Susceptibility to Poliomyelitis Virus of HeLa Cells Adapted to Growth in Horse Serum Medium. (21946)

KARL HABEL,* NANCY C. GREGG,* AND W. D. MCBRIDE.†

From the U. S. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, Bethesda, Md.

With the development of a subline of HeLa cells capable of growth in medium containing horse serum in place of human serum(1) it was of interest to determine its susceptibility to the effects of poliomyelitis virus.

Materials and methods. HeLa cells adapted to growth in human serum and the subline grown in horse serum were obtained from Dr. Virginia Evans in Dr. Earle's laboratory, National Cancer Institute, in the form of flask cultures on glass. Monkey kidney cultures were prepared according to the trypsinization method of Youngner(2). HeLa cells of both sublines were transferred from large bottles into tubes by mechanical washing of cells off glass with the proper growth media. 0.5 ml of a suspension containing approximately 75,000 cells per ml was inoculated into each tube. The tubes were incubated for 24-48 hours in a stationary position on their sides before use in the tests with virus. The growth medium consisted of 40% Earle's balanced salt solution, 20% ultrafiltrate from chick embryo extract, and 40% serum. At the end of the short growth period these cultures were changed to Eagle's synthetic medium containing 4% horse serum(3), which was quite efficient as a maintenance medium for both HeLa cell lines. Monkey kidney cultures were prepared in roller tubes by growing the original trypsinized kidney suspension in Melnick's "A" medium(4) for 4 days, at which time they were changed to a maintenance medium consisting of 75% Earle's balanced salt solution, 24% ox serum ultrafiltrate, and 1% horse serum. Poliomyelitis viruses used for quantitating the susceptibility of the cell lines were the Mahoney Type I, 6468 Type II (isolated in this laboratory from the stool of a case of paralytic polio), and the Saukett strain of Type III. Titrations of standard virus pools were carried out by inoculating 0.1 ml of each serial 10-fold dilution into each of 3 tissue culture tubes. Dilutions were carried out in Hanks' balanced salt solution. Cultures were read daily by microscopic examination for at least 7 days after inoculation. Stool emulsions prepared by extraction with balanced salt solution containing 2500 units each of penicillin and streptomycin, and clarified by centrifugation at 8000 r.p.m. for 30 minutes in a Spinco centrifuge, were used in a volume of 0.3 ml per tube. The tubes were then placed in the incubator for one hour, after which time the medium containing the stool emulsion was drained and replaced with maintenance medium.

TABLE I. Titrations of Standard Pools of 3 Types of Poliomyelitis Virus in Monkey Kidney Cells and HeLa Cells Grown in Horse Serum and Human Serum Media.

	Poliomyelitis virus type					
	Exp. 1			Exp. 2		
Tissue culture	I	II	III	I	II	III
HeLa						
(Human serum)	5.75*	4.5	5.75	5.75	5.75	5.75
(Horse ")	4.75	3.5	3.75	6.5	5.25	6.25
Monkey kidney	5.75	5.75	5.75	6.5	6.25	6.25

* Expressed as logs of 50% end-points of titrations per 0.1 ml.

Results. In Table I are given the 50% end-point titrations of each of the 3 types of poliomyelitis virus in the 3 different tissue cultures. Results of the first experiment in Table I indicated that the HeLa cells grown in the horse serum medium were less susceptible than were those grown in the human serum or the kidney cells. However, a second experiment indicated an equal susceptibility of all 3 tissue cultures.

In the first of these 2 experiments, on the 5th day following inoculation of the Type I virus at the 10^{-4} dilution when all tubes were showing marked cytopathogenic effect, super-

* National Microbiological Institute, Laboratory of Infectious Diseases.

† National Institute of Dental Research.

TABLE II. Multiplication of Poliomyelitis Virus in HeLa Cells Grown in Human Serum and in Horse Serum.

Tissue culture	Poliomyelitis virus type I	II	III
HeLa (Human serum)	5.75* 6.5	4.75	7.25
" (Horse ")	5.75 6.5	4.75	6.0

* Titration of 50% end-points in logs/0.1 ml in monkey kidney roller tubes.

nates were harvested from both the horse and the human serum HeLa cell lines. These were titrated in monkey kidney roller tubes. In the second experiment similar harvests were made from the 2 HeLa sublines on the 5th day after virus inoculation from the tubes which had received 10^{-5} dilution of Type I, 10^{-3} dilution of Type II, and 10^{-5} dilution of Type III virus. Again, these harvests were titered in monkey kidney roller tubes. The results of these comparisons of the ability of the 2 sublines of HeLa cells to grow poliomyelitis virus are indicated in Table II, and there is no significant difference between the 2, except perhaps in the one experiment where the human serum HeLa cells apparently gave greater multiplication of the Type III virus.

In an attempt to show the relative efficiency of the 2 sublines of HeLa cells in the isolation of polio virus from human materials, a total of 23 stool emulsions previously shown to contain polio virus, were inoculated into the various tissue cultures. These stool suspensions contained an equal number of materials originally positive in either monkey testicle or kidney cultures for each of the 3 types of poliomyelitis virus. In Table III are shown the results which indicate that the HeLa cells grown in horse serum are just as responsive to polio virus in human stools as those grown in human serum.

In Dr. Earle's laboratory this subline of HeLa cells grown in horse serum medium has been adapted to mass growth in a suspended cell shaker flask type of culture(1,5,6). Because of certain advantages for basic studies on interrelationships between virus and cell of such a culture containing large numbers of viable HeLa cells, multiplying while suspended in the culture medium, 2 experiments were carried out to determine polio virus multiplication in these suspended cells. The suspended cells were grown in Dr. Earle's laboratory. Horse serum adapted HeLa cells grown in stationary flasks were used as the inoculum for the shaker flasks. One flask contained over 400,000,000 cells (approximately 1.6 g wet weight) at the time of inoculation, and the second contained 1,000,000,000 cells (approximately 4 g wet weight).

TABLE III. Isolation of Poliomyelitis Viruses from Stools of Patients in HeLa Cells Grown in Human Serum and in Horse Serum.

Tissue culture	Positive stools/total tested Exp. 1	Exp. 2
HeLa (Human serum)	6/10	8/13
" (Horse ")	4/10	10/13
Monkey kidney	—	10/13

The total volume of each culture was centrifuged and the sedimented cells were resuspended in shaker flasks in 150 ml of Eagle's maintenance medium containing 4% horse serum. The first flask was inoculated with 3 ml of the 10^{-1} dilution of our standard Mahoney Type I virus, which has a titer of $10^{-7.5}$ per ml. A sample was removed immediately after mixing and again after 24 hours incubation. During incubation the flask was rotated at the rate of 9060 revolutions per hour and was aerated by flushing 4 times with 4 liters of 5% CO_2 in air. The second flask was inoculated with 0.1 ml of the 10^{-3} dilution of the same virus. This flask was rotated at 12,000 r.p.h. and aerated and sampled like the first. Assay of the samples from the first flask carried out in monkey kidney roller tubes showed an increase of virus titer from 10^{-5} per ml at zero hour to greater than $10^{-7.5}$ in 24 hours. Samples from the second flask titered at 10^{-2} at zero hour and $10^{-6.5}$ in 24 hours.

Discussion and Conclusions. Human serum is not readily obtainable in large volumes from a uniform source for use in tissue culture. Furthermore, the possible presence of specific antibodies introduces further difficulties in the use of cells grown in human serum media when working with certain human viruses. The adaptation of HeLa cells to growth in horse serum media eliminates these difficulties.

It would appear from these experiments

that HeLa cells adapted to growth in the presence of horse serum rather than in media containing human serum are quite susceptible to the cytopathogenic effect of all three types of poliomyelitis virus, and are as efficient in producing virus as the HeLa cells grown in the human serum. They likewise seem to be as efficient in their ability to demonstrate the presence of poliomyelitis virus in human stool suspensions. Preliminary evidence indicates that this subline of HeLa cell grown in mass suspended cultures is also capable of supporting the growth of poliomyelitis virus.

1. Perry, V. P., Evans, V. J., and Earle, W. R., *Science,* 1955, v121, 805.

2. Youngner, J. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 202.

3. Eagle, H., *ibid.,* 1955, v89, 96.

4. Black, F. L., and Melnick, J. L., *J. Immunol.,* 1955, v74, 236.

5. Earle, W. R., Bryant, J. C., and Schilling, E. L., *Ann. N. Y. Acad. Sci.,* 1954, v58, 1000.

6. Earle, W. R., Bryant, J. C., Schilling, E. L., and Evans, V. J., *Ann. N. Y. Acad. Sci.,* 1955, in press.

Received July 28, 1955. P.S.E.B.M., 1955, v90.

Glycemic Effects of Chlorpromazine in the Mouse, Hamster and Rat. (21947)

DAVID NORMAN AND WILLIAM A. HIESTAND.

From Laboratory of Animal Physiology, Department of Biological Sciences, Purdue University, Lafayette, Ind.

Various pharmacological effects of chlorpromazine (CPZ) have been reported in the literature among which are depression of body temperature, potentiation of morphine and barbiturates, anti-epinephrine as well as anti-acetylcholine action, and a depression of cellular activity or narcobiosis(1,2,3). The drug which structurally is 10-(3-dimethylaminopropyl)-2-chlorophenothiazine hydrochloride is similar in structure to promethazine but shows very little anti-histaminic property. In view of the various profound effects on the nervous system and cellular activity, the question arose as to the possible effects on blood sugar levels during the post-injection period when this substance is pharmacologically most active.

Materials and methods. The effect of intraperitoneal injections of chlorpromazine (Thorazine, S.K.F.) on blood sugar level of non-fasted and fasted white male mice of the Rockland (RAP) strain was first studied. Animals were injected with CPZ at 5 mg/kg body weight and blood samples were withdrawn *via* the orbital sinus(4), at intervals of 30 minutes, and at 1, 2, 3 and 5 hours later. Non-injected controls were bled at the same interval periods and blood sugar determinations similarly made. Since the response to CPZ injection in mice was a substantial wave of hyperglycemia, similar experiments were carried out with the hamster and rat. Also the glycemic effects of chlorpromazine in the alloxan diabetic mouse, and the effects, if any, of this substance on the hypoglycemic action of insulin were observed. Mice were separated into 2 groups, each group consisting of 16 animals. Group I was made diabetic by subjecting it to a 48-hour fasting period followed by injection of alloxan monohydrate, 75 mg/kg *via* the intracardiac route(5). After diabetes had been established, these animals were treated with CPZ and the glycemic effects determined. Group II mice received insulin injections in the following manner: Animals were fasted for 24 hours, injected with CPZ, 5 mg/kg, ip, and 2 hours later were injected subcutaneously with insulin, 1/6 unit per 18 g body weight. A minimum of 16 animals was used in all cases for each type of experiment with mice, hamsters and rats. Blood sugar determinations were made by the Somogyi-Nelson method(6).

Results. Normal male mice receiving CPZ