The highly uniform width, the well-rounded contours and the length of the shadows are characters suggesting that the headpieces of the bacteriophage are likewise probably short cylinders. The hexagonal shape in ordinary electron micrographs indicates a conical shape of the ends of the rods. In the absence of information of the density, size and water content of the bacteriophage in aqueous suspension, no estimate can be made of the possible degree of shrinkage on drying.

Summary. Electron micrographs of the rabbit papilloma and vaccinia viruses and the T_2 bacteriophage of *E. coli* were obtained with the metal-shadowing technic. The papilloma virus dried *in vacuo* for application of the metal appeared spheroidal in shape, flattened especially at the region of contact with the film. The vaccinia virus was greatly flattened and showed the presence of a denser internal material bulging beneath the surface. The bacteriophage was a tadpole-shaped structure with a headpiece of well-rounded contours and a stubby tailpiece. The findings were discussed with respect to their bearing on the shape of the viruses in the wet state.

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Attempts to Propagate Murine Poliomyelitis Virus on Various Intestinal Bacteria and Protozoa.*

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Studies of microbial metabolism have amply demonstrated that many bacteria and protozoa are endowed with a complex equipment of enzymes that are utilized for cellular propagation. It is therefore not surprising that numerous attempts have been made to grow certain pathogenic viruses in symbiosis with bacteria or other free-living cells.[1] Without passing on the merits of these observations, the relationship between the virus of poliomyelitis and the intestinal flora seemed to us of particular interest in this connection. As is well known, human virus—with sufficient neurotropic virulence to permit intracerebral transfer to monkeys—can often be isolated from the faeces of cases or healthy contacts. More important yet, normal albino mice harbor regularly in the intestinal tract neurotropic murine virus (Theiler virus) which apparently persists for the lifetime of the animal. Additional evidence of the remarkable affinity of poliomyelitis virus for the alimentary canal is furnished by experience with the mouse-adapted strain of human SK poliomyelitis virus which could be recovered from the faeces of 3 different infected hosts, natural or experimental, *i.e.*, man,[2] monkey[3] and guinea pig.[4] How a supposedly strictly neurotropic virus can maintain itself for any protracted period in the absence of living nervous material is dif-

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ficult to understand. The theory could therefore be advanced that the infectious agent may possess a vicarious enterotropism which permits viral propagation, at a saprophytic level, on enteric microorganisms normally present in the intestinal canal.

To test the validity of this hypothesis experiments were undertaken in which cultures of certain intestinal bacteria or protozoa (including some non-intestinal parasites)† were examined for their ability to serve as a substrate for *in vitro* propagation of murine poliomyelitis virus. A mouse-adapted strain of human poliomyelitis virus (MM) which grows well in embryonic mouse and fairly well in chick tissue cell culture was used in this work.[5] This virus was chosen because its extremely high virulence for mice by both intracerebral and intraperitoneal routes facilitated the detection of minimal amounts of virus; moreover, its marked resistance against physical and chemical agencies offered some assurance for survival of the virus under unfavorable environmental conditions. The microorganisms employed as test substrates for possible viral propagation were the following: (1) Mixed bacterial flora obtained from human faeces, (2) *B. coli communis,* (3) *Leptospira biflexa,* (4) *Entamoeba coli,* (5) *Trichomonas hominis,* (6) *Chilomastix mesneli,* (7) *Tetrahymena geleii,* (8) *Leishmania donovani,* (9) *Trypanosoma gambiense.* The general experimental procedure consisted of combining sufficient 10% viral mouse brain suspension with the different bacterial or protozoal cultures in their respective optimal media so as to yield a final 1:100 virus concentration. After proper incubation such mixtures were transferred to new culture media in amounts constituting a further 10-fold dilution of virus with serial passages being maintained for several subsequent generations. For control purposes, 10% viral brain suspension, in identical proportions, was added to the respective sterile culture media which, after similar incubation, were transferred serially to new culture media, thus yielding progressively increasing 10-fold dilutions of virus alone. The 2 sets of tubes, *i.e.,* microbial cultures and controls, were examined at each passage for the presence of viable organisms by microscopic count and for the presence of virus by the injection of albino mice. In the case of the enteric bacteria it was necessary to use Seitz filtrates for injection, whereas the protozoal culture and control tubes could be injected safely without previous filtration. All injections were carried out intracerebrally with 0.03 cc of test fluid, except for cultures of *Entamoeba coli, Chilomastix mesnili* and *Trichomonas hominis* which on account of their concomitant bacterial flora were tested by intraperitoneal injection of 0.1 cc amounts. The injected mice were carefully observed for the development of characteristic symptoms. Tissues of animals which died without definite signs of paralysis were further checked by subpassage to new mice for final identification of the virus. The results are shown in Table I.

It will be seen from Table I that none of the tested bacteria or protozoa of increasingly differentiated structure were capable of supporting neurotropic MM virus beyond the number of passages which maintained virus in control tubes, with the possible exception of the *Trichomonas hominis* culture which was therefore studied in more detail. Thus, active virus was clearly demonstrable in the protozoal-bacterial culture up to and including the fourth serial passage, whereas virus alone in the uninoculated medium failed to survive beyond the initial passage in repeated tests. The impression was first gained that virus had actually multiplied to some ex-

† Grateful acknowledgement is made of the receipt of the protozoal cultures from the following sources: *Entamoeba coli, Trichomonas hominis* and *Chilomastix mesneli* from Dr. Di Guisti, Dept. of Preventive Medicine, College of Medicine, New York University; *Tetrahymena geleii* from Dr. Kessler, College of Physicians and Surgeons, Columbia University; *Leishmania donovani* from the National Institute of Health, Washington, D.C.; *Trypanosoma gambiense* from Dr. D. Weinman, Department of Comparative Pathology and Tropical Medicine, Harvard University Medical School.

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TABLE I.

Attempts to Adapt Murine Poliomyelitis Virus to Various Microorganisms by Serial Culture Passages.

Organism	Culture medium with 10% MM virus added	Transfer: Interval days	Transfer: Temp. °C	Presence of virus in serial culture passages: I 10-2	II 10-3	III 10-4	IV 10-5	V 10-6	VI 10-7	VII 10-8	VIII 10-9	IX 10-10	X 10-11*
Mixed bacterial intestinal flora	9.5 cc saline + 0.5 cc 2% bacto-peptone	7	26	—	—	—							
—	" " "	"	"	+	—	—							
B. coli communis	" " "	"	"	?	—	—							
—	" " "	"	"	+	—	—							
Leptospira biflexa	van der Walle's modification of Vervoort-Korthof medium	"	"	+	—	—	—						
—	" " "	"	"	+	—	—							
Entamoeba coli	Egg slant with liver extr. sol. and powdered rice	2	37	+	—	—	—						
—	" " "	"	"	?	—	—	—						
Trichomonas hominis	Egg slant with liver extr. sol.	"	"	+	+	+	+	—	—	—			
"	" " "	"	"	+	+	+	+	—	—	—	—	—	—
" (heat-killed)	" " "	"	"	+	+	+	—	—	—	—	—	—	—
—	" " "	"	"	+	—	—	—	—	—	—			
—	" " "	"	"	+	?	—	—	—	—	—			
Chilomastix mesnili	Cleveland Collins medium and human serum	"	"	?	—	—	—						
—	" " "	"	"	+	—	—	—						
Tetrahymena geleii	2% bactopeptone	7	26	+	+	+	+	—	—	—	—		
"	" " "	2	"	+	+	+	+	?	—	—	—		
"	" " "	"	"	+	+	+	+	?	—	—	—	—	—
—	" " "	7	"	+	+	+	—	—	—	—	—		
—	" " "	2	"	+	+	+	+	—	—	—	—		
—	" " "	"	"	+	+	+	+	?	—	—	—	—	—
Leishmania donovani	NNN medium and Senekji's broth	7	"	+	+	?	—	—					
—	" " "	"	"	+	+	+	—	—					
Trypanosoma gambiense	Brutsaert & Henrard's modification of van	"	"	+	+	+	—	—					
"	Razgha's medium												
—	" " "	"	"	+	+	?	—	—	—				
—	" " "	"	"	+	—								
—	" " "	"	"	+	+	+	—	—	—				

* Theoretical concentrations of virus resulting from dilution.

tent. However, further experiments in which virus was added to a heat-killed *Trichomonas* culture showed that active virus could likewise be carried through 3 serial passages. It would therefore appear that no viral propagation had occurred in these tests and that the presence of the protozoan with its bacterial flora had merely served to render the unfavorable medium more favorable for survival of the originally inoculated virus.

Conclusions. Propagation of human or simian strains of poliomyelitis virus in any kind of culture medium is extremely difficult whereas certain murine strains will grow well in tissue culture containing embryonic mouse brain. A number of enteric bacteria and protozoa as well as some highly differentiated non-intestinal protozoa were examined for their ability to permit propagation of MM murine poliomyelitis virus in culture. No evidence of viral propagation was found as determined by mouse inoculation. The data do not encourage the assumption that neurotropic poliomyelitis virus in stool or sewage may be maintained in symbiosis with free living microbial cells. However the limited extent of this work does not exclude the possibility that experiments with other marine or faecal organisms under different experimental conditions might be more successful.

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Effect of Thiourea on Development of the Sea Urchin Arbacia Punctulata.

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Introduction. The development of the rat,[1-3] the frog,[4-6] and the fish[7] has been influenced by treatment with thiourea. These developmental changes have been attributed to a state of functional hypothyroidism resulting from an interference of thiourea with the production of normal thyroid hormone.[8-10] It has been suggested that the drug may act by inhibiting the peroxidase[11] or the cytochrome oxidase[12] activity of the thyroid gland. From additional studies,[13] however, it appears that thiourea inhibits an oxidative enzyme other than cytochrome oxidase. Recently it has been shown, that low dosages of thiourea incorporated in the food medium produced defects in developing Drosophila,[14]

* With the technical assistance of Miss Myrna Helfman.

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