and 3, used in the preparation of Salk vaccine. They were the strain pools 54, 55 and 56 distributed to each of the 28 laboratories participating in the 1954 poliomyelitis vaccine field trial. The Parker strain of Type 1 virus was obtained from Dr. David Bodian(4) in the 52nd monkey kidney tissue culture passage. It received 4 additional rhesus monkey kidney passages in our laboratory. The Cleveland 80-4 strain of Type 1 virus, obtained from Dr. Albert Sabin(5), was isolated in HeLa

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cells from a child with "coryza." It received 3 passages in HeLa cells followed by 2 rapid serial passages and 3 terminal dilution passages in cynomolgus monkey kidney cells. In our laboratory it received 3 additional rhesus monkey kidney passages. The YSK strain of Type 2 virus was obtained from Dr. Albert Sabin(6) in 52nd monkey kidney passage. It was an avirulent variant produced by rapid serial passage and terminal dilution technics in monkey kidney tissue cultures. It received 3 additional rhesus monkey kidney passages in our laboratory. Virus titrations were carried out in roller tubes of rhesus monkey kidney cells using half log dilutions, 4 tubes per dilution, and the infectivity endpoints were calculated by the method of Reed and Muench. Growth curves were obtained in milk dilution and Povitsky bottle cultures of conjunctiva and kidney cells. Both cell types were prepared for the growth curve studies in 2 types of media, (1) Chang's medium (CM) consisting of human serum 20%, Hank's balanced salt solution 75%, chick embryo extract 5%, and crystalline soybean trypsin inhibitor 0.001%, and (2) Eagle's medium (EM) consisting of horse serum 20%, Eagle's medium 80%, and crystalline soybean trypsin inhibitor 0.001%. To both media were added penicillin (100 μ), streptomycin (100 μg) and nystatin (25 μg). Monkey kidney cell cultures were cultivated in a nutrient medium consisting of solution 199 (99.5%) and horse serum (0.5%). Prior to infection cell cultures in milk dilution bottles were washed 5 times with 8 ml of balanced salt solution to remove the serum. On the basis of an estimated 10-fold dilution of serum with each washing the residual serum concentration was less than 1 part in 500,000. The bottles were then inoculated with 1 ml of poliovirus with a titer of $10^{5.9}$ to $^{7.7}$ $TCID_{50}$ per ml. Nine ml of solution 199 was added immediately and a sample removed for titration ("Seed" sample) after which the bottle was incubated one hour at 37°C. The medium was then removed, the culture was washed 10 times with 8 ml of balanced salt solution to remove unabsorbed virus and the bottle was then refed with 10 ml of solution 199. Samples for titration were removed immediately after this washing procedure ("0" hour sample) and at intervals of 1, 4, 8, 24, 48 and 72 hours. Povitsky bottles were washed 10 times with 100 ml of balanced salt solution prior to infection. The bottles were then inoculated with 25 ml of poliovirus. Five hundred ml of Eagle's medium was added immediately and samples for titration were removed at varying intervals ranging from 24 to 138 hours. Growth curves of the Parker, Mahoney, MEF-1 and Saukett strains in trypsinized monolayer cultures of first generation rhesus monkey kidney cells cultivated in milk dilution bottles served as controls.

Results. The results of titrations of 3 separate growth curves of Parker strain carried out in milk dilution cultures of conjunctiva cells are presented in Table I. After the adsorption period and the washing, between $10^{0.83}$ and $10^{1.7}$ $TCID_{50}$ of residual virus were left in the 3 experiments.

The graphic summary of the replicate growth curves of the Parker strain carried out in the 2 cell lines cultivated in CM is presented in Fig. 1. Similar triplicate growth curves of the Mahoney, MEF-1, Saukett, Cleveland and YSK poliovirus strains were essentially identical and could be superimposed on the curves of Fig. 1. For brevity these 30 growth curves are omitted. Fig. 2 shows single growth curves of Parker, MEF-1 and Saukett strains carried out in milk dilution bottle cultures of conjunctiva and kidney cells cultivated in EM. These two cell lines had been cultivated in EM containing 20% horse serum for a period of 4 months and still

TABLE I. Three Growth Curves of Poliovirus Type I, Parker Strain in Conjunctiva Cells. (Chang's medium.)

Time	Reciprocal of log $TCID_{50}$/.25 ml Exp. 1	Exp. 2	Exp. 3
Seed	5.25	5.25	5.7
0 hr	1.75	.83	1.33
1	1.75	1.75	1.7
4	2.33	2.75	2.7
8	5.0	5.33	5.83
24	5.67	6.7	6.75
48	6.7	7.2	6.75
72	7.17	6.5	7.0

Seed virus at 37°C = $10^{4.33}$ $TCID_{50}$ after 72 hr.

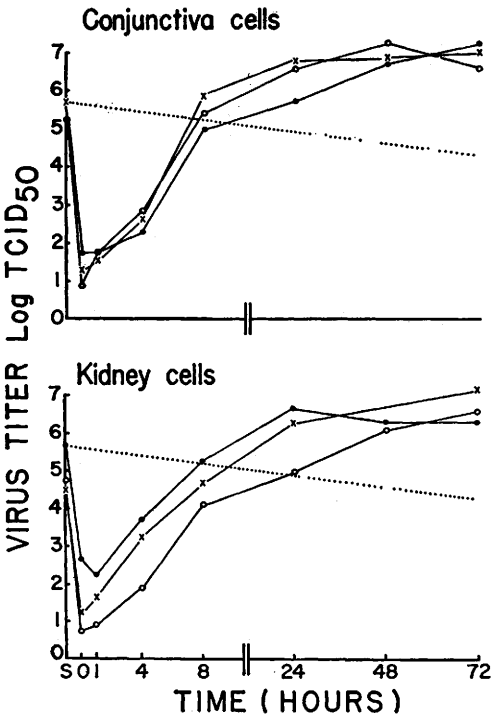

FIG. 1. Three replicate growth curves of Parker strain of Type 1 poliovirus in human conjunctiva cells and human kidney cells cultivated in milk dilution bottles in Chang's medium with 20% human serum. The dotted line represents seed virus incubated at 37°C in the absence of cells. "S" is the sample removed immediately after inoculation and "O" is the sample taken after period of adsorption and washing to remove excess virus.

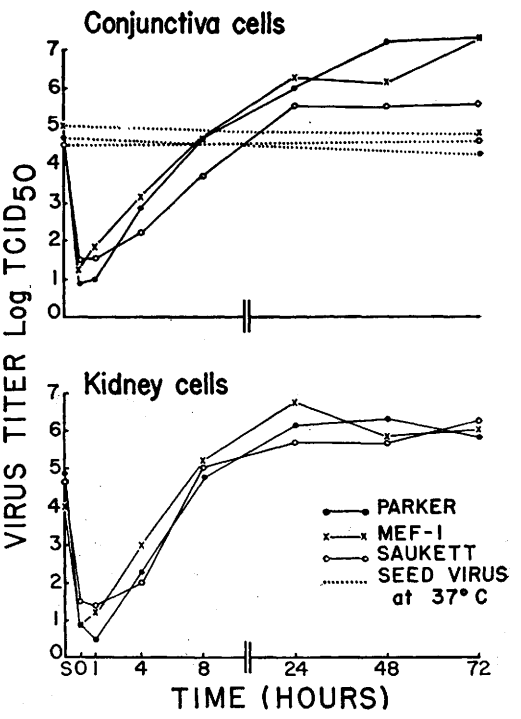

FIG. 2. Growth curves of polioviruses in human conjunctiva and human kidney cells cultivated in milk dilution bottles in Eagle's medium with 20% horse serum.

supported the growth of polioviruses as well as the parent cell lines which had been maintained in CM containing 20% human serum.

Control growth curves of the Parker, Mahoney, MEF-1 and Saukett strains carried out in milk dilution cultures of trypsinized monkey kidney cells are shown in Fig. 3. The maximal virus titers occurred between 48 and 72 hours and were $10^{6.62}$ for the Parker strain, Type 1, $10^{7.75}$ for Mahoney Type 1, $10^{8.0}$ for MEF-1 Type 2, and $10^{7.13}$ for Saukett Type 3. Fig. 4 summarizes the single growth curves of Parker, MEF-1 and Saukett strains carried out in Povitsky bottle cultures of conjunctiva cells cultivated in EM.

Discussion. There is a remarkable similarity between the general appearance and shape of the 49 growth curves described here (involving 6 virus strains, 3 media, 3 cell types and 2 sizes of culture bottles). The 3 replicate growth curves of the Parker strain shown in Fig. 1 indicate the reproducibility of the curves as well as the range of variation of the

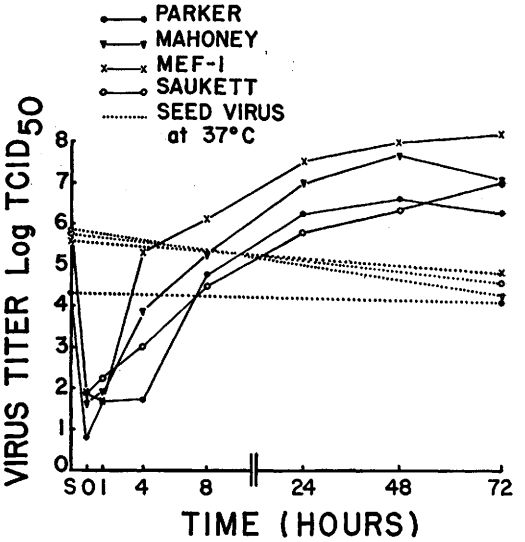

FIG. 3. Growth curves of polioviruses in monkey kidney cells cultivated in milk dilution bottles in medium 199 (99.5%) and horse serum (0.5%).

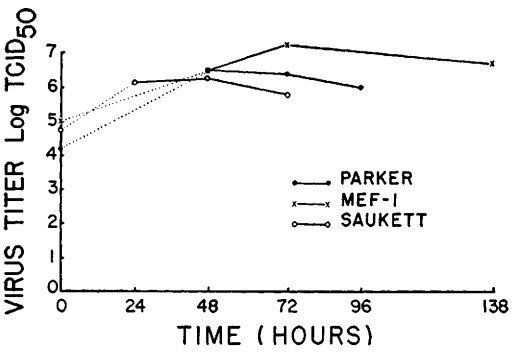

FIG. 4. Growth curves of polioviruses in human conjunctiva cells cultivated in Povitsky bottles in Eagle's medium with 20% horse serum.

method. There is usually a significant release of new virus by 4 hours. During the next 4 hours the virus release is very rapid and 20% of the curves reach the maximal titer by 24 hours, 65% by 48 hours. During the 72 hour period of observation there is no diminution of titer as a rule. No significant differences were observed between the 2 human cell strains or between these and monkey kidney cells. The yield of virus was approximately $10^{7.0}$ $TCID_{50}$/0.25 ml for all 6 viruses. This compares favorably with the virus titers of the Mahoney, MEF-1 and Saukett strains used in preparation of Salk vaccine for the 1954 trials where Povitsky bottle cultures of monkey kidney cells yielded virus pools with titers of $10^{5.5}$ to $10^{7.0}$ $TCID_{50}$(7).

Growth curves of poliomyelitis viruses obtained in monkey testis and kidney and strain HeLa have been described by Ledenko(8), Farrell(9), Melnick(10), Dulbecco(11), Lwoff(12), Scherer(13) and Ackerman(14).

The human conjunctiva and kidney cell lines as carried in our laboratory appear to satisfy all of the desirable technical criteria for mass production of polioviruses. They are easily grown on glass and can be passed serially in mass cultures. They can be carried in series in the absence of all human serum and retain their ability to support the proliferation of polioviruses. The relatively simple medium devised by Eagle or Parker's medium(199) with added horse or calf serum are adequate for prolonged cultivation. These sera can be removed by washing without damage to the cells. Finally, the growth curves of 6 poliovirus strains representing all 3 types give virus yields equal to those obtained in monkey kidney cells. The uniformity of the growth curves for all 6 strains suggests that the cells would support in a similar manner growth of other strains. These experimental growth curves have been obtained in mass cultures rather than on a test tube scale and the Povitsky bottles represent the container size which has been used for commercial mass production of poliovirus.

Summary. Tissue cultures of Chang's human kidney and human conjunctiva cells carried *in vitro* for 17 and 20 months, respectively, were tested for their ability to support growth of polioviruses. Three Type 1 strains, 2 Type 2 strains and 1 Type 3 strain were studied. Growth curves in the 2 human cell lines were compared with growth curves in first generation rhesus monkey kidney cells. The yield of viruses and the shapes of the growth curves were similar for all 3 tissue cultures. Human conjunctiva cells and human kidney cells which were cultivated 4 months in the presence of horse serum instead of human serum retained their ability to support the proliferation of polioviruses.

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Effect of Ethionine on Blood and Depot Lipids in Experimental Nephrotic Hyperlipemia.* (22378)

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The pathogenesis of nephrotic hyperlipemia is unclarified. An experimental approach to that problem has been facilitated since it was shown(1) that the renal disease induced in rats by the injection of anti-kidney sera obtained from rabbits simulates the nephrotic syndrome as observed in infants and children. Feinberg(2) and co-workers recently have shown that the oral administration of dl-ethionine markedly reduced the concentration of fatty acids, phospholipids and cholesterol in the blood of normal dogs. It thus seemed of interest to investigate the effect of this agent on the hyperlipemia that is regularly associated with the experimentally induced nephrotic syndrome in rats.

Procedures. Methods used to induce the nephrotic syndrome in rats have been described(1). All animals were kept in single cages, were fed Friskies and had unrestricted access to water. Determination of carcass and liver fat was carried out in rats that were fasted for 16-18 hours. They were exsanguinated from the heart under ether anesthesia. The liver fat concentration was determined by the method described by Payne (3) on approximately 1 g of liver tissue. The gastrointestinal tract was freed of its content by washing with saline solution. The entire carcass was then put through a meat grinder, dried at 100°C to constant weight (4-6 days) and ground to homogeneity with mortar and pestle. Three aliquot samples weighing approximately 1.5 g were placed in screw-cap bottles, covered with 50 cc petroleum ether and shaken intermittently for 48 hours. These were filtered under vacuum in fritted glass funnels lined with filter paper. The procedure was repeated until the filtrate was colorless. If not clear, the filtrate was centrifuged before evaporation and the fat residue was determined gravimetrically. 3.5 to 7.5 mg of dl-ethionine‡ per 100 g rat was given to 28 nephrotic rats by intraperitoneal injection twice daily for 5-21 days. A 2% saline solution of ethionine in 0.9% NaCl was used. Three additional rats were treated for 5 days with 13 mg ethionine and 12 mg methionine per 100 g rat per day. These agents were given in one syringe and their concentration was approximately equimolar.

Results. The effect of ethionine on blood lipids was studied in 12 nephrotic rats. A precipitous decline of the markedly increased total lipid and cholesterol values to normal or subnormal values was noted in all animals that received 11 mg or more of ethionine per 100 g rat per day (Fig. 1). When 7-10 mg was given, this effect was less regularly observed. The decrease occurred during the first 3-5 days of ethionine administration and subsided within a few days after its administration was discontinued. This effect was noted in male as well as in female rats. No other effect on the nephrotic renal disease was noted. Proteinuria, blood pressure and further course of the disease were not affected, and the microscopic appearance of the renal lesions was not different from that in

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