systems involving membranes permeable to solute as well as to solvent. It is shown that the pressure difference necessary to prevent a net osmotic movement of fluid across a leaky membrane may be smaller by several orders of magnitude than the pressure difference necessary to accomplish the same effect with a semipermeable membrane; *i.e.*, a membrane to which the van't Hoff equation applies. It is also shown that, contrary to what would be expected on the basis of reasoning from the van't Hoff equation, the osmotic transport rate across a leaky membrane is a function not only of the solute concentration difference but also of the nature of the solute molecules. Further, it is demonstrated that net transport of liquid may occur across leaky membranes separating iso-osmotic solutions of different non-electrolytes. 2. In all these cases, the use of equations derived for the equilibrium state across a semipermeable membrane (*e.g.* the van't Hoff equation) would lead to decidedly erroneous results. 3. These observations demonstrate the use of van't Hoff's equation in a recent analysis of capillary permeability to be incorrect.

1. Van't Hoff, J. H., *Z. f. physikal. Chem.*, 1887, v1, 481.

2. Laidler, K. J. and Schuler, K. E., *J. Chem. Physics*, 1949, v17, 851.

3. Berkeley, Earl of, and Hartley, E., *Proc Roy. Soc. A*, 1909, v82, 271.

4. Carr, C. W., and Sollner, K., *J. Gen. Physiol.*, 1944, v28, 119; Gregor, H. P., and Sollner, K., *J. Phys. Chem.*, 1946, v50, 53.

5. Grim, Eugene, Ph.D. Thesis, Univ. of Minn., 1950, p247.

6. Pappenheimer, J. R., Renkin, E. M., and Borrero, L. M., *Am. J. Physiol.*, 1951, v167, 13.

7. Flexner, L. B., Cowie, D. B., and Vosburgh, G. J., *Cold Spring Harbor Symp. Quant. Biol.*, 1948, v13, 88.

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Mack Virus—Serum and Gamma Globulin Neutralization of Unidentified Agent Isolated From Suspected Nonparalytic Poliomyelitis.* (20307)

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We wish to describe certain observations on an unclassified virus isolated from tissue-cultures of the feces of a child (Mack) who developed thermostable specific antibodies in convalescence. We believe that the child's illness was caused by the agent which is tentatively referred to as *Mack* virus. The 5-year-old girl's illness occurred during a poliomyelitis outbreak in Cincinnati during August, 1947, with symptoms of headache, pain and "stiffness" of the neck, temperature of 103°F and pleocytosis (40 leucocytes per cu mm, 65% lymphocytes.) Recovery was complete in a few days. Although objective nuchal-spinal rigidity was not detected the clinical findings were compatible with a short-lived aseptic meningitis syndrome. For additional clinical and epidemiological details the reader is referred to patient No. 7 (K.M.) included in a study reported by Sabin and Steigman(1).

Characteristics of Mack virus in tissue-culture—isolation and titration. The roller tube tissue-culture system described by Robbins, Weller, and Enders(2) for poliomyelitis virus was used with some modification. Normal rhesus and cynomolgus monkey testicles were used as the source of fibroblastic proliferation. Natural and synthetic(3) nutrient media which are used in cultivating poliomyelitis virus were used equally well in these studies. The presence of the *Mack* virus in the culture system is indicated by cytopathogenic effects upon the fibroblastic outgrowths which are specifically inhibited by appropriate

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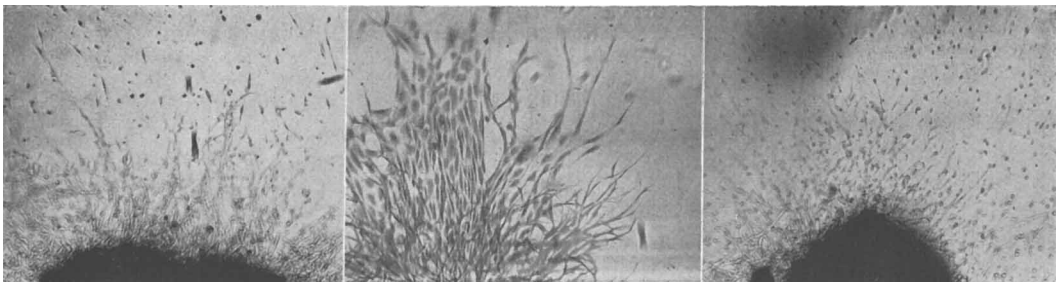

FIG. 1. Cytopathogenic effect of *Mack* virus indistinguishable from that of poliomyelitis on the fibroblastic outgrowth. To left is Type 1 poliomyelitis (Brunhilde); to right is *Mack* virus; central panel is control. Cultures were prepared simultaneously. ×70.

antisera. The character of the fibroblastic degeneration induced by the *Mack* virus cannot be distinguished morphologically from that induced by poliomyelitis. That the virus was derived from the patient and not from the tissues or natural nutrient media used was assured not only by the patient's specific serological response but also because of the ease of repeated isolation in different experiments. The virus was demonstrated readily in the fibroblastic outgrowth of the only human tissue studied—a highly anaplastic neuroblastoma removed from an infant; it failed to induce cytopathogenic effects upon cultures of adult mouse testicles. In the presence of *Mack* virus, fibroblastic degeneration appeared on the third day and was generally complete by the seventh day. Seven serial passages *in vitro* representing 17 changes of nutrient media at 4-day intervals have been made to date. Culture fluids titrated *in vitro* have an infectivity endpoint averaging $10^{-3.5}$. The endpoint is expressed as the $TCID_{50}$ (50% tissue-culture infective dose). Fig. 1 depicts the fibroblastic degeneration induced by *Mack* virus *in vitro* and the morphologically indistinguishable degeneration induced by poliomyelitis.

Neutralization of Mack virus by human sera, gamma globulin, and monkey immune serum. All sera were kept frozen except when used in tests, at which time they were heated at 56°C for 30 minutes. Acute and convalescent sera of the patient from whom the virus was recovered were diluted 5-fold in balanced salt solution and added to ± 100 $TCID_{50}$ of *Mack* virus and to a member of each of the 3 known antigenic types of poliomyelitis virus. The mixtures, kept either at room temperature or at 37°C for one hour and then overnight at 4°C were inoculated into pairs of actively growing culture tubes. Controls for the sera, viruses and tissue utilized were included in the test. It will be noted that while no alteration in poliomyelitis antibody appeared, the patient's convalescent serum in a dilution of 1:1250 inhibited the fibroblastic degeneration due to *Mack* virus, whilst the 1:2 dilution of acute serum failed to do so (Table I). Specific immune serum was prepared by the intramuscular injection of a rhesus monkey with infected tissue culture fluids suspended in mineral oil adjuvants without mycobacteria(4). This serum tested *in vitro* with *Mack* virus inhibited fibroblastic degeneration when diluted 1:1250 while undiluted normal rhesus serum failed to do so. The monkey immune serum failed to influence the fibroblastic degeneration induced by members of the 3 antigenic types of poliomyelitis virus which were specifically inhibited by their own respective antisera (Table II). The sera of 6 normal adults with no known contact with *Mack* virus were tested qualitatively for neutralizing antibody; 3 of the 6 exhibited ability to neutralize ± 1000 $TCID_{50}$ of *Mack* virus in tests in which 1:2 dilutions of serum were utilized.

Lyophilized pooled human gamma globulin† (without preservative) was redissolved and tested qualitatively for its capacity to inhibit

† Generously made available from Sharp and Dohme's Research Division by Dr. R. B. Pennell who reported that the material was processed from 9014 donors bled between May 1 and 21, 1952, in Baltimore, Boston, New York and Philadelphia.

TABLE I. Specificity of Immune Response of Patient to *Mack* Virus *In Vitro*.

Patient serum, days after onset	Serum neutralization titers in tissue-cultures of indicated viruses*†			
		Poliomyelitis type:		
	Mack	1 (Mahoney)	2 (MEF-1)	3 (Saukett)
1	1:2	<1:50	<1:50	<1:50
28	1:1250	"	"	"

* Each bearing ± 100 $TCID_{50}$ of virus.

† Final readings on 6th day when all virus control tubes showed +++ fibroblastic degeneration.

TABLE II. Failure of *Mack* Virus to be Neutralized *In Vitro* by 3 Antigenically Distinct Poliomyelitis Antisera.

Monkey antisera‡		Cytopathogenic effect of indicated viruses in tissue-cultures*†				
		Poliomyelitis type:				
		1	2	3	Mack	None
Poliomyelitis (serologic types)	1	0	+++	+++	+++	0
	2	+++	0	+++	+++	0
	3	+++	+++	0	+++	0
Mack		+++	+++	+++	0	0
None		+++	+++	+++	+++	0

* Each bearing ± 1000 $TCID_{50}$ of virus.

† Final readings on 6th day when all virus control tubes showed +++ fibroblastic degeneration.

‡ Final dilution 1:4.

the fibroblastic degeneration induced by *Mack* virus *in vitro*. A 1:64 dilution of the gamma-globulin (which corresponds to a 1:3 dilution of an unconcentrated serum) when tested against decimal dilutions of *Mack* virus revealed a neutralization index of 320. In other tests using ±1000 $TCID_{50}$ of virus and 1:64 dilution of gamma-globulin fibroblastic degeneration had not appeared on the 5th day by which time severe degeneration had occurred in the virus control cultures. By the 7th day degeneration became apparent in the cultures containing gamma-globulin and the ±1000 $TCID_{50}$ of virus.

Animal infectivity tests. Undiluted first and third passage culture fluids of *Mack* virus inoculated cerebrally and/or subcutaneously into rabbits, guinea pigs, adult and suckling mice, and hamsters have produced no observable illness. Cortisone(5) was injected daily in the last species without evident effect. *Further tests in suckling mice.* We observed that the *stools* of the index patient failed to infect 36 suckling mice. It is also known(6) that this patient developed no neutralizing antibodies to a strain of Coxsackie virus isolated at that time and in the same locale of another patient. In view of the fecal isolation *in vitro* of an unclassified cytopathogenic virus (Weider) by Robbins *et al.*(7) and subsequently identified as Boston type Coxsackie (8), additional tests were undertaken in suckling mice; 40 others were inoculated with first and third passage tissue culture fluids of *Mack* virus, and none exhibited any illness. *Further tests in monkeys.* The patient's feces were originally described as harboring poliomyelitis virus of low pathogenicity for monkeys. This was based upon the fact that of 3 monkeys inoculated intranasally, one exhibited lesions considered to be poliomyelitis and confined to the olfactory portal(1). Tissue culture fluids were, therefore, tested by a variety of inoculation routes in monkeys, some of whom received daily intramuscular injections of cortisone. Five monkeys received repeated nasal instillations, 4 bilateral thalamic inoculations, 3 sciatic injections, 2 intraspinal injections, and 2 pharyngeal injections. All remained well for the observation period of 30 days. In further tests the patient's frozen original feces (from which the

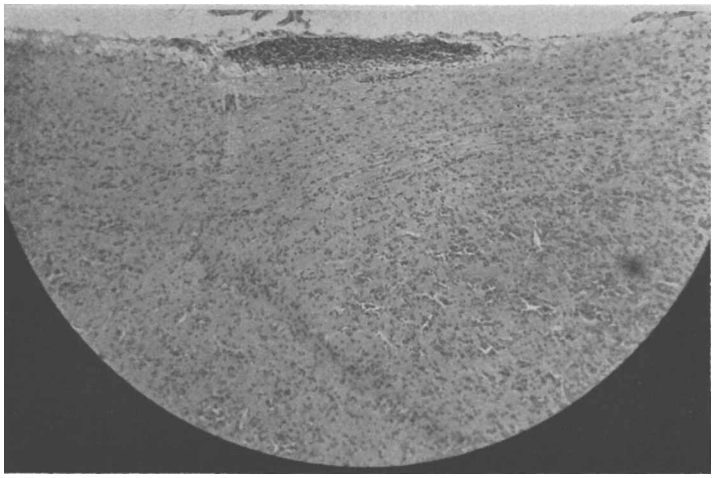

FIG. 2. Olfactory bulb of monkey inoculated nasally with *in vitro* infective *Mack* culture fluid. The round cell infiltrate shown was found on serial sections to be confined to the lamina fibrorum nervi. The mitral cell layer was normal hence not diagnostic of poliomyelitis virus. ×260.

Mack virus could be repeatedly isolated *in vitro*) were inoculated nasally and thalamically into 4 monkeys and pharyngeally into 2 others. Daily intramuscular injections of 25 mg of cortisone 2 days before and for 7-12 days after inoculation were given. These animals remained well for the observation period of 30 days.

Neurohistological studies in monkeys. The same feces which yielded *Mack* virus were earlier considered by Sabin and Steigman as containing "poliomyelitis virus of very low virulence." Their evidence was derived from serial sections of the olfactory bulbs of a monkey q.v.(1). Detailed study of CNS sections of the 22 monkeys inoculated with *Mack* virus (v. supra) revealed no lesions pathognomonic of poliomyelitis(9). Similar study of the olfactory bulbs of the 5 monkeys inoculated intranasally with tissue-culture infective fluids and the 4 additional monkeys inoculated nasally with the feces did not reveal lesions pathognomonic of poliomyelitis. The interesting lesion depicted in Fig. 2 does not meet the criteria for poliomyelitic infection of the olfactory bulbs. There is a dense ovoid infiltrate of round cells in the layer of nerve fibers but the mitral cell layer does not appear affected.

Other properties of Mack virus. The agent described passed readily through sintered glass (VF) and Seitz EK filters; was not destroyed by contact with ether and when treated with protamine was recovered in the supernatant phase. Exposure to a temperature of 56°C for 30 minutes destroyed infectivity *in vitro*. It was resistant to freezing and thawing, penicillin, and streptomycin. These properties are generally shared by the poliomyelitis and the Coxsackie viruses. Fecal specimens treated with ether and/or the antibiotics in poliomyelitis studies may be useful in revealing similar agents.

Discussion. That the patient who yielded *Mack* virus was not simultaneously infected with poliomyelitis virus is suggested by the histological study of monkeys reported herein together with failure of the patient to reveal alteration of neutralizing antibody levels to the 3 known immunological types of poliomyelitis virus in convalescence. The recovery of this unclassified virus and the appearance of specific neutralizing antibodies in convalescence should constitute noteworthy though not compelling evidence of a causal relationship. The findings herein described together with evidence of antibodies to *Mack* virus in adults and in pooled human gamma-globulin lends credence to the belief that the agent described might be parasitic in human beings. Its recovery from a febrile child with pleocytosis during the course of a poliomyelitis outbreak is not without interest. To determine the importance of this agent in disease and the

types of clinical illness it might produce would require a combined clinico-epidemiologic and laboratory approach patterned after the studies of Huebner *et al.* for the Coxsackie viruses(10).

Failure to infect laboratory animals, while useful in eliminating a consideration of certain known viruses, curtails the opportunity for histopathological studies. Although no antibody studies of patients, normal individuals or gamma-globulin have been reported, it would appear that other unidentified agents cytopathogenic for human(8) and monkey (11) fibroblasts but lacking animal infectivity may be recovered from human feces. Their description suggests that the morphology of the cytopathogenicity is generally distinguishable from that of poliomyelitis virus. Another unidentified agent isolated in our laboratory produces *in vitro* morphological effects readily distinguished from those of *Mack* virus and to which it is not related immunologically(12).

The recently introduced method for the cultivation of poliomyelitis virus, by which means only *Mack* virus was discovered, may lead to the isolation of similar agents whose relationship to each other and to human disease will require elucidation. It is likely that fecal isolation attempts at virus recovery in tissue cultures will be done most commonly in association with poliomyelitis-like illnesses such as the "aseptic meningitis syndrome." The evidence reported herein would appear to exclude a concurrent infection with either poliomyelitis or Coxsackie virus in the patient. In the presence of multiple viruses in a given fecal specimen each capable of producing cytopathogenic effects the situation becomes more perplexing. Although only a few Coxsackie viruses cause fibroblastic degeneration (11), many strains of poliomyelitis virus have this property, as does the *Mack* virus. The individual identification of multiple cytopathogenic viruses simultaneously present in a given fecal specimen might be influenced by their relative ascendancy of growth curves or by the interference phenomenon and deserves study.

Summary. 1. A virus (*Mack*) has been isolated *in vitro* from a patient's feces previously reported as containing poliomyelitis virus of very limited pathogenicity for monkeys. The virus is immunologically distinct from poliomyelitis, and does not produce pathognomonic lesions of poliomyelitis in monkeys. The patient failed to develop a rise of poliomyelitis neutralizing antibodies in convalescence. 2. The virus has been cultivated in monkey and human tissue cultures in which is produced fibroblastic degeneration morphologically indistinguishable from that caused by prototype poliomyelitis viruses. Suckling and adult mice are unaffected as are guinea pigs, rabbits, and hamsters. 3. The donor of the virus was a febrile child with pleocytosis who developed specific neutralizing antibodies in high titer in convalescence. Similar antibodies have been detected in adult sera and in a pool of human gamma globulin.

1. Sabin, A. B., and Steigman, A. J., *Amer. J. Hyg.*, 1949, v49, 176.

2. Robbins, F. C., Weller, T. H., and Enders, J. F., *J. Immunol.*, 1952, v69, 673.

3. Wood, W., Franklin, A. E., Clark, E. M., Duncan, D., and Rhodes, A. J., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 434.

4. Freund, J., *Ann. Rev. Microbiol.*, 1947, v1, 291.

5. Shwartzman, G., and Fisher, A., *J. Exp. Med.*, 1952, v95, 347.

6. Melnick, J. L., Ledinko, N., Kaplan, A. S., and Kraft, L. M., *J. Exp. Med.*, 1950, v91, 185.

7. Robbins, F. C., Enders, J. F., Weller, T. H., and Florentino, G. L., *Am. J. Hyg.*, 1951, v54, 286; also personal communication.

8. Contreras, G., Barnett, V. H., and Melnick, J. L., *J. Immunol.*, 1952, v69, 395.

9. Committee on Nomenclature of Nat. Found. for Infantile Paralysis, *Science*, 1948, v108, 701.

10. Huebner, R. J., Beeman, E. A., Cole, R. M., Beigelman, P. M., and Bell, J. A., *New Eng. J. Med.*, 1952, v247, 249, 285.

11. Melnick, J. L., and Agren, K., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 621.

12. Unpublished observations.

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