Poliomyelitis Virus Antibody in Different Lots of Human Serum Gamma Globulin.* (20756)

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The use of human serum gamma globulin in studies on the prophylaxis of poliomyelitis(1, 2) has created an interest in the antibody content of different samples of this substance. Published reports dealing with the quantitative determination of poliomyelitis antibody in gamma globulin preparations are those of Bodian(3) and Rhodes and Clark(4). Bodian, in tests on one lot of human serum gamma globulin employing a neutralization test in monkeys with 100 PD_{50} of virus, found antibody for all three immunologic types; 50% neutralizing titers of $10^{-2.0}$, $10^{-2.4}$, and $10^{-2.5}$ were reported for immunologic types 1, 2, and 3 respectively. Rhodes and Clark reported on the neutralizing substance demonstrable in mice for Type 2 poliomyelitis virus (Lansing); their studies included several samples of both placental and serum gamma globulins. Fifty-percent neutralizing titers of these preparations were about $10^{-3.5}$ using 8 to 85 LD_{50} doses in mice.

The present report deals with the antibody content of seven different lots of human serum gamma globulin tested in roller tube cultures of monkey kidney tissue; neutralizing activity against viruses representing the three immunologic types of poliomyelitis virus was determined.

Materials and methods. Gamma globulin. All human serum gamma globulin solutions used were from the supplies prepared for the American National Red Cross. Lot 116-1 represents the gamma globulin of serum collected from more than 20000 individuals by the American National Red Cross during World War II. This lot had previously been tested by Bodian(5) for Type 2 antibody in mice. The remainder of the preparations were kindly made available by Dr. W. McD. Hammon and had been used by him and his associates in their field trial studies(1,2). *Viruses employed.* The strains of virus used in these studies were Mahoney (Type 1), MEF-1 (Type 2), and Saukett (Type 3); the history of these strains and their adaptation to tissue culture have been described previously(6).

Neutralization tests. Preparation of cultures. Antibody determinations were carried out in roller tube cultures of cynomolgus monkey kidney tissue. Cultures were prepared with kidney fragments grown for 6 or 7 days under perforated cellophane in roller tubes; the medium employed for obtaining healthy epithelial outgrowth consisted of synthetic mixture 199(7,8) to which was added horse serum to a final concentration of 2% and penicillin and streptomycin in amounts of 100 units and 0.1 mg/ml respectively. The details of preparation of cultures and media will be presented in detail elsewhere. After 6 or 7 days of incubation, the medium used to obtain outgrowth of epithelial cells was removed and replaced by 1.5 ml of a 1:4 dilution of synthetic mixture 199 in Hanks' balanced salt solution (pH 7.4) to which was then added 0.5 ml of inoculum containing virus or virus-globulin mixtures. *Virus-globulin mixtures.* Serial 4-fold dilutions of gamma globulin were prepared using diluted mixture 199 (1:4) as diluent. To each dilution were added equal volumes of virus. Mixtures were incubated for one hour at room temperature before inoculation into cultures. The 0.5 ml inoculum for each culture contained 0.25 ml of virus dilution containing approximately thirty-two 50% tissue culture infecting doses ($TCID_{50}$) mixed with 0.25 ml of each dilution of gamma globulin. At least four cultures were used for each dilution. All samples of gamma globulin were tested simultaneously for antibody against each of the immunologic types of virus in order to minimize variations that may be attributable to testing on different occasions. Results were based on microscopic observations of cultures on the 3rd or 4th day following inoculation. All tubes showing typical signs of virus-induced degeneration were con-

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TABLE I. Neutralization of 3 Immunologic Types of Poliomyelitis Viruses by Different Lots of Human Serum Gamma Globulin.

Viruses (32 $TCID_{50}$)	Gamma globulin (lot #)	Gamma globulin dilutions* 64	256	1024	4096	16384	50% neut. end-point	Neg. log 50% neut. end-point
Type 1†	116–1		1/4‖	4/4	3/4	4/4	483	2.7
	157–3		0/4	2/4	4/4	4/4	1024	3.0
	169		0/4	3/4	4/4	4/4	634	2.8
	170		0/4	2/4	4/4	5/5	1024	3.0
	172		0/4	0/4	4/4	4/4	2048	3.2
	173		0/4	2/4	3/4	4/4	1392	3.1
	174		0/4	2/4	4/4	4/4	1024	3.0
							Avg	3.0
Type 2‡	116–1	0/4	0/4	4/4	4/4		512	2.7
	157–3	0/4	0/4	4/4	4/4		512	2.7
	169	0/4	1/4	4/4	4/4		404	2.6
	170	0/4	0/4	4/4	4/4		512	2.7
	172	0/0	0/4	3/4	4/4		634	2.8
	173	0/4	0/4	4/4	4/4		512	2.7
	174	0/4	0/4	4/4	4/4		512	2.7
							Avg	2.7
Type 3§	116–1	0/8	5/8	6/8	8/8		256	2.4
	157–3	0/8	2/8	6/8	8/8		512	2.7
	169	0/8	4/8	6/8	7/8		404	2.6
	170	0/8	1/8	8/8	8/8		458	2.6
	172	0/8	2/8	7/8	8/8		445	2.6
	173	0/8	3/8	8/8	8/8		338	2.5
	174	0/8	6/8	8/8	8/8		160	2.2
							Avg	2.5

* Expressed as reciprocal of the dilution.
† Mahoney Pool 34-SC7. Titer: $10^{-1.3}$.
‡ MEF-1 " 22 . " $10^{-3.0}$.
§ Saukett " 241-1 . " $10^{-4.5}$.
‖ Numerator = No. of tubes showing cytopathogenic changes. Denominator = No. of tubes inoculated.

sidered infected. Titers were expressed as the dilution of serum added to the virus before inoculation into cultures and were calculated as the 50% neutralizing end points according to the method of Reed and Muench(9). A standardized hyperimmune serum and a sample of normal monkey serum were tested along with the gamma globulin samples; in addition, each test included a virus titration and uninoculated control cultures.

Results. The detailed results obtained are presented in Table I. It is apparent that all lots of gamma globulin tested contained antibody against the three immunologic types of poliomyelitis virus and in relatively uniform proportions. Titers of the different lots for Type 1 antibody were all within a range of 0.5 log; for Type 2 antibody the differences were within a range of 0.2 log; and, for Type 3 antibody, within 0.6 log. The arithmetic average of the logarithms of the titers of the seven lots for each of the immunologic types indicated that the dilution capable of protecting 50% of the cultures against Type 1 virus was $10^{-3.0}$, for Type 2 virus was $10^{-2.7}$, and for Type 3 virus was $10^{-2.5}$.

It is interesting to note that lot 116-1 was reported by Bodian in 1951 to give a neutralization end-point of $10^{-2.8}$ when tested in mice against thirty-two PD_{50} of Lansing virus. In the present study, tests performed in roller tube cultures of monkey kidney revealed that the titer of this pool was essentially the same ($10^{-2.7}$).

Assuming that the gamma globulin preparations employed represent about a twenty-three-fold concentration of the plasma content of this substance(3), it is possible to calculate the antibody content of the original plasma pools. Basing the calculation on the average neutralizing titers against the three types, it may be estimated that the average antibody

values for the original plasma pools were $10^{-1.6}$ (1:44) against Type 1 virus, $10^{-1.3}$ (1:22) against Type 2 virus, and $10^{-1.1}$ (1:13) against Type 3 virus.

Summary. Neutralizing antibody content of 7 lots of human serum gamma globulin against the 3 immunologic types of poliomyelitis viruses were studied in roller tube cultures of monkey kidney tissue. Antibody against the 3 immunologic types of viruses was found to be present in all lots tested. The average of 50% neutralization end points in tests of the individual lots of gamma globulin for type 1 virus was $10^{-3.0}$, for Type 2 virus $10^{-2.7}$, and for Type 3 virus $10^{-2.5}$.

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Potentiating Effect of Iproniazid on the Pharmacological Action of Sympathomimetic Amines.* (20757)

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The antitubercular compound iproniazid (1-isonicotinyl-2-isopropyl hydrazine) was shown by Zeller *et al.*(1,2) to produce a marked *in vitro* and *in vivo* inhibition of mammalian monamine oxidase (MO) while isoniazid (isonicotinic acid hydrazide) was almost without effect. In accordance with these results Schayer demonstrated that iproniazid, but not isoniazid, reduces the deamination of C^{14} labeled epinephrine injected into rats(3). Since MO oxidizes and inactivates several other sympathomimetic amines such as phenylethylamine and tyramine(4,5,6) and thus may limit their pharmacological activity, the question arose as to whether or not the blocking of mammalian MO by iproniazid would change the response to these monamines. In this study the reaction of the nictitating membrane of the cat to the administration of various amines before and after the application of iproniazid is described.

Methods. Cats were anesthetized with pentobarbital and prepared for recording contractions of the right nictitating membrane and the carotid blood pressure. The injections of the drugs, except iproniazid and isonazid, were made into a saline infusion cannula and then flushed into the femoral vein with 2.5 to 3 ml of normal saline. Injections made in this way were prepared in 1 ml volume. Since iproniazid was found to cause equal enzyme inhibition whether given intravenously or intraperitoneally, 0.18 millimoles/kg were administered intraperitoneally. Isoniazid was administered in the same amount and manner. Phenylethylamine, tyramine and epinephrine were given in equal pressor doses to determine the contraction of the normal nictitating membrane; following this iproniazid was administered, and a period of 1 to 2 hours allowed to elapse to permit the enzyme to become inhibited. The three drugs were again administered in the same dosages as before. MO activity was determined for liver and brain homogenates ac-

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