tine approximately tripled the weights of the induced granulomas over those resulting from cotton alone.

1. Christian, J. J., *Am. J. Physiol.*, 1955a, 181, 477.
2. ———, *ibid.*, 1955b, v182, 292.
3. ———, *Ecology*, 1956, v37, 258.
4. Louch, C. D., *J. Mammal.*, 1958, v39, 109.
5. ———, *Ecology*, 1956, v37, 701.
6. Dougherty, T. F., *Ann. N. Y. Acad. Sci.*, 1953, v56, 748.
7. Taubenhaus, M., Amromin, G. D., *J. Lab. Clin. Med.*, 1950, v36, 7.
8. Aronson, S. M., Shwartzman, G., *Am. J. Path.*, 1953, v29, 381.
9. Stoner, R. D., Godwin, J. T., *ibid.*, 1953, v29, 943.
10. Whitney, D. M., Anigstein, L., *Tex. Rep. Biol. Med.*, 1953, v11, 561.
11. Pollard, M., Wilson, B. R., *ibid.*, 1955, v13, 126.
12. Boring, W. D., Angevine, D. M., Walker, D. L., *J. Exp. Med.*, 1955, v102, 753.
13. Chitty, D., *Ecology*, 1954, v35, 227.
14. Meier, R., Schuler, W., Desaulles, P. *Experentia*, 1950, v6, 469.
15. Meyer, R. K., Stucki, J. C., Aulsebrook, K. A., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 624.
16. Robert, A., Nezamis, J. E., *Acta Endocrinol.*, 1957, v25, 105.
17. Davis, D. E., Read, C. P., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 269.

Received August 25, 1958. P.S.E.B.M., 1958, v99.

Some Distinctive Biological Characteristics of Echo-10 Virus.* (24359)

G. D. HSIUNG (Introduced by R. H. Green)
Section of Epidemiology and Preventive Medicine, Yale University, School of Medicine, New Haven, Conn.

It has been reported(1,2) that kidney cells from various monkey species had different degrees of susceptibility to enteric viruses, and host cell range was suggested as a useful screening procedure for grouping certain viruses and facilitating their identification. Recently this study has been extended to include the susceptibility of kidney cells from non-primates to a variety of enteric viruses isolated from man and animals. The present report is concerned with ECHO-10 virus which behaves differently from all other enteroviruses that were tested in its ability to grow in non-primate as well as primate tissue cultures. Similar results have been found only with certain enteroviruses isolated from animal species other than man.

Material and methods. A. *Tissue culture.* Kidneys were obtained from the following: 1) Primates: rhesus (*Macaca mulatta*), patas (*Erythrocebus patas*) and capuchin (*Cebus capuchinus*) monkeys. 2) Domestic animals: swine, cat, dog, and calf. 3) Laboratory animals: rabbit and guinea pig. All kidneys were decapsulated aseptically and trypsinized by the method of Rappaport(3). The trypsinization fluids were centrifugated and the packed cells resuspended in growth media containing Hanks' salt solution, 0.5% lactalbumin hydrolysate and various amounts of calf serum, depending upon the animal species used. Details of the preparation of the different monolayer cultures will be published separately. B. *Virus strains*: The strains used were rhesus monkey kidney culture passage materials, and included the following: Poliovirus types 1, 2, 3; Coxsackie virus A-9, B 1-5; ECHO virus types 1-14,** ECMO (enteric cytopathogenic monkey orphan) virus (2) groups A 7853, SV_{23}†; Group B SV_{16}†; Group C SV_6†; ECBO (enteric cytopathogenic bovine orphan) virus‡ isolated by Moll and Finlayson(4). All ECHO-10 virus titra-

* Aided by a grant from the National Foundation.

† SV_{23}, SV_{16}, SV_6 virus strains were made available by Dr. R. N. Hull, Lilly Research Laboratories, Indianapolis, Ind.

‡ ECBO virus was supplied by Dr. T. Moll, State College of Washington, Dept. of Veterinary Microbiol., Pullman.

** All strains used were the prototype suggested by the ECHO viruses Committee (*Science*, 122 (3181): 1187-1188, 1955) except ECHO virus types 3 and 9. In these instances the Berardi and Bourn strains were respectively used.

TABLE I. Cytopathic Effect of ECHO-10 and Other Enteric Viruses in Kidney Cultures Derived from Different Animal Species.

Virus	Plaque producing ability in rhesus	CPE in kidney cultures of: Primates: Rhesus	Patas	Capuchin	Non-primates: Swine	Cat	Dog	Calf	Rabbit	Guinea pig
Human enteroviruses										
ECHO*										
Type 10	—	+	+	+	+	+	+	±	±	+
" 1, 3, 4, 9, 11, 13 } Group A(2)	+	+	—	—	—	—	—	—	—	—
" 2, 3, 5, 6 } Group A(2)	—	+	—	—	—	—	—	—	—	—
" 7, 8, 12 Group B(2)	+	+	+	—	—	—	—	—	—	—
Coxsackie										
Group A 9	+	+	—	—	—	—	—	—	—	—
" B 1-5	+	+	+	—	±	—	—	—	—	—
Poliomyelitis										
Type 1-3	+	+	+	—	—	—	—	—	—	—
Animal viruses										
ECMO(2)										
Group A (7853, SV_{23})	—	+	+	+	+	+	+	+	+	+
" B (SV_{16})	+	+	+	—	—	—	—	—	—	—
" C (SV_6)	+	+	—	—	—	—	—	—	—	—
ECBO(4) (Moll)	+	+	+	N.D.	±	—	±	+	±	+

CPE = Cytopathic effects. + = Positive; ± = Questionable; — = Negative; N.D. = Not done

* All strains used were the prototype suggested by the ECHO viruses Committee (*Science*, 122 (3181): 1187-1188, 1955) except ECHO virus types 3 and 9. In these instances the Berardi and Bourn strains were respectively used.

tions and neutralization tests were made in tube cultures of patas monkey kidney cells. Antiserum was obtained from rabbits immunized with the prototype ECHO-10 (Lang strain) virus. Neutralization tests were performed by mixing 0.5 ml of virus samples containing approximately $100TCD_{50}$ with 0.5 ml of antiserum at different dilutions; the mixtures were incubated at room temperature for one hour before inoculation of culture tubes, 3-4 per dilution.

Results. A summary of comparative susceptibility of primary kidney cultures derived from different animal species to ECHO-10 virus as well as to other enteric viruses isolated from man and animal is shown in Table I. ECHO-10, unlike any of the other human enteric viruses, grew well in cultures derived from the 3 monkey species tested, as well as swine, cat, dog, guinea pig, and to some extent rabbit and calf. A similar host cell range was found for group A ECMO virus, and the ECBO strain of Moll.

In *primate kidney cultures* from patas and rhesus monkeys, ECHO-10 produced a different type of degeneration than other enteroviruses. Similar observations have been reported by Ramos-Alvarez and Sabin(5) for this agent in cynomolgus monkey kidney cultures. Although the cytopathic effect (CPE) was distinct in both rhesus and patas cultures in fluid medium, no plaques were obtained from monolayers of either species under agar. ECHO-10 was thus the only one of the enterovirus group (poliovirus types 1-3, Coxsackie virus A-9, B 1-5, ECHO virus types 1-14), that caused readily recognizable destruction of patas cells but was unable to produce plaques. In addition, capuchin kidney cultures were found susceptible only to ECHO-10 among the enteroviruses tested, as observed previously(6).

In *non-primate kidney cultures*, ECHO-10 caused CPE in all species tested (Table I). Figs. 1-4 show the typical changes in infected cat and guinea pig cultures. Similar changes with this agent in swine kidney cultures have been noted by Guerin and Guerin(7). Virus

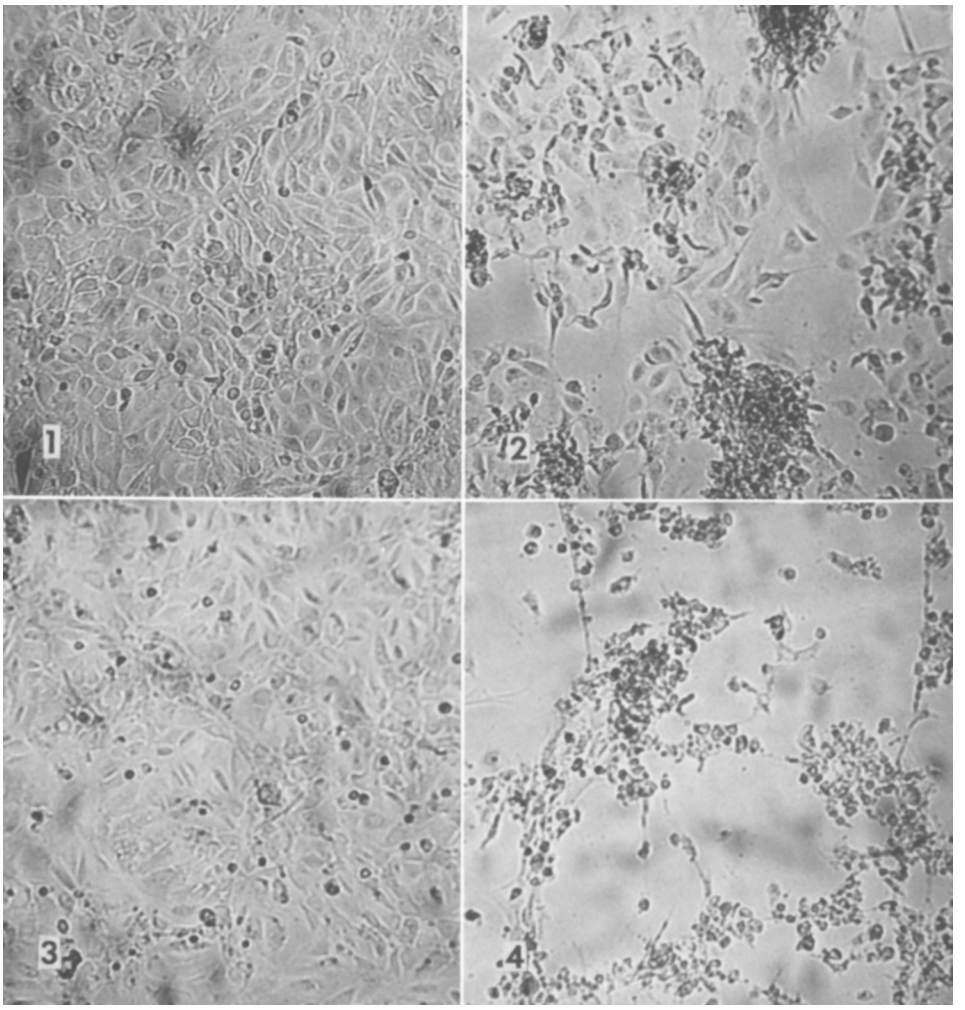

FIG. 1. Cat kidney culture control (17 days old).
FIG. 2. Cat kidney culture + ECHO-10 (first cat passage material) 13 days after inoculation.
FIG. 3. Guinea pig kidney culture control (12 days old).
FIG. 4. Guinea pig kidney culture + ECHO-10 virus 5 days after inoculation.

titers obtained from non-primate kidney cultures (ranging from 10^4 to $10^{4.5}TCD_{50}$ per ml, 7-9 days after inoculation) were essentially the same as those of the primate cultures when titrations were made in patas monkey kidney cell tubes. The identity of the 3rd passage in guinea pig and the 4th passage in cat cultures was established by neutralization tests which indicated that these materials were antigenically indistinguishable from prototype rhesus passage ECHO-10 virus.

Discussion. Since ECHO-10 did not produce plaques either on rhesus or on patas monolayers, this virus was earlier placed in Group A of the ECHO viruses(2), even though distinct cytopathic changes were obtained in patas cultures. A similar failure to produce plaques in spite of cytopathic capabilities was also noted in ECMO virus of the Group A strains(2) and the adenoviruses tested (types 1 and 2) (to be published). It is noteworthy that in addition to these biological characteristics in primate cultures, the ability of ECHO-10, ECMO Group A and adenoviruses to produce CPE in non-primate kidney cells is also common to all.

As suggested by the Enterovirus Committee(8), if the term ECHO is to be used as

more than a temporary repository for viruses awaiting identification, ECHO-10 probably should be classified elsewhere. The features which distinguish it from others in the ECHO group are its large size, its distinctive cytopathogenic effect, and the disease with which the virus has been associated. In addition, the host cell range of ECHO-10 has been demonstrated to differ from those of the other enteroviruses. Its ability, in common with certain animal viruses, to multiply in non-primate kidney cultures may furnish a clue for the classification of these viruses with regard to their origin and evolutionary development.

Summary. A comparative susceptibility study of kidney cultures derived from primates and non-primates, indicates that ECHO-10 virus has distinctive biological characteristics which differentiate it from the other human enteroviruses.

1. Hsiung, G. D., Melnick, J. L., *J. Immunol.,* 1957, v78, 137.
2. ———, *Ann. N. Y. Acad. Sci.,* 1958, v70, 342.
3. Rappaport, C., *Bull., W. H. O.,* 1956, v14, 147.
4. Moll, T., Finlayson, A. V., *Science,* 1957, v126, 401.
5. Ramos-Alvarez, M., Sabin, A. B., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 655.
6. ———, *Am. J. Public Health,* 1956, v46, 295.
7. Guerin, L. F., Guerin, M. M., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 322.
8. Committee on the Enteroviruses, *Nat. Found. for Infantile Paraly., Am. J. Public Health,* 1957, v47, 1556.

Received August 25, 1958. P.S.E.B.M., 1958, v99

Methods of Harvesting Mammalian Cells Grown in Tissue Culture.* (24360)

W. E. MAGEE, M. R. SHEEK, B. P. SAGIK (Introduced by R. W. Heinle)
Divisions of Chemical Research and Biological Research, The Upjohn Co., Kalamazoo, Mich.

Recent publications(1,2) have noted the deleterious effects of trypsinization as a means of harvesting cells. Our own experience with HeLa cells and first passage chicken embryo cells has extended these observations.

Methods. HeLa cells were grown in a medium (HLCa) consisting of Hanks' balanced salt solution(3) plus 0.5% lactalbumin hydrolysate and 20% calf serum under an atmosphere of 8% CO_2 in air. Chick embryo fibroblast cells were grown in 60 ml of HLCa + 2 mM/ml glutamine and vitamins (Microbiological Associates Inc.) in stoppered Roux bottles at 37°C. The cell monolayers were washed 3 times with Hanks' balanced salt solution and harvested by one of the following methods: a. 5 ml of 0.25% Bactotrypsin (Difco) solution was added to each bottle for 5-10 minutes to lift the cells. b. The cells were washed with Puck's saline(4) and 5.0 ml of 0.01% recrystallized trypsin in Puck's saline was added to each bottle to lift the cells. c. 5 ml of Hanks' balanced salt solution was added to each bottle and the cells scraped with a rubber policeman. d. 5 ml of alcohol:ether (3:1) was added to each bottle and the denatured cells removed by scraping. The cells from each treatment were subsequently heated in alcohol:ether to extract lipid material. The cell residue was dissolved in 1 N KOH at 37°C(5) and analyzed for protein (6), RNA(7) and DNA(7,8,9) by colorimetric methods. A comparison was made of the damage to the cells harvested by scraping or trypsinization by vital staining with 0.01% neutral red and by determination of the efficiency of replating the cells(10). The cells were diluted in HLCa medium to give 100 cells/ml. One ml of these cells were plated in 90 mm petri dishes in 10 ml HLCa and the colonies counted after 14 days.

Results. Significant protein losses were noted with trypsinization. However, extremely high efficiencies of replating were obtained from those single cells lifted by trypsin, while scraping resulted in damage and low viability to single cells. Small clumps of cells that resulted from the scraping procedure stained with neutral red and were capable of growth and division (Table I).

The leakage of protein and free amino acids of the cells during harvest was investigated

* Grateful acknowledgement is made of the tireless assistance of R. M. DesArmier and R. W. Sodergren.