tein caloric basis. Sunde(4) has provided data from chick tests which yield little or no correlation of growth with calory-protein ratio, a low correlation with calory-protein product and a good correlation, of about 0.8, of growth with the non-protein calory-protein product. Data with rats have been provided by Sibbald *et al.*(5). While the weights reported are averages of initial and final weights, the growth results are highly correlated with the product of digestible nonprotein calories and digestible protein intakes, (coefficient 0.92). Other examples have been studied with similar results. The relations discussed above no longer apply when the genetic or physiologic capacities of the animal have been exceeded.

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Comparative Behavior of 16 ECHO Virus Types in Fibroblast-like and Epithelial-Like Human Cell Strains.* (23742)

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One of the characteristics of the ECHO viruses as a group is their ability to proliferate in tissue cultures of rhesus (*Macaca mulatta*) monkey kidney cells(1). Neither cultured kidney cells from other species of monkeys, nor cultured strains of human cells have appeared to be uniformly susceptible to all of the ECHO viruses(1,2,3). This communication reports a systematic study of the ability of 16 ECHO virus types to proliferate in human cell strains of different morphology as well as from different malignant and non-malignant sources.

Materials and methods. Cell strains. One fibroblast-like (Fb-L) and 6 epithelial-like (Ep-L) human cell strains have been investigated. Four of the Ep-L strains have been previously described(4). Of these, Detroit-30A was derived from carcinomatous ascitic fluid, Detroit-32 from bone marrow of a patient with carcinoma, Detroit-98 from normal bone marrow, and Detroit-116P from lymphomatous pleural fluid. Although some of the ECHO viruses appear to proliferate in HeLa cells(1), especially after adaptation(3), the HeLa strain was included in this study for purposes of comparison. The 2 additional cell strains, Detroit-196 Fb-L and Detroit-196 Ep-L were established as follows. A skin biopsy from a patient with urticaria pigmentosa was minced, washed with basal medium, Eagle (5) (BME), and incubated in 0.25% trypsin solution (Difco, 1:250). The resulting cell suspension was again washed and approximately 10^5 cells were suspended in 8 ml of growth medium (BME 80%, human serum 20%) and deposited into a 3-ounce prescription bottle. After incubation at 36°C for 40 days, fibroblast-like cells were distinguishable among the cellular debris and within the next 10 days a network of Fb-L cells covered the wall of the bottle. These were subcultured according to procedures previously described (4). An inoculum of 4×10^5 cells in 3-ounce bottles yielded averages of 2×10^6 cells in 8 days. After the Detroit-196 Fb-L cells had undergone 6 serial subcultures over a period of 2½ months, islands of polygonal cells appeared in several bottles of the 6th passage. These polygonal cells outgrew the fibroblasts and thus a substrain of epithelial-like cells emerged which has been continuously main-

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TABLE I. Histories of Human Cell Strains.

Cell strain	Tissue source	Diagnosis of patient's disease	Biopsy material containing malignant cells	Life of strain* (mo)
Detroit-196 Fb-L	Skin	Urticaria pigmentosa	None	13
-196 Ep-L	(Derived from 196 Fb-L cultures)	" "	"	10
- 32 "	Sternal marrow	Carcinoma, primary site undetermined	Pleural fluid	30
- 98 "	" "	Refractive error; no malignancy detected	None	26
- 30A "	Ascitic fluid	Carcinoma of the breast	Mass in breast; ascitic fluid	33
-116P "	Pleural fluid	Lymphosarcoma	Pleural fluid	25

* To Nov. 1, 1957.

TABLE II. Behavior of 16 ECHO Viruses in Cultures of Detroit-196 Fb-L and -196 Ep-L Strains of Human Cells.

ECHO virus type	TCD_{50}* in M.K. cultures	Dilution of inoculum	Passage in Detroit-196 Fb-L			Passage in Detroit-196 Ep-L		
			1st CPE	2nd CPE	3rd TCD_{50}†	1st CPE‡	2nd	3rd TCD_{50}†
1	7.2	1:100	+	+	5.8	+	§	—
2	6.8	"	+	+	4.6	+		—
3	6.2	"	+	+	4.6	+		—
4	7.5	"	+	+	5.5	+		—
5	7.5	"	+	+	5.7	+		—
6	6.8	"	+	+	5.0	+		3.2
7	6.6	"	+	+	6.4	+		—
8	5.6		+	+	6.5	+		—
9	4.5	1:100	+	+	4.5	—		
10	4.5	"	—			+		2.5
11	5.7	"	+	+	4.5	+		—
12	6.5	"	+	+	4.4	—		
13	6.4		+	+	5.5	+		—
14	7.3	1:100	+	+	5.4	+		—
15	1.5	Undiluted	+	+	3.2	—		
16	4.5	1:100	+	+	4.8	—		

* Expressed as log dilution/0.1 ml, based on cytopathogenic effect (CPE).
† As assayed in monkey kidney cultures.
‡ Presence of virus was determined by CPE in monkey kidney cultures.
§ Blind passage.
‖ Undiluted in 196 Fb-L.

tained by serial subculture. Meanwhile, in parallel cultures, polygonal cells did not appear, and cells serially subcultured from these have retained their fibroblast-like morphology for over a year. This phenomenon offered an opportunity to study viruses simultaneously in 2 different morphologic types of cells derived from the same source. Table I lists the origin of each Detroit cell strain, pertinent data on the human donor and the period during which each strain has been maintained.

Viruses. The ability of ECHO viruses,† types 1 through 16, to produce cytopathogenic changes or to multiply in each of the described cell strains was investigated. Tube cultures of each of the cell strains were prepared by implantation with approximately 1 x 10^5 cells in 1 ml of the growth medium. The Ep-L strains formed cellular monolayers in 2 days, the Fb-L strain in 5-6 days. Each virus (in previously assayed fluids from infected monkey kidney cultures) was diluted as indicated in Table II, and 0.1 ml layered on the cells of each of 2 cultures. After an

† The prototype ECHO viruses were kindly furnished by J. L. Melnick, W. McD. Hammon, and from the laboratory of A. B. Sabin by M. Ramos-Alvarez.

TABLE III. Behavior of 16 ECHO Viruses in Cultures of 5 Human Epithelial-Like Strains.

ECHO virus type	TCD_{50}* in M.K. cultures	Dilution of inoculum	Passage† in human cell strains: Detroit-30A 1	2	3	Detroit-32 1	2	3	Detroit-98 1	2	3	Detroit-116P 1	2	3	HeLa 1	2	3
1	7.2	1:100	—			+	—		—			—			+	+	4.6
2	6.8	"	+	—		+	—		—			—			+	—	
3	6.2	"	+	—		+	—		+	—		+	—		+	+	5.2
4	7.5	"	+	—		+	—		+	—		+	—		+	—	
5	7.5	"	+	—		+	—		+	—		—			+	—	
6	6.8	"	+	+	—	+	+	—	+	—		+	+		+	+	6.3
7	6.6	"	+	+	—	+	—		+	—		+	+		+	+	3.6
8	5.6	"	—			—			—			—			+	+	4.4
9	4.5	"	—			+	—		—			—			+	—	
10	4.5	"	+	—		+	—		+	—		+	+	4.7	+	+	1.6
11	5.7	"	+	—		+	—		+	—		—			+	—	
12	6.5	"	+	—		+	—		+	+	2.6	—			+	+	1.6
13	6.4	"	—			—			+	—		—			+	+	—
14	7.3	"	+	+	—	+	—		+	—		+	—		—		
15	1.5	Undiluted	—			—			—			—			—		
16	4.5	1:100	—			—			—			—			—		

* Expressed as log dilution/0.1 ml.
† As determined by CPE or back titration in monkey kidney cultures.

adsorption period of 15-20 minutes, 1.0 ml of BME containing 5% calf serum was added to each inoculated culture as well as to uninoculated control cultures. This medium contained no inhibitors for the 16 ECHO viruses. All cultures were incubated at 35°C and were observed daily. As soon as definite cytopathogenic effects occurred, the culture fluids were removed and frozen. If cytopathogenic effects were not apparent, the culture fluids were frozen at the time that the uninoculated control cultures showed evidence of degeneration. Except where noted in Tables II and III, such fluids were centrifuged, diluted 1:100, and similarly carried through 2 additional passages in each cell strain. Where indicated, the culture fluids collected at each passage were tested undiluted, or back-titrated, in monkey kidney cultures for presence or titer of virus.

Results. Table II shows that each of the ECHO virus types, with the single exception of ECHO type 10, was cytopathogenic for Detroit-196 Fb-L cells. In first passage in these cells, 15 ECHO virus types produced distinct cytopathogenic effects within 1-3 days and usually all of the cells in the cultures were affected within the next 1-2 days. These intervals were generally shortened in 2 successive serial passages, but throughout the 3 passages on the Detroit-196 Fb-L strain, it was noted that ECHO types 7 and 11 consistently produced cytopathogenic effects more rapidly than the others, whereas ECHO type 15 was consistently slower. The culture fluids recovered from the 3rd serial passage were back-titrated in monkey kidney cultures. The titers, listed in Table II, show that there is no doubt that multiplication of virus had taken place. No attempt was made to ascertain the significance of differences in these titers before and after 3 passages in the Detroit-196 Fb-L strain. The failure of ECHO virus type 10 to proliferate in these cells did not appear to be due to the pH of the culture fluids or to the lability of the virus as has been shown in the case of monkey kidney cultures(6,7). However no multiplication of ECHO virus type 10 could be demonstrated in Detroit-196 Fb-L cells when the medium was maintained either at pH 7.2 or at pH 8.0.

In contrast to their fibroblast-like counterpart, the Detroit-196 Ep-L strain appeared susceptible to only 2 of the ECHO viruses, type 6 and type 10. Cytopathogenicity was difficult to determine in these particular epithelial-like cells, partly because this strain does not form confluent monolayers on glass and partly because uninoculated control cultures often exhibited areas of cellular degeneration during the test period. Consequently, culture fluids of the first virus passage were

transferred undiluted to monkey kidney cultures, and cytopathogenicity in the latter served as an indicator system. As shown in Table II, 12 of the ECHO viruses were present in Detroit-196 Ep-L cells at the end of one passage. Culture fluids were then "blindly" passed through a second passage. Of the third passage culture fluids, only those of the ECHO type 6 and type 10 series produced cytopathogenic effect in monkey kidney cells, and these at dilution end-points of $10^{-3.2}$ and $10^{-2.5}$, respectively. The total dilution through 3 passages makes it likely that these titers represent some multiplication and are not due to residual inocula.

Table III illustrates the behavior of the 16 ECHO viruses in epithelial-like strains, Detroit-30A, -32, -98, -116P, and HeLa. In the Detroit strains, most of the viruses had disappeared by the end of the second passage. ECHO virus type 12 was present in low titer in the third passage culture fluids of Detroit-98, and ECHO type 10 in appreciable titer in third passage fluids of Detroit-116P. Of the Ep-L strains tested, HeLa appeared to be susceptible to the greatest number of ECHO virus serotypes, namely types 1, 3, 6, 7, 8 and possibly types 10 and 12, although multiplication of the latter 2 types might be questioned because of the very low titers.

Discussion. Surveys of the virus susceptibilities of human cell strains have been carried out in this laboratory with a dual purpose in mind: to further characterize cells of similar morphology but perhaps differing in other biologic properties, and also to determine whether or not various cell strains might be useful for the cultivation of viruses hitherto handled inconveniently in the laboratory. Studies of the comparative behavior of viruses in human cell strains have usually involved epithelial-like (Ep-L) cells(8-13). One is impressed that such cell strains, irrespective of their tissue sources, are more or less uniformly susceptible to a wide variety of animal viruses and uniformly refractory to others.

Recently it has been shown that many human cell strains now in existence might be classified into 2 groups on morphologic grounds: a) Ep-L cells, of which the HeLa strain may be taken as the prototype; and b) the more recently described "smaller round or irregularly shaped" cells that "do not form islands or sheets unless cultures become crowded"(14). These differences in cell morphology are matched by marked differences in their viral spectra(13). In addition, certain human fibroblast-like (Fb-L) strains are susceptible to viruses that have not been previously propagated in Ep-L strains(15). Thus it appears that differences in morphology are of significance with respect to virus susceptibility. This concept is emphasized by the present study where it is shown that a human Fb-L strain differs markedly from human Ep-L strains in its susceptibility to ECHO viruses. Detroit-196 Fb-L was susceptible to all but one of the ECHO virus serotypes whereas among 6 human Ep-L strains, 2 were completely refractory, and 4 had a narrow and variable susceptibility range.

The wide susceptibility range of the Detroit-196 Fb-L strain is of practical importance since it is an example of a cultured human cell type that may be useful for studies of ECHO viruses.

The marked differences in ECHO virus susceptibility between the Detroit-196 Fb-L strain and its Ep-L derivative would be of particular interest if it could be determined whether or not the Fb-L cell had undergone a transformation of the kind described in detail by Berman, *et al.*(16), or if the Ep-L cell was selected from a mixed cell population by a mechanism similar to that proposed by Puck, *et al.*(17).

Within the group of Ep-L cell strains themselves, there were differences in susceptibility. These are the first marked differences we have seen between these particular Detroit Ep-L strains other than minor morphologic variations.

Summary. The susceptibility ranges of a fibroblast-like and 6 epithelial-like human cell strains to 16 ECHO virus types have been described. All of these ECHO viruses except type 10 multiplied in the fibroblast-like cell strain (Detroit-196 Fb-L). In contrast, an epithelial-like strain (Detroit-196 Ep-L) evolving from the Fb-L strain, as well as 5 additional Ep-L cell strains, either were re-

fractory or had narrow susceptibility ranges. The significance of differences in morphology with respect to virus susceptibility was discussed.

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Fiber Formation in Suspension Cultures of L Strain Fibroblasts.* (23743)

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While numerous workers have reported fiber formation in primary explant cultures of fibroblasts, as indicated in reviews by Bloom and Fell(1,2) there have been no such reports concerning monolayer or suspension cultures of stable cell strains. In this paper, conditions are described under which L strain mouse fibroblasts in agitated cultures give rise to membranous structures containing numerous discrete fibers. A preliminary study of the origin and nature of these fibers is reported herein.

Materials and methods. Strain L 929 fibroblasts(3) were maintained in stationary bottle cultures in synthetic mixture 199† supplemented with 1.0% Bactopeptone‡ (199P). To prepare suspension cultures cells were scraped from the glass, dispersed by vigorous pipetting and diluted to a concentration of 1.5-2.5 x 10^5 cells/ml in medium 199P. One hundred milliliters of the suspension were put into a 250 ml Erlenmeyer flask. Control cultures were prepared by suspending an equal number of cells in medium 199P supplemented with 5% horse serum (199P-HS). Both the experimental flasks and the controls were stoppered and placed on a rotary action shaker§ (100 rpm) at 35°C(4). After several hours incubation delicate membranes appeared on the surface of the serum-free medium. A number of these membranes were pipetted onto cover glasses. Such preparations were fixed either in Zenker-acetic or in 10% formalin, and were stained with Regaud's modification of the Masson trichrome technic or by Foote's modification of the Bielschowsky silver method for argyrophilia (5). Membranes from several flasks were also collected by centrifugation and fixed in 10% formalin. These were minced in a Waring blendor and the resulting material

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