TABLE I. Urinary Sodium and Potassium Excretions. 5 subjects in each series.

Normal	Total urinary Na excretion Acetazoleamide	% increase
4.4 MEQ	58.4 MEQ	1225
10.5	76.2	635
49.0	82.3	68
15.5	40.2	58
54.6	121.7	123
	Avg % increase	440
Normal	Total urinary K excretion Acetazoleamide	% decrease
12.3 MEQ	4.9 MEQ	60
7.1	3.8	47
7.2	2.4	67
8.4	1.6	81
11.0	5.8	47
	Avg % decrease	60

tolerance tests). The first glucose tolerance test was used as a control. A second test was performed 2½ to 3½ hours after the administration of acetazoleamide. These tests showed a tendency for the acetazoleamide to increase the oral glucose tolerance. The administration of acetazoleamide 2½ to 3½ hours prior to the intravenous administration of glucose did not result in as marked a degree of change in the glucose tolerance curve. After administration of acetazoleamide to patients receiving oral glucose tolerance tests a diminution of the urinary potassium excretion was observed.

1. Maren, T. H., *Trans. N. Y. Acad. Sc.,* 1952, v15, 53.

2. Fegler, J., *Nature,* 1944, v153, 137.

3. Jacobs, M. H., and Stewart, D. P., *J. Gen. Physiol.,* 1941-1942, v25, 539.

4. Galeone, A., and Dal Santo, D., *Acta Med. Scand.,* 1952, v143, 266.

5. Davenport, H. W., *Am. J. Physiol.,* 1941, v133, 257.

6. Feldberg, W., Keilin, D., and Mann, T., *Nature,* 1940, v146, 651.

7. Davies, R. E., and Edelman, J., *Biochem. J.,* 1951, v50, 190.

8. Birnbaum, D., and Hollander, F., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 23.

9. Ashby, W., Garzoli, R. F., and Schuster, E. M., *Am. J. Physiol.,* 1952, v170, 116.

10. Hawk, P. B., Oser, B. L., Summerson, W. H., *Practical Physiological Chemistry,* 12th Ed., The Blakiston Co., 1937, 520.

11. Berliner, R. W., Kennedy, T. J., and Orloff, J., *Am. J. Med.,* 1951, v11, 274.

12. Friedberg, C. K., and Halpern, M., *Fed. Proc.,* 1952, v11, 49.

13. Sollman, T. H., *A Manual of Pharmacology,* 7th Ed., W. B. Saunders and Co., 1948, 556.

Received May 17, 1954. P.S.E.B.M., 1954, v86.

Possible Occurrence of Multiple Antigens in Type 2 Poliomyelitis and GDVII Mouse Encephalomyelitis Viruses.* (21147)

ROBERT RUSTIGIAN, PHYLLIS HAVENS, AND MARY KOLNER.
(Introduced by S. A. Koser.)

From the Department of Bacteriology and Parasitology, University of Chicago.

The occurrence of at least two distinct antigens has been established for a number of mammalian viruses(1) and for *B. coli* T2 bacteriophage(2) by complement fixation and precipitin tests. The viruses for which such antigens have been separated and characterized are of relatively large size ranging from about 100 mμ to 450 mμ. However, very little information is available concerning the presence of multiple antigens in viruses considerably smaller in size. The foot and mouth disease virus, one of the smallest known viruses, has been found to have 2 complement fixing components(3,4); one associated with virus particles (infectivity) which measures about 20 mμ in diameter on the basis of sedimentation studies, and the other a smaller component of 6.5 mμ(4).

This report concerns the possible presence of 2 complement fixing antigens in GDVII mouse encephalomyelitis virus, estimated to be

* This investigation was supported by a research grant from the Elaine Settler Polio Foundation, Chicago, Ill.

TABLE I. Comparative Complement Fixing Activity of Saline-Ether and Acetone-Ether Extracts of Infant Mouse CNS Infected with MEFl Virus.

Serum	Infant mouse passage	Antigen: Saline-ether Serum titer	Antigen: Saline-ether Antigen titer	Antigen: Acetone-ether Serum titer	Antigen: Acetone-ether Antigen titer
Type 2 anti-MEFl mouse pool 105105	67, 69	1:256 or >	1:2	1:512	1:8
Type 2 anti-Lansing monkey 35308	74	1:128	1:1	1:128	1:16
Type 2 anti-Lansing monkey 95305	76	1:4096	1:12	1:4096	1:48
Type 2 human	76	<1:8	0	1:128	1:12

Infectivity titer of saline-ether antigens for 3- to 4-wk-old mice following intracerebral inoculation ranged from $10^{-4.9}$ to $10^{-5.5}$ ID_{50} per .03 ml inoculum for 8 suspensions from 67th through 76th infant mouse passages. Infectivity titer for 4 suspensions of acetone-ether antigens varied from $10^{-1.3}$ to $10^{-3.7}$ ID_{50}.

7 to 12 mμ in size by ultrafiltration(5,6), and type 2 poliomyelitis virus, 12 to 23 mμ by ultrafiltration, and 23 to 30 mμ by sedimentation and electron microscopy(6,7). The occurrence of distinct antigens in these small viruses would be of fundamental significance. In addition, knowledge of the antigenic structure of poliomyelitis virus is of practical importance in view of the variation of complement fixing antibody in poliomyelitis patients (8) and application of the complement fixation reaction in epidemiological studies(9).

Materials and methods. Infant mouse brains and upper spinal cords infected with MEFl poliomyelitis and GDVII viruses served as the source of complement fixing (CF) antigens. The MEFl virus was kindly supplied by Dr. Casals in its 63rd infant mouse passage. Antigens were prepared from the 67th through 76th passage. GDVII virus, maintained in young adult mice, was highly virulent for infant mice. The incubation period varied from 2 to 7 days in the first 3 passages, but on subsequent passages it was, almost without exception, 2 to 3 days. Signs of disease were marked weakness, cyanosis, death, and occasionally paralysis. The *antigens* were prepared as follows: Following intracerebral inoculation of 2- to 3-day-old mice, the brains and cords were harvested from mice with paralysis 2 to 4 days after inoculation with MEFl virus, and marked weakness or paralysis after inoculation with GDVII virus. The infected CNS tissue was extracted with either saline-ether(10) or acetone-ether(11) and then used as antigen following centrifugation at an average relative centrifugal force of approximately 13,500 g for 30 minutes. Fifty to 100 brains and cords were used for the saline-ether and usually 200 to 400 for the acetone-ether extract antigens. *Type 2 poliomyelitis* sera were prepared by immunization of monkeys with monkey spinal cord infected with the Lansing strain and adult mice with the infant mouse adapted MEFl virus. Sera were also obtained from poliomyelitis patients. The GDVII sera were prepared by immunization of mice with adult mouse virus. All sera were inactivated at 60°C for 20 minutes for the CF tests. The *CF tests* were performed employing two 50% units of complement. Readings were made in a Coleman Junior Spectrophotometer at a wave length of 550 mμ. For titration of antigen, the antigen was diluted and the serum held constant. Serum titers were established by the reciprocal procedure. In addition, a large number of tests were carried out by using various dilutions of antigen against various dilutions of serum. The tests were set up in 0.3 ml volume (0.1 ml serum, complement and antigen), held at 5 to 10°C for 16 to 24 hours and followed by the addition of 0.2 ml sensitized cells, and incubation at 37°C for 30 minutes. The volume was then brought up to 1.5 ml with chilled veronal buffer, centrifuged at 2000 rpm for 5 minutes and readings taken. Titration end points were defined on the basis of 55% or less hemolysis. The MEFl and GDVII antigens were used interchangeably as controls and in a number of tests infant mouse adapted dengue virus(12) was also used as control antigen. Infectivity titrations were made in 3 to 4 weeks old mice using 10-fold

TABLE II. MEFl Complement Fixing Activity of Supernatant and Sediment Fractions Obtained by Centrifugation of Acetone-Ether Antigen at 35000 RPM for 75 Min.

Antigen	Infectivity	Complement fixation* Antigen titer	Serum titer
Saline-ether			
Control	$10^{-5.4}$	1:12	1:4096
Supernatant (¾ vol)	$10^{-2.4}$	1:1	1:16
Sediment (pellet and bottom ¼ supernatant)	$10^{-5.8}$	1:6	1:4096
Acetone-ether			
Control	$10^{-3.7}$	1:32	1:4096
Supernatant			
Upper ½ vol		1:8	1:1024
Lower ½ "		1:24	1:4096
¾ "	$10^{-1.6}$	1:16	1:4096
Sediment			
Pellet		1:32	1:4096
Pellet and bottom ¼ supernatant	$10^{-4.4}$	1:32	1:4096

* Anti-Lansing monkey serum 95305.
Blank = No test.

dilutions and the ID_{50} estimated by the method of moving averages(13).

Results. Table I illustrates the relationships of CF activity between saline-ether and acetone-ether extract antigens of MEFl virus. Although comparable serum titers were obtained with both antigens and hyperimmune sera, the acetone-ether antigens were of greater potency as evidenced by the 4-fold and higher antigen titers. The saline-ether antigen differed further in that it failed to give any significant reaction in human serum from a poliomyelitis patient infected with type 2 virus. It also failed to react significantly with sera from 3 additional patients (type 1 infection) which gave serum titers of 1:64 to 1:128 with the acetone-ether antigen. On the other hand the infectivity titer of the acetone-ether antigens was 6% and less that of the saline-ether antigens.

The effect of high speed centrifugation on the CF activities of both antigens was of much greater interest. Supernatants of acetone-ether extracts obtained by centrifugation at 35000 rpm (average g = 80730) in a Spinco Model L Ultracentrifuge had antigen titers 25 to 50% that of the controls even though 99% of the infectivity was removed and the sediment resuspended to volume was comparable to the controls in both CF activity and infectivity. In contrast, supernatants of saline-ether antigens were without or had only slight CF activity. Table II summarizes such an experiment. The sediment fraction of the acetone-ether antigens consistently gave stronger reactions than the supernatant fraction. This was evident not only on the basis of antigen titers, but also by the fact that the sediment gave slight but definite optimal ratio effects which was not obtained with the supernatant fraction (Table III).

The GDVII saline-ether antigen, in contrast to the same type of preparation of MEFl virus, had marked CF activity following centrifugation which removed over 99% of infectivity and hemagglutinating activity† (Table IV); and acetone-ether extraction resulted in a 16

TABLE III. "Box" Titration of 35000 Supernatant and Sediment Fractions of MEFl Acetone-Ether Antigen.

Antigen	Dilution	% hemolysis serum dilutions† 1:16	1:32	1:64	1:128	1:256	1:512
Supernatant	1:1	0	2.2	11.7	*	41.3	77.9
	1:2	0	5.8	31.3	74.3	88.8	95.2
	1:4	0	15.2	44.4	73.1	85.7	92.8
	1:8	25.8	59.3	80.7	91.3	96.7	95.8
	1:16	92.5	93.2	92.5	94.4	98.3	
Sediment	1:1	0	0	0	0	35.8	90.8
	1:2	0	0	0	0	14.3	87.3
	1:4	2	0	.9	.3	4.8	72
	1:8	2.3	1.4	6.0	5.1	27.9	69.2
	1:16	16.8	26.9	38.3	57.3	64.7	89.6
	1:32	74.3	75.2	91.3	91	92.8	93.3

Supernatant = ¾ vol. Sediment = Pellet and bottom ¼ supernatant.
* Excess sheep cells.
† Anti-Lansing serum 65301.

TABLE IV. Effect of Centrifugation at 40000 RPM (Avg g = 105400) for 2 Hr on Infectivity and Hemagglutinating and Complement Fixing Activity of Saline-Ether Extract of Infant Mouse CNS Infected with GDVII Virus.

Control			40,000 supernatant			40,000 sediment		
Infectivity titer	HA titer*	CF antigen titer†	Infectivity titer	HA titer	CF antigen titer	Infectivity titer	HA titer	CF antigen titer
$10^{-7.0}$ or >	1:819,200	1:128	$10^{-4.0}$	1:800	1:64	$10^{-7.0}$ or >	1:819,200	1:128

* Hemagglutination titer: Final dilution of supernatant.
† Serum: anti-GDVII mouse pool 65205.

TABLE V. Comparison of Complement Fixing Activity of Control, 40000 Supernatant and Sediment Fractions of Saline-Ether and Acetone-Ether Extracts of GDVII Virus.

	Control		Supernatant		Sediment	
Antigen	Antigen titer	Serum* titer	Antigen titer	Serum titer	Antigen titer	Serum titer
Saline-ether	1:64	1:256	1:16	1:128	1:32	1:128
Acetone-ether	1:512	1:128	1:512	1:128	1:512	1:256

* Mouse serum pool 95304.

to 32-fold concentration in CF activity of the sediment and supernatant fractions (Table V).

Studies with sera from poliomyelitis patients revealed significant differences in the CF titers obtained with the supernatant and pellet fractions of MEFl acetone-ether antigens. Of further interest is the fact that paired sera from 10 of 11 patients from whom types 1 and 3 viruses were isolated, gave titers of less than 1:16 with the supernatant fraction even though in some instances the titers with the pellet fraction were as high as 1:128 to 1:256. On the other hand, either the "acute" or "convalescent" sera from 3 patients from whom type 2 virus was isolated, had titers of 1:32 to 1:128 with the supernatant fraction, as compared to maximum titers of 1:64 to 1:512 with the pellet fraction. Obviously the data is too limited for definite conclusions. Moreover, these differences in such non-hyperimmune sera may, in part, reflect differences in the relative CF potency of the supernatant and pellet fractions.

Discussion and summary. There are several possibilities which may account for complement fixing activity present in the supernatants of acetone-ether but not in saline-ether extracts of MEFl infant mouse virus obtained by centrifugation at 35000 rpm for 75 minutes. The acetone-ether extraction not only inactivates a large amount of infectivity, but at the same time decreases the density of such inactivated virus or breaks down a portion of the virus particles into smaller units. The CF activity of the supernatant, on the other hand, may represent a second antigen evident with acetone-ether but not saline-ether extracts because of concentration effects. In addition, it is possible that acetone-ether but not saline-ether treatment releases from the virus particles a second antigen. Finally, inhibitor substance(s) which partially interfere with CF activity of MEFl virus may be removed by acetone-ether treatment. With GDVII virus it would appear that a physical separation of 2 CF antigens was realized, one associated with infectivity and hemagglutination, and the other of smaller size with comparatively little or no infectivity and hemagglutinating activity. Studies are now in progress to determine whether the supernatant CF activity of MEFl acetone-ether extract antigen represents an antigen qualitatively different from the sediment fraction and also to obtain further evidence for the multiplicity of antigens in GDVII mouse encephalomyelitis virus.

† It should be noted that GDVII infant mouse virus gave 32- to 128-fold higher hemagglutinating titers and approximately 10-fold higher infectivity titers than adult mouse virus(14).

1. Smadel, J., *Serological Reactions in Viral and Rickettsial Infections,* in *Viral and Rickettsial Infections of Man* edited by T. M. Rivers, J. B. Lippin-

cott Co., Philadelphia, 2nd edition.

2. Lanni, F., and Lanni, Y. T., *Cold Spring Harbor Symposium on Quant. Biol.*, 1953, v18, 159.

3. Traub, E., and Pyle, G., *Z. Immun. Forsch.*, 1943, v104, 158.

4. Bradish, C. J., Brooksby, J. B., Dillon, J. F., and Norambuena, M., *Proc. Roy. Soc. London*, 1952, v140, 107.

5. Theiler, M., and Gard, S., *J. Exp. Med.*, 1940, v72, 49.

6. Melnick, J. L., Rhian, M., Warren, J., and Breese, S. S., Jr., *J. Immunol.*, 1951, v67, 151.

7. Sabin, A. B., Hennessen, W. A., and Warren, J., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 359.

8. Svedmyr, A., Enders, J. F., and Holloway, A., *Am. J. Hyg.*, 1953, v57, 60.

9. Goldblum, N., and Melnick, J. L., *J. Exp. Med.*, 1952, v96, 175.

10. Rustigian, R., Pappenheimer, A. M., and LeBlanc, T., *J. Immunol.*, 1951, v67, 225.

11. Casals, J., Olitsky, P. K., and Anslow, R. O., *J. Exp. Med.*, 1951, v94, 123.

12. Sabin, A. B., *Bact. Rev.*, 1950, v14, 225.

13. Thompson, M. R., *ibid.*, 1947, v11, 115.

14. Wolfson, L., and Rustigian, R., unpublished studies.

Received May 24, 1954. P.S.E.B.M., 1954, v86.

Relationship of Ulcer Pain to pH and Motility of Stomach and Duodenum. (21148)

EDWARD R. WOODWARD AND HERBERT SCHAPIRO.

From Surgical Service, Veterans Administration Center, Los Angeles; Department of Surgery, U.C.L.A.; and Department of Medical Physiology, University of Southern California.

The exact cause of the pain of peptic ulcer has been the subject of controversy for many years. Following Carlson's(1) description of contractions of the stomach synchronous with ulcer pain, this pain was related to motility until Palmer(2-4) found that, in patients with active peptic ulcer, the instillation of 0.5% hydrochloric acid by gastric tube reproduced the pain, and immediate relief followed aspiration. Palmer concluded that ulcer pain resulted from direct chemical irritation of pain fibers in the base of the ulcer crater. Bonney and Pickering(5) confirmed Palmer's work. Ruffin and coworkers(6) reported observations on 56 peptic ulcer patients given an acid barium mixture during fluoroscopic examination. When pain was produced, persistent spasm of either stomach or duodenum occurred. If no pain developed, normal gastric motility and emptying were seen although the acid barium mixture was actually demonstrated in the ulcer crater.

Rollins and Friedlander(7) recorded kymograph tracings of either gastric or duodenal motility in patients with duodenal ulcer while ulcer pain was produced by the intragastric injection of 0.5% HCl. The administration of hexamethonium decreased motility, but did not relieve the ulcer pain. Palmer(8) studied 26 ulcer patients known to have had a positive acid test. Administration of anticholinergic drugs failed to alter the pain response in 23. Dragstedt and coworkers(9) investigated 5 patients with duodenal ulcers and with positive acid tests. Following surgical division of the vagus nerves, intragastric administration of hydrochloric acid produced the characteristic pain response for a period of 5-6 days postoperatively.

In view of the conflicting findings reported, it was decided to make further observations on patients with peptic ulcer pain under conditions whereby simultaneous motility records and pH determinations were obtained from both stomach and duodenum.

Method. Patients selected for study had active but uncomplicated duodenal ulcers. A triple-lumen tube was passed under fluoroscopic control into the duodenum; one lumen was used for inflating a balloon which recorded duodenal motility; another was used for periodic aspiration of duodenal contents; and the third lumen was used for the administration of N/10 HCl. A double lumen tube was then introduced into the pyloric antrum under fluoroscopic control. One lumen was