THE MAMMALIAN CELL-VIRUS RELATIONSHIP

V. Susceptibility and Resistance of Cells in Vitro to Infection by Coxsackie A9 Virus*

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HeLa cell cultures have been reported previously to be resistant to infection by Coxsackie A9 virus (1, 2). Similar results have been reported for a number of other stable cell strains derived from human tissues (3–6). In contrast, this virus has been reported to infect primary cell cultures of human and monkey cells (1, 3, 7–9). The resistance of HeLa cells to A9 virus infection was shown by Takemoto and Habel (10) to be relative, since virus-carrier cultures could be produced. Even in the presence of relatively large amounts of virus and in the absence of antibody in the medium, only a small proportion of the cells were infected at any time. We have reported previously that natural resistance of non-primate cells to enterovirus infection is related to the absence of cellular receptors active in the primary adsorption of these viruses (11–13). Evidence that resistance to Coxsackie A9 infection had been acquired by human cells in continuous culture prompted similar studies on the mechanism of resistance to this virus.

The present investigation demonstrates that established human cell cultures are also resistant because of absence of receptor, and it will be shown that enteroviruses differ with regard to cell receptor specificity and cofactor requirements for viral attachment.

Materials and Methods

Cell Origin and Cultivation.—Cell strains established in continuous culture from human, rabbit, and mouse tissue, and maintained at the University of Minnesota as previously described (11, 14) were studied. The human epidermoid carcinoma strain KB (15) also was studied. Human cell strains were propagated in 20 per cent human serum (HuS), diluted in Hanks's balanced salt solution (BSS), supplemented with 0.1 per cent yeast extract as YEM medium (16). Primary cultures of human, monkey (MK), dog, cat, pig, and rabbit kidney or

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skin were prepared from trypsinized cell suspensions and cultivated in 10 per cent calf serum diluted with BSS containing 0.5 per cent lactalbumin hydrolysate. Primary cultures of human amnion (PAm) and chorion cells were prepared as described by Dunnebacke and Zitcer (17). Five cell lines were established in continuous culture from human amnion, chorion, and fetal kidney, as accomplished by Zitcer and Dunnebacke (18). These cells were maintained in 20 per cent human serum, 70 per cent YEM, and 10 per cent Scherer's maintenance solution until complete degeneration occurred, or until established rapidly growing cell strains were obtained. After 2 to 5½ months' incubation, colonies of cells representative of the 5 lines were isolated and strains derived from these colonies have been propagated through 65 to 85 passages during a period of 2 years.

Viruses.—Coxsackie A9 virus strains PB (prototypic), Grigg, and Wiederhold were supplied kindly by Dr. Grace Sickles, Dr. G. D. Hsiung, and Dr. J. L. Melnick, respectively. Virus pools were prepared as fluids from infected primary cultures of monkey kidney or human amnion. Other viruses included HeLa-grown type 1 (Mahoney) poliovirus obtained originally from Connaught Laboratories, Toronto; Coxsackie B1 (Conn. 5) and Coxsackie B3 (Nancy) viruses purchased from the American Type Culture Collection, and Coxsackie B5 (Faulkner) virus received by courtesy of Dr. G. Dalldorf.

Virus Assay.—Replicate monolayer PAm or MK cultures containing about 2×10^6 cells were employed for counting of plaques visualized 48 to 72 hours after infection of cultures by pouring off agar overlay medium and staining cell monolayers with crystal violet (19). Monolayers were inoculated with virus in 0.1 ml. of fluid. Numbers of free virus particles or infected cells (infectious centers) were counted as plaque-forming units (PFU) per milliliter, as previously described (11).

Infection of Cells with Ribonucleic Acid.—Ribonucleic acid (RNA) was extracted from Coxsackie A9 virus by the phenol procedure of Gierer and Schramm (20), and inoculated into cultures in 0.1 ml. amounts of 2 M magnesium sulfate (21).

EXPERIMENTAL RESULTS

Susceptibility of Cells in Primary and Continuous Culture to Coxsackie A9 Virus.—In order to determind whether resistance to infection by Coxsackie A9 virus was a property common to stable human cell lines, replicate bottle cultures containing 1 to 3×10^6 cells were rinsed 3 times to remove traces of human serum, and inoculated with 0.1 ml. of BSS containing 10^7 PFU of A9 virus. After 2 hours, the inoculated cultures were incubated at 37°C. under 10 per cent calf serum in YEM. Cultures were observed daily for evidence of cytopathic effect (CPE) for 10 days. Results (Table I) indicated that while all primary cultures (seeded with cells directly dispersed from tissue) of human and monkey cells were destroyed rapidly, all non-primate cultures and human cells of strains in continuous culture showed no visible deterioration. None of the human cell strains in continuous culture revealed obvious CPE despite subjection of each strain to as many as 5 blind passages of each of 3 strains of A9 virus. Assay of passage culture fluids by inoculation of monkey kidney cultures showed titers of less than 10^4 PFU per ml.; *i.e.*, less than 1 PFU per hundred cells.

Capacity of Cells in Primary and Continuous Culture to Adsorb Coxsackie A9 Virus.—To determine whether cellular capacity to adsorb A9 virus was in-

volved in susceptibility, adsorption of virus from 0.1 ml. of BSS by monolayer cultures was determined quantitatively. Bottles were rocked periodically to ensure uniform exposure of cells. Two hours after inoculation, 10 ml. of cold BSS was added to each bottle to halt adsorption by dilution and was removed for assay of unattached virus. Extreme care was taken to rinse thoroughly all monolayers prior to viral inoculation to remove traces of human serum. Some

TABLE I

Susceptibility of Various Cell Cultures to Cytopathic Effect of Coxsackie A9 Virus

Cells	Type of culture	Species origin	Cytopathic effect
Amnion	Primary	Human	+
Kidney	"	"	+
Chorion	"	"	+
Skin	"	"	+
Kidney	"	Monkey	+
Minnesota HeLa	Continuous	Human	0
Detroit-6	"	"	0
Minnesota Harris (Pulvertaft)	"	"	0
Maben (Frisch)	"	"	0
Strain EE (Minnesota 55-12-1) esophageal epithelium	"	"	0
Conjunctiva (Chang)	"	"	0
Liver (Chang)	"	"	0
KB (Eagle)	"	"	0
Kidney	Primary	Dog	0
"	"	Calf	0
"	"	Swine	0
"	"	Rabbit	0
Strain FF (Minnesota 56-8-8) papilloma	Continuous	"	0
Strain CRE (Minnesota 57-8-19) skin	"	"	0
Strain DRF (Minnesota 56-8-6) skin	"	"	0
Strain L (Earle)	"	Mouse	0

cultures were disrupted by freezing and thawing at the termination of the adsorption period to reveal unproductively adsorbed virus. Cell-associated virus usually represented no more than 1.0 per cent of the inoculum as has been found previously with poliovirus (11). Only cells responding to infection by visible CPE (Table II) adsorbed virus significantly. Human cell strains in continuous culture and non-primate cells did not adsorb detectable amounts of virus from the supernatant fluid.

Susceptibility to Coxsackie A9 Virus of Cells in Primary and Continuous Culture from the Same Tissue Source.—It was of interest to determine whether

Coxsackie A9-resistant cell strains could actually be derived from pretested susceptible A9 virus-adsorbing primary human cells. Replicate bottle cultures were prepared from trypsinized cell suspensions from 8 human amniotic membranes, 2 chorionic membranes, and 1 fetal kidney. Sample cultures were tested for susceptibility to A9 virus as revealed by cytopathic effect, and for capacity

TABLE II

Adsorption of Coxsackie A9 Virus by Cell Cultures

Type of culture*	Species origin	No. of cell lines tested	Cytopathic effect	Virus adsorbed from 0.1 ml. inoculum in 2 hrs. at 37°C.‡
				per cent
Primary	Human	5	+	>90
"	Monkey	2	+	>90
Continuous	Human	8	0	<10§
Primary	Rabbit	1	0	<10
"	Calf	1	0	<10
Continuous	Rabbit	3	0	<10
"	Mouse	1	0	<10

* Approximately 2×10^6 cells per monolayer.

‡ Washed monolayers inoculated with 2×10^7 PFU virus as revealed on monkey kidney control monolayers.

§ No adsorption within limits of method.

TABLE III

Susceptibility of Derived Continuous Cultures of Human Cells to Coxsackie A9 Virus Infection

Cell source	No. cultures tested	Cytopathic effect on primary cultures	Per cent virus adsorbed from 0.1 ml. in 2 hrs. at 37°C.	No. cell lines established	Cytopathic effect on established lines	Per cent virus adsorbed from 0.1 ml. in 2 hrs. at 37°C.
Human amnion	8	+	>90	3	0	<10
Human chorion	2	+	>90	1	0	<10
Human fetal kidney	1	+	>90	1	0	<10

to adsorb virus. Ten uninoculated bottle cultures were maintained with repeated feeding. Colonies of growing cells appearing in these cultures were trypsinized and the cells passed. Cell sublines representative of 3 amnions, 1 chorion, and 1 kidney were tested for cytopathic response to A9 virus and adsorption capacity. While the original primary cultures actively adsorbed A9 virus and were destroyed rapidly (Table III), the derived sublines like other human cells in continuous culture neither adsorbed virus nor responded with CPE.

When cells of the derived human sublines were exposed to Coxsackie

A9 virus at a multiplicity of 10 PFU per cell, washed thoroughly, dispersed, counted, and plated for assay of productive infection (infectious centers), only a small proportion of the cells produced plaques (Table IV), in comparison

TABLE IV

Proportion of Cells Giving Rise to Plaques after Exposure to A9 Virus at High Multiplicity

Cell culture	Type of culture	Cells producing plaques*
		per cent
Monkey kidney	Primary	76
Human amnion	"	80
" "	Continuous	<1
Chorion	"	<1
Kidney	"	<1
HeLa	"	<1

* Washed monolayers exposed for 60 minutes to 10 PFU per cell, rinsed, dispersed with trypsin, and plated on rinsed primary amnion cell monolayers.

TABLE V

Effect of Calcium and Magnesium Ions on Adsorption of Coxsackie A9 Virus by Primary Amnion Cultures

Adsorbing medium	Average PFU adsorbed after 30 min.	Virus adsorbed compared to control
		per cent
BSS (control)	60	100
PS*	12	20
PS + 10^{-1} M $CaCl_2$	5	8
PS + 10^{-3} M $CaCl_2$	66	100
PS + 10^{-4} M $CaCl_2$	36	60
PS + 10^{-3} M $MgCl_2$	62	100
PS + 10^{-3} M $CaCl_2$ + 10^{-2} M EDTA‡	6	10
PS + 10^{-3} M $MgCl_2$ + 10^{-2} M EDTA	8	13
PS + 10^{-2} M EDTA	2	3

* PS = 0.02 M phosphate-buffered NaCl solution.

‡ EDTA = tetrasodium ethylenediaminetetraacetate.

with the large proportion of infectious centers yielded by virus-exposed monkey kidney and human amnion cells in primary culture.

Effect of Divalent Cations on Adsorption of Coxsackie A9 Virus by Primate Cells in Primary Culture.—Addition of divalent cations to physiological saline does not affect the rate of poliovirus adsorption (12). Preliminary experiments indicated that greater amounts of A9 virus were adsorbed to either MK or PAm cells in the presence of BSS than were adsorbed from phosphate buffered saline (PS) containing no Ca or Mg ions. Coxsackie A9 virus for adsorption by human

amnion cells in primary monolayer culture was prepared in BSS as control, or in 0.02 M phosphate-buffered sodium chloride solution containing calcium or magnesium ions with and without ethylenediamine sodium tetraacetate (EDTA). The depression of virus adsorption resulting from substitution of phosphate-buffered salt solution for BSS was reversed in the presence of 10^{-3} M calcium or magnesium chloride (Table V). This enhancement of A9 virus adsorption was inhibited by EDTA and was not observed with 10^{-1} M calcium ion. Further experiments showed that divalent cations in optimal concentration enhanced adsorption of all 3 strains of A9 virus, but not of types 1, 2, 3 polio-

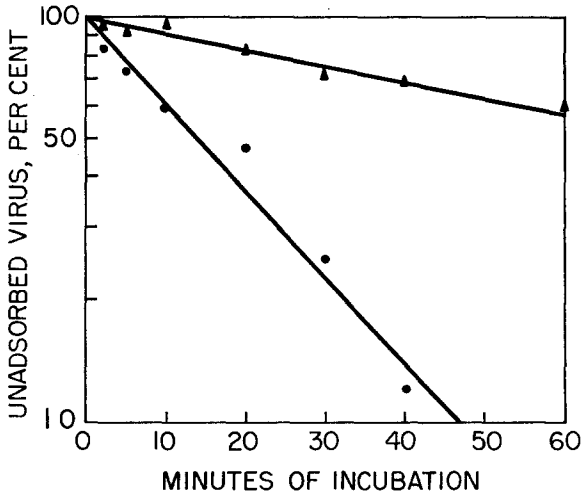

FIG. 1. Effect of calcium on adsorption of Coxsackie A9 virus by amnion cells in primary culture as measured by numbers of plaques formed on replicate monolayers. Results are given for buffered NaCl solution + 10^{-3} M Ca^{++} (black circles) and for buffered NaCl solution only as control (black triangles).

virus or Coxsackie B1, B3, or B5 viruses. After 2 minutes, A9 virus adsorbed by cell monolayers from BSS could not be freed by rinsing the cells with phosphate-buffered salt solution or sucrose solution and only a small and variable amount was removed when 10^{-2} M EDTA was employed. Fig. 1 shows the kinetics of adsorption of A9 virus by human amnion cells in primary culture, from phosphate-buffered salt solution with and without 10^{-3} M calcium ion.

Inactivation of Coxsackie A9 Virus by Debris of Cells in Primary or Continuous Culture.—Poliovirus is rapidly inactivated when added to cell debris prepared from susceptible but not insusceptible cells (12). It was of interest to learn whether other enteroviruses also reacted with debris from susceptible cells and whether human cells in continuous culture might contain Coxsackie A9 receptor. Debris was prepared from suspensions of 5×10^6 human, monkey, or rabbit cells per ml. of BSS by 5 cycles of freezing and thawing as previously

TABLE VI

Inactivation of Coxsackie A9 Virus by Debris from Disrupted Cells

Source of cell debris	Type of culture	Virus inactivation*		
		Coxsackie A9	Poliovirus type 1	Coxsackie B1, 3. 5
Human amnion	Primary	+‡	+	+
Monkey kidney	"	+	+	+
HeLa	Continuous	0§	+	+
KB	"	0	+	+
Human amnion	"	0	+	+
Human chorion	"	0	+	+
Human kidney	"	0	+	+
Monkey kidney + human kidney	Primary + continuous	+	+	+
Rabbit papilloma	Continuous	0	0	0
Heated amnion‖	Primary	0	0	0

* Viruses were incubated at 37°C. for 2 hours in BSS (control) or in 1 ml. BSS containing debris from 5×10^6 disrupted cells. After 2 hours mixtures were diluted 1:100 and 0.1 ml. samples were plaque-assayed for remaining virus.

‖ Debris from amnion cells heated to 56°C. for 30 minutes.

‡ +, 80 per cent or more loss of viral infectivity.

§ 0, no measurable loss of viral infectivity.

TABLE VII

*Effect of Calcium Ions on Inactivation of Coxsackie A9 Virus by Cell Debris from Primary Human Amnion Cultures**

Reaction medium	PFU per 0.1 ml, after 2 hrs. at 37°C.	Virus inactivated
		per cent
PS + 10^{-3} M $CaCl_2$ (control)	105, 105	0
PS	105, 101	0
PS + cell debris	119, 115	0
PS + 10^{-3} M $CaCl_2$ + cell debris	6, 9	92
PS + 10^{-3} M $CaCl_2$ + 10^{-2} M EDTA + cell debris	106, 111	0

* Virus was incubated at 37°C. for 2 hours in 1 ml. 0.02 M phosphate-buffered NaCl solution, with or without 10^{-3} M $CaCl_2$, or in 1 ml. 0.02 M phosphate-buffered NaCl containing washed debris from approximately 5×10^6 amnion cells with or without 10^{-3} M $CaCl_2$.

described (12). Resultant debris was incubated for 2 hours at 37°C. with about 10^5 PFU of A9 virus in 1 ml. of BSS. After 2 hours, mixtures were diluted 1:100 and 0.1 ml. samples assayed for plaques produced by residual infectious virus. Debris was tested similarly for ability to inactivate type 1 poliovirus and Coxsackie B1, B3, and B5. Results are shown in Table VI. Only debris prepared from monkey or human cells in primary culture inactivated A9 virus

Susceptibility of monkey kidney or human cells in primary culture thus was correlated with the presence of a specific structural receptor. Debris from monkey kidney cells in primary culture mixed with debris from human kidney cells in continuous culture was able to inactivate A9 virus, to indicate that inactive debris did not inhibit the activity of receptor substance. Since debris from all primate cells could inactivate type 1 poliovirus and Coxsackie B1, B3, and B5 viruses, the suceptibility of cells in continuous culture to A9 virus appeared related to lack of specific A9 receptor substance. This and other receptor substance of human amnion cells was inactivated by heat at 56°C. for 30 minutes, as shown by loss of ability to inactivate Coxsackie A9, type 1 poliovirus, and the other Coxsackie viruses.

TABLE VIII

Reactivation of Coxsackie A9 Virus Inactivated by Cell Debris from Human Amnion Primary Cultures

Virus (mixture A) incubated 1 hr. at 37°C. in:	Mixture A incubated 1 hr. at 37°C. after 1 per cent dilution into:	PFU per 0.1 ml. average	Virus inactivation	Virus reactivation
			per cent	*per cent*
BSS (control)	TBG*, pH 7.5	180	0	
" "	TBG, pH 4.5	200	0	
" "	10^{-2} M EDTA	182	0	
BSS + cell debris	TBG, pH 7.5	6	97	
" " "	TBG, pH 4.5	220		100
" " "	10^{-2} M EDTA	150		83

* TBG, 1 per cent gelatin in BSS, buffered with 10^{-2} M tris(hydroxymethyl)aminomethane-HCl.

If active debris contains specific receptor for A9 virus attachment then inactivation of A9 virus should be enhanced by divalent cations. Results presented in Table VII indicated that calcium ions were required for inactivation of A9 virus by debris from primary amnion cultures. To demonstrate enhancement unequivocally, it was necessary to wash cell debris in phosphate-buffered salt solution containing 10^{-3} M EDTA by centrifugation in the cold for 20 minutes at 25,000 *g*.

Reversal of Inactivation of Coxsackie A9 and Type 1 Poliovirus by Cell Debris.— Previous attempts by numerous means to recover poliovirus eclipsed by virtue of being inactivated by cell debris were unsuccessful (12). These results suggested that the reaction between virus and receptor did not represent reversible attachment but physical or chemical alteration with permanent impairment of infectivity. Coxsackie A9 virus inactivated by debris from primary cultures of human amnion cells was recovered by treatment of the inactive virus-debris mixture with tris-buffered gelatin solution at pH 4.5 (22) or 10^{-2} M EDTA (Table VIII). Cell monolayers rinsed with these solutions immediately

TABLE IX

*Dissociation of Coxsackie A9 Virus Adsorbed onto Monolayers of Human Amnion Primary Culture**

Treatment of monolayers after adsorption of virus	PFU per 0.1 ml.	Virus-forming plaques
		per cent
A. Maintained at 1°C. for 2 minutes		
1. Rinsed with pH 7.5 TBG‡	150, 175, 160	100
2. Rinsed with pH 4.5 TBG	29, 35, 32	20
B. Maintained at 37°C. for 60 min.		
1. Rinsed with pH 7.5 TBG	152, 146, 161	96
2. Rinsed with pH 4.5 TBG	165, 156, 148	100
C. Cell monolayers rinsed before virus adsorption with:		
1. pH 7.5 TBG	161, 149, 152	100
2. pH 4.5 TBG	176, 149, 160	100

* Triplicate cell monolayers were exposed for 2 minutes at room temperature to sufficient virus to produce a countable number of plaques. Adsorption was halted by rinsing monolayers 3 times with cold BSS and bottles were either immediately chilled to 1°C. or placed in 37°C. incubation prior to rinsing with TBG buffered solutions.

‡ TBG = 1 per cent gelatin in BSS, buffered with 10^{-2} M tris(hydroxymethylaminomethane).

TABLE X

Reaction of Type 1 Poliovirus Inactivated by HeLa Cell Debris

Treatment of virus-debris mixture	Poliovirus reactivated
	per cent
pH 7.5 TBG*, 1 hr. at 37°C.	0
pH 4.5 TBG, 1 hr. at 37°C.	0
pH 2.5 PBG‡, 1 hr. at 37°C.	93
pH 10, BBG§, 1 hr. at 37°C.	7
5 vol. ether, shake 5 min. at 4°C.‖	8
5 vol. fluorocarbon, shake 5 min. at 4°C.¶	0
Dilute 1:100 in distilled H_2O, 1 hr. at 37°C.	6
Dilute 1:100 in EDTA, 10^{-2} M, 1 hr. at 37°C.	0

Type 1 poliovirus incubated for 1 hr. at 37°C. in either 1 ml. BSS or 1 ml. BSS containing debris from 5×10^6 HeLa cells (mixture A). Mixture A treated as indicated after 1 per cent dilution. Virus assayed by diluting treated mixtures appropriately in pH 7.5 TBG and plating 0.1 ml. on replicate primary amnion monolayers.

* TBG, 1 per cent gelatin in BSS, buffered with 0.01 M tris(hydroxymethylaminomethane-HCl).

‡ PBG, 1 per cent gelatin in BSS, buffered with 0.05 M phthalate-HCl.

§ BBG, 1 per cent gelatin in BSS, buffered with 0.05 M H_3BO_3-KCl-NaOH.

‖ Treatment of virus control by shaking with ether for 5 minutes at 4°C. caused 35 per cent virus inactivation.

¶ Treatment of virus control with fluorocarbon (freon 112 or genatron) caused 25 per cent virus inactivation.

after exposure to A9 virus for 2 minutes also released adsorbed A9 virus. Virus could not be recovered similarly from monolayers after 1 hour incubation at 37°C. with adsorbed virus (Table IX). Type 1 poliovirus was dissociated from HeLa cell debris at pH 2.5 (Table X), but significant virus could not be recovered by treatment at alkaline pH (pH 7.5-10.0), ether extraction which destroys the activity of poliovirus receptor (12) or by extraction with fluorocarbon which has been found to dissociate poliovirus from neutralizing antibody (23).

Infection of Primate Cells in Primary or Continuous Culture by Coxsackie A9 Virus Ribonucleic Acid.—It has been reported (13) that naturally insusceptible cells and animals, which lack virus receptor, can be infected after exposure to

TABLE XI

Production of Coxsackie A9 Virus by Cell Cultures Exposed to Viral Ribonucleic Acid (RNA)

Cell culture	Type of culture	Virus PFU per culture vessel at 20 hrs. after exposure to RNA*
HeLa	Continuous	7×10^4
Human kidney	"	3.3×10^4
Human amnion	"	2.9×10^5
Human chorion	"	2.5×10^5
KB	"	2.3×10^5
Human amnion	Primary	2.7×10^4
Monkey kidney	"	1.0×10^4
Any of above inoculated with ribonuclease-treated RNA		0

* Monolayer cultures of about 2×10^6 cells were treated with 0.1 ml. RNA; cultures were disrupted by alternate freezing and thawing 24 hours after infection. Disrupted cells and medium were pooled and assayed for plaque-forming activity.

enterovirus ribonucleic acid. Monolayers of monkey kidney and human amnion cells in primary culture and of human cells in continuous culture were exposed for 15 minutes to ribonucleic acid extracted from Coxsackie A9 virus. Control cultures were exposed to RNA pretreated with ribonuclease. After incubation for 24 hours, cultures were disrupted by cyclical freezing and thawing, pooled with medium, assayed on human amnion cultures for plaques produced by infectious A9 virus. Although shown unable to respond to intact A9 virus, human cells in continuous culture produced infectious A9 virus after exposure to RNA (Table XI). The continuously cultivated cells were even more receptive to RNA infection than the primary cell cultures. Intact A9 virus produced by continuously cultivated human cells infected with RNA was again unable to infect cells of the same strains, as occurred when non-primate cells were infected with RNA from other enteroviruses (13).

DISCUSSION

Compared to monkey kidney or human amnion, chorion, fetal kidney, and skin cells in primary culture, human cells in continuous culture and non-primate cells were insusceptible to infection by Coxsackie A9 virus even with blind passage. No cytopathic effect was produced by heavy exposure of numerous cell strains of various tissue origin to A9 virus, and infectious virus after adsorption was found associated with a negligible proportion of exposed cells. Since insusceptible cell strains neither adsorbed A9 virus extensively nor yielded debris able to inactivate this virus significantly, resistance to infection was correlated with lack of a specific receptor mechanism as has been demonstrated for non-primate cell resistance to enteroviruses (11–13). Like the poliovirus receptor (12), activity of the A9 receptor was independent of cell integrity. Five strains of human cells established in continuous culture from tested fully susceptible human cells in primary culture were shown to lack this receptor substance and to be resistant to A9 virus infection. Loss of this receptor substance by human cells in continuous culture contrasted with retention of susceptibility to infection and receptor substance for type 1 poliovirus and Coxsackie B1, B3, and B5 viruses was an indication that cell receptors may be specific for certain enteroviruses. The specific A9 receptor substance lost by human cells with continuous culture also differed from the receptors for type 1 poliovirus and Coxsackie B1, B3, and B5 viruses in that Coxsackie A9 virus adsorption was enhanced by divalent cations and reversed at pH 4.5 (or by chelation of divalent cation). Thus, the process of enterovirus penetration and eclipse appears to be initiated by binding between virus and cell receptor, which does not result immediately in alteration of the viral particle as happens with some bacteriophages (24). Adsorption of A9 virus by intact susceptible cells could not be reversed after 1 hour at 37°C., showing that either firmer binding had occurred or eclipse had been initiated with resulting impairment of infectivity. The recovery of debris-inactivated enteroviruses indicates that vital activity of host cells may be needed in order for true eclipse to take place. Thus, the receptor inactivation of enteroviruses is not in discord with the viropexis theory of Fazekas de St. Groth (25) as was previously considered (12).

These results show that susceptibility of primate cells to Coxsackie A9 virus as with poliovirus and other enteroviruses (12), is dependent on a specific structural receptor. As with these other viruses (13) ribonucleic acid extracted from A9 virus is able to infect naturally insusceptible primate cells, which produce A9 virus again unable to infect cells of the same type. Present findings are of particular interest because possession of the susceptibility-determining A9 receptor substance was limited to primate cells in primary culture. Establishment of continuously cultivated cell lines from fully susceptible human cell cultures was accompanied by loss of this primate-specific receptor. It is not known whether loss of this receptor reflects (*a*) inability of the continuously

propagated cells to synthesize the receptor component, or (*b*) selective propagation of receptorless cells among susceptible cells of the primary populations. It should be recalled, however, that a small proportion of insusceptible, nonprimate cells can be infected by exposure to a very high multiplicity of poliovirus (13). In addition, adaptation of Coxsackie A9 virus to HeLa cells (10) has resulted in modified "virulence" for mice as well as monkey kidney cell cultures, *i.e.*, by production of slower CPE and smaller plaques. This behavior is accompanied by a slower rate of adsorption to monkey kidney cells. Thus, the degree of susceptibility of cells to enteroviruses apparently correlates with their rate of viral attachment. At the present time, these cell receptors can not be regarded as rigidly specific for particular enteroviruses but must be considered as possessing a range of viral affinities.

The resistance of established human cell strains to A9 virus appears to differ from the resistance of patus monkey kidney cells which, although naturally resistant, adsorb A9 virus and can become infected after x-irradiation (26). The possibility of resistance mechanisms independent of cellular adsorption capacity has been shown also by Vogt and Dulbecco (27), who found that HeLa cells selected for resistance to poliovirus could adsorb virus with a decreased probability that adsorption would initiate infection. Since primary cultures of human adult, in contrast to embryonic kidney cells, have been reported to be resistant to Coxsackie A9 virus (28), it remains to be seen whether the A9 receptor substance lost by human cells in continuous culture might be the result of cellular maturation. The human cell strains derived and tested in this laboratory were of fetal origin, and amnion and chorion cells are embryonic in type.

SUMMARY

All primary cell cultures tested from numerous human and *rhesus* monkey tissues were susceptible to Coxsackie A9 virus infection and were found to contain a specific A9 virus receptor. Ability of these cells to adsorb actively virus correlated with the presence of this receptor. Attachment of A9 virus to cell receptor was specifically enhanced by calcium and magnesium ions. Human cells established in continuous culture from pretested susceptible cultures were found to have lost A9 virus susceptibility and A9 receptors but not receptors for adsorption of type 1 poliovirus or B1, B3, B5 Coxsackie viruses. These cultures were able also to produce infectious A9 virus after exposure to viral RNA. Coxsackie A9 virus as well as type 1 poliovirus, inactivated following reaction with debris prepared from susceptible cells, could be recovered fully in infectious form by treatment of the inactive systems at low pH. Recovery of infectious A9 virus was also possible with a chelating compound. Coxsackie A9 virus could also be dissociated by means of low pH after adsorption onto susceptible cells for 2 minutes at 1°C., but not after 1 hour incubation at 37°C.,

indicating that viral host cell activity may be required in order for irreversible enterovirus attachment to take place.

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