Propagation *in vitro* of Polioviruses. VIII. Effect of pH on Virus Yield and Cell Metabolism.* (22743)

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Susceptible animal cell cultures infected by any of a variety of cytolytic viruses undergo progressive cellular degeneration(1). The pH of the culture medium is decreased concomitantly by cell metabolic activity. The purpose of this study was to measure quantitatively the effects upon virus yield and upon host cell metabolism of purposeful alterations in pH of the medium. Strain HeLa cells and poliovirus, Type 1, strain Mahoney, were employed to learn the effect of increments in pH from 6.3 to 8.0.

Materials and methods. Host cells: Strain HeLa cells were employed, cultivated, and maintained as described(2). The growth medium was composed of 40% human adult serum and 60% Hanks' balanced salt solution (HuS-40, BSS-60). *Assay for virus*: Virus titrations were conducted with 5 replicate HeLa cell cultures at each successive geometric dilution. Titers computed as most probable numbers of tissue culture infectious units (TCIU) from the frequency of positive tubes agreed within the range ± 0.3 log. For virus titrations the human serum growth medium was removed from cell cultures by 3 rinsings with BSS and replaced with 10% chicken serum, 90% modified maintenance solution (MMS) medium(3). *Virus*. Poliovirus, Type 1, strain Mahoney, was employed as supernatant fluid from passage 65 in cultures of HeLa cells, stored at —70°C. A small amount of virus (usually 15 to 30 TCIU) was employed in each experiment so that several cycles of virus synthesis could be followed and thus small effects would be magnified. *Manometric method*. The procedure for the cultivation of HeLa cells in Warburg flasks and the measurement of respiration has been described(4). *Glucose determination*. The Nelson-Somogyi photometric method of estimating glucose was employed(5). Readings were made in a Beckman DU spectrophotometer at 650 mμ. *Buffers*. For pH studies the MMS medium was fortified with additional buffers. Sodium hydrogen phosphite(4), 0.01 M, tris (hydroxymethyl) aminomethane (THAM), 0.01 M and sodium hydrogen phosphate, 0.01 M were employed. When buffers were employed an equivalent molar concentration of sodium chloride was omitted from the medium so as not to increase tonicity. The required pH was obtained by the addition of 0.15 M hydrochloric acid or sodium hydroxide.

Results. Effect of pH on poliovirus yield in stationary HeLa cultures. The first experiment was designed to test the effect of pH in range from 6.3 to 7.3 upon the replication of poliovirus in stationary cultures of strain HeLa cells. Supplementary buffers were found necessary to maintain the pH. Several buffers were tested for toxicity for strain HeLa cells. Table I shows that phosphate, 0.02 M, phosphite, 0.02 M and THAM, 0.01 M, did not significantly alter the respiration of HeLa cells over an observation period of 78 hours. Medium, ChS-10, MMS-90 containing 0.01 M each of phosphite, phosphate and THAM was prepared. Aliquots of the medium were adjusted to the required pH by the addition of 0.15 M HCl or NaOH, and were made up to equal volume

TABLE I. Effect of Various Buffers on Respiration of Strain HeLa in ChS-10, MMS-90.*

Buffer	Molarity	Oxygen uptake, μl Hr of incubation 24	53	78
Control		212	475	751
Phosphate	.02	232	540	783
Phosphite	.02	235	550	800
THAM	.01	225	535	810

* 840,000 cells/flask.

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TABLE II. Effect of Variable pH upon Poliovirus Yield in Strain HeLa Cell Cultures.

pH			log TCIU/ml	
Initial	24 hr	37 hr	24 hr	37 hr
6.3	6.3	6.3	2.0	2.0*
6.5	6.5	6.5	2.5	3.5†
6.7	6.7	6.7	3.5	5.1‡
6.9	6.9	6.9	3.7	6.0
7.1	7.05	7.0	4.0	6.1
7.3	7.25	7.2	4.1	6.0

* Control bottles without virus demonstrated toxicity; over 50% of cells off glass, remaining cells rounded and granular.

† Control bottles without virus demonstrated toxicity; approximately 25% of cells off glass, remaining cells rounded and granular.

‡ Control bottles without virus demonstrated toxicity; few cells off glass, some rounding of cells.

with 0.15 M NaCl. Approximately 3.0 mg/ml of glucose were employed in the medium. Duplicate planar bottle cultures of strain HeLa were prepared at each pH increment and infected with approximately 25 TCIU of poliovirus. All cultures were incubated at 36°C. To better maintain the pH, 20% of the medium was replaced every 6 hours. The experiment was terminated at the first evidence of cytopathogenicity so that variations in the number of HeLa cells would not limit virus yields. Results of titrations of supernatant fluids harvested at 24 and 37 hours are shown in Table II. The virus yield from cells incubated at pH 6.7 or lower was significantly retarded or reduced. The possibility existed that decreased yield of virus observed at the lower pH levels might be due to a greater instability of the virus at these pH levels. Aliquots of media at pH 6.5, 6.8, 7.0 and 7.5 were prepared with $10^{3.8}$ TCIU/ml of virus and allowed to incubate at 36°C for a period of 42 hours. Titrations to determine the stability of poliovirus at these pH levels were performed. Titers all fell within the range $10^{3.5\pm0.3}$ TCIU/ml of virus. Maintenance of the original titer indicated that the virus was stable at the pH increments employed. *Effect of pH on HeLa cell metabolism.* To determine what effect alteration of pH had upon the metabolism of HeLa cells, quadruplicate Warburg flasks were prepared at each of 4 pH levels and 2 flasks of each series were infected with approximately 25 TCIU of virus. Medium, MMS-90, ChS-10, fortified with 0.01 M each of phosphate, phosphite and THAM, was employed. The results of three separate experiments are summarized in Table III. With alterations in pH from approximately 7.5 there resulted a decrease in oxygen uptake and a decrease in glucose utilization. The results at an initial pH of 6.9, 7.6 and 8.0 are shown in Fig. 1.

Discussion. Results of these experiments showed that the yield of poliovirus, Type 1, Mahoney strain, in HeLa cells was dependent upon the pH of the medium in which the host cells were maintained. The decreased yield of virus at decreased or increased pH levels was related to a decreased metabolic state of the cell cultures; such cultures failed to be maintained as shown by the decreasing rate of oxygen uptake. Fogh(6) investigated the effects of pH variation on absorption of poliovirus by monkey kidney cells; he concluded that possible differences in virus absorption at pH levels between 6.0 and 8.0 did not materially affect the latent period or virus yield. This evidence and evidence for the stability of poliovirus further supports the conclusion that reduced virus yield at low or high pH levels results from a decreased metabolic rate of the host cell. The present experiments also emphasize the importance of pH as a factor affecting metabolic rate of host cells and thus of virus yield. It is apparent that

TABLE III. Effect of Alterations in pH upon Poliovirus Yield and upon Metabolic Activity of Strain HeLa Cell Cultures.

pH		Glucose utilized,* μg/ml/10 hr/100,000 cells	Oxygen uptake,* μl/10 hr /100,000 cells†	Virus yield, log TCIU /ml
Initial	Final			
6.6	6.6	0	4.3	3.8
6.8	6.6	8	5.6	4.9
7.0	6.8	15	6.6	5.7
7.2	6.9	18	9.0	6.4
7.1	7.1	21	8.0	6.6
7.4	7.3	24	8.7	6.4
7.6	7.4	21	9.5	6.4
7.7	7.5	21	10.0	6.6
7.7	7.6	28	10.0	6.2
7.8	7.6	28	9.8	6.4
7.9	7.7	33	9.5	6.0
8.0	7.8	13	7.9	5.1

* Avg value obtained over period of observation.

† Initial live cell population.

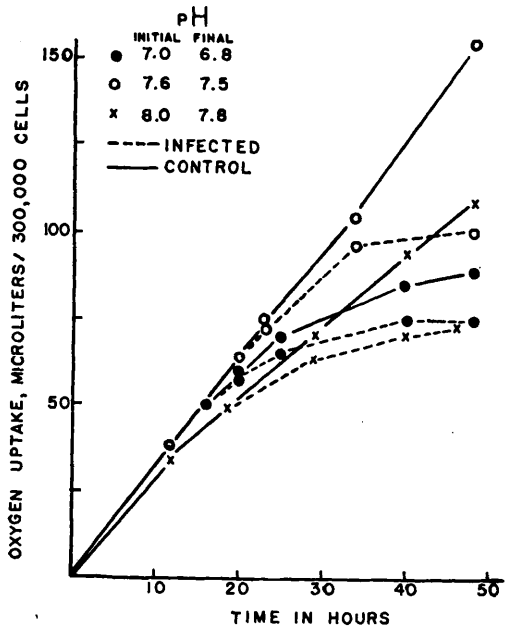

FIG. 1. Oxygen uptake of infected and control HeLa cell cultures at initial pH of 7.0, 7.6 and 8.0.

virus titrations should be carried out in adequately buffered media and that reduction of medium pH below 7.0 during incubation should be corrected. It is also apparent that in screening potential chemotherapeutic or chemoprophylactic agents the effect of pH should be considered. A test agent could act upon cells to increase glycolysis. The resultant increased acid production would thus lower virus yield to simulate specific interference with virus production.

Summary. The pH of tissue culture medium was altered purposefully to measure quantitatively the effects upon virus yield and host cell metabolism. By use of strain HeLa cells and poliovirus Type 1 (strain Mahoney) for growth in media of pH from 6.3 to 8.0 it was established that virus yield was affected by the pH of the culture medium. Decreased virus yield at decreased or increased pH levels was related to a decrease in metabolic rate of host cell cultures.

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Chronic Catheterization of the Renal Artery: Technic for Studying Direct Effects of Substances on Kidney Function.* (22744)

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During the course of studies on the pathogenesis of sodium retention in experimental congestive heart failure(1) it was shown that sodium retention occurred in dogs with normal basal glomerular filtration rates, suggesting the presence of increased tubular reabsorption of sodium. In order to obtain more direct information concerning the increased tubular reabsorption of sodium, a method has been developed for chronic catheterization of one renal artery, with collection of urine from each kidney individually. Such a technic affords the opportunity of studying the direct effect of salt solutions, hormones and drugs injected directly into the renal artery of the unanesthetized dog, using the opposite kidney as a control. Furthermore such experiments are relatively uncomplicated by extrarenal factors, and allow the separation of renal from

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