TABLE III. Microbial Counts.

Treatment*		(7/14) Excreta Coliform, cells/g × 10^6	(7/14) Excreta Yeast-mold, cells/g × 10^4	Termination (7/27) Ceca with contents Coliform, cells/g × 10^7	Termination (7/27) Ceca with contents Yeast-mold, cells/g × 10^5
Basal	a	11.0	125	2.23	2.86
	b	4.3	.24	1.18	4.09
Fasted	a	1.3	3.7	2.21	2.95
	b	.75	1.4	.385	1.52
Protamone	a	3.6	1.0	3.50	2.12
	b	15.0	1.1	.931	4.22
Fasted with Protamone	a	1.3	.18	1.24	2.09
	b	1.7	20.0	1.40	—

* Treatment shown was prior to subdivision for B_{12} response trial. "a" subgroups were controls, "b" subgroups B_{12}-supplemented.

chicks given dietary vit. B_{12} to higher cecal contents of vit. B_{12}; and in chicks not given dietary vit. B_{12} to lower liver contents of vit. B_{12} than those found in respective controls. It is suggested that the ability of iodinated casein to increase the sensitivity of chicks to dietary vit. B_{12} is due in part to an interference with intestinal absorption of the vitamin.

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Comparison of Methods for Recovering Poliomyelitis Viruses from Human Sources.*† (20997)

HERBERT A. WENNER AND C. ARDEN MILLER.
(With the technical assistance of Paul Kamitsuka and Noel Jarnevic.)
From the Hixon Memorial Laboratory, Department of Pediatrics, University of Kansas School of Medicine, Kansas City, Kansas.

The substitution of new methods for old requires an appraisal of their comparative merits. A large number of published papers attest to the usefulness of tissue culture methods for growth and immunological identification of poliomyelitis viruses, and for serum antibody surveys in the human population (1,2). In many studies stock viruses were used after adaptation to tissue culture from monkey or rodent sources. A few studies (3-6) have explored the usefulness of tissue culture methods in recovering poliomyelitis virus in specimens obtained directly from human beings.

In casting out among several methods most amenable for use in our laboratory it was quickly evident that some comparative information was required if confidence were to be

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TABLE I. Frequency of Recovery of Poliomyelitis Viruses in Tissue Culture.

Source	No. of samples	No. P	No. N	% P	Type 1	Type 2	Type 3	CP+, no type
Feces	70	47	23	67.2	43	2	2	6
Oropharynx	34	12	22	35.3	11	1		0

CP+ = Cytopathogenic in tissue culture.
P = Positive; N = Negative.

had in results obtained. The comparative information was obtained in a study of samples of feces and oropharyngeal exudate obtained from 104 individuals. The results obtained with these specimens studied in tissue cultures and in monkeys are presented in this report.

Materials and methods. Early experiences using monkey testicular roller tubes were salutary; however, with additional experience extending to other methods, monkey kidney explants were selected for general use in studies of poliomyelitis viruses. During the past year the HeLa cell type has been added to the tissue culture group. The plan of the present study was to compare recovery rates obtained with kidney and HeLa cell type tissue cultures inoculated with fecal and oropharyngeal samples obtained from human beings. Selected specimens were studied simultaneously in testicular roller tubes and in monkeys. *Feces.* Seventy fecal samples were obtained from children and adults during an illness of (a) paralytic or (b) non-paralytic (suspect) poliomyelitis, or (c) resident of a household where one or more of the members was known or suspected to be ill of poliomyelitis. Individuals identified in the first 2 categories were usually sampled during the first 2 weeks of illness. There were exceptions related to several community studies where samples from patients and familial associates were obtained 3 to 6 weeks after onset in the index case. *Throat swabs.* Thirty-four samples of oropharyngeal exudate were obtained in 1946, and stored since that time in a dry ice cabinet. The samples were obtained from patients admitted to the Children's Hospital with clinical signs of paralytic or non-paralytic poliomyelitis. *Preparation of specimens.* Feces were triturated, using sterile precautions to make a 20% suspension in distilled water. The crude extract was spun in a centrifuge (1500 rpm/10 minutes). The supernatant fluid was divided into 2 aliquots. One was used for inoculation of monkeys; the other for inoculation of tissue cultures. The latter aliquot was spun in a PR-2 International Refrigerated Centrifuge (13,000 rpm/30 min.). The supernatant fluid was usually free of bacteria. The virus was eluted from throat swabs, using a method previously described(7). Penicillin and streptomycin were added to the eluate prior to inoculation into tissue culture tubes. *Tissue cultures.* Three types of cell explants were used: (a) monkey testis, (b) monkey kidney, and (c) squamous cell carcinoma cells (HeLa) derived from a human uterine cervix. Methods used in initiating and maintaining growth of these tissues in artificial cultures have been described(8-10). Roller tubes were employed with monkey tissues. HeLa cells were grown in stationary tubes. After satisfactory outgrowth of cells each of 4 cultures of the same tissue were inoculated with 0.1 cc of the specimen under test for poliomyelitis virus. Inoculated tubes were incubated at 37°C. Tubes were read for cytolytic changes every other day. Monkey kidney and HeLa tubes were kept until the 6th day, monkey testis tubes until the 12th day, when the tests were regarded as finished. If cytolysis occurred, serial pasage was made 2, 3, or 4 times in order to exclude non-specific degenerative changes(4). The reproducibility of cytolysis having been ascertained, neutralization tests were done in order to define the strain type, using standard prototype serums prepared against 3 known immunologic types (11). *Monkeys.* Thirty-three stool samples were tested in monkeys. Twenty-four samples were tested each in 2 monkeys; 9 samples were tested each in one monkey. Stool extracts were inoculated intraperitoneally(12), and intranasally(13) in rhesus or cynomolgus monkeys. The presence or absence of poliomyelitis in monkeys was based on the ap-

TABLE II. Recovery of Poliomyelitis Viruses in 3 Different Types of Tissue Culture.

Source	Type of cells used: Monkey testicular — No. tested	No. P	% P	Monkey kidney — No. tested	No. P	% P	HeLa — No. tested	No. P	% P
Feces	35	18	51.4	70	43	61.4	63	39	61.9
Oropharynx	11	1	9.1	33	11	33.3	31	3	9.9

P = Positive.

pearance of paralysis, and the presence or absence of histologic lesions characteristically found in the central nervous system of the experimental animal.

Results. The over-all recovery rates of poliomyelitis virus from feces and orpharynx appear in Table I. Fifty-nine strains were recovered, 47 from fecal and 12 from oropharyngeal samples. The frequency distribution of types of poliomyelitis viruses are given also in Table I. Six fecal samples were cytolytic on continuous passage in HeLa cell type. Cytolysis was not abolished by specific poliomyelitis antiserum. The latter 6 samples have not been included among the positive tests.

Comparative merit of 3 types of tissue cultures. Data in regard to the relative and comparative sensitivity of each of the 3 tissue culture types appear in Table II. A significant difference was not observed in the capacity of the 3 respective tissue cultures to detect poliomyelitis virus in feces. Differences were observed in recovery of virus from throat swabs. Monkey kidney epithelium cultures provided virus recoveries 3 times as often as either testicular or HeLa cell cultures.

Among 104 samples tested 103 were tested in kidney, 94 were tested in HeLa, and 46 were tested in testicular tissue cultures. Virus was recovered from 55 samples (53.4%) in kidney, from 44 samples (45.7%) in HeLa, and from 19 samples (41.3%) in testicular cultures. In 4 instances virus was detected with either testicular (1x) or HeLa (3x) cell types and not with kidney cultures. In 7 instances virus was detected with kidney, but not with testicular (1x) or HeLa (6x) cell types.

Recovery rates and clinical category. The over-all rates of recovery of poliomyelitis virus in feces and oropharyngeal samples obtained from human beings with apparent or inapparent infection with poliomyelitis virus appear in Table III. The comparative merits of the 3 types of tissue cultures in the recovery of virus according to clinical category appear in Table IV. The over-all recovery rates signify a satisfactory indicator system(s). The high recovery rates of virus with feces obtained from patients with non-paralytic illnesses, and from family contacts support the adequacy of tissue culture methods in detecting poliomyelitis viruses. In regard to comparative recovery rates appreciable differences were not observed in fecal recovery rates obtained with any of the tissue cultures used. The observed low rates with testicular cultures cannot be considered significant because of the small number of samples. In regard to recovery of virus from the oropharynx, the rates obtained with kidney epithelium exceeded those obtained with either

TABLE III. Results in Respect to Clinical Category.

Source	No. of samples	Paralytic: No. tested	No. P	% P	Non-paralytic: No. tested	No. P	% P	Familial contacts (asymptomatic or minor illness): No. tested	No. P	% P	Unclassified† : No. tested	No. P	% P
Feces	70	33	28	85	11	6	55	24	11	46	2	2	100
Oropharynx	34	23	10	43	6	1	17				5	1	20

* With minor illness or no history of illness.
† Hospital records unavailable.
P = Positive.

TABLE IV. Percentile Distribution of Positive Tests in Respect to Type of Tissue Culture.

Clinical category	Fecal extracts						Oropharyngeal exudate					
	Testis		Kidney		HeLa		Testis		Kidney		HeLa	
	No. P	% P	No. P	% P	No. P	% P	No. P	% P	No. P	% P	No. P	% P
Paralytic	11/16	69	25/31	81	24/28	86	1/7	14	10/22	45	4/21	19
Non-paralytic	3/ 8	37	6/11	54	4/ 8	50	0/3		1/ 6	17	1/ 5	20
Familial associates	4/11	36	10/24	41	8/23	35						
Unclassified*			2/ 4	50	3/ 4	75	0/1		1/ 5	20	0/ 5	

* Paralytic or non-paralytic patients.
11/16 = of 16 samples 11 were positive for poliomyelitis.
P = Positive.

HeLa or testicular cultures by a ratio of 3:1.

Recovery rates in tissue cultures and in monkeys. Thirty-three fecal samples were tested in monkeys. Of these samples 22 were positive and 11 were negative in tissue culture. Among the 22 positive tissue culture samples 13 were positive and 9 were negative in monkey tests. Among 11 specimens which were negative in tissue culture, 2 contained virus capable of producing experimental poliomyelitis in monkeys.

Discussion. Any one of the 3 tissue culture methods used was satisfactory for the detection of poliomyelitis virus in fecal samples obtained from patients experiencing clinical poliomyelitis. An 85% recovery rate from feces and a 43% recovery rate from oropharyngeal samplings signify an adequate indicator system in comparison with previous observations on corresponding studies in monkeys(14,15). However, there were some observed differences in the capacity of the tissue cultures to reveal virus under the conditions employed in the study. The data suggested that monkey kidney epithelium cultures were more sensitive in revealing poliomyelitis virus. To us the differences appear to be related to inocula containing comparatively fewer infective particles of virus (*i.e.*, throat swabs compared with feces) some of which may have been bound with antibody(16); or, as was pointed out by Syverton(17) another factor, difficult to properly evaluate in 2 tissue culture types because implants were used, is the comparative number of cells per unit volume available to the virus particles.

TABLE V. Comparative Tests on the Relative Sensitivity of Monkeys and Tissue Culture.

Clinical category	Monkey		Tissue culture P		Tissue culture N	
	P	N	P		N	
Paralytic	7	4	1		1	
Non-paralytic	—	3	—		3	
Familial associates	6	2	1		5	
Total	13	9	2		9	

P = Positive; N = Negative.

In regard to the comparative merits of monkeys and tissue cultures in the detection of poliomyelitis virus it is evident that each method has limitations. Samples were encountered containing strains of poliomyelitis viruses which grew in tissue culture without causing cytolysis, and which produced paralytic poliomyelitis in monkeys. Conversely, there were specimens containing virus demonstrable in tissue culture and not in monkeys. In this study the type of dissociation encountered most frequently fits in the latter category. Presumably, the specimens contained small amounts of virus, for in some of them, one or 2 tubes of the 4 inoculated showed cytolysis.

No claim can be made here that tissue culture methods are more sensitive than monkeys in detecting poliomyelitis virus in specimens obtained from human beings. The data are not strictly comparable insofar as the number of test trials is weighted in favor of the tissue culture methods. It is clearly demonstrated however, in view of present and past experiences(14,15) that the tissue culture methods are as good as, if not better than, monkeys in the detection of poliomyelitis virus in fecal and oropharyngeal specimens obtained from human beings.

Summary. One hundred and four fecal and oropharyngeal specimens obtained directly

from human sources were studied in 3 types of tissue cultures. The virus recovery rates among fecal samples were of the same order regardless of the type of tissue culture used. Under the conditions of the study, monkey kidney epithelial cell cultures were superior to HeLa or testicular cultures in the primary isolation of poliomyelitis virus from oropharyngeal specimens. A comparison of tests with fecal samples in monkeys and in tissue culture indicated that the latter was as good as, if not better than, monkeys in detecting poliomyelitis virus.

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Effects of Dietary Aureomycin and Sulfasuxidine on Phosphorylation in the Liver of Rats.* (20998)

W. E. CORNATZER AND DUANE G. GALLO.

From the University of North Dakota Medical School, Grand Forks, N. D.

Aureomycin has been shown to be a beneficial factor in the prevention of massive dietary necrosis in the rat(1). However, Lepper *et al.*(2) have reported that a reversible fatty infiltration of the liver frequently follows the administration of aureomycin intravenously or intraperitoneally in mice. The action of antibiotics in liver disease may be due to a direct chemical effect on the liver or to an antibacterial effect on intestinal flora. The sparing effect of antibiotics on the requirements of the rat for certain B vitamins has been shown to be mainly due to the alteration of intestinal flora(3). The supplementation of sulfasuxidine in a choline-deficient diet prevents cirrhosis(4). Both aureomycin and sulfasuxidine have been shown to delay the development of renal damage due to choline deficiency(5,6). However, no analyses were made on the phospholipide content of the liver following the administration of these antibiotics.

In view of the above findings, experiments were undertaken to ascertain if phospholipide

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