Infection of Monkey Kidney Tissue Cultures with Virus-Like Agents.* (21478)

ROBERT RUSTIGIAN, PAUL JOHNSTON, AND HELEN REIHART.
(Introduced by S. A. Koser.)
From the Department of Microbiology, University of Chicago.

The wide current interest in tissue cultures for the study of virus-host cell relationships and applied problems of virus diseases has emphasized the need of employing cells and tissues free of microbial agents. The hazard of introducing viruses into tissue cultures with contaminated media has been stressed recently (1). Of equal, if not greater importance, is the problem of the presence of viruses in tissue cultures as a result of unrecognized infection of cells and tissues employed as primary explants. Detection of such viral infections, in certain instances, may be complicated by the fact that no readily recognized changes are manifested. On the other hand, some viruses under the same conditions may subsequently interfere markedly with cellular growth as evidenced by inhibition of acid production or cytological changes.

In our attempts, in 1953, to adapt mouse-adapted Hawaii dengue virus(2) to roller tube cultures of rhesus monkey kidney, an unidentified agent was encountered which induced cytopathogenic changes in cultures of monkey kidney and cancer HeLa epithelial cells. The agent was filterable and could not be cultured in various non-living media. Subsequently, 3 additional agents with identical cytopathogenic characteristics were passed from uninoculated monkey kidney cultures prepared for poliomyelitis studies, to HeLa cell cultures. Enders and Peebles(3) have recently reported recovery of an agent from an uninoculated monkey kidney culture which appears to have the same cytopathogenic characteristics in monkey renal cultures as our agents have. However, other than a description of its cytopathic effect in monkey kidney cultures, no other data was given concerning their agent. In view of the wide use of monkey kidney cultures in virus studies it seems of importance to report our observations with these agents. These include their cytopathic effect in rhesus monkey renal tissue, in HeLa cell and certain other tissue cultures, the manner of their recovery and passage in tissue culture and other characteristics. In addition, evidence is presented that kidneys of apparently healthy monkeys are the source of these agents in monkey kidney tissue cultures and not the medium constituents.

Materials and methods. Tissue culture technics. A. *Roller tube cultures.* Technics of Robbins, Weller and Enders(4) were essentially followed for preparation of roller tube cultures. In addition to rhesus monkey kidney, cultures were prepared from monkey testes, human embryonic kidney, human embryonic skin and muscle† and mouse infant kidney. In early studies the culture medium consisted of Hanks-Simms solution (70%), beef embryo extract (10%) and horse serum (20%). Penicillin and streptomycin were added for final concentration of 100 units and 100 μg respectively. Later, bovine amniotic fluid was used in place of Hanks-Simms solution(5). Soybean trypsin inhibitor, in final concentration of .05 mg, was incorporated in medium for all cultures. One ml of medium was added to each culture tube containing 10 to 15 fragments of tissue. Culture fluids were changed every 3 to 4 days. At time of inoculation of agents, the medium was modified to contain 85% Hanks-Simms or bovine amniotic fluid, 10% beef embryo extract and 5% horse serum. B. *Stationary cultures* of monkey kidney were prepared by trypsinization technic of Youngner(6) with bovine amniotic fluid containing 5% beef embryo extract and 10% horse serum. Following standardization of cell suspensions(7) 0.5 ml containing about 500,000 cells was added to culture tubes.

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† We are grateful to Dr. W. P. Mavralis, Illinois Central Hospital, for making human embryo available for tissue culture.

When good cell growth occurred (7 to 9 days), the medium was changed to 95% bovine amniotic fluid and 5% horse serum. C. *HeLa cell* strain of cancer epithelial cells, kindly supplied by Dr. J. T. Syverton of the University of Minnesota, was cultured as described by Scherer, Syverton, and Gey(8) and Syverton (9). Culture tubes were seeded with approximately 40,000 cells in 1 ml, and in 4 to 5 days a layer of growth was obtained containing 90,000 to 160,000 cells. The nutrient fluid was then replaced with maintenance solution containing 10% chicken serum(8,10). With prolonged incubation of cultures in this fluid, degenerative changes were noted (granulation, rounding of cells). When this occurred the maintenance medium was replaced with nutrient fluid which restored normal morphology in 1 to 2 days. Accordingly, in experiments involving long incubation periods, nutrient fluid was substituted for maintenance medium and left on the cells for brief intervals. *Preparation and staining of cells for histologic study.* Examinations of stained tissue culture cells from infected monkey kidney and HeLa cell cultures were made. Each monkey kidney culture was prepared by placing a kidney fragment on cover slip with chicken plasma which was then clotted with chick embryo extract. The cover slip was then placed in a roller tube containing 10 to 15 fragments. To obtain HeLa cells for staining, a cover slip was placed in a Pritchard tube which was then inoculated with cell suspension and sealed with rubber stopper. Following infection of the cells the cover slips were removed, fixed in Zenker's solution containing 10% formalin, dehydrated and stained with hematoxylin and eosin.

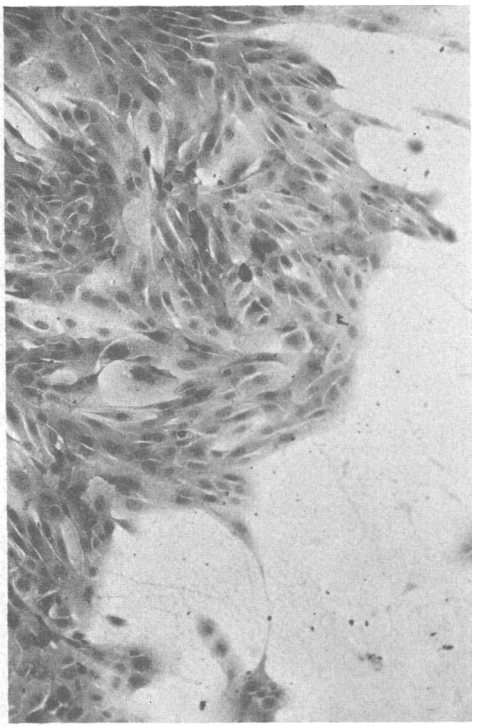

FIG. 1. Outgrowth of epithelial cells of uninoculated monkey kidney tissue culture 15 days after preparation (×90).

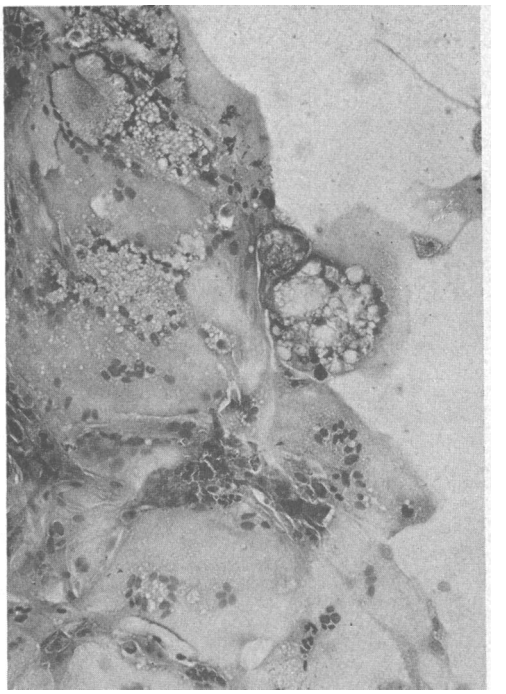

FIG. 2. Infection of epithelial cells of a monkey kidney culture 5 days after inoculation with agent MK-D showing vacuolated syncytial structures (×90).

Experimental. Cytopathic characteristics of agents in tissue cultures.‡§ A. *Monkey kidney epithelial cells.* Degeneration of epithelial cells of monkey kidney cultures containing

‡ We are indebted to Dr. Robert W. Wissler, Department of Pathology, for aid in interpreting cytological changes.

§ Cytopathic effects of agents are presented prior to manner of recovery to facilitate description of their recognition and passage in tissue culture.

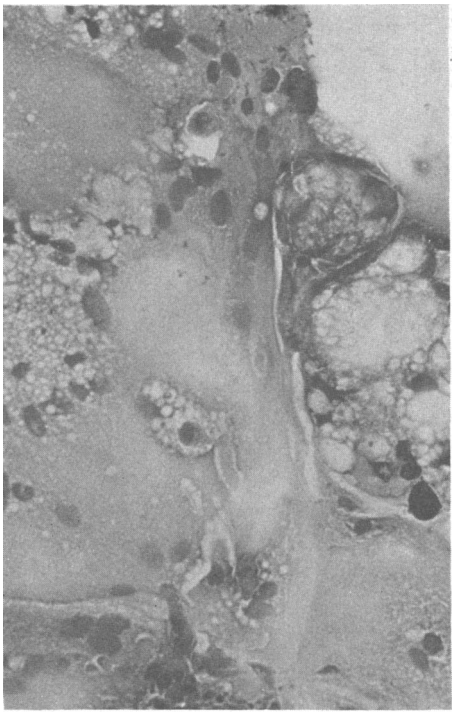

FIG. 3. Same as Fig. 2 at a higher magnification (×207).

these agents usually could be seen under low power magnification (100X) after 5 to 8 days. Cellular changes usually appeared first at or near the periphery of outgrowth of epithelial cells. There were areas in which individual cells were no longer visible but appeared to be made up of degenerated and perhaps "fused" cells, dull in appearance and with "ill defined" cell boundaries. Clusters of nuclei could be distinguished. The multiple nuclei, grouped together, against the dull background suggested large fused syncytial structures. There appeared, at the same time, variable sized vacuoles which in some instances were more prominent than the syncytial structures. With further incubation the number of "fused" and vacuolated areas increased until few if any patches of normal cells remained. Degeneration was slow, 1 to 2 weeks were required after onset for practically complete degeneration. Examination of stained preparations of infected monkey kidney cells confirmed these observations with unstained cells (Fig. 1, 2, 3). These reactions in monkey kidney cultures appear to be similar to those obtained by Enders and Peebles(3) with their monkey renal agent which they encountered during studies with agents from measles patients. The reactions also resemble those of their measles agents in human as well as monkey kidney cultures. The measles agents, however, differed in that they caused marked intranuclear changes.

B. *HeLa epithelial cells.* Following a similar incubation period degenerated areas containing cellular substances and clusters of nuclei were much more sharply defined than in monkey kidney cultures and could be seen macroscopically as lytic-like areas (Fig. 4, 5). Of much greater contrast to reactions in monkey kidney cultures was the absence of vacuolation. Small refractory masses which may have been vacuoles were seen at the time of marked degeneration, rare and difficult to define. Degeneration seemed to consist mainly of a gradual increase in size and number of lytic-like areas and their subsequent coalescence. At the same time multinucleated cellular masses representing either giant cells or syncytial structures became more prominent. It should be noted that, on occasions, small

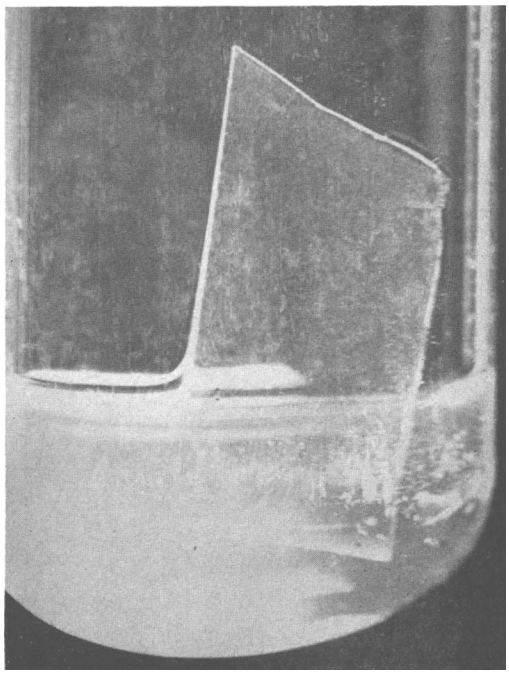

FIG. 4. Macroscopic appearance of normal film of unstained HeLa cells grown on a portion of a cover slip in a Pritchard tube.

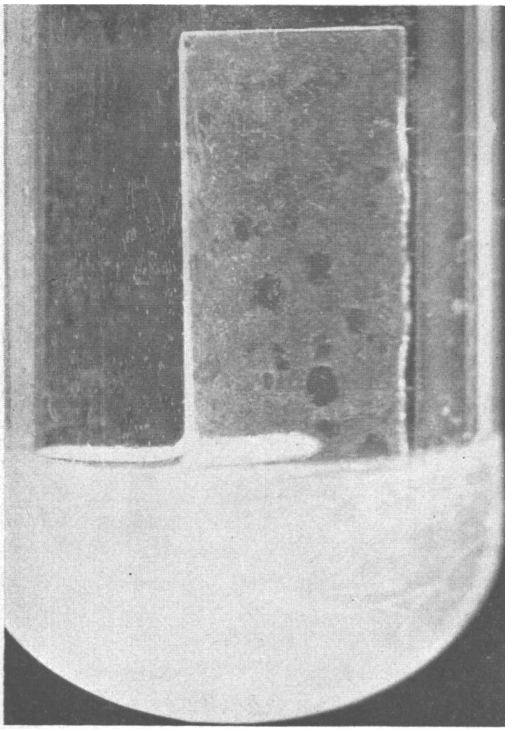

FIG. 5. Film of unstained HeLa cells on cover slip in Pritchard tube showing macroscopic appearance of well defined lytic-like areas 8 days after inoculation with agent MK-D.

lytic-like areas with giant-cell forms were observed in uninoculated HeLa cell cultures. However, they were never as marked as in infected cultures nor did they increase in size. Moreover, blind passage experiments with such cultures on two different occasions not only failed to enhance the reaction, but did not give rise to its consistent occurrence. Staining of infected HeLa cell cultures did not reveal any additional changes (Fig. 6,7,8).

Cytopathogenesis in other types of tissue cultures. The cytopathogenic effect of one agent in its 8th monkey kidney passage was tested in rhesus monkey testicular cultures, human embryonic kidney, human embryonic skin and muscle, and infant mouse kidney. In addition, pooled fluid from uninoculated monkey kidney cultures, from which one agent was recovered in HeLa cell cultures, was passed to human embryonic kidney and human embryonic skin and muscle cultures. Cytopathic effects were obtained in all instances except in mouse kidney cultures. Epithelial cells of human embryonic kidney showed degeneration as in monkey kidney cultures. However, degeneration of fibroblastic growth in monkey testicular and human skin and muscle cultures differed in that no syncytial structures nor vacuolation were observed, but the fibroblasts appeared to undergo a granular fragmentation.‖

Recovery of agent MK-D during attempts to adapt dengue virus to monkey kidney cultures. Following inoculation of the mouse-adapted strain of Hawaii dengue virus (137th mouse passage) into monkey kidney cultures, no cytopathic effects were observed for 16 days. Nevertheless, fluid harvested on 12th day from 3 cultures was pooled and passed into fresh 9-day-old monkey kidney cultures. With this second passage cellular changes, as described, occurred in all 5 inoculated cultures in 8 to 20 days. But of 3 uninoculated control cultures held for 33 days, one revealed

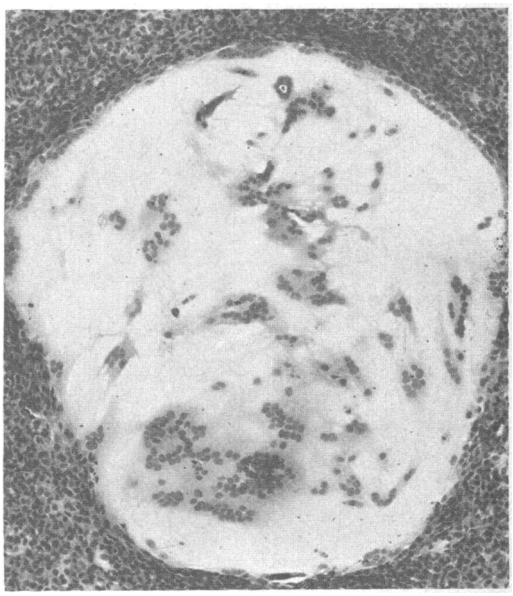

FIG. 6. Stained film of HeLa cells showing lytic-like area containing giant cells or syncytial structures 8 days after inoculation with agent MK-D. Note absence of vacuolation (×44).

‖ Dr. J. S. Youngner, Virus Research Laboratory, University of Pittsburgh, has kindly given us permission to state that he has also observed granular fragmentation in monkey testicular cultures with similar agents he recovered from uninoculated monkey kidney cultures.

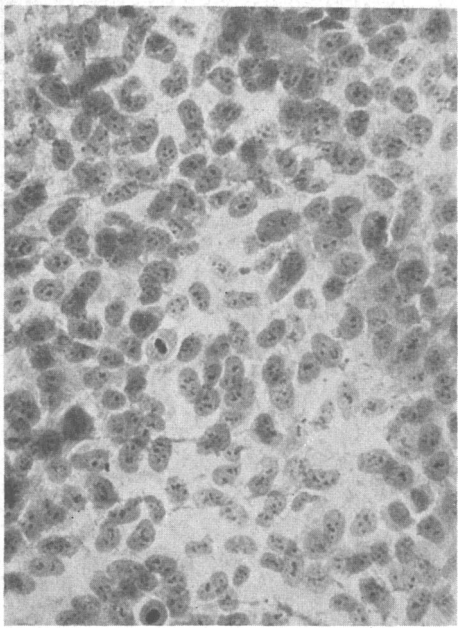

FIG. 7. High magnification of 14-day-old culture of stained normal HeLa cells (×207).

identical degeneration in 19 days. This culture, unfortunately, was discarded. With third passage 3 cultures gave reactions in 10 days, and by 4th passage the incubation period decreased to 6 to 8 days. Fifteen serial passages have now been made with this agent in monkey kidney cultures and starting with 8th monkey kidney passage, 9 serial passages in HeLa cell cultures. With the first tissue culture passage of dengue virus, infant mouse inoculations were made with fluids harvested at 4-day intervals and continued through 7th tissue culture passage. Fluids from 1st and 2nd tissue culture passages produced signs of disease compatible with dengue, but not fluids from subsequent passages. Further studies, including challenge experiments in mice with mouse dengue virus and neutralization tests with dengue serum conclusively demonstrated that the agent established in tissue cultures was not dengue.

Recovery of agents MK1, MK3, and MK4 from uninoculated monkey kidney cultures. Shortly after encountering agent MK-D in attempts to adapt dengue virus to monkey kidney cultures, syncytial masses and vacuolation were again observed in an uninoculated roller tube culture 12 days after its preparation. This culture and 16 others prepared from the same kidney were set aside for further study. By 15th day degeneration was marked in this culture and had appeared in 3 other cultures; and by 34th day in 7 of the 16 cultures. Fluids were harvested on 14th and 15th day from cultures showing degeneration, pooled and 0.2 ml inoculated into HeLa cell cultures. Lytic-like areas were present in 10 days. Fluids from these cultures were then harvested, pooled and 0.1 ml passed to fresh HeLa cultures. Similar degeneration with this second passage occurred in 6 days. Subsequent passages in HeLa cells were made with 0.1 to 0.2 ml of pooled fluids harvested at 1- to 2-day intervals for 4 to 6 days following definite signs of degeneration. The fluids were stored in a CO_2 cabinet for varying periods between passages. This agent, designated MK1 has now been passaged serially 10 times in HeLa cell cultures. *Agents MK3 and MK4* were recovered similarly from uninoculated cultures prepared from kidneys of different rhesus monkeys. Thus, MK3 was recovered from a series of uninoculated cultures in which 8 of 72 roller tubes revealed moderate to strong

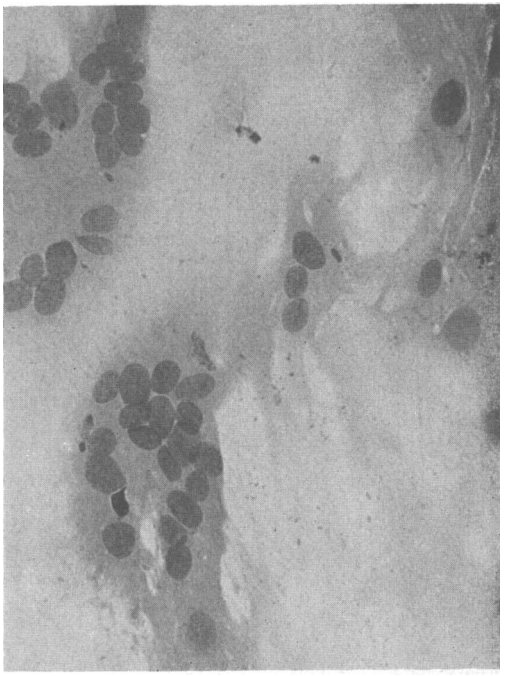

FIG. 8. High magnification of giant cells or syncytial structures in HeLa cell culture infected with agent MK-D (×207).

TABLE I. Frequency of Syncytial Structures and Vacuolation in Uninoculated Monkey Kidney Cultures Prepared from Kidneys of 12 Rhesus Monkeys.

Kidney series*	Date prepared	No. kidneys	Days of observation	Degeneration			Isolation of agent	Neutralization by homologous serum
				No.†	Degree	Time of appearance (days)		
21	11/13/53	1	34	7/16	++ to +++	12–24	+	N.D.§
22	12/ 5	1	23	0/25	±‡		—	+
23	12/19	1	55	0/13	"		N.D.	—
24	1/ 6/54	1	20	11/18	++ to +++	12–20	+	+
25	1/13	1	23	2/ 7	+++	15–21	N.D.	+
32	6/25	2	28	0/15	±‡		"	N.D.
39	8/11	4	34	13/41	++ to +++	19–24	+	"
42	8/ 2	4	28	3/14	*Idem*	20–23	N.D.	Monkey 1: + " 2: —
43	9/13	4	34	1/10	++	31	"	N.D.

* Kidney series 21 through 25, roller tube cultures; 32 through 43, stationary cultures with trypsinized cell suspension.

† Numerator = No. of cultures showing reaction; denominator = No. of cultures.

‡ Slight vacuolation in practically all cultures.

§ N.D. = Not done.

degeneration 12 days after preparation and MK4 from 11 of 41 stationary cultures with degeneration 20 days after preparation with trypsinized cell suspension. These 2 agents have now been serially passed 10 and 6 times respectively in HeLa cultures. MK3 following 10 HeLa cell passages caused vacuolation and formation of syncytial masses in monkey kidney cultures. Same results were obtained with MK4 after 6 HeLa cell passages. Information has been obtained which suggests that frequency of occurrence of these agents in uninoculated cultures may be relatively high. Thus in 6 of 9 series of cultures prepared from kidneys of different monkeys one or more of 7 to 41 cultures in each series held 20 to 55 days showed characteristic degeneration (Table I); and the recovery of an agent from cultures of 3 different series was successful. One attempt to recover an agent from uninoculated cultures of a kidney series which did not show degeneration was unsuccessful after 3 blind passages in HeLa cells and subsequent passage in monkey kidney cultures. We have consistently observed in practically all cultures prepared from different monkey kidneys occasional small vacuoles apparent from the onset of cell outgrowth. In some cultures they persisted, but in others they apparently disappeared with continued incubation.

Infectivity titer in HeLa and monkey kidney cultures. Infectivity titrations were made with 2 of the 4 agents in HeLa cell and monkey kidney cultures. Ten-fold dilutions of fluids from infected cultures were prepared and 0.1 ml of each dilution inoculated into 4 cultures. The TC_{50} was estimated by the method of moving averages(11). Titration in HeLa cells of HeLa cell line of agent MK-D (previously passed serially 8 times in monkey kidney cultures) in passage 2,3,4 and 8, gave infectivity titers of $10^{-3.0}$, $10^{-2.8}$, and $10^{-3.5}$ respectively. Eleventh monkey kidney passage fluid of the same agent titered to $10^{-3.0}$ in monkey kidney cultures. Four additional titrations were made with the same and earlier passage fluids of the monkey kidney line in monkey kidney cultures. In 3 of these no estimation of infectivity titer was possible because of occurrence of characteristic degeneration in uninoculated controls. It might be noted that agents MK1 and MK4 were recovered in HeLa cell cultures from 2 different series of these control cultures. In the 4th bacterial contamination occurred. Two titrations were also carried out with MK3 agent in HeLa cell cultures. Third HeLa cell passage fluid gave a titer of $10^{-1.0}$ but after 5 additional passages, it increased to $10^{-2.8}$.

Effect of agent MK-D in other hosts. No obvious signs of disease were produced in 30 to 60 days with agent MK-D in its 8th to 12th monkey kidney tissue culture passage in 8 monkeys and 8 rabbits inoculated intracere-

brally (0.6 to 0.8 ml) and intracutaneously (2 ml) or intraperitoneally (5 ml) and intramuscularly (2 ml). Intraperitoneal inoculation (1 ml) of one-day-old chicks and intracerebral inoculation (.03 ml) of young adult mice also failed to elicit obvious reactions. No lesions were formed on chorioallantoic membrane of 10- to 12-day-old chick embryos. However, in 2- to 3-day-old mice inoculated intracerebrally occasional deaths occurred with early and late passage fluids of the agent in monkey kidney culture. Blind passage experiments were then made to determine if more uniform results could be obtained. Following intracerebral inoculation of 7th passage fluid, 4 infant mice were killed after 7 days. Of the remaining 12 mice, 9 died within 3 weeks. Brains of 4 killed mice were passed as a 20% suspension (10% rabbit serum-saline) immediately after harvesting. With this second passage 5 mice were sacrificed at 7 days and of remaining 13, four were dead in 3 weeks. By the 4th rapid serial passage all of 17 infant mice inoculated were moribund or dead in 3 days. The brains of a number of these sick mice were passed with the same result. But with the next passage (6th) the incubation period increased to 10 days. Inoculations were repeated with 5th passage brain suspension and this time no infection occurred, and 3 blind passages with this suspension gave the same results. Fourth passage brain suspension was then tested again and it also failed to cause reactions. These findings have not been explained. However, in contrast to previous brain suspensions which were prepared and passed immediately after harvesting the brains, 5th passage brains had been stored for a month at −20°C prior to their passage. The second experiment with 4th passage brain suspension was made after 4 months storage in a CO_2 cabinet. It is possible that these relatively long storage periods may have been detrimental to subsequent activity of the agent in infant mice.

Other characteristics of agents. No bacterial growth occurred following inoculation of blood agar, veal infusion, and sodium thioglycollate broth with 2 of the 4 agents. No *Leptospira* were cultured after inoculation of agent MK-D in Fletcher's medium and baby chicks(12). Studies for pleuropneumonia organisms were kindly carried out by Dr. L. Dienes of the Massachusetts General Hospital, Boston, with negative results. *Agent MK-D* passed through a Selas (.03 porosity) but not through a Seitz (fine) filter. The Selas filtrate titered to 10^{-2} as compared to 10^{-3} of unfiltered control. Both monkey kidney and HeLa cell lines of agent MK-D were infectious for at least 4 months and agents MK1 and MK3 at least 2 months after storage in a CO_2 cabinet. After such storage and even for shorter periods, incubation periods were prolonged almost a week in some instances. Monkey kidney line of agent MK-D did not show as much variation in incubation period under these conditions as did its HeLa cell line or the HeLa cell lines of agents MK1, MK3 and MK4. In addition, the monkey kidney line of agent MK-D was stable, with no significant variation in incubation period, for at least 2 days at room temperature. However, after heating at 56°C for 30 minutes it failed to produce cytopathic effects in monkey kidney cultures for 21 days. The same results were obtained for agent MK4 in HeLa cell cultures.

Neutralization studies. Neutralization tests were made with agent MK-D and normal sera from rhesus monkeys, other animals and man. In addition, tests were performed with 2 lots of human gamma globulin and 1 lot of bovine and porcine gamma globulin and with sera prepared against a number of known viruses.¶ Tests were carried out by mixing an equal volume of undiluted fluid from infected HeLa cell cultures containing around 1000 to 3200 TC_{50} of agent with undiluted inactivated (56°C for 30 minutes) serum and 16% solutions of gamma globulins. In a few instances tests were made with monkey kidney line of same agent. Following incubation 1 hour at 37°C, 0.1 ml of each agent-serum mixture was inoculated into 3 or 4 HeLa cell or monkey kidney

¶ Human gamma globulins were kindly supplied by Dr. F. F. Johnson, Cutter Laboratories and porcine and bovine gamma globulins by Dr. Lawrence L. Lachat, Armour and Co.; rabbit "anti B" serum by Dr. Albert B. Sabin, guinea pig anti-LCM serum by Dr. Joel Warren and mouse anti-yellow fever serum and monkey anti-Sinbis, West Nile and Japanese B serums, by Dr. Max Theiler.

TABLE II. Neutralization Results with Agent D Tested against Normal Sera and Various Immune Sera.

Sera	Results, No. positive/No. tested	Range of incubation period
A. Agent		
controls	9/9	6 to 9 days
B. Normal		
Monkey	16/29	pos 13.7 to ∞* neg 6 to 11.3
" pool	1/1	>14
Human	0/20	7 to 9
" pool	0/1	7
" γ globulin	0/2	6 to 7
Rabbit	0/9	7 to 10
Mouse pool	0/3	6.3 to 8
Horse "	0/2	7 to 9
Chicken "	0/1	8
Bovine γ globulin	0/1	6
Porcine γ globulin	0/1	8
C. Immune		
1. Non-monkey		
Sabin B, yellow fever, LCM, dengue (Hawaii and Saigon), Columbia SK, GDVII, FA, Influenza A and B	0/1 (each type)	6.5 to 9
2. Monkey		
Sinbis	0/2	7 to 9
West Nile	1/1†	14.3
Japanese B	1/1†	13.7

* No cytopathic effect after 21 days.

† Delayed cytopathic effect obtained with these 2 sera was due, in all probability to the normal component since normal sera from these monkeys caused the same delay in reaction.

cultures. The agent, mixed with an equal volume of fresh fluid, and held under same conditions served as control. Limited tests were also made with other agents and normal monkey sera. A relatively large number of normal monkey sera, including some collected 3 years ago, caused a significant delay in development of cytopathic effect of agent MK-D (Table II). However, the neutralization effect with different sera varied, ranging from a delay of 5 days in average incubation period to complete neutralization for at least 21 days. In contrast, none of the normal sera from other species gamma globulins, and immune serums caused any significant neutralization. Five normal monkey sera which neutralized agent MK-D and 5 which failed to do so were tested against agent MK3 with the same results. Normal monkey serum pool neutralized all 4 agents. These findings provide evidence that the agents were derived from renal tissue of monkeys. Evidence that other tissue culture constituents are not the source of the agent is as follows: One agent was recovered at time Hanks-Simms solution was used instead of bovine amniotic fluid and the reverse was true for other 3 agents. Beef embryo extract has been routinely employed for cultivation of HeLa cells for over a year without resulting in progressive characteristic degeneration which occurred following inoculation of such cultures with the agents. Horse serum has been heated at 56°C for 30 minutes prior to its use as a medium constituent, and, as previously noted, 2 of the 4 agents so treated failed to produce cytopathic effects after 21 days. Finally 3 blind passages in HeLa cells and subsequent passage in monkey kidney cultures of fluids from one monkey kidney series failed to reveal an agent. But agents were recovered from cultures of 2 other monkey kidney series set up shortly prior to and after this series with the same lots of medium constituents.

Failure of MK-D agent to interfere with poliomyelitis viruses in monkey kidney cultures. In view of the importance of monkey kidney cultures in the study of poliomyelitis (13), an experiment was carried out to determine if agent MK-D would interfere with infectivity of poliomyelitis viruses in tissue culture. A number of 10-day-old monkey kidney cultures were inoculated with freshly harvested MK-D agent in its 14th monkey kidney passage. After 5 days definite foci of syncytial masses and vacuolation were present. Four such cultures were then inoculated with 3.2 TC_{50} and 4 additional cultures with 10 TC_{50} of type 2 virus, 4 each with 20 and 60 TC_{50} of type 1, and 4 each with 20 and 60 TC_{50} of type 3 poliomyelitis viruses. Rounding degeneration, characteristic of poliomyelitis viruses, was evident with no delay even in areas in close proximity to syncytial and vacuolation reactions. Moreover, the amounts of each type of poliomyelitis virus employed did not appear to interfere with further development of reaction by agent MK-D. In a number of cultures the reaction had spread further with appearance of rounding degeneration.

Further incubation resulted in complete rounding degeneration and disappearance of cells with only an occasional vacuolated area visible.

Summary. During attempts to adapt dengue virus to rhesus monkey kidney cultures, an unidentified agent which causes formation of syncytial masses and vacuolation in such cultures was encountered. Subsequently, 3 additional agents with similar cytopathogenic effects were passed and maintained in HeLa cell cultures from uninoculated monkey kidney cultures. Renal tissue and not the medium constituents is the source of the agent. Bacteriological studies with one agent were negative. The same agent passed through a Selas filter. Accordingly it is considered to be virus-like in nature. Similar experiments were not done with 3 other agents but because of certain common characteristics are believed to be of the same nature.

The authors are grateful to Mr. Joseph Joyner and Mr. Oliver Rampersaad for their technical assistance in certain phases of this study.

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In vivo Method for Studying Intestinal Motility in the Rabbit.* (21479)

JOHN T. FARRAR, MELVIN D. SMALL, AND FRANZ J. INGELFINGER.
(Introduced by R. W. Wilkins.)
From the Evans Memorial, Mass. Memorial Hospitals and Department of Medicine, Boston University School of Medicine.

The purpose of this report is to describe a method devised to record the effects of ionizing radiation on the intestinal motility of the rabbit. We believe that this method offers the following advantages: (1) anesthesia is avoided, (2) comparable, repeated studies are possible in the same animal, (3) the gut is in the usual position and its continuity is maintained, and (4) intra-enteric pressure is measured directly and can be differentiated from intra-peritoneal pressure.

Operative Procedure. Male white rabbits (2-3 kg) were used. The animals were anesthetized with pentothal sodium (30 mg/kg), administered intraperitoneally. A 12 cm midline ventral skin incision was made. Three polyethylene tubes (int. diam. .78 mm, ext. diam. 1.22 mm) were passed subcutaneously with a Kelly clamp from the abdominal incision to the back of the neck. An incision was made in the skin of the neck and the tips of the tubes exteriorized. Each tip was secured by forcing one arm of a U-shaped pin into the tube; the other arm was attached to a plastic sheet which was sutured to the skin. The pins were easily detached when the tubes were used for recording. The abdominal skin incision was then extended into the peritoneal cavity. A stab wound was made in the lesser curvature of the stomach, approximately 1 cm from the

* This study was performed under contract with the U. S. Atomic Energy Commission.