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Problems in the Laboratory Isolation of Simian Hemorrhagic Fever Viruses and Isolation of the Agent Responsible for the Sussex/69 Epizootic

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At least six epizootics of simian hemorrhagic fever have occurred at four different primate centers. Although these diseases could easily be transmitted to other monkeys of the *Macaca* species, difficulty has been encountered in isolating the causative virus in cell culture. The results of this study have shown that the isolation of simian hemorrhagic fever virus strains in cell culture is dependent upon the use of a susceptible MA-104 cell strain and that the ability of such strains to support the replication of these viral agents may vary. By using this information we have been able to isolate a viral agent in cell culture from materials derived from the Sussex/69 epizootic.

Simian hemorrhagic fever (SHF) is an explosive fatal disease known to affect only monkeys of the *Macaca* species and is characterized by anorexia, high fever, a severe hemorrhagic diathesis, and ultimately shock. Including the first recognized outbreaks in 1964, six epizootics have occurred at four primate centers. The epizootiology (4-7, 9, 11), clinical features (4, 5), pathology (1, 4), and virology (7, 9, 11, 12) of these outbreaks have been described in detail. We have received specimens from animals involved in four of these epizootics (namely, Sukhumi/64, Bethesda/64, Davis/67, and Sussex/69) and have consistently been able to reproduce the characteristic illness in rhesus (*M. mulatta*) monkeys (9; *unpublished data*).

Although the SHF viruses could easily be transmitted to monkeys of the macaque species, all attempts to propagate the agents in other laboratory animals have been unsuccessful, and extreme difficulty was encountered in isolating the agent in cell culture (12). Attempts to isolate SHF virus in cell cultures were initially unsuccessful until the MA-104 embryonic rhesus monkey kidney continuous cell line was used. Subsequently, by using that line, very similar agents were recovered from serum and organ suspensions taken from sick monkeys inoculated with material obtained from the Bethesda/64 (12) and the Sukhumi/64 (11) epizootics.

Other laboratories, however, have been unable to isolate the virus strains responsible for the Sukhumi/64 (4, 7) and Davis/67 outbreaks (C. D. Espana, *personal communication*). Also, we failed repeatedly, even when using MA-104 cell cultures, to isolate an agent from monkeys infected with the Davis/67 and Sussex/69 strains, although these latter agents have been shown to be serologically related to the prototype Bethesda/64 strain (*unpublished data*). In addition, we have experienced difficulty in cultivating the MA-104 cell culture-adapted virus in other cell lines derived from the same and related simian species (9, 12).

This paper deals with our efforts to resolve these problems. Our studies demonstrate the remarkable specificity that SHF virus strains have for growth in a single cell line. In addition, applying what was learned from these efforts, we have been successful in isolating the causative agent of the Sussex/69 epizootic.

MATERIALS AND METHODS

Viruses. Studies were conducted employing several different types of viral preparations. (i) Infectious sera containing the SHF agent in high titer were prepared by the intramuscular inoculation of 4- to 6-lb rhesus (*M. mulatta*) monkeys with 0.2 ml of infectious material (usually serum) diluted 1:10 in 0.5% bovine plasma albumin (BPA) in phosphate-buffered saline (PBS, 2). Because of the high incidence of terminal bacteremia, the inocula were filtered through a 450-nm membrane filter before inoc-

ulation into animals. The monkeys were housed in a negative-pressure isolator (10) for 5 to 6 days when, at the peak of illness but prior to the onset of severe dehydration and prostration, they were sacrificed by exsanguination. The blood, which clotted very poorly, was stored at 4 C for 6 to 8 hr and the serum was separated by centrifugation (900 × *g* for 20 min) and then frozen in samples at −70 C until used. Virus isolation in cell cultures was performed with samples which had been frozen and thawed only once. (ii) After isolation, stock virus pools were prepared from a harvest of infected cells and fluid when cytopathic effect (CPE) involved 75% of the cell sheet. When inoculated back into rhesus monkeys, low cell culture-passaged SHF virus produced the characteristic SHF syndrome. (iii) The BS-C-1 cell culture-adapted strain of the Bethesda/64 agent was also employed in these studies. This strain had been passaged twice in monkeys, eight times in the C-15 series of MA-104 cells, eight times in BS-C-1 cells, and twice in the C-1 series of MA-104 cells [i.e., M/2, MA-104 (C-15)/8, BS-C-1/8, MA-104 (C-1)/2]. Other virus preparations will be appropriately described in the text or in the tables which follow.

Tissue culture. Cell cultures were prepared either by Microbiological Associates, Inc., or by the Cell Biology Section of our Div. of Biologics Standards Laboratory by previously described methods (8). Cultures were received in our laboratory 3 to 5 days after initiation; growth medium was replaced with maintenance medium consisting of Earle's balanced salt solution containing 0.5% lactalbumin hydrolysate, 2% native (not inactivated) fetal bovine serum (Gibco), penicillin G (100 units per ml), streptomycin sulfate (100 μg per ml) and Mycostatin (50 units per ml). After the cell sheet had completely developed, the cultures were ready for use. The old maintenance medium was removed and cultures were inoculated (0.1 ml per tube); after adsorption at 36 C for 1.5 hr, fresh maintenance medium was added (when the source of virus was infectious sera, media containing 5% Selas-filtered native calf serum [Gibco] was used).

Chromosome studies. Chromosomal analysis was performed by David Fabrizio, Bionetics Research Laboratories, Kensington, Maryland 20795.

RESULTS

Reisolation of the prototype virus strain from the Bethesda/64 epizootic. Many unsuccessful attempts were made to isolate the Davis/67 and Sussex/69 strains of SHF in diverse cell cultures, including the MA-104 cell line at numerous passage levels, from fresh serum, frozen serum, ground organ suspensions, and trypsinized organ suspensions. On the fifth day after inoculation with infectious sera, passage of suspensions of these cell cultures into susceptible macaques did not result in disease; subsequent challenge of these same animals with infectious sera resulted in their death from typical SHF. Additionally, we were unsuccessful in our attempts to promote the adsorption of these agents onto coverslip cultures of MA-104 by high-speed centrifugation (3); no CPE was seen (12) nor, employing the immunofluorescence technique (11), could the presence of SHF viral antigen be detected.

Because the disease induced by the Davis/67 and Sussex/69 strains is clinically and pathologically indistinguishable from the prototype Bethesda/64 strain, and because monkeys dying late in the course of these diseases were found to contain complement-fixing and fluorescent antibodies to the Bethesda/64 virus, we were extremely dismayed at our inability to cultivate these agents in cell culture. We therefore attempted to reisolate the Bethesda/64 agent from the original material (stored at −70 C for 5 years) and from freshly prepared infectious rhesus sera. Despite multiple "blind passages," varying the media, the pipettes (plastic versus glass), antibiotics, age of cell culture, presence or absence of *Diplococcus pneumoniae* type 19 (which caused terminal bacteremia in approximately 40% of animals studied), and the presence or absence of simian virus 40 (which also was isolated from the blood and spleen of a single animal dying from SHF), we were unable to reisolate the prototype strain in cell culture.

Examination of our laboratory records for May 1966 (which was the last time an isolation of the Bethesda/64 SHF virus strain had successfully been made from infectious sera), showed that we had had three "series" of MA-104 cells in production, each introduced into the Cell Biology Section of our laboratory at different passage levels at different times. (The term "series" refers to different introductions of the same uncloned cell line into our laboratory and maintained as separate lines which have been given different laboratory accession [C] numbers.) One of these was the direct ancestor of the MA-104 cell line (C-1 series) which we were then using. Another had probably been employed in the initial isolations of the Bethesda/64 agents in 1966. Upon retrieving the latter series of MA-104 (C-15) cells from liquid nitrogen storage, we were once again able to repeatedly isolate the Bethesda/64 agent.

Once the prototype strain had been reisolated it became possible to critically evaluate the isolation system. It was repeatedly demonstrated that the addition of either 5% Selas-filtered calf serum or fractionated calf serum, from which the gamma globulin had been removed, to the culture medium increased our ability to recover wild-type SHF viruses from

infectious sera by a factor of 10 or more (Table 1). In addition, virus recovery was maximal in cultures maintained at 32 and 36 C; incubation at 39 C inhibited virus isolation. It was also shown that neither a single freeze-thaw cycle nor the presence or absence of 100 units of penicillin G per ml, 100 μg of streptomycin per ml, 50 units of Mycostatin per ml, and 50 units of tetracycline per ml affected the recovery of SHF virus from infectious sera. However, despite these and other minor refinements in the isolation system, neither the Davis/67 nor the Sussex/69 agents would propagate in either the C-1 or C-15 series of MA-104 cells.

Isolation of an agent from the Sussex/69 epizootic. A number of additional series of MA-104 cells were obtained at various passage levels from the originator of this cell line. In one of these (the C-66 series) the Sussex/69 agent was isolated. This agent had an identical CPE, and the presence of both cytoplasmic and nuclear SHF viral antigen was easily detected in infected cells by the immunofluorescence technique employing antibody against the prototype Bethesda/64 agent. The Sussex/69 agent appeared to be distinguishable from the prototype strain only by this peculiar difference in cellular susceptibility. We have, as yet, been unable to find a cell line which will support the growth of the Davis/67 virus strain.

TABLE 1. *Effect of bovine serum upon the isolation of SHF (Bethesda/64) virus from infectious serum*

Sera added to media[a]			Titer[b] in MA-104 cell series	
%	Serum	Lot no.	C-15	C-66
0	None		2.1	ND[c]
2	Fetal calf	1[d]	1.7	1.5
	Fetal calf	2[d]	ND	1.5
	Calf	1[e]	1.7	ND
	Calf	4[f]	ND	3.3
5	Fetal calf	1[d]	2.1	ND
	Calf	1[e]	3.0	3.0
	Calf	2[e]	ND	3.3
	Calf	3[g]	ND	2.8
	Calf	4[f]	ND	3.0

[a] Serum was added to maintenance medium (Earle's balanced salt solution containing 0.5% lactalbumin hydrolysate, 100 units of penicillin G per ml, 100 μg of streptomycin per ml and 50 units of Mycostatin after virus adsorption.

[b] Log_{10} $TCID_{50}$ per 0.1 ml of Bethesda/64 infectious serum; the third and fourth rhesus passages were inoculated into C-15 and C-66 series, respectively.

[c] Not done.

[d] Grand Island Biologics Co.

[e] Grand Island Biologics Co., Selas filtered.

[f] Hyland Laboratories.

[g] Flow Laboratories.

Comparative sensitivity of three MA-104 cell series to the Sussex/69 and Bethesda/64 strains of SHF virus. Figure 1 shows the results of successive passages of the Sussex/69 virus strain in the C-66 MA-104 series when each passage was simultaneously titrated in three MA-104 cell series (i.e., C-1, C-15, and C-66). On initial isolation from infectious serum (Fig. 1, passage 0), the Sussex/69 virus grew only in MA-104 C-66 series. After passaging in MA-104 C-66 series, Sussex/69 virus developed the capacity to grow in all three of the MA-104 cell series employed; growth in MA-104 C-1 series considerably lagged behind the other two MA-104 cell series.

The simultaneously determined sensitivities of the three MA-104 cell series to the Bethesda/64 agent is shown in Table 2. The Bethesda/64 virus appeared to grow equally well in both the C-15 and C-66 MA-104 cell series, regardless of the virus source. The C-1 MA-104 cell series appeared insensitive to the growth of virus from infectious serum and less sensitive to the BS-C-1 cell-adapted virus than the other two MA-104 cell series.

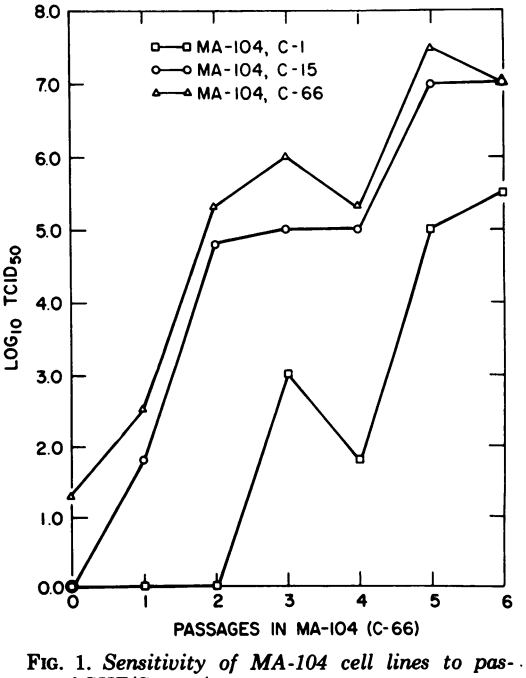

FIG. 1. *Sensitivity of MA-104 cell lines to passages of SHF/Sussex/69.*

Characteristics and properties of the three MA-104 cell strains: cytology. The C-1 and C-66 MA-104 cell strains were cytologically indistinguishable; although the C-15 strain appeared very similar it did have more multinucleated cells (a characteristic of MA-104), and it takes appreciably longer to trypsinize the cell sheet from glass.

Mycoplasma contamination. The C-1 and C-15 cell strains have long been contaminated with *Mycoplasma arginini* and *orale* in the Cell Biology Section of our Laboratory. The sensitivity of the C-66 cell strain to SHF viruses, however, was apparently unaffected by the presence or absence of these mycoplasma species.

Karyology. Both the C-15 (passage 99) and the C-66 (passage 65) cell series exhibited a range of chromosome number from 43 to about 100, with modal numbers of 58 and 64, respectively. The types of chromosomes observed included metacentrics, submetacentrics, acrocentrics, and possibly tetracentrics. There were approximately 5% polyploid breaks and structural rearrangements in both series. The C-1 cell series (passage 69) was remarkably different with a range of chromosome number from 50 to 160 and with a modal number of from 100 to 150. All types of chromosomes were observed. A great many cells showed aberrations and structural rearrangements including dicentrics, quadriradials, rings, fragments, chromosome and chromatid breaks, pulverization, and fragmentation. Species identification by chromosome analysis was not possible in any of these cell series.

TABLE 2. *Relative sensitivity of the MA-104 cell series to the Bethesda/64 strain of SHF virus*

MA-104 series	Passage	Age of culture (days)	Source of Bethesda/64 virus	
			Infectious sera[a]	BS-C-1 cell-adapted virus strain[b]
C-1	25	5	<0.5[c]	4.8
C-15	48	5	3.8	5.8
C-66	51	6	3.8	5.8

[a] Infectious rhesus serum in its fourth passage: M/4.

[b] Virus passaged 18 times in tissue culture after the second rhesus passage: M/2, MA-104 (C-15)/8, BS-C-1/8, MA-104 (C-1)/2.

[c] Titer expressed as $\log_{10}$ $TCID_{50}$ per 0.1 ml.

Sensitivity to the CPE of other viruses. Vaccinia, vesicular stomatitis, echovirus 10, and Chikungunya viruses were titrated in tube cultures of the three MA-104 cell series. The titer, as determined by CPE, of each virus pool and the type and progression of the CPE characteristic for each virus were identical in the three cell series employed.

Virus plaque morphology. There was a marked difference in the plaque size and clarity of the Bethesda/64 virus in the three MA-104 cell series: plaques in C-15 were largest, clearest, and most distinct; in the C-1 cell strain, plaques were the smallest and least distinct; plaques in the C-66 cell series were intermediate to these extremes. There were no observable differences between the plaque characteristics of Chikungunya or vaccinia viruses in the three MA-104 cell series.

Long-term experience with the isolation of the Bethesda/64 agent in three MA-104 cell series. Samples of a stock preparation of the Bethesda/64 virus in the form of infectious rhesus monkey serum were titrated in the three MA-104 cell series (when available) frequently over a period of 65 weeks (Fig. 2). Between the 5th to 11th week after the onset of these experiments, the sensitivity of the C-15 and C-66 MA-104 cell strains markedly decreased. We thought that this may have been related to increase in the passage level of the cell series. However, the sensitivity of these two cell series returned on the 12th week and remained high until the 23rd to 27th week when the sensitivity was again reduced to very low to undetectable levels. This fluctuation continued throughout the 65 weeks of the study. It was interesting to observe that when the sensitivity increased or decreased it appeared to do so for both the C-15 and C-66 cell series during roughly the same period. The sensitivity of the C-1 cell series remained very low to nil throughout most of the study period, except for a brief time at the 57th to 59th week. The fluctuation in the sensitivity of the three MA-104 cell series was unrelated to the passage level of the cells, to change in lots of basic components of the growth and maintenance media, to mycoplasma contamination, and to different technicians responsible for preparing the cell cultures.

Fluctuations in sensitivity of the MA-104 cell series did not occur to the BS-C-1 cell culture-adapted Bethesda/64 virus.

Sensitivity of other cell lines to SHF viruses. Because of the unusual cellular specificity of the SHF viruses, an attempt was

made to find susceptible tissue culture systems other than the MA-104 embryonic rhesus monkey kidney cell line. For this purpose various cell cultures were inoculated with undiluted virus (about $10^{5.8}$ median tissue culture infective doses per 0.1 ml) and 10^{-1} and 10^{-2} dilutions of the BS-C-1 cell culture-adapted Bethesda/64 virus. If no CPE developed by the 7th day, the tubes inoculated with undiluted and 10^{-1} virus dilution were harvested and passaged undiluted and at a 10^{-1} dilution into the C-66 strain of MA-104, which is highly sensitive to the virus strain employed. The results of this attempt are shown in Table 3.

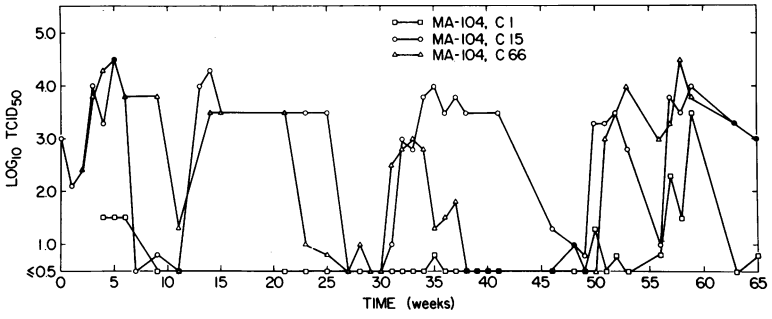

FIG. 2. *Sensitivity of MA-104 cell lines to SHF/Bethesda/64 infectious sera.*

TABLE 3. *Cell cultures capable of supporting the growth of the BS-C-1 cell culture-adapted Bethesda/64 SHF virus strain*[a]

Species of origin	Organ of origin	Passage (no.)	Identification	Results	
				CPE[b]	Growth[c]
M. irus embryo	Brain	1	C-43	0	+
	Spinal cord	1	C-64	0	+
	Lung	1	MA-354	0	+
	Kidney	0	C-49	0	+
	Muscle (quadriceps)	2	MA-355	+	+
	Tongue	1	MA-356	+	+
M. mulatta embryo	Lung	26	MA-101	0	+
	Kidney	9 to 100	MA-104	+	+
	Spleen	17	MA-108	±	+
adult	Liver	14	None	0	+
C. aethiops embryo	Kidney	Many	MA-134	+	+
C. aethiops adult	Kidney	0	Many	Variable	Variable
	Kidney	Many	BS-C-1	+	+
Human embryo	Tongue	3	C-25	0	+

[a] The following cell cultures were unable to support the growth of the BS-C-1 cell culture-adapted Bethesda/64 SHF virus strain: (i) *M. irus* (embryo): brachial plexus, passage (P) 1; choroid plexus, P1, 2, and 4; lung, P0 (primary); kidney, P1, 3 and 4; adrenal, P1; liver, P1; ovary, P3; thymus, P1; spleen, P2; lymph node, P1; stomach, P5; intestine, P1; uterus, P3; bladder, P1; heart, P2 and 11; tongue, P1; and thyroid, P4; (ii) *M. mulatta* (embryo): brain, P57; heart, P27; and skin and muscle, P34; (iii) *M. mulatta* (adult): kidney, P0; kidney (LLMK), P10; and kidney (BSTC-224), P30; (iv) *C. aethiops* (embryo): liver, P21; gonad, P20; lung, P24; skin and muscle, P17; and heart, P24; (v) *C. aethiops* (adult): kidney (Vero), P127; (vi) *E. patas* (adult): kidney, P1; (vii) *Cebus* (embryo): kidney, P1; lung, P3; small intestine, P4; colon, P4; (viii) *Human* (embryo): brain, P3; cornea P3; spleen, P4; thymus, P3; tonsil, P3; lung, P2; lung (WI-38), P28 and 30; liver, P3; kidney, P0; (ix) *Human* (adult): amnion, P0; HA amnion line, P27; F1 amnion line, P43; HEp-2, P24 and 76; HeLa, P15; and normal lymphocytes, P0; (x) *Human* (newborn): foreskin, P28; and (xi) miscellaneous animal species: calf kidney, P0; bovine embryonic kidney, P41; rat embryo, P25; rabbit embryo, P0; newborn rabbit kidney (MA-111), P24; adult rabbit kidney (LLC-RK1), P238; opossum kidney, P40; chicken embryo, P0; duck embryo, P0; mouse embryo, P0; hamster embryo, P0; hamster kidney (BHK-21), P41; guinea pig lung, P0 and kidney, P0.

[b] Cytopathic effects at day 7.

[c] Determined by passage at day 7 into MA-104 (C-66).

TABLE 4. *Comparison of the growth of SHF viruses in susceptible cell cultures*

Cell line	Series code	Passage	SHF/Bethesda/64				SHF/Sukhumi/64				SHF/Sussex/69				SHF/Davis/67	
			M/5[a]		M/2, MA104/9[b]		M/3[a]		M/1, MA104/14[b]		M/5[a]		M3/MA104/10[b]		M/5[a]	
			CPE	Growth (No CPE)	CPE	Growth (No CPE)	CPE	Growth (No CPE)	CPE	Growth (No CPE)	CPE	Growth (No CPE)	CPE	Growth (No CPE)	CPE	Growth (No CPE)
MA104	C-1	71	0.0[c, d]	No	6.8	ND[e]	0.0	No[f, g]	4.5	ND	0.0	No[f, g]	5.3	ND	0.0	No[g]
	C-15	91	3.3[c]	ND	7.0	ND	0.0	No[f, g]	5.5	ND	0.0	No[f, g]	6.5	ND	0.0	No[g]
	C-66	56	3.3[c]	ND	6.8	ND	0.0	No[f]	5.0	ND	1.0	ND	6.0	ND	0.0	No
MA355		6	0.0	ND	0.0	No	0.0	ND	0.0	No	0.0	ND	0.0	No	0.0	ND
MA356		5	0.0	ND	0.0	Yes	0.0	ND	0.0	Yes	0.0	ND	0.0	Yes	0.0	ND
MA134		64	1.3	ND	≥3.5	ND	0.0	No	≥1.5	ND	0.0	No	≥1.5	ND	0.0	No[g]
BSC-1		46	0.0	ND	0.0	Yes	0.0	ND	0.0	Yes	0.0	ND	0.0	Yes	0.0	ND
VMK		0	0.0	Yes	≥3.5	ND	0.0	No	≥1.5	ND	0.0	No[g]	≥1.5	ND	0.0	No[g]

[a] End points: initial culture, 28 days; first subculture, 18 to 22 days.
[b] End points: initial culture, 6 days; first subculture, 6 days.
[c] The subsequent passages of MA-104 used for subculturing had essentially the same titers.
[d] Titer expressed as $\log_{10}$ $TCID_{50}$ per 0.1 ml.
[e] Not done.
[f] Subcultured on day 10, all others subcultured on day 6.
[g] Subcultured in the same cell line and MA-104 (C-66), all others subcultured in MA-104 (C-66) only.

Those few cell cultures supporting virus growth were limited to a few tissues derived from susceptible species (*unpublished data*).

The six cell lines demonstrating CPE were then inoculated with infectious sera containing the agent responsible for each SHF epizootic, as shown in Table 4. The results essentially support the earlier observation of the unique sensitivity of the MA-104 cell line to the primary isolation of the Bethesda/64 and Sussex/69 SHF virus strains. Unfortunately, the newer cell lines found to support the growth of cell culture-adapted Bethesda/64 virus did not show promise for use in isolating SHF virus from infectious serum. The loss of sensitivity of the cynomolgus fetal muscle (MA-355) and fetal tongue (MA-356) to the cell culture-adapted Bethesda/64 virus, as compared to the data shown in Table 3, may reflect either a decreased sensitivity of the cell on further subculture or the fact that a different cell culture-adapted Bethesda/64 strain was employed in this latter experiment.

DISCUSSION

The unusual in vivo and in vitro host specificity of the SHF viruses undoubtedly contributed to the difficulties encountered in their initial isolations. Our experiments have shown that the ability to isolate these agents in cell culture was dependent upon not only the use of a susceptible cell line or culture, but also the use of that cell line or culture during a period while it is sensitive to the virus. There appeared to exist transient sensitivity fluctuations which may have been related to factors extrinsic to the cultured cells, because these fluctuations occurred transiently in two cell "series" at the same time. Our failure to find an extrinsic cause did not rule out the possibility that one existed.

It is also possible that there existed a more permanent type of intrinsic variation in cell sensitivity to one or more of the SHF virus strains examined. Variations are known to occur in cell lines or cultures during serial passaging. The failure of some strains of SHF virus to infect the different series of the same MA-104 continuous fetal rhesus monkey kidney cell line was, therefore, not unusual and was probably due to variations of an intrinsic cellular mechanism regulating the early stages of virus-host cell interaction. It is attractive to postulate that these variations of cellular susceptibility might be related to the degree of chromosomal disruption.

One cannot exclude the possibility that the more permanent type of intrinsic variation and the transient extrinsic sensitivity fluctuations were not manifestations of the same phenomenon, which may appear to be different because only the limits of the latter type could be measured by our present methodology.

Although it is well known that the prolonged in vitro cultivation of cell lines or cultures frequently may result in either an increase or decrease in susceptibility to various viruses, we are unaware of a similar instance where cellular sensitivity actually fluctuates. We presume that our inability to isolate an agent in cell culture from materials derived from the Davis/67 epizootic was either because we have not yet found a cell line (or cell strain) that is genetically capable of supporting replication of this virus (such as the C-1 MA-104 series for the Sussex/69 virus), or that the cell lines we have examined were temporarily insensitive when tested.

In 1969 an outbreak of SHF occurred in Sussex, Great Britain, from which we have now isolated the causative agent. This epizootic was similar to the previously reported outbreaks, and the experimentally induced illness in rhesus monkeys was indistinguishable both clinically and pathologically from the earlier epizootics (9). The agents responsible for all known epizootics thus far have been shown to be antigenically indistinguishable (*unpublished data*), and those virus strains that have been cultivated in cell cultures induce identical and characteristic CPE. It would appear, then, that the only distinction between these viral strains is their remarkable host specificity in cell culture.

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