The Vacuolating Virus, S.V.$_{40}$ (26128)

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Viruses are commonly carried by monkeys and may appear as contaminants in cell cultures of their tissues, especially the kidney. Included among these are B virus, foamy agent, measles-like virus, hemadsorption viruses, LCM virus, arborviruses, and a wide variety of miscellaneous viral agents(1-7). Hull *et al.*(2,3) have studied extensively viruses encountered in "normal" monkey renal cell cultures. Hull called them simian or SV viruses and classified them into 4 groups based on the kind of cytopathic change induced in monkey kidney cell cultures infected with the agents. Twenty-eight of these viruses were precisely separated serologically into types and, additionally, 24 unidentified viruses were recorded. Malherbe and Harwin(4) distinguished 7 distinct types among the simian agents or SA viruses they recovered from vervet kidney materials.

During the past 2 years, our virus research group* has repeatedly encountered a new simian virus of rhesus and cynomolgus monkey kidney origin. This agent was unique among simian viruses studied hitherto since it grew but did not cause a cytopathic effect in the rhesus or cynomolgus kidney cell cultures from which it was derived. Instead, it grew and caused marked cytopathic changes in cell cultures of a heterologous species, *i.e.*, the green monkey, *Cercopithecus aethiops* obtained from Equatorial East Africa [grivet, according to Sanderson(8)]. This agent has been called the "vacuolating virus" by us because of the prominent cytoplasmic vacuolation seen in infected cell cultures. Dr. Hull† suggested that this agent be given the "official" designation of S.V.$_{40}$ in C.P.E. group 4.

The discovery of this new virus, the vacuolating agent, represents the detection for the first time of a hitherto "non-detectable" simian virus of monkey renal cultures and raises the important question of the existence of other such viruses. The present report summarizes the current information available concerning the vacuolating agent. Certain of these data have been presented at the Second International Conference on Live Poliovirus Vaccines(9).

Materials and methods. Tissue cultures. Primary cell cultures were prepared from trypsinized cell suspensions and were grown in Melnick's lactalbumin-yeast extract medium(10) containing 2% heat-inactivated calf serum. All line cell cultures were grown in Eagle's basal medium(10) containing 10% heat-inactivated horse serum. During virus propagation, all cell cultures were maintained using medium 199(10) containing 2% heat-inactivated chicken serum and at initial pH 7.6. All culture fluids contained 100 μg of penicillin and 100 units of streptomycin per ml.

Propagation of vacuolating agent. The virus grew readily in cell cultures of grivet monkey kidney with production of cytopathic change. Such cultures were used routinely for the work described in this report. For ordinary purpose, the virus was passed in an initial dilution of 10^{-1} of infected tissue culture fluid using 0.2 ml of inoculum per 2.0 ml of maintenance medium. After 4 to 7 days incubation, at a time when at least 50% of the cell sheet showed cytopathic change typical of that of the vacuolating agent, the cultures were frozen and thawed once and the fluid was harvested. The virus was stored frozen in the dry ice refrigerator.

Virus titrations and serum neutralization tests. All virus titrations were performed using serial 10-fold dilutions of virus. The inoculum dose was 0.2 ml per roller tube culture containing 2.0 ml of maintenance medium. The end point was the highest initial

* The first such agent was recognized by Dr. L. McClelland in the course of safety testing of vaccines.

† Personal communication.

dilution of virus which caused degeneration of 25% or more of the cells in the cultures after 7 to 10 days incubation. In the neutralization tests, serial 2-fold dilutions of serum inactivated at 56°C for 30 minutes were incubated for one hour at 37°C with an equal volume of virus diluted to contain about 200 TCD_{50} per 0.2 ml. The inoculum was 0.2 ml of the virus-serum mixture and the end point was the highest initial dilution of serum which effected complete suppression of cytopathic change in cultures observed 7 to 10 days following inoculation.

Preparation of antiserum against the vacuolating agent. Young adult rabbits were injected intravenously with 1.0 or 2.0 ml of infected grivet monkey kidney culture fluid plus 1.0 ml of infected fluid emulsified in Arlacel-Drakeol adjuvant administered intramuscularly in a single dose or intracutaneously in divided doses. A final 1.0 ml dose of infected fluid was administered intravenously 3 weeks later and the animals were bled one week thereafter.

Results. Recovery of strains of vacuolating virus. Vacuolating virus was recovered from "normal" cell cultures of rhesus and cynomolgus monkey kidney and from the seed stocks of a variety of viruses which had become contaminated with the vacuolating virus during passage in cultures of rhesus and cynomolgus monkey kidney.

The vacuolating virus was recovered from contaminated virus seeds by passage of the virus seed, in the presence of its homologous antiserum, in cell cultures of *Cercopithecus aethiops* or grivet monkey. Under these conditions, the vacuolating virus "broke through" and was identified in serum neutralization tests using vacuolating virus antiserum prepared in rabbits. Recovery of the virus from "normal" rhesus or cynomolgus cell cultures was accomplished by simple passage, in grivet cell cultures, of the fluid from the rhesus or cynomolgus cultures which had been held for 7 to 10 days with maintenance medium. Vacuolating virus isolates were identified in serum neutralization tests using rabbit antisera prepared against prototype strains.

TABLE I. Origin of 8 Strains of Vacuolating Virus.

Strain	Recovered from	Infectivity titer of original material
776	Adenovirus type 1, seed stock	N.D.*
175	" " 5, " "	"
S 207	Sabin, type 1, live attenuated oral polio vaccine, strain LSC	$10^{-4.5}$
S 211	Sabin, type 2, live attenuated oral polio vaccine, strain P712	$10^{-3.7}$
S 215	Sabin, type 3, live attenuated oral polio vaccine, strain Leon	$10^{-4.5}$
953	Uninfected "normal" rhesus monkey kidney cell culture	$10^{-5.5}$
1095	*Idem*	$10^{-6.0}$
584	Uninfected "normal" cynomolgus monkey kidney cell culture	$10^{-5.5}$

* Not done.

More than 20 strains of vacuolating virus have been recovered in our laboratory. Table I shows the origin of 8 of the strains examined most extensively. The vacuolating virus has appeared as a contaminant in the seed stocks of all of types 1 through 7 adenovirus. These seeds had been prepared in cultures of rhesus or cynomolgus monkey kidney and were obtained from Dr. R. J. Huebner or were brought to this laboratory by one of us (M.R.H.) from Walter Reed Army Inst. of Research. Additionally, vacuolating virus was recovered from seed stocks of *Myxovirus parainfluenza* 1 and 3, from the SA virus, and from the respiratory syncitial agent received from Dr. R. Chanock and Dr. K. Habel.

Vacuolating virus strains S207, S211 and S215 were derived from types 1, 2 and 3, respectively, of Dr. Albert Sabin's live attenuated poliomyelitis vaccine. For recovery of vacuolating agent, the poliovirus was neutralized by homologous polio immune serum prepared in rabbits immunized with Syverton's HeLa cell lines of poliovirus propagated in human stable amnion cell culture. The infectivity titer of the vacuolating virus in each of the Sabin vaccines ranged from $10^{-3.7}$ to $10^{-4.5}$. These isolates of vacuolating virus were identified in serum neutralization tests with rabbit antisera against prototype strain

776. At the time of the initial isolations of vacuolating virus, the Sabin vaccines were also passaged in presence of a mixture of homologous poliovirus and vacuolating agent antiserum. The antiserum mixtures suppressed both the poliovirus and vacuolating agent and showed the absence of other detectable viruses which might have been present in the Sabin materials.

The essentially ubiquitous occurrence of vacuolating virus in the various virus seed stocks and vaccines suggested a high infection rate among normal rhesus and cynomolgus monkey kidney cell cultures. This was borne out in tests for virus in "normal" cell cultures. During a one-month period, 10 rhesus and 10 cynomolgus monkey kidney cell lots prepared for use in ordinary Salk vaccine production were examined for presence of the vacuolating virus by passage to grivet kidney cultures. Each lot of monkey kidney was derived from a pool of kidneys from 2 or 3 monkeys. None of these "normal" cell lots presented any cytopathic change suggestive of the vacuolating virus. Of 10 lots of rhesus kidney, 7 yielded vacuolating virus. One lot was infected with a foamy-like virus(1) and 2 lots appeared free of simian contaminants. Only one of the 10 cynomolgus cell culture lots revealed vacuolating virus. However, it was not possible to carry out definitive tests of these materials since 8 of the lots were heavily contaminated with what appeared to be foamy virus. Thus, the vacuolating agent, if present, may have been overgrown and excluded by the foamy virus.

It is striking that the titer of vacuolating agent was as high as $10^{-6.0}$ in the fluids from the normal rhesus kidney cell cultures, even though these cultures showed no cytopathic change suggestive of the presence of a virus.

Cultures of grivet monkey kidney prepared in our laboratories have proved remarkably free of the vacuolating agent. During use of more than 1000 lots of individual monkey kidney cell cultures, presence of the vacuolating agent was suspect in only 4 lots and proved for only 2 lots.

Properties of vacuolating virus. Cytopathic effect. Fig. 1 shows the outstanding cyto-

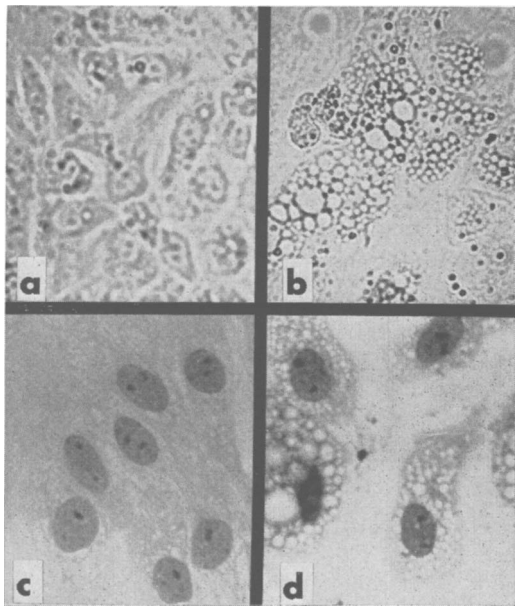

FIG. 1. Cytopathic changes in infected kidney cell cultures of *Cercopithecus aethiops* monkeys caused by strain 776 of vacuolating virus. a. Uninoculated control culture, unstained, day 6, 188 × mag. b. Infected culture, unstained, day 6, 188 × mag. c. Uninoculated control culture, day 6, 442 × mag., stained with H & E. d. Infected culture, day 6, 442 × mag., stained with H & E.

pathic changes caused by vacuolating virus in grivet kidney as observed in wet and in stained culture preparations. Grivet kidney cell cultures infected with about 1000 TCD_{50} of vacuolating virus show beginning cytopathic changes on day 3 or 4, when some of the cells may appear rounded or shrunken and the cytoplasm may be darkened. A few cells may show beginning vacuolation of the cytoplasm. With increased time, typical changes develop, consisting predominantly of ballooning of cells which are free or spread out on the glass together with intense vacuolation of the cytoplasm of such cells. These vacuoles are highly refractile in wet preparations and appear as "holes" with intensely stained boundaries in hematoxylin and eosin stained materials. In cultures stained with acridine orange and observed by ultraviolet fluorescent microscopy, the vacuoles likewise appear to be "holes" in the cytoplasm. We have not been able to demonstrate any stainable substance within the vacuoles nor has it been possible to demonstrate inclusion bodies

TABLE II. Antigenic Relationships of Vacuolating Virus Strains as Measured in Serum Neutralization Tests.

Virus	Serum titer 776	175
776	1:4096 or >	1:800
175	1:4096 or >	1:1600
1095	1:4096 or >	1:2048
953	1:4096 or >	1:4096 or >
584	1:4096 or >	1:4096

in such infected cells. Nuclei of infected cells may appear normal or disorganized internally. Limited fusion of the cytoplasm of adjoining infected cells may occur but, predominantly, each cell maintains its own integrity. Thus, the syncitium or giant cell formation, so characteristic of measles, foamy virus and certain other virus infections, is not prominent in vacuolating virus infection and this serves as a distinguishing feature. Terminal cytopathic changes occur in the cultures 5 to 10 days post-inoculation when the infected cells aggregate together and detach from the glass leaving behind only small islands of normal or degenerated cells. The cytopathic changes caused by vacuolating virus appear to be quite distinct from those described for Hull's 4 C.P.E. groups of simian viruses or for Malherbe's and Harwin's 7 simian agents.

Antigenic relationships. All strains of vacuolating virus studied to date have comprised a single immunologic group. Antisera against vacuolating virus strains 776 and 175 neutralized the homologous viruses and all of 3 additional strains as well (Table II). Additionally, and not shown in the table, all 3 of the Sabin vaccine isolates (S207, S211, S215) were neutralized by these sera.

Strain 776 of vacuolating virus was not neutralized by antiserum against C.P.E. group 4 strain $S.V._{6}$, $S.V._{26}$ or $S.V._{29}$ antisera furnished by Dr. Hull. Additionally, it was not neutralized by Myxovirus parainfluenza 1 or 3 antisera or by such SA, $S.V._{5}$ or foamy virus antisera as were available to us. Vacuolating virus strains 776, 175, 953, 1095 and 584 were neutralized by pooled antisera against Hull's group 1B ($S.V._{20, 23, 25, 27}$) and the last three strains by group 3 ($S.V._{12, 28, 59}$). None was neutralized by group 2 ($S.V._{2, 16, 18, 19}$) or by group 4 ($S.V._{5, 6, 29}$) antisera. In these tests, only a negative finding had significance since the antisera employed were prepared using virus grown in rhesus or cynomolgus monkey kidney cell cultures and these might also have contained vacuolating virus.

Host range. A preparation of vacuolating virus strain 776 which titered 10^{-5} in grivet kidney culture was tested in the various primary and line cell cultures shown in Table III. The virus was cytopathic for patas kidney and for rhesus monkey testicle, but these cells were about 30 to 300 times less sensitive than grivet kidney. Vervet monkey kidney has also been reported highly susceptible to the vacuolating virus(11). Grivet and vervet monkeys are separate races of the species *Cercopithecus aethiops.*

No definitive cytopathic change referrable to the vacuolating virus was observed in any of the remaining primary or line cell cultures, shown in the table, when observed for eight to twelve days. The virus persisted for at least 7 days in most of the cultures without increase in titer. Limited proliferation might have occurred. Titers as high as $10^{-8.5}$ have been obtained with vacuolating virus grown in grivet kidney.

The susceptibility of suckling mice and of embryonated hen's eggs to the vacuolating virus was tested. Suckling mice, inoculated intracerebrally, subcutaneously or intraperitoneally failed to develop illness within 21

TABLE III. Titration for Cytopathic Effect of Vacuolating Virus Strain 776 in Various Cell Cultures.

Kind of cell	Titer based on cytopathic end point, 8 days
Primary cell cultures	
Grivet monkey kidney	$10^{-4.5}$
Patas " "	$10^{-3.0}$
Rhesus " testicle	$10^{-2.0}$
Rhesus " kidney	$<10^{0}$
Rabbit kidney	$<10^{0}$
Human amnion	$<10^{0}$
Line cell cultures	
HeLa	$<10^{0}$
Stable amnion	$<10^{0}$
Chang liver	$<10^{0}$
Girardi human heart	$<10^{0}$

TABLE IV. Filterability of Vacuolating Virus Strain 776.

Filter used	Infectivity titer of filtrate
Seitz, S1	$10^{-3.75}$
Selas 03 candle	$10^{-3.25}$
Unfiltered, control	$10^{-5.00}$

TABLE V. Heat Stability at 56°C, 1 Hour, of Infectivity of Vacuolating Virus Strain 776.

Virus menstruum	Infectivity titer
Medium 199	
Heated 1 hr, 56°C	$10^{-4.0}$
Not heated	$10^{-5.5}$
Medium 199 with 2% normal rabbit serum	
Heated 1 hr, 56°C	$10^{-3.75}$
Not heated	$10^{-5.5}$

days following primary inoculation of vacuolating virus or following subpassage of brain or carcass carried out 7 days following primary inoculation. Embryonated hen's eggs inoculated after 8 days incubation *via* the allantoic or amniotic routes failed to develop manifest signs of disease within 6 days primary incubation. Additionally, none of the harvested fluids contained demonstrable hemagglutinins for chick erythrocytes.

Miscellaneous physical and biological properties of the vacuolating virus. The vacuolating agent was readily filtered through bacterial sterilizing filters (Table IV). The agent is relatively heat stable compared with most viral agents (Table V). Fig. 2 presents data relating to inactivation‡ at 37°C with 1:4000 formalin of the vacuolating agent. The slope of the inactivation curve indicates a slightly less rapid inactivation rate than that ordinarily found for poliovirus under the same conditions. Table VI presents a summary of known biological and physical properties of the vacuolating agent.

Occurrence of antibody against vacuolating virus in human and monkey sera. Table VII summarizes the results of tests for antibody against the vacuolating virus in sera of human subjects and in normal monkey sera. None of the normal or pre-vaccination sera

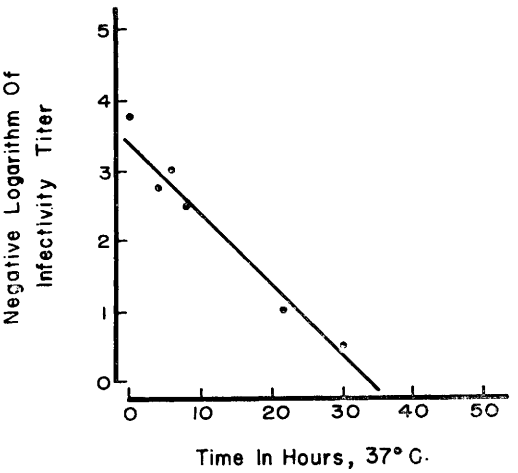

FIG. 2. Inactivation kinetics, vacuolating virus strain 776 with 1:4000 formalin at 37°C.

TABLE VI. Summary of Biological and Physical Properties of Vacuolating Virus.

1. Widespread "inapparent" virus in normal rhesus and cynomolgus kidney cell cultures. May also occur as an "apparent" contaminant of grivet kidney cell cultures.
2. Cytopathic for grivet kidney, vervet kidney, patas kidney and rhesus testicular cells in culture. Fails to cause cytopathology in a variety of other cells in culture.
3. Fails to cause manifest illness or death in newborn mice or in embryonated hen's eggs.
4. Known strains comprise a single serotype.
5. Filterable through Seitz S1 and Selas 03 filters.
6. Infected cultures or culture fluids fail to hemadsorb or to cause hemagglutination of guinea pig, chicken, or human "O" erythrocytes at 4°C or at 25°C.
7. Resists treatment for 18 hr with an equal volume of diethyl ether.
8. Infectivity destroyed by treating with 1:4000 formalin at 37°C.
9. Relatively heat stable. Heating at 56°C for 1 hr reduces infectivity titer about 30-fold.
10. Stable on storage at −20°C and at −70°C.
11. Induces homologous neutralizing antibody in rabbits immunized with the agent.
12. Sensitive to photodynamic inactivation with visible light in presence of certain dyes(14).
13. Preliminary findings indicate that agent is infectious for man when given by respiratory route(14).

‡ We are indebted to E. Dwyer for these data.

TABLE VII. Tests for Neutralizing Antibody against Vacuolating Virus Strain 776 in Various Human and Monkey Sera.

Description of sera	No. positive / No. tested
Military recruits:	
Received 2 doses of killed adenovirus vaccine	
Pre-vaccination sera (diluted 1:5)	0/9
Post-vaccination sera (diluted 1:5 and 1:20)	9/9 *
Received 2 doses of placebo	
Pre-injection sera (diluted 1:5)	0/7
Post-injection sera (diluted 1:5)	0/7
Children:	
Received 2 doses of Salk poliomyelitis vaccine	
Lot 43038—Pre-vaccination sera (diluted 1:5)	0/7
Post-vaccination sera (diluted 1:5 and 1:20)	5/7 †
Lot 43058—Pre-vaccination sera (diluted 1:5)	0/6
Post-vaccination sera (diluted 1:5)	1/6
Fed Sabin live oral polio vaccine on 6 occasions, serum diluted 1:5	0/5 ‡
Normal children, not fed Sabin vaccine	0/5 ‡
Human gamma globulin, Philadelphia Serum Exchange, lot G50B, diluted 1:50	0/1
Normal grivet monkey sera (5 lots) (undiluted and 1:5)	0/14
Normal rhesus monkey sera (diluted 1:5)	12/18

* 8/9 titered 1:20 or >. † 3/7 titered 1:20 or >. ‡ Sera tested for Dr. A. Sabin.

from any of the human subjects contained antibody against the vacuolating virus nor was such antibody present in a lot of human gamma globulin. This suggests that natural infection in man with the vacuolating virus, if it occurs at all, is infrequent. However, all 9 persons given 2 doses of formalin-killed adenovirus vaccine prepared in rhesus or cynomolgus monkey kidney cell culture by one of us (M.R.H.) at the Walter Reed Army Inst. of Research(13), developed antibody against the vacuolating virus. Similarly, 7 of 13 persons given 2 doses of Salk poliomyelitis vaccine developed antibody against the vacuolating virus. These results are wholly consistent with the high frequency of vacuolating virus in monkey renal cell cultures, and indicate also the apparent high potency of vacuolating virus antigen in these vaccine preparations. None of 5 persons developed antibody against the vacuolating virus when fed Sabin live polio vaccine on 6 occasions. These data and those obtained more recently by Sabin(14) suggest lack of infectiousness of the vacuolating virus when administered by the oral route. This is in contrast to the apparent ability to infect man when the virus was administered by the respiratory route (12).

None of 14 normal sera from grivets representing 5 different shipments contained antibody against the vacuolating virus. By contrast, 12 of 18 sera from rhesus monkeys proved to have antibody to the virus. These findings are consistent with the very infrequent finding of vacuolating virus in grivet kidney cultures and the high prevalence in rhesus kidney.

Discussion. The discovery of the vacuolating virus represents the first demonstration of a hitherto hypothetical "non-detectable" simian virus, *i.e.*, a virus present in monkey kidney cell cultures but not detectable by current procedures. All of the simian viruses which were recovered previously and which were of monkey kidney origin were detected in kidney cell cultures of the same monkey species, either in the primary culture itself or on sub-passage. In the present instance, the vacuolating virus was detected only following passage in the kidney cell cultures of a heterologous host. The demonstration of this first "non-detectable" simian virus raises the question of whether other as yet undetected

viruses might also await demonstration. Such experience, although new to simian viruses and monkey kidney, is neither new nor unique to other viruses and cell culture systems.

The discovery of the vacuolating virus has special significance during the contemporary period in relation to live poliovirus vaccine. As shown in this report, all 3 types of Sabin's live poliovirus vaccine, now fed to millions of persons of all ages, were contaminated with vacuolating virus.

It is well established that when 2 different viruses compete in the same cell culture or animal host system, the end result may be interference with growth of one or both of the viruses, enhancement of pathogenic effect, or no immediate demonstrable effect. The interference effect is documented in numerous publications(15). The recent demonstration by Dalldorf and Weigand(16) that monkeys may become paralyzed following cerebral injection of both Coxsackie A14 and live attenuated type 1 poliovirus but not following either alone, serves as a cogent example of mutual potentiation of pathology by 2 viruses in consort.

The presence of the contaminating vacuolating virus in such live poliovirus vaccine raises the question, at least, of the validity of tests for monkey neurovirulence (*cf.* 17) following cerebral, spinal or muscular inoculation(18,19) and of the tests for "genetic" markers of poliovirus(20) such as the t, MS, d and others carried out in tissue culture and subject to such competitive effect. The question may also be raised whether observed alterations in neurovirulence and other "genetic" marker properties attending passage of live attenuated poliovirus through the human gut might reflect, in part at least, alterations in the poliovirus-vacuolating virus ratio in such materials submitted for test.

The essentially ubiquitous occurrence of vacuolating virus in seed stocks of a multiplicity of the "newer" respiratory viruses, as recorded herein, raises the question of the possible influence of vacuolating virus on the outcome of tests carried out in human volunteers to determine the pathogenicity of these respiratory agents for man. Similarly, the possible influence of vacuolating virus on results of diagnostic CF tests employing antigens prepared in rhesus or cynomolgus monkey kidney requires review.

The failure, to date, to detect excreted vacuolating virus or development of antibody against the agent among persons fed live poliovirus vaccine(14) strongly suggests lack of massive proliferation of the virus in the human intestinal tract under the conditions employed although possible limited proliferation under these circumstances cannot be excluded. Alternatively, demonstration of antibody development among persons given vacuolating virus *via* the respiratory tract(12) provides strong evidence for infectivity of the virus in man when given by this route. The lack of significant harmful effect in man, in the short term, is well established in results of studies in millions of volunteers fed to date. Less can be said concerning a possible long-term effect. For example, the highly theoretical question has already been raised (21) concerning the possibility of oncogenic properties of viruses of unknown quality, such as the vacuolating virus, especially when administered to babies. Induction of tumors in hamsters following infection by parenteral or extraparenteral routes, early in life with polyoma-infected mouse tissue(22,23) presents an experimental analogy for the hypothetical situation in man. While such possibility is only theoretical and certainly remote, the determination must await the outcome of the experiments which may be decades in process. The argument(24) that human subjects continually consume viruses in foods with no apparent ill effect is lacking in substance since raw monkey kidney is not ordinarily part of the human diet.

In the practical sense, the vacuolating agent appears to be just "one more" of the troublesome viruses to be conquered in the quest for vaccines which are safe and effective when used in man. For preparing killed vaccines as, *e.g.*, Salk poliomyelitis vaccine, the matter is simply dealt with by virtue of the susceptibility of the vacuolating virus to the inactivating action of formaldehyde. For live virus vaccines, the simple solution seems

to involve elimination of the agent from the seed stocks and to discard any contaminated lots of the material.

Summary. The newly discovered vacuolating virus, $S.V._{40}$, appears to be a common and essentially ubiquitous contaminant of rhesus monkey kidney cell cultures and a likely common contaminant of cynomolgus kidney cultures in which it develops to high titer without evident cytopathic change. The virus grows readily in grivet, vervet and patas kidney and in rhesus testicle cultures causing a unique cytopathology characterized by ballooning and intense vacuolation. Some biological and biophysical properties of the virus are described. Principal interest in and importance of the vacuolating virus arise from its demonstrated presence in all 3 types of Sabin's live poliovirus vaccines and in numerous respiratory virus seed lines employed in human volunteer inoculation experiments. Demonstration of the virus has raised the question of safety following administration to human subjects, especially infants, and of possible lack of validity of conclusions reached from experiments to measure neurovirulence or "genetic" markers of attenuated poliovirus vaccine strains and to determine the pathogenicity of respiratory viruses for man. The vacuolating virus is effectively dealt with in killed virus vaccines by virtue of its susceptibility to formalin. The optimal solution to the live virus vaccine problem appears to lie in elimination of the virus from such preparations.

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