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.

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

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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

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TABLE I. Influence of Harvest Conditions on HeLa Cells.

Treatment	% change*			% viability	
	Protein	RNA	DNA	Neutral red	Plating efficiency
Trypsin (a)	− 7.8	− 5.7	− 6.7	85	99
	− 6.6	− 2.9	− 1.8		
	−23.3	−13.5	−23.0		
	−15.9	− 5.8	− 6.4		
	−13.2 ± 7.4	− 7.0 ± 4.6	− 9.5 ± 9.3		
Scraping (c)	− 7.4	− 5.7	− 4.3	67†	27‡
	− 2.8	+8.5	+15.9		
	+9.2	+7.2	− .2		
	+2.6	− 8.7	+ 3.6		
	.4 ± 6.7	.3 ± 8.6	3.7 ± 7.4		

Protein, RNA, DNA were determined by micromodifications of the Folin-Ciocalteu method (6), orcinol method(7) and diphenylamine reaction with acid precipitated DNA(7,8). Percent viability was determined by counting those cells taking up dye in 0.01% neutral red in balanced salt solution and by plating by the procedure of Puck(10). The table summarizes 4 experiments.

* Relative to alcohol : ether treatment (d), per cell.

† Counting includes both single cells and those in clumps.

‡ Lowered efficiency is exaggerated by clumps being counted as progenitors of a single colony. Most clumps consisted of 3-6 cells. Thus there was nearly 100% efficiency of clump plating.

with chick fibroblast cells. The cells were grown in complete medium in bottles in the presence of either cystine-S^{35} or randomly labeled glutamic acid-C^{14} to obtain labeled cellular protein. In the experiment with glutamic acid-C^{14} the tracer was added at various times during growth of the cells to determine if protein synthesized early was more or less susceptible to the action of trypsin than that synthesized late in the growth period. The cells were washed and harvested by the methods described above. Total radioactivity in the cells was obtained by summing that found in the harvest solution, first wash, alcohol-ether extract and total alkaline digest (Table II). Sixty-five percent of the S^{35} that was taken up by the cells in the period of growth between 48 and 72 hours was removed by trypsin, while scraping liberated 48% of the total. The cells receiving C^{14} glutamic acid at the beginning of their growth period showed a 24% loss upon harvest with trypsin (method a) and a 17% loss with .01% trypsin in Puck's saline (method b). Similar results were obtained whether the cells were labeled with glutamic-C^{14} early or late in their growth. From 50 to 80% of the leaked radioactivity could be precipitated with perchloric acid and was probably protein. Both harvest methods liberated isotope far in excess of that which

TABLE II. Leakage of Isotope from Chick Fibroblast Cells during Harvest.

Hours receiving isotope	% loss into harvest solution and 1st wash			
	Scraping into Hanks' BSS	Bactotrypsin in Hanks' BSS	Recrystallized trypsin in Puck's saline	Bactotrypsin in Puck's saline
Glutamic acid-C^{14}				
0–42*	39	24		
0–34	30	24	17	21
34–42	30	36		
34–48	42	34		
Cystine-S^{35}				
48–72†	48	65		

Glutamic acid-C^{14} was added to each bottle for the indicated period. Cell sheet was washed 3 times with appropriate salt solutions at 48 hr and the cells harvested. Cystine-S^{35} was added to each bottle at 48 hr. Cell sheets were washed with Hanks' salt solution and harvested at 72 hr. Each bottle contained 5.2×10^7 cells.

* Maximum loss from additional wash, 4%. † Maximum loss from additional wash, 8%.

would be expected from an additional wash of the cells. There was less loss when the cells were lifted with trypsin in Puck's saline containing no divalent ions (perhaps due to the shorter exposure necessary). Differences between colorimetric and isotopic data may reflect differences in the cells used and/or different sites damaged. Scraping can be expected to conserve extracellular material, *i.e.* cementing matrix, while causing damage to individual cells. Trypsinization may remove such extracellular material as well as alter the cells' permeability. This latter change must be reversible in many cases, however, as such cells are capable of dividing, whereas individual cells damaged by scraping can no longer do so. This loss of metabolic products could assume considerable importance in studies of cellular metabolism with isotopes.

Summary. HeLa cells and chick embryo fibroblasts were grown on glass and harvested under various conditions. Trypsinization and scraping were each compared with alcohol-ether denaturation. Trypsinization was found to be less efficient as a means of obtaining material for chemical analysis although less damaging to cell viability than scraping. Alcohol-ether denaturation *in situ* was the preferred means of harvesting cells for chemical analysis.

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Anti-heart Serum and Reactivity of Different Cell Types in Tissue Culture.* (24361)

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This report presents information regarding specificity of antisera and reactivity of cell types studied in tissue culture, to clarify the cellular mechanisms involved in the immediate hypersensitive reaction. In other work an antiserum to chick embryo heart cells was produced in guinea pigs and the cytologic reactions of chick heart fibroblasts in tissue culture to this antiserum were studied with phase (1) and electron microscopy(2). Antiserum was active at high dilutions, heat-labile, complement-like factors were necessary for its immediate cytotoxic actions, and it was inactive against mouse fibroblasts. Cortisol (17-hydroxycorticosterone) did not inhibit this cellular reaction. In the present report further evidence for species specificity and for lack of organ or cell type specificity of the antiserum is presented.

Methods. Fibroblasts and epithelial cells were grown in tissue culture by modification of methods previously used(1). The following nutrient solution was used after several were tried: human cord serum (Difco), 35%; chick embryo extract, 15%; and Hank's balanced salt solution, 50%. The cells were tested as before(1) with the exception that fresh normal guinea pig serum was added reg-

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