ELECTROPHORETIC STUDY OF THE INTERACTION OF RADIOACTIVE POLIOVIRUS WITH COMPONENTS OF CULTURED CELLS

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Received for publication 8 January 1963

ABSTRACT

THORNE, H. V. (University of Minnesota, Minneapolis). Electrophoretic study of the interaction of radioactive poliovirus with components of cultured cells. J. Bacteriol. **85**:1247–1255. 1963.—The interaction of P_{32}-labeled poliovirus with the postnuclear fraction of HeLa cell homogenates was first examined using radioactivity to quantitate the reaction. The effects of virus and debris concentration, suspending medium, temperature, and time on the reaction were determined. Binding was independent of salt concentration up to 1.0 M and unaffected by calcium and magnesium ions at 10^{-3} M. The postnuclear particulate components of homogenates of mammalian cells in culture were examined by sucrose gradient zone electrophoresis at pH 7.2 with a simple apparatus which permitted several simultaneous analyses. The distributions of components for different cell types were distinct and appeared to be influenced by conditions of culture. Addition of radioactive poliovirus to the homogenate before analysis was used to identify components with virus-binding activity. Activity of HeLa and human esophageal epithelium cell homogenates was found mainly in membranous fractions of relatively low electrophoretic mobility. Components with a broader spread of mobility were moderately active in cultured human amnion, but uncultured amnion had almost undetectable virus-binding activity and a distinct distribution of components. Rat heart cells and L cells did not bind poliovirus, but binding components were present in both ERK-1 and CRE rabbit lines.

Particulate substances which neutralize the infectivity of a number of enteroviruses were demonstrated in homogenates of virus-susceptible cells by Holland and McLaren (1959*a*) and McLaren, Holland, and Syverton (1960); similar neutralizing activity against Newcastle disease virus (NDV), Western equine encephalomyelitis (WEE), vaccinia viruses (Quersin-Thiry, 1961), and the virus of foot-and-mouth disease (Thorne and Cartwright, 1961) also has been reported. Holland and McLaren (1959*a*) noted a close correlation between susceptibility of the intact cell and cell-debris activity, and, from this and their observation that enteroviral ribonucleic acid (RNA) would infect insusceptible nonprimate cells, Holland, McLaren, and Syverton (1959) concluded that the components active in neutralizing virus were possibly the specific cellular receptors for enteroviruses. Isolation and characterization of these components and examination of corresponding components in the intact cell in the process of interacting with the infective virus particle is obviously necessary to test this hypothesis. Recently Holland and McLaren (1961) fractionated cell homogenates by differential centrifugation and found that the majority of the neutralizing activity for type 1 poliovirus and Coxsackie B_1 virus occurred in a microsomal fraction sedimenting between 7,000 and 38,000 × *g*. In the present work, a different approach to characterization and fractionation, employing electrophoresis and density gradient centrifugation, has been taken. This paper describes the application of electrophoresis to the analysis of homogenates of both poliovirus-susceptible and -insusceptible cells, using purified radioactive virus to mark components which bind virus. A second paper (Thorne, *in preparation*) presents electrophoretic analyses of cell fractions prepared by centrifugation. A summary of the results has been presented (Thorne, 1962).

MATERIALS AND METHODS

Cell cultures. HeLa, human esophageal epithelium (EE; Syverton and McLaren, 1957), and human primary amnion cells were cultured as monolayers in Eagle's basal medium (90%)

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plus 5% calf serum and 5% human serum, except where stated otherwise. The mouse L cell line, the rabbit CRE and ERK-1 lines, and a rat heart line established by L. C. McLaren were cultured as monolayers in 90% Eagle's basal medium plus 10% calf serum, 10% rabbit serum, and 10% human serum, respectively. Other cells were cultured or prepared as described in Results. Penicillin (100 units/ml), streptomycin (150 units/ml), and neomycin (50 mg/ml) were added to all culture media.

Virus. Type 1 (Mahoney) poliovirus was grown in HeLa monolayers in Eagle's basal medium with double-strength amino acids and vitamins. The virus suspension was harvested after 24 hr at 37 C, clarified by centrifugation, and stored at −20 C. Virus labeled with P_{32} was grown in citrate-buffered medium containing 25 to 50 μc/ml of radioactive orthophosphate, and was purified by chromatography on a diethylaminoethyl (DEAE) cellulose column (15 × 1 cm) by the method of Hoyer et al. (1959). The column fractions containing virus were dialyzed against several changes of 0.02 M phosphate buffer (pH 7.2) for 48 hr at 4 C, or centrifuged twice at 100,000 × *g* for 60 min (Spinco SW-39 rotor) to remove radioactive phosphate. It was generally necessary in the latter case to band the final labeled virus suspension in a 5 to 20% (w/v) sucrose density gradient (100,000 × *g* for 50 min in a SW-39 rotor) to remove radioactive orthophosphate and slowly sedimenting radioactive components resulting from virus breakdown apparently occurring during the course of the purification (Thorne, *to be published*). These virus preparations were then stored at 4 C. Virus infectivity was assayed using HeLa monolayers by the modification of the plaque technique described by Holland and McLaren (1959*b*), with 0.1-ml inocula, threefold dilutions, and two monolayers per dilution.

Preparation of cell debris. Monolayers were washed twice with Hank's balanced salts solution (BSS), and the cells were scraped from the glass and suspended in BSS at a concentration of about 5 × 10^7 cells/ml. To avoid possible modification of cell components, trypsin was not employed to remove cells from glass. The cells were disrupted by homogenization in a Potter-Elvehjem homogenizer with a Teflon pestle at 0 C. [Four cycles of freezing (at −70 C) and thawing gave a similar final preparation.] Nuclei and large cell fragments were deposited by centrifugation for 10 min at 700 × *g*. The supernatant was removed, and the deposit was rehomogenized and centrifuged as before. The combined supernatants were centrifuged for 20 min at 25,000 × *g*, and the sedimented material was resuspended in phosphate buffer. After resedimentation at 25,000 × *g*, the deposit was suspended in phosphate buffer at a concentration corresponding to about 10^8 cells/ml, and stored at 4 C. This material contains the majority of the neutralizing activity of the disrupted cells (Holland and McLaren, 1959*a*), and is referred to subsequently as the 25,000 × *g* fraction.

Measurement of neutralizing activity of debris. The debris fraction (0.1 ml) was incubated with 0.1 ml of virus suspension [10^6 to 10^7 plaque-forming units (PFU)] at 25 C for 1 hr, and the unneutralized virus was assayed by plating out appropriate dilutions of the mixture; a control consisting of 0.1 ml of virus suspension and 0.1 ml of phosphate buffer was included in the assay.

Measurement of radioactivity. The samples were added to planchets, mixed with a drop of detergent, dried, and counted with a Tracerlab gas-flow end-window counter to at least 1,000 counts.

Zone electrophoresis. Particles of the size and nature of most of those present in cell homogenates can only migrate in an unsupported liquid medium, and, therefore, the zone method employing a sucrose density gradient introduced by Braake (1955) was employed.

A simple form of apparatus which permitted several simultaneous analyses using identical columns was constructed (Fig. 1). The electrophoresis tubes (45 cm long, 1 cm internal diameter) were closed at the lower ends with external rubber stoppers through which passed short glass tubes (diameter, 7 mm); these tubes were sealed at the lower ends with taut cellophane membranes, and before the introduction of the gradients into the main columns were filled with 20% sucrose (w/v) in phosphate buffer to provide an electrical connection between the gradient and the lower electrode vessel (anode). In addition to simplifying the construction, the use of cellophane eliminated convection problems due to electro-osmotic flow encountered with agar bridge connections. A linear sucrose density gradient was then formed in each column from 15 ml of 5% and 15 ml of 20% sucrose in phosphate buffer, using a device of the type described by Britten

and Roberts (1960). A second linear gradient, 2.5 to 0%, was then formed above the first from 2.5 ml of 2.5% sucrose in phosphate buffer and 2.5 ml of phosphate buffer. The sample to be analyzed, containing 4 to 5% sucrose, was introduced by screw-controlled feed, at the junction of the two gradients, from a syringe with a 2.5-in. no. 22 needle. After removal of the syringe, the remainder of the column was filled with phosphate buffer. Connection to the upper electrode vessel (cathode) was obtained through a short glass tube sealed at the lower end with cellophane and filled with phosphate buffer. The electrode vessels contained phosphate buffer, and current was supplied through platinum-wire electrodes from a power supply of 0 to 1,000 v (E-C Apparatus Co.). For parallel analyses, a measurement was made to check equality of current in the columns. The columns were jacketed and maintained at constant temperature in the range from 8 to 12 C by circulation of water. At the end of the run, a no. 20 hypodermic needle with an attached rubber tube and pinch clamp was inserted through the stopper at the bottom of the column, and fractions corresponding to 1 cm of tube length were collected as from a burette for measurement of optical density and radioactivity.

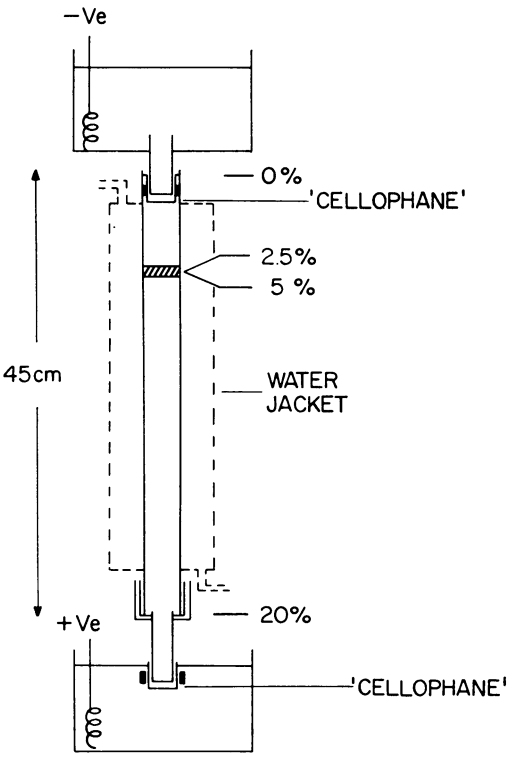

FIG. 1. *Sucrose gradient zone electrophoresis apparatus.*

RESULTS

Analysis of labeled virus preparations. Virus preparations were examined for the presence of contaminating radioactive components by both sucrose and cesium chloride density gradient centrifugation and by zone electrophoresis. As noted above, some preparations contained slowly sedimenting components, which were removed by density gradient centrifugation before use of the virus. Analyses of a typical final preparation are shown in Fig. 2. The ratio of PFU to counts/min was of the order of 10^3:1. The preparations showed no evidence of breakdown to smaller radioactive components when stored at 4 C in phosphate buffer for periods up to 8 weeks. However, components of lower sedimentation constant than the intact virus, finally representing up to 70% of the total radioactivity, gradually appeared in stock preparations which were allowed to come to room temperature when samples were removed.

Influence of various factors on binding of virus. To assess the validity of using radioactive virus to locate cell components with binding activity in centrifugation and electrophoresis experiments, some quantitative features of the reaction between radioactive virus and the HeLa 25,000 × *g* fraction were studied. Virus and debris suspensions were mixed together in a total volume of 0.2 ml in Lusteroid centrifuge tubes (0.328 × $1\frac{5}{16}$ in.) coated with silicone (Siliclad) and incubated for 30 min at 25 C with intermittent shaking. After addition of 1.5 ml of phosphate buffer, or appropriate diluent, the tubes were centrifuged for 20 min at 30,000 × *g*. The supernatants and the resuspended deposits were assayed for radioactivity.

Debris concentration. Results for two different preparations (Fig. 3) showed that the amount of virus bound increased with and was initially proportional to the debris concentration. The use of silicone-treated tubes reduced to the order of 10% losses of virus caused by adsorption to tube walls; without silicone, losses of up to 50% of the input virus were encountered. Most binding experiments were performed at 25 C, since up to 50% breakdown of virus was observed after in-

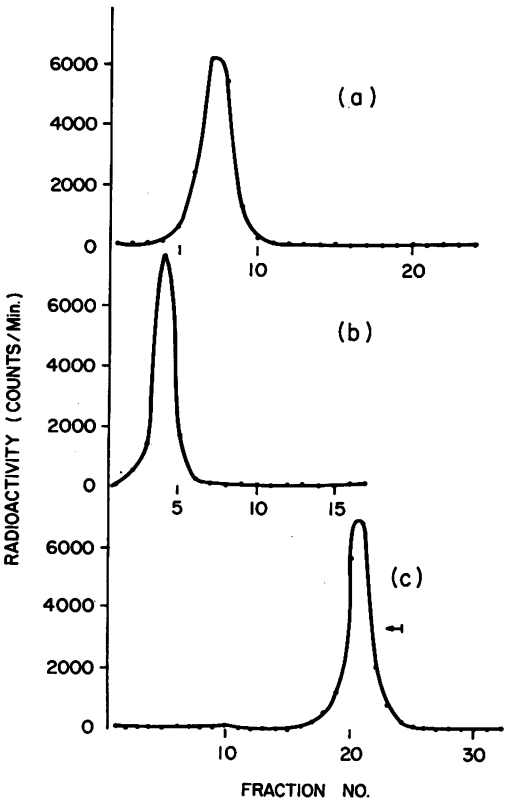

FIG. 2. *Centrifugal and electrophoretic analysis of P_{32}-labeled poliovirus. (a) CsCl density gradient centrifugation, 18 hr, 35,000 rev/min, SW-39 rotor; mean density, 1.33. (b) Sucrose gradient centrifugation, 40 min, 100,000 × g, SW-39 rotor, 5 to 20% gradient. (c) Sucrose gradient zone electrophoresis, 0.02 M phosphate buffer, pH 7.2, 15 hr, 5 ma, 9 C; 1-cm fractions; arrow indicates origin.*

cubation alone at 37 C in glass tubes (Thorne, *to be published*). No breakdown was observed at 25 C in the periods of time used.

Virus concentration. For the virus inputs used (2×10^6 to 2×10^8 PFU), the amount of virus bound was proportional to the virus input (Fig. 4). The same amount of virus was bound in either phosphate buffer or phosphate buffered saline (Dulbecco and Vogt, 1954).

Medium. At pH 7.2, equal binding occurred in 0.15 M NaCl, 0.15 M NaCl + 10^{-3} M Ca + 10^{-3} M Mg ions, 1.0 M NaCl, and 0.02 M phosphate buffer. The amount of virus bound was, therefore, unaffected by ionic strength in the 0.05 to 1.0 range or by the presence of calcium and magnesium ions.

Temperature. The amount of virus bound after 60 min was measured at 25 and 0 C with 0.15 M NaCl as the suspending medium; about twice as much virus was bound at 25 C (1,470 counts/min) as at 0 C (870 counts/min).

Time. Debris-virus mixtures were incubated at 25 and 37 C, and samples were removed, diluted 20-fold with cold phosphate buffer, and centrifuged for 20 min at 30,000 × *g*. At both 25 and 37 C, the kinetic curves (Fig. 5) were similar to the curves for 25 C obtained from infectivity measurements (Holland and McLaren, 1959*a*) in the occurrence of a persisting unadsorbed virus fraction. The initial rate of adsorption was greater at 37 C than at 25 C.

Comparison of neutralizing and binding activity. Within the limits of error, agreement between the amount of virus bound, as determined by measurement of infectivity, and of bound radioactivity was obtained for whole debris preparations. However, debris from insusceptible cells and HeLa cell fractions with weak neutralizing activity was found to adsorb considerable radioactivity when measured as described. Resuspension and washing by centrifugation showed that this virus was only loosely bound. The results with debris from several cell fractions are presented elsewhere (Thorne, *in preparation*).

Recovery of bound virus from cell debris with sodium dodecyl sulfate (SDS). As also found by Holland and McLaren (1961), addition of SDS destroyed the neutralizing activity of cell debris against poliovirus. Treatment of HeLa cell debris-virus mixtures incubated at 37 C for up to 4 hr with 0.4% SDS resulted in recovery of 50% or more of the input virus infectivity (Table 1). Density gradient centrifugation of P_{32}-labeled virus-debris mixtures incubated at 25 C and then solubilized showed only a single peak, sedimenting at the same rate as untreated virus. These procedures were used to determine the state of virus particles bound by cell components and in locating components with binding activity in centrifugal and electrophoretic fractionations (Thorne, *in preparation*).

Electrophoretic analysis of homogenates of susceptible cells. HeLa, EE, and primary human amnion (21 days in culture) cell homogenates were analyzed. Cells scraped from the glass were suspended in BSS or 0.25 M sucrose + 0.01 M phosphate buffer (pH 7.2), homogenized at 4 C, and centrifuged at 700 × *g* for 10 min to sedi-

ment nuclei and large particle debris. The remaining particulate components were then washed free from soluble cell components by two centrifugations of 2 hr at 105,000 × *g*, and were suspended in a small volume of phosphate buffer or 0.25 M sucrose in phosphate buffer. A sample of this suspension corresponding to 10^6 to 10^7 cells/ml was incubated with a small volume of P_{32}-labeled poliovirus for 30 min at 25 C in a siliconed tube, and brought to a sucrose concentration of 4 to 5% before addition to the sucrose gradient in a volume of 0.2 to 0.5 ml. The analyses were by descending electrophoresis, and a current of 5 ma or less per column was used for all analyses quoted. At currents of 10 ma or higher, with columns of the diameter used, there was a tendency for convection currents to be produced in the lower part of the column. At the end of the run, fractions (0.95 ml) were collected, and optical density at 260 mμ (to locate particulate components) and radioactivity (to locate free virus and virus-binding components) were measured. Representative analyses are shown in Fig. 6. Optical density measurements showed that all of the cell types contained several negatively charged components, some minor in quantity, covering a range of mobility. (The presence of virus had no effect on the observed distribution.) Rigorous identification of the cell components represented in the peaks was not attempted, but the peak with maximal optical density in HeLa and EE cells largely represented material with mitochondrial sedimentation characteristics (Thorne, *in preparation*), and the component of next lower mobility was material largely derived from the microsomal fraction of the cell. In some preparations, particulate material, possibly free lipid, of zero or low positive mobility was observed, which was very variable in quantity in different preparations. The nuclear fraction was excluded from these analyses because the large mass which it contributes to the extract limits the amounts of the other components which can be analyzed. Since separation of this fraction may result in loss of other components, distortion of the real distribution of components is possible. Similarly, incomplete disruption of cells may be differential in effect on the distribution of components isolated. A further effect may arise from solubilization and extraction of materials from components which may produce changes in composition and electrokinetic properties.

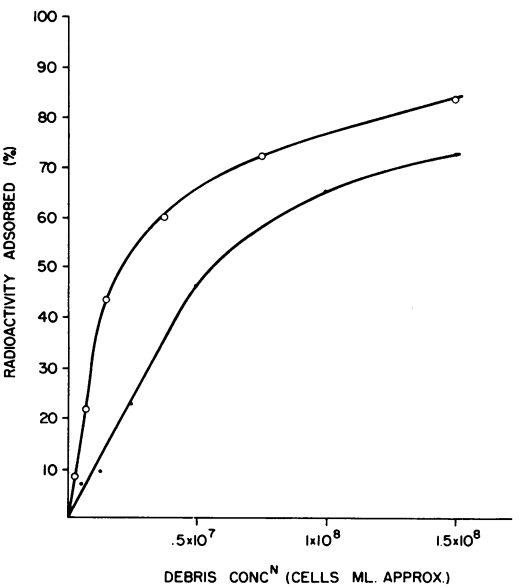

FIG. 3. *Influence of debris concentration on the fraction of virus radioactivity adsorbed. Virus input:* ● = 4×10^8 *PFU;* ○ = 9×10^6 *PFU.*

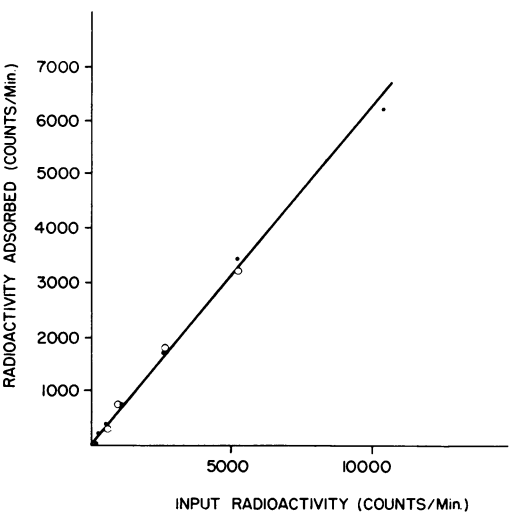

FIG. 4. *Influence of virus concentration on the amount of virus radioactivity adsorbed. Debris concentration* = 4×10^7 *cells/ml;* ● = *phosphate buffer;* ○ = *phosphate buffer* + *sucrose.*

However, if this occurred, the reproducibility of the mobilities in different preparations of the same cell line indicated that the degradation was reproducible and rapidly completed.

In contrast to the optical density patterns, the

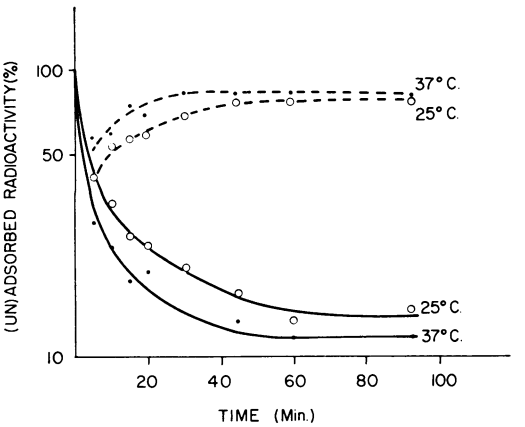

FIG. 5. *Kinetics of binding of virus radioactivity in phosphate buffered saline at 25 C (○) and 37 C (●). Debris concentration = 6 × 10⁷ cells/ml; virus input = 2 × 10⁸ PFU; solid line indicates unadsorbed radioactivity; broken line, adsorbed radioactivity.*

TABLE 1. *Recovery of infectivity from HeLa cell debris-virus mixtures with sodium dodecyl sulfate (SDS)**

Time	Debris + virus	Debris + virus + SDS	Debris + SDS + virus
min	*PFU*	*PFU*	*PFU*
0	—	—	—
30	3.0×10^3	3.5×10^5	—
90	4.3×10^3	2.7×10^5	2.9×10^5
180	5.8×10^3	4.0×10^5	—
240	4.7×10^3	3.8×10^5	3.3×10^5

* Cell debris and virus were incubated together at 37 C, and, at the times indicated, samples were removed to determine the amount of virus neutralized. A second sample was treated with SDS (final concentration, 0.4%) and then assayed for infectivity. The control consisted of debris first solubilized with 0.4% SDS to which the same concentration of virus was added. Input was 4.0×10^5 PFU.

radioactivity analyses for HeLa and EE cells showed only a single major peak of binding activity which migrated with a maximum in the position of the slow-moving "microsomal" peak. (The slowest peak of radioactivity represents uncomplexed virus.) A similar distribution of binding activity was obtained after electrophoresis in the absence of virus, followed by measurement of binding activity of collected fractions. At high cell concentrations, one or two faster-moving minor binding components which were only partially resolved from the leading edge of the main peak were apparent. The breadth of the main peak showed that the binding material was heterogeneous in electrophoretic properties, since diffusion rate alone was too low to account for the amount of broadening observed. (In the absence of current, less than a twofold broadening of the starting zone occurred during the time taken for an analysis.)

Two preparations of cultured amnion cells examined (Fig. 6 and 9) showed much less binding activity than HeLa and EE preparations, and the activity was distributed over at least two components.

Influence of culture conditions on the electrophoretic pattern of HeLa cells. Differences were apparent among homogenates from HeLa 229 cells cultured under different conditions as follows: (i) 12 passages in 10% human serum (Fig. 7, curve 1); (ii) 8 passages in 10% human serum, 3 passages in 10% calf serum-10% human serum (Fig. 7, curve 2); (iii) unknown number of passages, but at least 20 in 20% calf serum (Fig. 7, curve 3); (iv) 8 passages in 10% human serum, at least 20 in 5% human serum-5% calf serum (Fig. 6).

Although components of a similar range of mobility were found, the distribution of components varied. In particular, iv gave a much sharper profile concentrated near the major component than the other lines. All lines gave a single major peak of binding activity; that for the calf serum HeLa line iii was significantly slower than the others.

These experiments indicate that culture conditions may affect both the relative amounts and the electrokinetic properties of different cellular components.

Electrophoresis of homogenates of "insusceptible" cells. As a control for the previous experiments and to examine the possibility that electrokinetic properties alone might determine the binding of poliovirus by cell components, nonprimate cell lines were analyzed (Fig. 8). The four cell types gave distinct patterns, which were also distinguishable from those of HeLa and EE. The two rabbit lines were particularly noticeable, because of the occurrence in each of a rapidly moving major peak of turbidity which could be seen to separate from slower components very early in the run.

Examination for the presence of binding activity showed no active components in L cells or the rat cell line, but both rabbit lines showed small, but significant, activity present in the slowest moving component. It is possible that this activity was due to contamination of both these lines with human cells, since an independently carried CRE line originating from the same parent culture was found by antigenic analysis to be so contaminated (*personal communication*, R. R. A. Coombs to K. G. Brand), and human cell contamination of the ERK-1 line appears to have been substantiated (Brand and Syverton, 1960). The binding components in both lines were slower in mobility and of much weaker activity than the major binding components in HeLa; however, since culture conditions seem to modify the properties of cell components, the significance of these differences cannot be determined.

The similar mobilities of the main binding peaks in HeLa and EE and the nonbinding peaks of turbidity in rat and L cells showed that electrokinetic properties alone did not determine binding of the virus.

Electrophoresis of cultured and uncultured human amnion cells. In contrast to the susceptibility of the cultured cells, many uncultured primate tissues, including monkey kidney tissue and human amniotic membrane, do not support multiplication of poliovirus or, when homogenized, neutralize virus infectivity (McLaren, Holland, and Syverton, 1962; Holland, 1961). The possibility that this change to susceptibility on culture may be due to the production of components previously absent has been suggested for monkey kidney by Quersin-Thiry (1961) and human amnion by Holland (1961). Electrophoretic analysis of both cultured and uncultured human amnion cells gives some support to this interpretation. Cell suspensions were prepared from human amniotic membranes with 0.25% trypsin in BSS at 37 C, and, after washing free from trypsin with BSS, half of the cells were stored frozen (at −20 C) in BSS and the remainder were cultured as monolayers for 14 days in Eagle's medium containing 5% calf and 5% human serum. At this time, homogenates minus the nuclear fraction were prepared for analysis as described for HeLa cells. Figure 9 shows two distinct distributions of components with major peaks separated by about 3.5 cm. Consistent

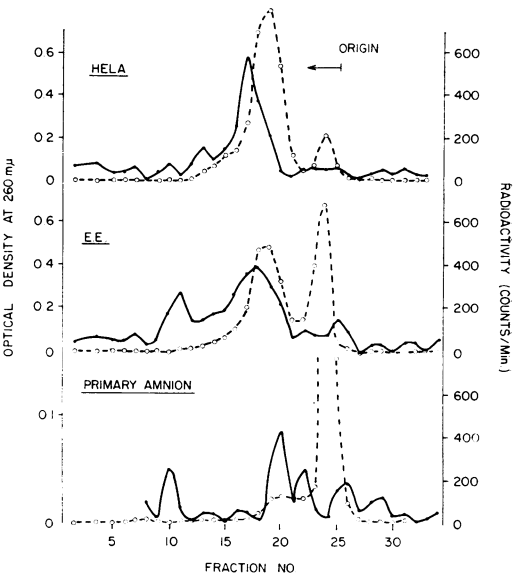

FIG. 6. *Electrophoresis of HeLa, EE, and primary human amnion postnuclear homogenates. Solid line, optical density; broken line, radioactivity; 7 hr at 5 ma per column.*

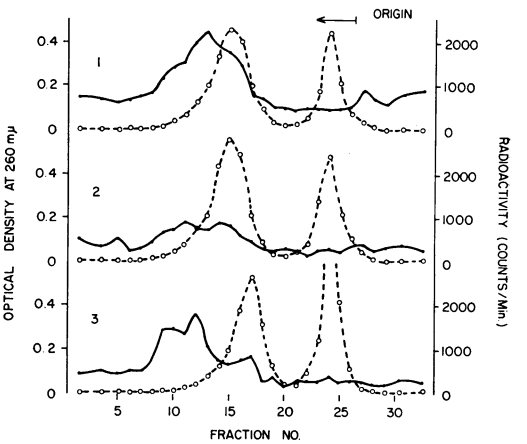

FIG. 7. *Electrophoresis of postnuclear homogenates of differently cultured HeLa cells. Solid line, optical density; broken line, radioactivity; 14 hr at 4 ma per column.*

with the virus-neutralizing activity of cultured amnion cell debris, binding activity (although of a low order) was found in the cultured cells. However, unlike HeLa and EE activity, it was distributed among three turbidity peaks. The slowest of these was significantly slower than the main binding peaks in both HeLa and EE

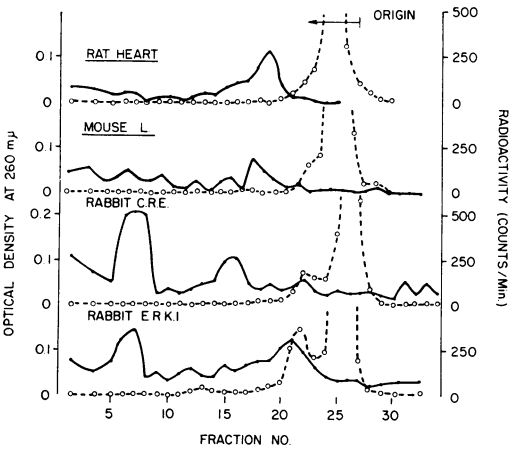

FIG. 8. *Electrophoresis of rat heart, mouse L, and rabbit CRE and ERK-1 postnuclear homogenates. Solid line, optical density; broken line, radioactivity; 7 hr at 5 ma per column.*

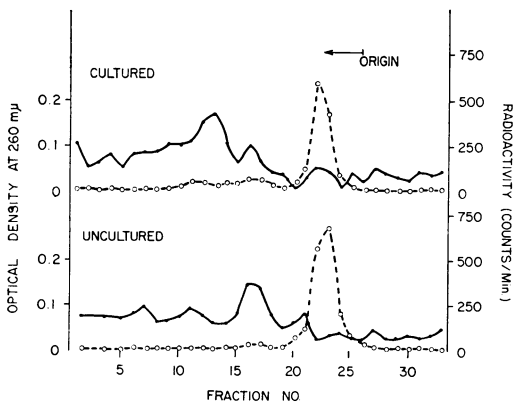

FIG. 9. *Electrophoresis of post-nuclear homogenates of cultured and uncultured human amnion cells. Solid line, optical density; broken line, radioactivity; 15 hr at 4 ma per column.*

cells. Only very slight binding activity was observed in the uncultured cells which coincided with the slowest peak of activity in the cultured cells. Differences between the two cell types were therefore apparent, but further experiments are necessary to determine their relationship to differences in viral susceptibility. It was also observed that uncultured cells were much less resistant to disruption than cultured cells; the possibility that this might be due to the use of trypsin in the preparation of uncultured cell suspensions was not investigated.

DISCUSSION

Provided precautions to control or minimize nonspecific virus adsorption are taken, the measurement of bound radioactivity can be used to detect and assay cell components which interact with poliovirus. Although fractions ultimately must be tested for biological activity, radioactive assay permits a more extensive initial examination of components produced by fractionation procedures.

Zone electrophoresis on sucrose gradients, first introduced for the study of soluble proteins and viruses (Braake, 1955), provides a simple means for investigating the electrokinetic properties of particulate components of tissue cells and for fractionating small amounts of material. Significant differences in mobility of different cell components and of components probably of the same type within different cells were observed. By extending the duration of the analyses or varying the ionic conditions, greater resolution than was achieved in the present work should be possible.

Limited investigation of the effects of different growth media on HeLa cells indicates that variations in composition may produce alterations not only in the relative amounts of the cell components but also in their electrophoretic properties. Such changes might be correlated with the varying susceptibilities to poliovirus reported for different HeLa lines.

The use of radioactive virus to reveal binding demonstrates electrophoretic heterogeneity of the active fractions in susceptible cells. In addition to the minor binding components found, the breadth of the main virus-binding peak in HeLa and EE cell homogenates indicated the presence of more than one component. The possibility that the heterogeneity arose during preparation should be considered, but no differences could be distinguished between homogenates prepared in BSS or 0.25 M sucrose. A second possibility, that the heterogeneity was only apparent and due to the adsorption during preparation of one active component by several inactive components of different mobilities, seems unlikely since the major binding peak occurred in the same position irrespective of the method of extraction and even in whole-cell and nuclear fractions (Thorne, *in preparation*), where the large mass of more rapidly migrating material

present might be expected to carry along appreciable activity.

The examination of nonprimate cells illustrated further the differentiation of cell types possible by the electrophoretic technique and the detection with radioactive virus of components with weak binding activity. The presence of poliovirus-binding components in nonprimate cells may, in general, indicate contamination with human cells, and, therefore, this method provides a simple and sensitive means for the detection of such contaminants.

Since virus firmly adsorbed by debris can be recovered in a fully infectious form at low pH (McLaren et al., 1960) or by extraction with SDS, it is evident that the neutralization process is distinct from that involved in the adsorption of virus by intact cells at 37 C. According to Joklik and Darnell (1961), over 50% of the virus adsorbed by HeLa cells spontaneously elutes in a noninfectious form at 37 C, and the remainder loses infectivity by some interaction with the cell involving slow breakdown of the virus. Identification and comparison of the cell components involved in these reactions is required before the pathway of the virus which initiates infection can be discerned.

ACKNOWLEDGMENTS

I thank Opal Skaren, Sally Gibson, and Andrew Hudson for excellent assistance. I also wish to record my appreciation of the courtesy shown me by the late J. T. Syverton and the members of his department. I am especially indebted to L. C. McLaren for pleasant discussions and advice.

This study was aided by a grant from The National Foundation.

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