The portal vein in 28 dogs was wrapped with Polythene and partially occluded with a tantalum band. The veins were removed for gross and microscopic examination 3 to 60 days later. Extensive venous collateral channels developed in the gastrohepatic mesentery and along the posterior peritoneum, passing into the renal, rectal, and caval veins. In 4 of 6 dogs venous anastomoses were adequate to sustain life following excision of the portal vein 5 days after partial portal occlusion.

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Neutralization of Three Immunological Types of Poliomyelitis Virus by Human Gamma Globulin.* (17399)

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Recent studies of antigenic groups of poliomyelitis virus have shown the existence of at least 3 distinct types.[1,2] Two of these are widespread in their distribution, but the third is represented thus far by only one strain (Leon) isolated in Los Angeles, California in 1937.[3] In an attempt to determine whether this strain is perhaps unusual in its occurrence, or is sufficiently widespread to be considered of importance in the epidemiology of this disease, neutralization tests with human gamma globulin were caried out using this strain as well as representatives of the other two antigenic types, Lansing and Brunhilde.[1]

Methods. The viruses used have been described in detail before,[1] and the pools used are designated as Brunhilde III, Lansing VIII, and Leon I. These virus pools are of high titer, and consist of 10 or more cords of rhesus monkeys killed on the first day of paralysis. Only lumbar and cervical enlargements were used. Aqueous suspensions were prepared with a Waring blendor, and aliquots sealed in glass ampules and stored on dry ice. Titrations of these pools are shown in Table I.

The globulin solution used was a sample supplied by the courtesy of the American National Red Cross who distribute it for measles prophylaxis. The sample used was prepared by E. R. Squibb and Sons, and information regarding it was kindly supplied by Dr. J. W. Palmer of the Squibb Company. The human plasma pool used in the preparation of the globulin pool from which this sample was derived totalled 3,000 liters and consisted of the surplus plasma returned to the American Red Cross by the armed forces after the war. The plasma is therefore representative of about 20,000 to 50,000 individuals who contributed blood to the Red Cross during the war. These individuals lived predominantly on the East Coast and in the Great Lakes Area. A small fraction of the plasma was also collected in the Far West. The plasma was originally dried and subsequently reconstituted. The primary fractionation was made following method 6 of Cohn, *et al.*;[4] the subfractionation was made according to method 9 of Oncley, *et al.*[5] The preparation of the globulin fraction from plasma does not appear to result in deterioration of antibody.[6] The effect of prolonged storage of the plasma in the dried state might conceivably have had a detrimental effect on antibody levels, although this seems doubtful in view of the

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1 Bodian, D., Morgan, I. M., and Howe, H. A., *Am. J. Hyg.*, 1949, **49**, 234.

2 Kessel, J. F., and Pait, C. F., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 315.

3 Kessel, J. F., Moore, F. J., and Pait, C. F., *Am. J. Hyg.*, 1946, **43**, 82.

4 Cohn, E. J., Strong, L. E., Hughes, W. L., Jr., Mulford, D. J., Ashworth, J. N., Melin, M., and Taylor, H. L., *J. Am. Chem. Soc.*, 1946, **68**, 459.

5 Oncley, J. L., Melin, M., Richert, D. A., Cameron, J. W., and Gross, P. M., Jr., *J. Am. Chem. Soc.*, 1949, **71**, 541.

6 Enders, J. F., *J. Clin. Invest.*, 1944, **23**, 510.

TABLE I.
Titrations of Virus Pools Used.

Virus and pool No.	Dilutions of cord suspensions in water							
	10-1	10-2	10-3	10-4	10-5	10-6	10-7	PD50*
Brunhilde III		24/24†	24/25	14/14	4/6	2/6	0/6	10-5.6
Lansing VIII	9/9		13/14	4/6	3/6	0/6		10-4.7
Leon I	15/15	8/8	16/16	8/8	8/8	6/10	3/10	10-6.3

* PD50 calculated with paralytic rates taken on a per cent basis.
† Number paralyzed over number inoculated (accumulated inoculations).

TABLE II.
Neutralization of Poliomyelitis Type Viruses by Human Gamma Globulin.

	Viruses (100 PD50)	Globulin dilutions			NMS*
		10-1	10-2	10-3	
A. 6/7/49	Brunhilde	0/2†	1/2	2/2	3/3
	Lansing	0/2	0/2	2/2	3/3
	Leon	0/2	0/2	2/2	2/3
B. 8/24/49	Brunhilde	0/2	2/4	2/2	
	Lansing	0/1	1/3	2/2	
	Leon	0/2	0/4	2/2	

		Globulin dilutions				Neutralization index§
		10-1	10-2	10-3	PD50‡	
Total A + B	Brunhilde	0/4	3/6	4/4	10-2	10,000
	Lansing	0/3	1/5	4/4	10-2.4	25,000
	Leon	0/4	0/6	4/4	10-2.5	30,000

* Normal monkey serum.
† Number monkeys paralyzed over number inoculated.
‡ Calculated by the method of Reed and Muench.[8]
§ Defined here as the antilog of the sum of the exponents of the PD50 of the virus used and the PD50 dilution of globulin. The neutralization index is thus based on the titer of the virus in aqueous solution, and the assumption is made that virus dilutions and globulin dilutions are equivalent.

results to be described.

Our sample consisted of 2 ml of a solution containing 16.8% of gamma globulin, as compared to 0.74% contained in normal plasma, so that the gamma globulin is not only purified but also concentrated to about 23 times the level of normal plasma. It should contain most of the antibodies of adult plasma. The original solution used in the tests to be described was diluted with physiological saline to prepare 1 to 10, 1 to 100, and 1 to 1000 dilutions. These were used immediately for the first series of neutralization tests and the remaining solution stored at 4° C until used in the repeated tests.

The diluted globulin solution was mixed in equal proportions with virus suspensions to prepare the material for inoculation. The mixtures contained 100 PD50 of virus in 0.4 ml, in each instance. The mixtures were allowed to stand at room temperature for 2 hours, and then at 4° C for 2 hours, before inoculation. Each monkey was inoculated into the left thalamus with 0.4 ml of the globulin-virus mixture according to the method described in detail elsewhere.[7]

All monkeys were observed for 3 weeks for signs of poliomyelitis. Animals which did not show typical signs, including severe paralysis, were then prepared for histopathological examination of brain and spinal cord. The results recorded were based on the results of these procedures. No instances of non-paralytic poliomyelitis were revealed by the

[7] Bodian, D., Morgan, I. M., and Schwerdt, C. E., *Am. J. Hyg.*, 1950, **51**, No. 1.

[8] Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

histological studies.

Results. Results are shown in Table II. They indicate that the neutralizing capacity of gamma globulin in this sample is very high for each of the 3 antigenic types of poliomyelitis virus. The results of the two sets of tests, made with separate virus aliquots, show agreement and indicate that the concentrated gamma globulin can neutralize about 30,000 PD50 of the Lansing and Leon viruses, assuming that virus and globulin dilutions are comparable. Storage of the globulin solution at 4° C for almost 3 months does not appear to have impaired the neutralizing potency.

Discussion. The most interesting result of these tests is that pooled adult gamma globulin may contain as much antibody against Leon virus, as against the representatives of the other two types, which are known to be widespread in distribution.[1,2] Since relatively few strains have thus far been differentiated as to type, it is possible that more representatives of the Leon type will be isolated in the future. The question may be raised as to whether this type may have been more or less restricted to the Far West, where Leon was isolated in 1937, especially since our sample of gamma globulin contained some material from that area. In view of the fact that only a small fraction of the plasma pool was obtained from individuals in the Far West, it is doubtful that the Leon antibody was derived only from this source. Since the titer of Leon antibody was equal to that of the Lansing and superior to the Brunhilde virus, there seems every reason to suppose that the Leon virus type is, or was, of importance in the epidemiology of poliomyelitis. It is of further interest that antibody against Brunhilde virus, the representative of the type currently found to be most prevalent,[1] seemed to be present in somewhat less concentration than antibody against the other two types. It is obvious, however, that this preliminary study should be extended to determine in greater detail not only the geographical distribution of type-specific antibodies in human sera, but also the distribution in time and in various age groups. It is also possible to speculate that viruses of the Brunhilde type, although more prevalent than those of other types, may be inferior as antigens.

Antibody against Lansing poliomyelitis virus has previously been demonstrated in human gamma globulin.[6,9] The fact that antibodies against all 3 known antigenic types of poliomyelitis virus exist in high concentration in gamma globulin adds further emphasis to the report of Bahlke and Perkins,[10] who found that relatively large amounts of this material were ineffective in the treatment of preparalytic poliomyelitis. Although elaborate controls were exercised in their study, and although it is now clear that high polyvalent antibody levels are present in gamma globulin, their conclusion that serum therapy, in any form, is ineffective in poliomyelitis still seems to require some qualification. First, the virus, or viruses, responsible for the cases they attempted to treat was not identified as to type, so that a remote possibility exists that specific antibody was not present in the gamma globulin used. Moreover, unconcentrated hyperimmune monkey serum contains levels of antibody approximately the same as those in human gamma globulin.[11,12] Preparation of gamma globulin from hyperimmune serum could therefore be expected to produce antibody levels about 20 times greater than those found in human gamma globulin.

Summary. A sample of gamma globulin, refined and concentrated about 23-fold from pooled adult human plasma, was tested for neutralizing antibodies against representatives of three distinct antigenic types of poliomyelitis virus, Brunhilde, Lansing, and Leon. The neutralizing potency was high against all three viruses. A dilution of the globulin solution of 1/100 neutralized 100 PD50 of virus in almost every instance. A dilution of 1/1000 failed to neutralize 100 PD50 of virus.

[9] Kramer, S. D., quoted in [10].

[10] Bahlke, A. M., and Perkins, J. E., *J.A.M.A.*, 1945, **129**, 1146.

[11] Morgan, I. M., *J. Immunol.*, 1949, **62**, 301.

[12] Howe, H. A., *Am. J. Hyg.*, in press.

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