luted to 15.0 ml has been satisfactory. The dilution will vary with different lots of the material.) 1.0 ml of the mixture of diluted prothrombin, unknown plasma and thromboplastin is added to 2.0 ml of a substrate containing 1 part of a 1% $CaCl_2$ solution, 1 part of imidazole (1.72 g in 90 ml of 0.1 N HCl, this solution then made to 100 ml with water), 2 parts of a 15% acacia solution (calcium-free) and 5 parts of saline. After 10 minutes, 0.4 ml samples of this final mixture are added at accurately timed intervals of 2 minutes to 0.1 ml samples of a 1% fibrinogen solution and the clotting times plotted as described by Ware and Seegers(2). By comparing the curve with those obtained from normal control plasmas the Ac-globulin content of the unknown plasma may be calculated in per cent of normal.

The use of human stored plasma as a source of prothrombin is subject to the same criticism that applies to any process which does not provide for the absolute control of all factors other than the one being measured. Assays in our laboratory for Ac-globulin in the whole stored plasma show less than 0.1% of the normal amount. Antithromboplastin is diminished and in some instances destroyed by freezing(6). Approximately 75% of the normal antithrombin remains, and less than 25% of the physiologic concentration of fibrinogen. Heparin is uncontrolled. All of the above factors including, of course, prothrombin are diluted 20 to 24 times in the final substrate. All of these considerations must be kept in mind but need not interfere with the utility of the method in most instances. A number of comparisons have been made with purified prothrombin† and the crude prothrombin substrate herein described. The Ac-globulin curves are readily reproducible and compare favorably with those obtained with the use of the purified material.

Summary. Oxalated human plasma deficient in Ac-globulin as a result of storage can be used as a source of prothrombin in the 2-stage assay of Ac-globulin.

6. Tocantins, L. M., Personal Communication.

† Dr. Walter H. Seegers kindly supplied us with purified prothrombin.

Received September 4, 1950. P.S.E.B.M., 1950, v75.

A Complement-Fixation Test for Poliomyelitis Virus. (18184)

JORDI CASALS AND PETER K. OLITSKY.*

From The Rockefeller Institute for Medical Research, New York.

For several years this laboratory was concerned with a practical serological test for determination of infection with poliomyelitis virus, apart from that of neutralization. Conventional hemagglutination and precipitin tests failed, as did those by means of complement fixation (CF) which occasionally offered incomplete reactions not totally specific and not in any satisfactory way sufficient as a dependable, practical procedure(1). Hitherto Loring *et al.*(2) stated that CF could be observed, but a degree of non-specificity obscured certain results. Roberts(3) brought forward a flocculation test with a complex antigen; its value has not yet been established by other workers. During this investigation the MEFl strain of poliomyelitis virus(4)

* Acknowledgement is made to Major Leslie C. Murphy, to Major Ralph O. Anslow, to Joan Fitz-Gerald and to Henry Koch for their invaluable aid. Thanks are due to Dr. David Bodian, Johns Hopkins University, for his generous gift of monkey sera.

1. Casals, J., and Olitsky, P. K., in *Diagnosis of Viral and Rickettsial Infections,* edited by F. L. Horsfall, Jr., Columbia University Press, New York, 1949, Chapter 6, pp. 57-82.

2. Loring, H. S., Raffel, S., and Anderson, J. C., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 385.

3. Roberts, E. C., *Public Health Rep.,* 1949, v64, 212.

was used, this strain being of the Lansing type but responding with a more uniform reaction and exhibiting a higher LD_{50} titer in mice than the Armstrong-Lansing strain (5).

The belief arose through these studies on a CF test, that were the virus secured in higher concentration and, perhaps, in a different milieu than adult brain, a successful antigen might be achieved. This belief was apparently justified by the results here described, by which a specific CF test could be successfully and easily performed.

It was found that newborn W-Swiss mice, 1 to 4 days old, relatively resisted the virus after its intracerebral injection. With increasing age to about 21 days, they showed a gradually developing susceptibility to reach then the familiar complete susceptibility of adults. A report by Sabin(6) of similar findings with Lansing virus then appeared which could therefore be confirmed in practically all respects. However a step further was taken; an attempt was made to increase the pathogenicity of the MEFl virus for the relatively resistant infant mice by serial intracerebral passage which included "blind" passages from the 6th to the 9th. The experiment was successful and will be described in detail in another article. The result was that a virus was obtained which was highly active in newborn mice and was of a relatively higher LD_{50} titer than that yielded by adults.†

With this infant-mouse adapted strain of MEFl virus as antigen, the following sera have yielded positive results so far: (a) mouse, immunized with MEFl virus, (b) cotton rat, immunized with the Armstrong strain of Lansing virus, (c) monkey, convalescent after infection with MEFl strain and (d) monkey, immunized with MV strain.

Antigen. The source of material for the preparation of antigen was brain and cord tissue from newborn mice infected intracerebrally with MEFl virus. Serial passages of this virus in 3- and 4-day-old mice, carried out every 3 or 4 days, resulted in an adapted strain which is now in its 32nd passage. The characteristics of the adapted strain of virus were: (1) from 20 to 80%—average 50% of the mice inoculated, were paralyzed or dead by the 5th day, and 90 to 100% by the 10th day; (2) the LD_{50} titer—using 4-week-old mice for titration—was $10^{-4.6}$ in passage 15, $10^{-4.5}$ in passage 23, and $10^{-4.8}$ in passage 28. By comparison, the LD_{50} titer of the standard MEFl virus when propagated in 3- to 4-week-old mice had an average value in 18 titrations of $10^{-3.3}$ with a spread of $10^{-2.9}$ to $10^{-4.2}$.

Preparation of the antigen. 3- and 4-day-old W-Swiss mice were injected intracerebrally with 0.02 ml of a 10^{-1} dilution of virus obtained from the 21st to 24th and the 25th to 28th passages. Mice that were paralyzed on the 2nd to the 5th day following injection were sacrificed and the brain and cord removed. When enough material was available—from 15 to 20 g obtained from 75 to 100 mice—an antigen was prepared using essentially a method previously described (1,7), consisting of repeated extractions with acetone and ether. Following the last ether extraction, the dry material was resuspended in physiological saline solution in proportion of 1 part of diluent to 1 part of wet weight of CNS tissue. The supernatant obtained following centrifugation at 10,600 rpm for 1 hour constituted the antigen; merthiolate in final dilution of 1:10,000 was added and the

4. Schlesinger, R. W., Morgan, I. M., and Olitsky, P. K., *Science,* 1943, v98, 452.

5. Yager, R. H., Olitsky, P. K., and Lahelle, O., *J. Bact.,* 1940, v58, 112.

6. Sabin, A. B., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 394; see also Dalldorf, G. in *Pathogenesis and Pathology of Viral Diseases,* edited by J. G. Kidd. Columbia University Press, New York, 1950, Chapter 4, pp. 31-46.

† The success of the adaptation of the MEFl strain to newborn mice led to a trial with other neurotropic viruses. Thus far B virus has been successfully adapted to such newborn animals, with the result that it possessed a uniform reaction; *Herpes simplex* virus was found after such passage to have an LD_{50} titer in excess of that obtained in adults—a report will follow. Other neurotropic viruses, including Brunhilde and Leon strains of poliomyelitis virus are being studied for possibilities of such adaptation.

7. Casals, J., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 339.

antigen was kept in a mechanical freezer at −20°C. The yield of antigen was approximately 2/3 ml per g of tissue. Antigens similar to the above have been prepared with normal uninfected newborn mice and with such mice infected with the viruses of Japanese (J.B.E.) and Western equine encephalitis (W.E.E.) which were used as controls.

Sera. Immune mouse serum was prepared on separate occasions by injection of infant-mouse adapted virus into adult mice. The latter, which were 2 months old when immunization was begun, were given 3 intraperitoneal injections of virus in dilution 10^{-1}; each injection of 0.5 ml was given at weekly intervals. These mice were bled 10 days after the last injection. Immune sera against W.E.E. and J.B.E. viruses were prepared in a similar manner, with the respective viruses being propagated in infant mice. Prior to the injection of active W.E.E. and J.B.E. viruses, the mice received 2 intraperitoneal doses of formalin-inactivated virus.

Cotton-rat immune serum was prepared over 1 year before the experiments on adaptation of the MEFl virus to infant mice were begun and was kept frozen at −20°C. The Armstrong strain of Lansing virus was used; the animals were immunized with cotton-rat propagated virus, injected once intramuscularly and 3 additional times intraperitoneally; pools of serum were obtained 3 weeks after the first injection and 2 months later. Normal cotton-rat sera were used as controls; in one series of these, 3 of 4 were found to be slightly anticomplementary.

Monkey sera. The sera from 2 convalescent monkeys infected with the MEFl strain were available. These sera had been received seven years ago and had been kept frozen. Also available were pools of sera from monkeys immunized with the Brunhilde, Leon, and MV strains—the MV being a Lansing type virus—which were kindly supplied by Dr. David Bodian. Normal monkey sera were used as controls.

Test. The CF test was performed as previously described(1,7) employing overnight incubation at 2-4°C and a total volume of 0.6 ml per tube. All necessary controls were

TABLE I. Showing a Complement-Fixation Test with Newborn Mouse Brain Antigen and Various Antisera with Controls.

	Antigens																											
	MEFl					J.B.E.					W.E.E.					Normal tissue					Saline							
Serum in dil. (reciprocal)	2	4	8	16	32	64	128	2	4	8	16	32	64	128	2	4	8	16	32	64	128	2	4	8	16	32	2	4	8
Monkey 1, MEFl	4	4	4	2	0																	0	0	0	0	0	0	0	0
Monkey 2, MEFl	4	4	2	0	0																						±	0	0
Monkey, Brunhilde	0	0	0	0	0			±	0	0	0	0			0	0	0	0	0								0	0	0
Monkey, Leon	0	0	0	0	0			0	0	0	0	0			0	0	0	0	0								0	0	0
Monkey, MV	3	3	2	±	0			0	0	0	0	0			0	0	0	0	0								0	0	0
Monkey, normal	0	0	0	0	0			0	0	0	0	0			0	0	0	0	0								0	0	0
Cotton rat, Pool 1, Armstrong-Lansing	4	4	4	4	4																	0	0	0	0	0	0	0	0
Cotton rat, Pool 2, Armstrong-Lansing		4	4	4	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						0	0	0
Cotton rat, Pool, normal	4	4	0	0	0	0	0	4	3	0	0	0	0	0	3	1	0	0	0	0	0						3	1	0
Mouse, Pool 1, MEFl	4	4	4	4	4																	0	0	0	0	0	0	0	0
Mouse, Pool 2, MEFl	4	4	4	4	3-4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0						0	0	0
Mouse, Pool, W.E.E.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	4	4	4	4	4	4						0	0	0
Mouse, Pool, J.B.E.	0	0	0	0	0	0	0	4	4	4	4	4	4	4	0	0	0	0	0	0	0						0	0	0
Mouse, Pool, normal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Numbers 0 to 4 represent absence of and degree of complement-fixation; J.B.E., Japanese B encephalitis; W.E.E., Western equine encephalitis.

TABLE II. Showing a "Box-titration" of Antigen in Graded Dilutions Tested with Serum in Graded Dilutions to Determine Titer of Antigen.

MEF1 antigen (dilutions)	Serum: Cotton rat, in dilutions (reciprocal)								Serum: Mouse, in dilutions (reciprocal)							
	2	4	8	16	32	64	128	256	2	4	8	16	32	64	128	256
Undiluted	4	4	4	4	3-4	3	±	0	4	4	4	4	4	3	±	0
½	3	3	3	2-3	2	2	2	±	2	1	1	1	1	±	0	0
¼	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0
⅛	1	±	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1/16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Normal brain, undil.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

included in each test. It is emphasized that all sera included in each test were tested against 1 or 2 control antigens. In addition, 2 different lots of MEF1 antigen have been prepared and 2 different groups of mice were immunized. The test could be repeated 2 and 3 times with the different sets of materials. Table I shows the results of such tests.

With the exception of sera that were anticomplementary, the specificity of the reaction was manifested. Furthermore, in a test with the Brunhilde and Leon types of hyperimmune monkey serum, it appeared that the result was type specific, *i.e.*, MEF1 antigen only fixed complement in the presence of the Lansing-type antibody. Additional tests with convalescent sera, to be reported, will concern the possibility of cross-reactions between the serological types.

A titration in which serial dilutions of serum were tested against serial dilutions of antigen is shown in Table II.

Table II indicates that the antigen as prepared has a low titer, that is, undiluted antigen reacted with mouse serum, and 1:2 dilution with cotton-rat serum. Incomplete reactions were observed in higher dilutions. Methods for increasing this titer are now being investigated.

Summary. The results of experiments here reported show that the MEF1, a Lansing type of poliomyelitis virus, can be adapted to infant mice in which the virus develops an LD_{50} titer in excess of that found, as a rule, in adult mice. In the medium of newborn mouse brain, and in such an increased titer, the virus now lends itself to the preparation of a suitable antigen for specific and reproducible complement fixation.

Received October 11, 1950. P.S.E.B.M., 1950, v75.

Further Studies of the Beneficial Effect of Glutathione on X-irradiated Mice.* (18185)

W. H. CHAPMAN AND E. P. CRONKITE

From The Naval Medical Research Institute, Bethesda, Md.†

The beneficial effect of glutathione and cysteine on the course of radiation illness has been previously reported(1-3). Glutathione

* The opinions and assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the Navy Department.

† Project NM 006 012.05.01.

1. Chapman, W. H., Sipe, C. R., Eltzholtz, D. C., Cronkite, E. P., and Chambers, F. W., Jr., *Radiology,* 1950, in press.

2. Patt, H. M., Tyree, E. B., Staube, R. L., and Smith, D. E., *Science,* 1949, v110, 213.

3. Patt, H. M., Smith, D. E., Tyree, E. B., and Staube, R. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 18.