The isolation of enteroviruses is an important laboratory problem, and the establishment of cultures sensitive for these agents is a significant problem. The authors come to a definite conclusion on this point and recommend both monkey kidney and human amnion tissue cultures.

COMPARISON OF VARIOUS TISSUE CULTURES FOR THE ISOLATION OF ENTEROVIRUSES

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A STUDY of the enteroviruses isolated in various tissue cultures should aid in the selection of procedures and be of special value where the choice of methods is limited. The number of isolations of polio, ECHO, and Coxsackie Group B viruses from samples of sewage each tested in different tissue cultures is reported here.

Over 400 strains of enteroviruses were isolated from about 500 samples of sewage examined during a four-year period. The samples were collected by swab technic[1] and prepared as previously described.[2] One-tenth ml volumes of the samples were added to 0.5 ml of nutrient in five tubes of each of the following types of tissue cultures: HeLa cells,[3] monkey kidney epithelium,[4] and human amnion tissues.[5] Five-tenth ml volumes of the samples were added to single bottle cultures of monkey kidney epithelium to which an agar overlay was added.[6] A few samples were also tested in tube cultures of Detroit-6 epithelium.[7] The cultures were examined daily for virus-induced degeneration or for plaque formation (PF). The presence of virus was confirmed by serial passage in homologous tissue cultures, and isolates were identified serologically by neutralization tests.

Results

The number and variety of agents isolated in HeLa cells were so low that their use was discontinued after three years. No agents were isolated in Detroit-6 cells, although parallel inoculations of other tissue cultures yielded Coxsackie, ECHO, and polioviruses. The over-all rate was greatest in amnion tissue culture, with isolations of ECHO viruses predominating; the frequency of cytopathogenic agents isolated in monkey kidney tissue culture did not differ greatly from that of plaque formers (Table 1).

While the varieties of agents isolated in monkey kidney and amnion tissue cultures were similar (12-15 serologic types), the relative frequency of isolation in the two tissue cultures was very different. The isolation rate of polioviruses, for example, was 50 to 80 times greater, and of Coxsackie Group B viruses, three to five times greater in monkey kidney than in amnion tissue cultures. On the other hand, the over-all isolation rate of ECHO viruses was five to ten times greater in amnion than in monkey kidney tissue cultures.

Ninety-three per cent of the isolations made in amnion tissue cultures were

Table 1—Isolation of Enteroviruses from Parallel Inoculations of 518 Sewage Samples into Four Different Tissue Cultures

Enterovirus Types	Number of Isolations			
	Tube Culture			Bottle Culture
	HeLa*	Monkey Kidney	Human Amnion	Monkey Kidney
Polio				
I	5	32	1	45
II	0	0	0	3
III	2	18	0	34
Total	7	50	1	82
Coxsackie B				
2	0	4	0	1
4	0	28	2	19
5	19	21	8	9
Total	19	53	10	29
ECHO				
1	0	6	0	2
3	0	1	43	0
4	0	2	6	0
6	0	4	1	1
7	0	5	11	2
8	0	3	0	3
9	0	3	4	2
10	0	0	1	0
11	0	1	30	0
12	0	2	27	3
13	0	1	2	0
18	0	0	3	0
19	0	0	3	0
Total	0	28	131	13

* Isolations from 366 samples.

ECHO viruses. Isolations in monkey kidney tissue cultures were distributed as follows: ECHO viruses 15 per cent; polioviruses 52 per cent; Coxsackie Group B viruses 32 per cent. Of the 26 isolations made in HeLa cells, three-fourths were Coxsackie Group B viruses and one-fourth were polioviruses.

The number of isolations in a tissue culture is an index of the sensitivity of that culture to enteroviruses, although it is influenced by differences in the affinity of strains for certain cultures. A sensitivity value, one-tenth the number of isolations per 518 samples, was assigned to each tissue culture (Table 2). HeLa cell and amnion tissue cultures had the lowest values for isolations of ECHO and polioviruses, respectively. Monkey kidney and amnion tissues had highest values for isolations of polio and ECHO viruses, respectively.

When the sensitivity to serologic types of viruses was compared (Table 3), additional distinctions were clear. It suggested that the sensitivity of HeLa cells to Coxsackie Group B Type 5 virus was greater than that of monkey kidney tissue, and that HeLa cells were also more sensitive to polioviruses than was amnion tissue.

The sensitivity of monkey kidney tissue cultures to Coxsackie virus Group B Type 4 was higher than to Type 5;

this was especially obvious in the bottle cultures, perhaps because of clearer plaque formation by strains of B-4 virus. The ECHO viruses Types 1 and 8 were isolated only in monkey kidney tissue cultures. Tube cultures of monkey kidney tissue had higher values than amnion for ECHO viruses Types 6 and 9, which do not form distinctive plaques in monkey kidney monolayers.

The sensitivity of amnion cultures to certain ECHO virus types—3, 11, and 12—was as much as 40 times higher than that of monkey kidney tissue cultures. It was also higher for Types 4, 7, and 13. The few strains found of Types 10, 18, and 19 were isolated in amnion tissue cultures only. The amnion cultures were also more sensitive to Type 5 than to Type 4 strains of Coxsackie virus Group B.

The monkey kidney bottle cultures were markedly sensitive to the polioviruses, probably because the agents form large distinct plaques. Conversely, the insensitivity of bottle cultures to other agents may be due to the tendency of these agents to form less distinct plaques.

From the hundreds of samples tested in each of the four different tissue cultures, few parallel isolations were made. For example, although the sensitivities of HeLa cell and monkey kidney tube cultures were similar for Coxsackie Group B Type 5 virus, according to the over-all frequency of isolation in these tissues, parallel isolations of the agent from the same sample in the two tissue cultures occurred in only 16 per cent of the successful attempts. Sixty-eight per cent of the strains isolated in HeLa cells were from samples in which parallel attempts in monkey kidney tissue culture were unsuccessful, and conversely, 57 per cent of the strains isolated in monkey kidney and 25 per cent of those in amnion tissue cultures were from samples in which parallel attempts in HeLa cells were unsuccessful. These apparent discrepancies in isolation attempts may be attributed to: (1) inocula which are known to contain barely perceptible amounts of virus (<1 to 4 PFU/0.1 ml inoculum); or (2) specificity of strains as well as serologic types for different tissue cultures.

The former is a likely explanation for the discrepancies between parallel attempts at isolation in bottle and tube cultures of monkey kidney tissue. Only 16 per cent of the isolations of poliovirus Type 3 were made in parallel attempts in both tube and bottle cultures; 60 per cent were made in bottle cultures only, and 25 per cent in tube cultures only. Thirty-three per cent of the poliovirus Type 1 isolations were

Table 2—Sensitivity of Tissue Cultures to Enteroviruses*

Tissue Culture	Sensitivity Value		
	Enterovirus		
	Polio	Coxsackie B	ECHO
Tube			
HeLa cells†	1.0	2.7	<0.1
Monkey kidney	5.0	5.3	2.8
Human amnion	<0.1	1.0	13.0
Bottle			
Monkey kidney	8.2	2.9	1.3

* 1=10 isolations/518 samples.
† Values based on prediction from 366 samples.

Table 3—Sensitivity of Tissue Cultures to the Serologic Type of Enteroviruses*

Tissue Culture	Sensitivity Value																	
	Serologic Type of Enterovirus																	
	Polio			Coxsackie B		ECHO												
	I	II	III	4	5	1	3	4	6	7	8	9	10	11	12	13	18	19
Tube																		
HeLa cells†	0.7	<0.1	0.3	<0.1	2.7	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Monkey kidney	3.2	<0.1	1.8	2.8	2.1	0.6	0.1	0.2	0.4	0.5	0.3	0.3	<0.1	0.1	0.2	0.1	<0.1	<0.1
Human amnion	0.1	<0.1	<0.1	0.2	0.8	<0.1	4.3	0.6	0.1	1.1	<0.1	0.4	0.1	3.0	2.7	0.2	0.3	0.3
Bottle																		
Monkey kidney	4.5	0.3	3.4	1.9	0.9	0.2	<0.1	<0.1	0.1	0.2	0.3	0.2	<0.1	<0.1	0.3	<0.1	<0.1	<0.1

* 1 = 10 isolations/518 samples.
† Values based on predictions from 366 samples.

made in parallel attempts in both tube and bottle cultures, 43 per cent in bottle cultures only, and 24 per cent in tube cultures only. The larger inoculum may account for the more frequent isolations in bottle cultures.

Discussion

These differences in sensitivity of tissue cultures to the enteroviruses emphasize the importance of choosing the proper culture. Both monkey kidney and amnion tissue cultures are needed if polio, Coxsackie Group B, and ECHO viruses are sought. Amnion is the most satisfactory tissue culture for isolation of ECHO viruses, except for Types 1 and 8, and bottle cultures of monkey kidney tissue are most satisfactory for polioviruses. If the choice is limited to one isolation method, tube cultures of monkey kidney tissue would yield the greatest variety of viruses. HeLa cells are satisfactory for isolation of viruses only when Coxsackie virus Group B Type 5 is anticipated. The comparison refers, of course, only to cytopathogenic and plaque-forming agents, not to mouse-pathogenic viruses. It should be noted that the comparison is based on results of original isolations of virus only and makes no attempt to rate the cultures according to their sensitivities to established strains. Although the data were obtained from isolations from sewage, it is likely that the different sensitivities of the tissue cultures listed hold also for isolations from other sources.

The selectivity of original isolates of ECHO viruses Types 1 and 8 for monkey kidney tissue suggests a closer relationship between these types than among other ECHO virus types, as is inferred also from their known serologic cross reactions. Similarly, Types 3, 11, and 12 might be suspected of close interrelationships.

The peculiar refractivity of HeLa

cells to ECHO viruses suggests that tissue affinity for these viruses may be a function more of pathologic type (normal, malignant) than of origin (human, monkey).

Summary

The number of enteroviruses isolated from sewage samples in parallel tests in four kinds of tissue cultures was used to establish the relative sensitivities of the cultures to the agents. Monkey kidney tissue cultures were more sensitive to polioviruses and to ECHO viruses Types 1, 6, and 8 than were the other kinds of cultures; HeLa cell cultures were the most sensitive to Coxsackie virus Group B Type 5, and amnion tissue cultures were the most sensitive to the other ECHO viruses. The use of both monkey kidney and human amnion tissue cultures is recommended for the isolation of enteroviruses.

REFERENCES

1. Moore, B. The Detection of Paratyphoid Carriers in Towns by Means of Sewage Examination. Month. Bull. Min. Health & Pub. Health Lab. Serv. 7:241–248 (Nov.), 1948.
2. Kelly, S. M. Detection and Occurrence of Coxsackie Virus in Sewage. A.J.P.H. 43:1532–1538 (Dec.), 1953.
3. Scherer, W. F.; Syverton, J. T.; and Gey, G. O. Studies on the Propagation in vitro of Poliomyelitis Viruses. IV. Viral Multiplication in a Stable Strain of Human Malignant Epithelial Cells (Strain HeLa) Derived from an Epidermoid Carcinoma of the Cervix. J. Exper. Med. 97:695–709 (May), 1953.
4. Dulbecco, R., and Vogt, M. Plaque Formation and Isolation of Pure Lines with Poliomyelitis Viruses. Ibid. 99:167–182 (Feb.), 1954.
5. Zitcer, E. M.; Fogh, J.; and Dunnebacke, T. H. Human Amnion Cells for Large-Scale Production of Polio Virus. Science 122:30 (July 1), 1955.
6. Hsiung, G. D., and Melnick, J. L. Plaque Formation with Poliomyelitis, Coxsackie, and Orphan (Echo) Viruses in Bottle Cultures of Monkey Epithelial Cells. Virology 1:533–535 (Dec.), 1955.
7. Stulberg, C. S.; Berman, L.; and Ruddle, F. H. Detroit-6 Strain of Human Epithelial-like Cells: Virus Susceptibilities. Proc. Soc. Exper. Biol. & Med. 89:438–441 (July), 1955.

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"1/1000: a New National Sample of the Population of the United States" is the title of a leaflet describing a new arrangement under which the Bureau of the Census plans to make selected materials from the 1960 census available on a cost basis for research purposes. The materials, which consist of "reels of magnetic tape or a set of punchcards," will be ready during the latter part of 1962.

Requests for the leaflet and further information to Richard M. Scammon, Director, Bureau of the Census, U. S. Department of Commerce, Washington, D. C.