jected into rats, guinea pigs, and rabbits, and distribution of C^{14}, 30 minutes after injection was studied. Activity was widely distributed and the results were variable; but in most experiments, activity (cts/min/mg tissue) was higher in the posterior pituitary gland than in any other tissue. Activity in the anterior pituitary, liver, and kidney was higher than that in muscle and brain.

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1. Riegel, B., Hartop, W. H., Kittinger, G. W., *Endocrinology,* 1950, v47, 311.

2. Joliot, F., Courrier, R., Horeau, A., Sue, P., *C. R. Acad. Sc.,* 1944, v218, 769.

3. Courrier, R., Horeau, A., Marois, M., Morel, F., *ibid.,* 1951, v232, 776.

4. Jensen, J. M., Clark, D. F., *J. Lab. Clin. Med.,* 1951, v38, 663.

5. Taurog, A., Harris, G. W., Tong, W., Chaikoff, I. L., *Endocrinology,* 1956, 59, 34.

6. Courrier, R., Zizine, L., *C. R. Acad. Sc.,* 1956, v242, 315.

7. Bush, I. E., *Schweiz. Med. Woch.,* 1955, v27, 645.

8. Ulrich, F., Long, C. N. H., *Endocrinology,* 1956, v59, 170.

9. Firschein, H. E., DeVenuto, F., Fitch, M. M., Pearce, E. M., Westphal, U., *ibid.,* 1957, v60, 347.

10. Peterson, R. S., Wyngaarden, M. B., Guerra, S. C., Brodie, B. B., Bumin, J. J., *J. Clin. Invest.,* 1955, v34, 1779.

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Use of Human Kidney Cultures in the Study of Enteroviruses.* (25335)

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Use of human kidney tissue in suspended cell cultures for propagation of polioviruses was reported by Weller *et al.*(1); however, studies on Coxsackie and ECHO viruses have been conducted almost entirely in other tissue culture systems, chiefly rhesus monkey kidney. It was previously reported that kidney tissue cultures from relatively few monkey species have proven satisfactory for studies of the entire family of enteroviruses(2,3), and cultures prepared from non-primate kidney tissues have consistently proved unsatisfactory for propagation of these viruses(4). For these reasons the susceptibility spectrum of primary human kidney cell cultures to the 3 groups of enteroviruses (poliomyelitis, Coxsackie and ECHO viruses) was investigated.

Materials and methods. Source of material. Human kidney specimens were obtained following surgical removal or at autopsy. In the latter case only those kidneys were used which had been removed within a few hours of death, with the capsule intact and, in so far as possible, with aseptic precautions, to avoid gross contamination. After examination by the pathologist, kidneys were delivered to our tissue culture laboratory in a sterile jar which contained Hanks balanced salt solution and antibiotics. *Preparation of human kidney (HK) cultures.* The human kidney tissues were trypsinized in the same manner used for preparation of monkey kidney cultures(5). The trypsinized cell suspensions were centrifuged and the packed cells resuspended in growth medium (Hanks salt solution, 0.5% lactalbumin hydrolysate, 10% medium #199, and 20% calf serum) at a final cell concentration of 3-5 x 10^5 cells/ml. Three-ounce prescription bottles and tubes (16 x 150 mm) were seeded with 10 ml and 1 ml, respectively and incubated at 37°C. When a confluent cell sheet was obtained, usually within 3-5 days, the growth medium was removed and replaced with maintenance medium containing Earles salt solution, 0.5% lactalbumin hydrolysate, and 2% calf serum. In cases where cell growth appeared to be poor, the cultures were usually changed with fresh growth medium 3-5 days after seeding and

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TABLE I. Cell Growth of Human Kidney Cultures.

Age (yr)	Specimens obtained hr after: Surgery	Death	No. grown /No. cases	% showing growth
Adult (37–76)	<2		5/5	100
" (28–87)		3– 6	4/6	67
		10–24	2/17	12
Baby (0–2)		3– 6	5/6	84
		8–24	32/38	84
		>24	0/2	0

incubated until a confluent cell sheet was obtained. *Virus strains.* Poliovirus, types 1, 2, 3, (virulent and attenuated strains), Coxsackie virus, types A-9, B 1-5 and ECHO virus types 1-14, were studied. Stock strains were all derived from rhesus monkey kidney cultures. Stool samples, as a source of virus, were obtained from apparently healthy individuals as well as from those suffering from a variety of febrile illnesses. Mouse brain suspension containing known Coxsackie A-9 virus was used.

Results. Growth of HK cultures. Table I summarizes experiences with growth of cultures prepared from human kidney tissue of 74 individuals. The cells from kidneys removed at operation grew in cultures with considerable ease. Success was attained in growing 4 out of 6 (67%) autopsy specimens from adults obtained less than 6 hours after death; whereas, only 2 of 17 (12%) kidney tissues were grown when the kidney was removed more than 10 hours after death. On the other hand, kidney tissues from babies, especially premature babies, yielded good cell cultures, even though the specimens were collected 10-24 hours after death. All cultures, either derived from adult or from baby kidney cells, were kept in satisfactory condition for as long as 2 months when the cultures were changed with maintenance medium weekly. In no instance were latent viruses observed.

Comparative susceptibility of rhesus (Rh) and HK cultures to enteroviruses. Poliovirus types 1, 2, and 3 (both virulent and attenuated strains) produced marked cellular degeneration in adult and baby HK cultures and in Rh cells 18 hours after inoculations (Table II). Plaque titers as well as virus yields were almost the same in both cell systems (Table II). Baby HK cultures were highly sensitive to Coxsackie and ECHO viruses and displayed distinct cytopathogenic changes (Figs. 1 and 2), similar to those produced by polioviruses. Virus yields in baby HK cultures were as good or better than those produced in Rh cells. However, certain adult HK cultures did not show similar responses when inoculated with Coxsackie A 9, Coxsackie B 1-5 or ECHO viruses (except ECHO virus types 7 and 10). Plaque titers were lower in adult HK cultures than in Rh cultures inoculated with these viruses (Table II). Incomplete cellular destruc-

TABLE II. Cytopathogenic Effect and Virus Titers of Poliomyelitis, Coxsackie and ECHO Viruses in Rhesus Monkey and Human Kidney Cultures.

Virus	CPE at time indicated after infection: Rh	HK (B)	HK (A)	Virus titer log (PFU/ml) measured in cultures of: Rh	HK (A)	Virus yields (PFU/ml) in cultures of: Rh	HK (B)
	(18 hr)	(18 hr)	(18 hr)				
Poliovirus type 1 (also types 2 & 3)	+++	+++	+++	7.8	7.8	7.7	7.8
	(2 days)	(2 days)	(14 days)				
Coxsackie Group A-9 (also Group B 1-5)	+++	+++	±	7.7	6.0	7.5	7.9
ECHO type 1 (also types 2,3,4,5,6,8, 9,11,12,13,14)	+++	+++	±	7.6	5.8	7.5	7.3
ECHO type 7	+++	+++	++++	6.7	6.6	7.8	7.1
" " 10	+	+++	++++	N.D.	N.D.	N.D.	N.D.

Rh = Rhesus monkey kidney cultures. HK (A) = Human (adult) kidney cultures. HK (B) = human (baby) kidney cultures.

+, A few cells degenerated. +++, ¾ of the cell sheet degenerated. ++++, Complete degeneration. ±, Questionable. N.D., Not done.

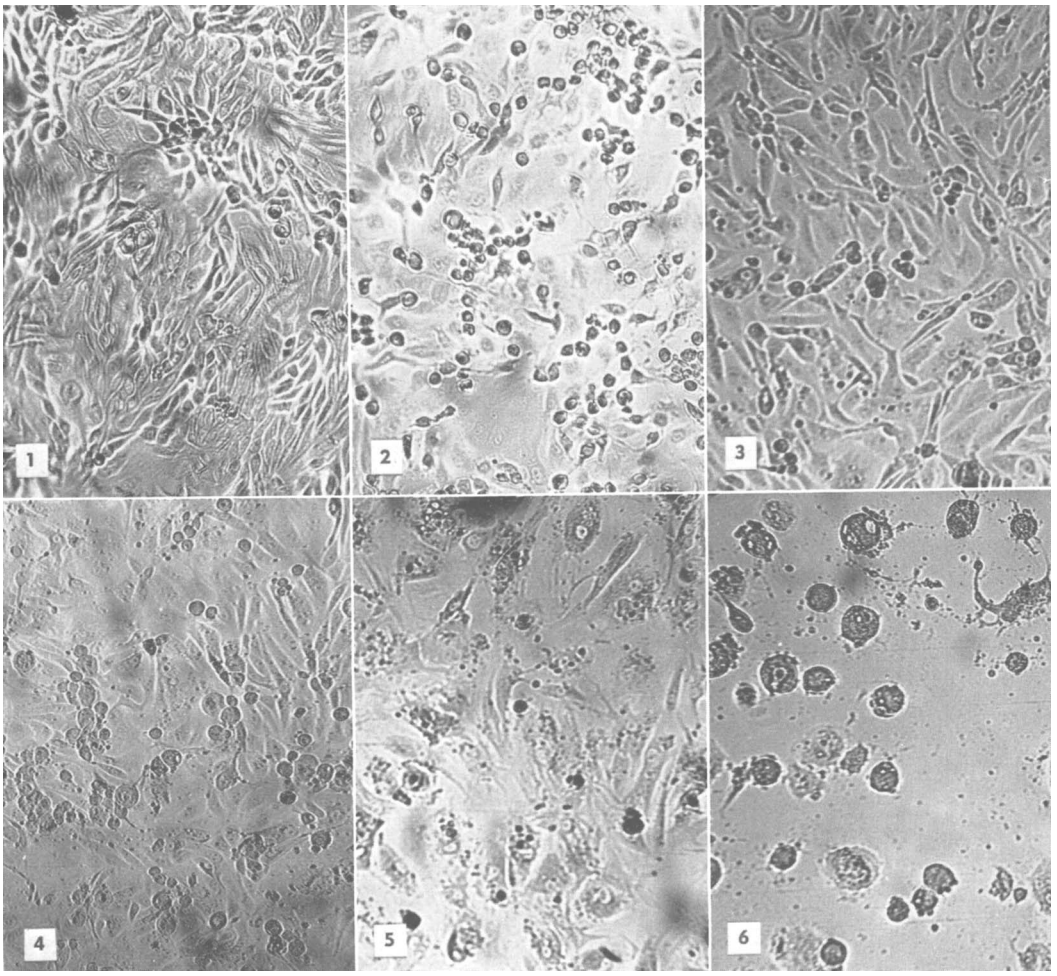

FIG. 1. Human (baby) culture control (7 days old).

FIG. 2. Human (baby) kidney culture infected with Coxsackie A9 virus, 0.1 ml of undiluted tissue culture fluid (18 hr after infection).

FIG. 3. Human (adult) kidney culture control (7 days old).

FIG. 4. Human (adult) kidney culture infected with Coxsackie A9 virus, 0.1 ml of undiluted tissue culture fluid (18 hr after infection).

FIG. 5. Human (adult) kidney culture infected with Coxsackie A9 virus (same sample as in Fig. 4 except 25 days after infection).

FIG. 6. Human (adult) kidney culture infected with Coxsackie A9 virus (as shown in Fig. 5) challenged with poliovirus type 1 (24 hr after challenge).

tion was observed 1-2 days after infection (Figs. 3 and 4) and then the cultures gradually recovered, although the viruses persisted in the fluid as long as 14-27 days. When an adult HK culture infected with Coxsackie A 9 virus 25 days previously (Fig. 5) was challenged with type 1 poliovirus, complete cellular destruction occurred within 24 hours (Fig. 6). Certain other enteroviruses, for example Coxsackie B5, which like Coxsackie A9, gave only limited degeneration in adult HK cultures, did, however, interfere with multiplication of subsequently inoculated poliovirus. Details of these interference experiments will be published.

Isolation of enteroviruses in HK cultures. Sensitivity of rhesus and human kidney cultures for primary isolation of enteroviruses directly from human stool or from mouse brain suspensions was also compared. Examples of such isolations are illustrated in Table III. Coxsackie A9 virus was readily isolated from mouse brain suspension in both Rh and baby HK cultures but not in adult HK cells. Rhe-

TABLE III. Isolation of Coxsackie and ECHO Viruses in Rhesus Monkey and Human Kidney Cultures.

Material	Days after inoc.	CPE at different virus dilutions: Rh Undil.	Rh 10^{-1}	Rh 10^{-2}	HK (A) Undil.	HK (A) 10^{-1}	HK (A) 10^{-2}	HK (B) Undil.	HK (B) 10^{-1}	HK (B) 10^{-2}	Virus identified
Mouse brain suspension	4	+++	++	++	0	0	0	0	0	0	Coxsackie A-9
	10				0	0	0	+++	+	0	
Stool #C suspension	4	0	0	0	0	0	0	0	0	0	ECHO-2
	10	+++	+++	+	0	0	0	+++	++	+	
Stool #WB suspension	4	0	0	0	0	0	0	+	0	0	" -11
	10	±	0	0	0	0	0	+++	+++	++	

See Table II for legend.

sus monkey kidney cultures and baby HK cultures were equally satisfactory for isolation of ECHO virus type 2 from stool suspensions. The baby HK cultures were highly sensitive for isolation of ECHO-11 (WB) virus from stool suspensions, whereas Rh cells were less susceptible to infection by this strain of virus. From 19 stool samples 8 (42%) yielded ECHO-11 (WB) virus when inoculated into baby HK cultures but ECHO-11 (WB) was isolated from only 3 (16%) when these 19 specimens were inoculated into rhesus monkey kidney cells. Furthermore, the 3 positive results with ECHO-11 virus obtained in rhesus cultures were also positive in baby HK cultures and were achieved only on stool samples containing high titers of virus. In no instance did adult HK cultures show distinct cytopathic changes when inoculated with these samples.

Discussion. Human kidney tissue obtained at autopsy from individuals of all ages usually are suitable for preparation of cultures if the specimens are processed within 6 hours after death. Difficulties encountered in prompt collection of specimens often limit the number of samples available. Consequently, there is a shortage of tissue suitable for preparing cultures.

The reason for the differences in susceptibility of adult and baby HK cultures to Coxsackie and ECHO viruses is obscure. This difference was not observed with polioviruses, but a similar phenomenon was noted by Henderson and Taylor(6) in studies of sandfly fever viruses in tissue culture. Our observation of the differences in sensitivity of kidney cells from young and mature individuals suggests that these differences may be the result of a type of cellular immunity present in tissues from adults. Dalldorf *et al.*(7) found that suckling mice were highly susceptible to Coxsackie viruses but adult mice were resistant to this group of agents. Recently McLaren and Sanders(8) have shown that this influence of age on susceptibility to Coxsackie viruses is due to the "accessibility" of host cells to the virus. In view of this, it seems possible that kidney cells from infants may be more accessible to Coxsackie and ECHO viruses than kidney cells obtained from older individuals.

The advantage of using baby HK cultures for isolation of ECHO viruses, for example ECHO-11 (WB) virus, is that these cultures apparently are more sensitive than monkey kidney cells, and, therefore, may facilitate detection of some of the elusive viruses of human infections.

Summary. 1) Human kidney cultures were prepared successfully from tissues obtained following surgical removal or at autopsy from individuals of all ages, when specimens were collected promptly. 2) Baby kidney cells were superior to adult both for culture growth and for isolation and propagation of enteroviruses. 3) Poliovirus types 1, 2 and 3 (virulent and attenuated strains) grew well in both adult and baby HK cultures. In contrast, Coxsackie A9, B 1-5 and all ECHO viruses tested (except types 7 and 10) induced distinct cellular degeneration only in baby HK cultures, while incomplete destruction with low virus titers were obtained in cultures derived from certain adult human kidneys. 4) The advantages of using baby HK cultures for isolation of certain ECHO viruses are discussed.

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1. Weller, T. H., Enders, J. F., Robbins, F. C., Stoddard, M. B., *J. Immunol.*, 1952, v69, 645.

2. Committee on Enteroviruses, Nat. Found. for Infantile Paralysis, *Am. J. Pub. Hlth.*, 1957, v47, 1556.

3. Hsiung, G. D., Melnick, J. L., *J. Immunol.*, 1957, v78, 137.

4. Hsiung, G. D., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 387.

5. Melnick, J. L., *Diagnostic Procedures for Virus and Rickettsial Diseases*, 2nd Ed., 1956, APHA, N.Y. City, 115.

6. Henderson, J. R., Taylor, R. M., *Am. J. Trop. Med. & Hyg.*, 1959, in press.

7. Dalldorf, G., Sickles, G. M., Plager, H., Gifford, R., *J. Exp. Med.*, 1949, v89, 567.

8. McLaren, A., Sanders, F. K., *J. Hyg.*, 1959, v57, 106.

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Zone Electrophoretic Studies of Proteins and Glycoproteins of Bovine Serum and Synovial Fluid. (25336)

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The electrophoretic patterns of bovine serum and synovial fluid were determined as part of a broader study in the human. The scarcity of information concerning bovine synovial fluid glycoproteins prompted this report.

Methods. Undialyzed samples of serum or synovial fluid were subjected to electrophoresis at room temperature, on Whatman 3 mm paper, 3 cm. wide, in barbital buffer of ionic strength 0.075, at pH 8.6, for 16 hours at a constant current of 0.6 milliampere/strip. The Spinco model R series C, paper electrophoresis apparatus was used. After oven drying at 110°C, for 15 minutes, glycoproteins were additionally fixed to the papers and cleared of buffer salts by a wash in absolute alcohol. Paper strips were dyed for proteins with amidoschwarz 10 B dye and for glycoproteins by a modification of the periodic acid-Schiff technic(1). The dyed paper strips were analyzed in a Spinco model RA densitometer, using Wratten filters No. 58 and 22 for proteins and an interference filter with maximal transmission at 561 mu for glycoproteins. Synovial fluids were digested by 140 turbidity reducing units of bull testicular hyaluronidase at 37°C for 1½ hours prior to electrophoresis to remove the viscous anomaly. Weight of protein applied to each paper strip was equivalent to 10 microliters of a 4.5 g/100 ml solution of protein. For glycoprotein determinations 30 microliters of serum and 50 microliters of synovial fluid were applied to the paper. Protein concentration was determined by biuret method and protein-bound hexose by modification of the anthrone tryptophane reaction(2).

Material. Serum from 13 cows was obtained immediately after slaughter. Synovial fluid was aspirated from third joint of foreleg at the same time. In eight animals bilateral joints were aspirated making a total of 21 specimens of synovial fluid. This material was obtained through the courtesy of Hormel Co., Austin, Minn.

Results. Table I describes the mean protein and glycoprotein patterns of 13 bovine sera. Mean values from 13 human control sera are included for comparison. There was

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