Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 85 APRIL, 1954 No. 4

SECTION MEETINGS

CLEVELAND, O.	
University Hospitals	February 8, 1954
DISTRICT OF COLUMBIA	
George Washington University	April 1, 1954
ILLINOIS	
Chicago Medical School	February 16, 1954
MINNESOTA	
University of Minnesota	March 24, 1954
SOUTHWESTERN	
University of Texas Medical School	March 5-6, 1954

Monolayer Tissue Cultures. II. Poliomyelitis Virus Assay in Roller Tube Cultures of Trypsin-Dispersed Monkey Kidney.* (20940)

J. S. YOUNGNER. (Introduced by Jonas E. Salk.)

From the Virus Research Laboratory, University of Pittsburgh School of Medicine.

The purpose of this report is to present data on the utilization of trypsin-dispersed monkey kidney cells prepared according to a modification(1) of the method of Dulbecco and Vogt(2) for titration of poliomyelitis virus in roller tube cultures. Data are presented on yield of satisfactory culture tubes and on the experience with titrations of poliomyelitis viruses in such cultures as compared with cultures containing tissue fragments.

Methods and materials. Preparation and standardization of trypsin-dispersed cell suspension, as well as materials employed, are the same as described earlier(1), including Medium D used to obtain outgrowth. At the time of virus inoculation, *Medium E,* composed of a mixture of 97.5 ml of Synthetic mixture 199 and 2.5 ml of 5% $NaHCO_3$/100 ml, was employed to replace medium D. Penicillin and streptomycin were added to a concentration/ml of 100 units and 0.1 mg, respectively. Medium D may be used to advantage when longer survival of cultures is desired, but was omitted in these studies to avoid any possible influence of the horse serum component on virus activity. *Preparation of roller tube cultures of kidney fragments.* Kidney fragments about 1-2 mm in diameter were prepared by mincing with scissors and were planted without exposure to trypsin. Two types of kidney fragment cultures were utilized: (a) kidney fragments grown under perforated cellophane(3), and (b) kidney fragments grown directly on the glass wall of the roller tube. From 5 to 10 fragments of kidney cortex were introduced into each tube and outgrowth obtained by

* Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

TABLE I.
Culture Tube Production from 18 Successive Batches of Trypsin-Dispersed Cell Suspension.

Date	Cell susp. stored 4°C (days)	Dil. of cell susp.	Approx. No. cells/tube (in 0.5 ml)	No. tubes prep.	No. tubes discard	% usable
			×1000			
7/17/53	0	1:100	314	290	0	100
9/ 3	0	1:200	350	199	9	95
4	0	1:200	500	40	2	95
		1:400	250	91	14	84
8	0	1:100	600	174	1	99
11	0	1:300	310	64	1	98
16	0	1:260	300	100	2	98
22	0	1:200	270	101	4	96
25	0	1:300	400	300	0	100
29	3	1:200	380	46	2	95
11/ 9	1	"	600	300	5	98
12	1	"	300	306	15	95
16*	1	"	470	312	26	91
20*	1	"	500	216	29	87
23*	1	"	550	300	30	90
12/ 3*	1	"	550	595	78	86
8	1	"	620	600	86	85
14*	1	"	310	300	34	88
15*	1	"	570	231	16	93

* Kidneys from rhesus monkeys employed in the preparation of batches marked with asterisk. All other batches prepared with tissue from cynomolgus monkeys.

incubation while rolling at 36°C; nutrient consisted of 2 ml of Medium D/tube. After 6 or 7 days a zone of healthy epithelial outgrowth surrounded one or more fragments in each tube. At that time, the medium used to obtain outgrowth of epithelial cells was removed and replaced by Medium E, and inoculations carried out as described below. *Inoculation of culture tubes with virus.* Using either 0.1 ml or 0.5 ml as inoculum, prepared using Medium E as diluent, the final volume of medium was brought to 2 ml in each tube with Medium E. Following inoculation, they were placed in a roller drum, since it has been found that with rolling, virus-induced cellular degeneration appeared more rapidly and non-specific degenerative changes occurred less frequently than when inoculated cultures remained stationary. Microscopic examination for signs of virus-induced destruction was carried out at 3 and 6 days following inoculation. Tubes showing typical signs of virus-induced degeneration were considered infected regardless of the degree of severity of the cellular changes. Titers were calculated according to the method of Reed and Muench(4) and were expressed as the number of 50% infectious doses (ID_{50})/ml of original virus pool, based on the dilution of virus added to the cultures. *Viruses employed.* The strains of virus used in these studies were Mahoney (Type 1), MEF-1 (Type 2), and Saukett (Type 3); the histories of these strains and their adaptation to tissue culture have been described previously(5).

Results. Yields of satisfactory cultures from successive batches of trypsin-dispersed cell suspension. Table I contains a summary of the results obtained with culture tubes prepared from 18 batches of trypsin-dispersed cell suspension. It can be seen that 85-100% of cultures were usable when freshly prepared suspension or suspension stored for one day at 4°C was employed; in one instance, suspension stored for 3 days was utilized. With certain batches of cell suspension, viability has been maintained after storage for as long as 7 days, the longest period studied. Suspensions have been shipped by air to cities 300 to 900 miles away, and have been transported by train, both without refrigeration; after arrival, suspensions were found to be usable for providing satisfactory cultures. The majority of cultures discarded were unsatisfactory because of incomplete sealing of

TABLE II. Summary of Data Comparing Infectivity of Poliomyelitis Viruses in Different Types of Kidney Cultures.

Cultures employed	Neg. log ID_{50} doses per ml — Type 1 Mahoney (pool #50)	Type 2 MEF-1 (pool #60)	Type 3 Saukett (pool #70)
Roller tubes			
Trypsin-dispersed cells	6.9	6.6	6.7
Fragments on glass	6.2	5.9	5.8
Fragments under cellophane	5.5	5.4	5.6
Petri dishes			
Trypsin-dispersed cells	7.7*	7.1	7.1

* Negative log of the number of plaque-forming particles/ml.

the tubes due either to defective caps or to a cracked lip on the screw-capped tube. Such incomplete seals permitted the pH to rise and remain above 8.0, thus preventing growth.

In tubes with complete closure, the pH of the medium dropped to 6.8 on the 6th or 7th day of incubation when 0.5 ml volume of medium was employed. With experience it was found that this pH drop could be used as a reliable indication of a usable tube without the necessity for microscopic examination. It was usually sufficient to discard all alkaline tubes and spot check microscopically only every 10th tube showing an acid pH. With amounts of medium greater than 0.5 ml/tube, the pH drop could not be used to aid in selection of usable cultures. For work requiring more precise observations all tubes were examined microscopically prior to inoculation.

Assay of poliomyelitis viruses in roller tube cultures of trypsin-dispersed monkey kidney cells. Comparison with titers obtained using kidney fragment cultures. Two to four replicate titrations of pools of virus strains representing each of the 3 immunologic types of poliomyelitis virus were carried out in 6- or 7-day-old roller tube cultures of trypsin-dispersed monkey kidney cells as well as in cultures of monkey kidney fragments; 4 to 10 cultures were used for each virus dilution. In each instance, higher virus titer was noted in roller tube cultures of trypsin-dispersed kidney cells than in the cultures employing fragments. The data summarized in Table II indicate that from 1.0 to 1.5 logs more virus activity was demonstrable when dispersed-cell cultures were used than when cultures of kidney fragments under cellophane were employed. The sensitivity of roller tube cultures of kidney fragments on glass was from 0.7-0.9 log less than that of cultures prepared with trypsin-dispersed cell suspension.

Using a technic similar to that recommended by Dulbecco and Vogt(2), plaque forming particle (p.f.p.) counts were done with the 3 virus pools employed in these studies. As a matter of interest, the counts obtained are included in Table II. In all cases, the number of plaque-forming particles/ml of original virus pool was somewhat greater than the number of ID_{50} doses/ml. The details of the method employed and a more detailed comparison of ID_{50} titers in roller tubes and p.f.p. counts in petri dishes will be made in a subsequent report.

Discussion. In attempting to understand the differences in sensitivity to virus encountered in the different kinds of cultures, the following factors may apply. In kidney fragment cultures, a proportion of tissue fragments do not grow out and with inocula containing only a few infectious particles, it is conceivable that these tissue fragments may adsorb and remove enough infectious particles so that multiplication of virus may not occur. With trypsin-dispersed cell cultures, the epithelial monolayers contain no large tissue fragments and all but a few of the cells are viable and susceptible, presenting a much reduced opportunity for adsorption of a virus to inert tissue material; it is likely that such cultures provide a greater opportunity for contact between virus and a susceptible cell, a factor of importance when few infectious virus particles are concerned.

Summary. The preparation of roller tube cultures of trypsin-dispersed monkey kidney cells and their utilization for the assay of poliomyelitis viruses has been described. Data have been presented that show that cultures prepared in the manner described are more sensitive indicators of the presence of polio-

myelitis virus infectivity than are cultures prepared from minced kidney fragments.

1. Youngner, J. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 202.
2. Dulbecco, R., and Vogt, M., *J. Exp. Med.*, 1954, v99, 167.
3. Youngner, J. S., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 697.
4. Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, v27, 493.
5. Youngner, J. S., Ward, E. N., and Salk, J. E., *ibid.*, 1952, v55, 301.

Received February 15, 1954. P.S.E.B.M., 1954, v85.

Role of Reticulo-Endothelial System and Disposition of Dietary Cholesterol in Various Mammalian Species.* (20941)

LAWRENCE FEIGENBAUM, SANFORD O. BYERS, AND MEYER FRIEDMAN.
From the Harold Brunn Institute, Mount Zion Hospital, San Francisco, Calif.

In a recent report from this laboratory(1), it was shown that the hepatic R-E cell is essential in the disposition of *exogenously* derived cholesterol in the rat. This was demonstrated not only by microscopic detection of cholesterol in the Kupffer cell within 6 hours following administration of cholesterol, but also by the production of hypercholesteremia and persistence of a chylomicronemia in rats given parenteral injection of various colloidal suspensions believed capable of interfering with hepatic reticulo-endothelial cell function.

In view of these results, it was decided to determine whether this hypercholesteremia following interference with the integrity of the R-E system could be duplicated in other species than the rat. Therefore, the present study was undertaken in which rabbits and dogs, as well as rats, received India Ink intravenously together with high cholesterol feedings.

Effect of intravenous administration of India Ink on plasma cholesterol and total lipid of animals on high cholesterol feedings. Plasma samples of all species studied were taken before and 48 hours after the beginning of the experiment. These were analyzed for their total cholesterol and total lipid content by methods previously described(2,3). In addition, most of the samples were examined microscopically under darkfield illumination for the presence of chylomicra.

Rats. Methods. Male rats of the Long-Evans strain, approximately 15 weeks old (avg wt 244 g), were used. Two series of rats were studied. Series A (15 rats) was given 100 mg of cholesterol in 3 cc of olive oil by stomach tube per day for 2 days. Series B (13 rats) was given 100 mg of cholesterol in 3 cc of olive oil by stomach tube intubation per day, *plus* 0.5 cc of 20% India Ink intravenously twice daily for 2 days.

Results. The control rats given cholesterol feedings alone, showed a rise in plasma cholesterol (Table I) but the average plasma cholesterol concentration at 48 hours was still only 64 mg/100 cc and the plasma lipid concentration was not found to change. However, when India Ink was injected into the rats of Series B in addition to the oral administration of cholesterol, a striking increase in both their plasma cholesterol and total lipid occurred. Thus, the average plasma cholesterol rose from 52 mg % before the experiment to 100 mg/100 cc 48 hours after the beginning of the experiment. The average plasma lipid content rose from 242 to 322 mg/100 cc in the same period of time.

As observed earlier(1), chylomicra invariably were absent from the sera of the control rats and always present in great numbers in the sera obtained at 48 hours from ink-injected rats. The latter sera, also, were invariably turbid.

* This work was aided by grants from the Life Insurance Medical Research Fund and the National Heart Institute, National Institutes of Health, Public Health Service.