and glucose substrate are not in themselves sufficient for normal metabolism of excised gills in 1 mM sodium chloride. While calcium ions are reported to increase cell adhesiveness (4) addition of amounts equivalent to that found in blood did not materially increase O_2 consumption.

The results obtained with rutin tend to confirm the current opinion(5) that this substance has no particular influence on the maintenance of the intercellular cement.

The increased tolerance to dilute solutions exhibited by excised gills at 30°C over that found at 37°C adds support to the contention of Zeidman(2) that increased cellular adhesion with lower temperatures is a factor of importance to poikilothermic animals. This seems to be true, at least for fresh-water forms.

Summary. Excised gills of goldfish cannot maintain a respiratory rate as high in 1 mM sodium chloride as gills in 125 mM sodium chloride at 37°C even though the intact fish can live indefinitely in these solutions. Studies on the oxygen consumption of gill tissue supplemented with histological observations, indicate that the gill is protected not only by increasing the sodium chloride concentration to 125 millimolar, but by reducing the temperature to 30°C. However, the tissue is not significantly benefitted by the addition of either calcium or rutin to 1 mM sodium chloride.

4. Hober, R., Physical Chemistry of Cells and Tissues, Philadelphia, Blakiston, 1945.

5. Clark, W. G., and MacKay, E. M., *J.A.M.A.*, 1950, v143, 1411.

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Passive Immunity in Poliomyelitis: II Lansing Antibody Content of Human Gamma Globulins.* (18496)

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Work is being carried out in these laboratories on some of the problems of passive immunity in experimental poliomyelitis, for which the Lansing strain of virus serves as a convenient model. On the one hand, attempts are being made to produce in monkeys, horses, and rabbits large quantities of high titer antiserum that can be subjected to fractionation. A report on one aspect of this part of the study has been prepared, describing the production of immune serum in the horse(1). On the other hand, search is also being made for a potent source of Lansing antibody from human sources, for it has been known for some years that gamma globulin prepared from pools of human plasma contains this antibody, as demonstrated by virus neutralization or passive protection experiments in mice(2,3). More recently, Bodian (4), carrying out virus neutralization tests in monkeys, has extended these earlier observations and shown that plasma gamma globulin contains antibodies for not only the Lansing, but also the Brunhilde and Leon types, in high titer. He found, for example, that gamma globulin diluted 1:100 neutralized 100 PD_{50} of virus.

This paper describes virus neutralization tests and passive protection experiments carried out in mice, with plasma as well as placental gamma globulin of human origin.

Materials. The placental globulin (Lederle) was purchased commercially. This product contains at least 16% gamma globulin and is sold for the prevention or modification of measles. The plasma gamma globulin was fur-

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1. Rhodes, A. J., Clark, E. M., and Shimada, F. T., *Canad. J. Publ. Hlth.*, 1951, v42, 23.

2. Enders, J. F., *J. Clin. Invest.*, 1944, v23, 510.

3. Stokes, J., Jr., *Yale J. Biol. Med.*, 1944, v16, 415.

4. Bodian, D., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 259.

nished by the American National Red Cross. This product likewise contains at least 16% gamma globulin and is recommended for measles prophylaxis. Both of the gamma globulin preparations contained 1:10,000 merthiolate.

Methods. Virus neutralization tests. The neutralization test that we have used is similar to that employed in the studies of Morgan(5), and the technical details have been fully described(1). Briefly, serial 10-fold dilutions of gamma globulin were prepared in normal saline. To these dilutions were then added equal volumes of Lansing virus mouse pool. The pool was used in various dilutions, so that the final amounts of virus in the mixtures inoculated in mice varied from 8 to 85 LD_{50}. After one hour of contact at room temperature, groups of 10 mice (males 14-16 g weight) were inoculated cerebrally with 0.03 ml quantities of the virus-globulin mixtures. The mice were observed for 5 weeks and all deaths occurring after the day following inoculation were regarded as specific. The 50% neutralizing capacity of the globulin was then calculated by plotting the probits of the percentage mortalities in the groups of mice against the globulin dilutions in logarithms, drawing a straight line and reading off the globulin dilution where 50% mortality might be expected. It should be realized that the dilutions of globulin mentioned in this paper refer to the dilutions before admixture with an equal volume of virus.

Passive protection experiments. In these experiments, various materials containing Lansing antibody, along with suitable controls, were inoculated peritoneally (0.3 ml) in 14-16 g male mice. Twenty-four hours later the mice were challenged by the cerebral route with 5 or 25 LD_{50} of Lansing virus, and thereafter were observed for 5 weeks.

Results. Virus neutralization tests. The results of 16 titrations of lot No. 2167 of placental gamma globulin on 11 different working days are given in Table I. The average 50% neutralizing titer was $10^{-3.5}$, the lowest figure in any titration being $10^{-2.5}$ and the highest $10^{-4.0}$. These differences are probably not greater than can be explained by the operation of chance, having regard to the rather widely spaced dilutions of globulin used. It will be apparent that the neutralizing titer was little affected by the amount of virus added to the virus-globulin mixtures, as shown for instance in the tests performed on 26 October, 1950. It has also been demonstrated that heating gamma globulin to 56°C for 30 minutes does not lower the antibody titer (Experiment of 1 November, 1950).

The 7 lots of plasma gamma globulin were prepared from very large pools of human plasma, each representing at least 20,000

TABLE I. Estimation of 50% Neutralizing Titers of Placental Gamma Globulin.

Date of test	Reference No. of sample tested	Amt of virus in neutralization tests (in LD_{50})	50% neutralizing titer
21 Sept. '50	2167-56A	85	$10^{-2.5}$
29 "	"	85	$10^{-4.0}$
3 Oct.	"	85	$10^{-3.75}$
18 "	"	85	$10^{-3.35}$
20 "	2167-86A	85	$10^{-2.8}$
20 "	"	17	$10^{-3.46}$
24 "	"	17	$10^{-3.65}$
26 "	"	85	$10^{-3.53}$
26 "	"	25	$10^{-3.0}$
26 "	"	12	$10^{-3.75}$
26 "	"	8	$10^{-3.5}$
1 Nov.	" (heated 56°C ½ hr)	17	$10^{-3.75}$
1 Nov.	2167-86A (unheated)	17	$10^{-3.75}$
2 "	2167-86A	17	$10^{-3.75}$
8 "	"	17	$10^{-3.55}$
10 "	"	17	$10^{-3.82}$

TABLE II. Lansing Virus Neutralization Antibody Titers of 7 Samples of Human Gamma Globulin (Plasma).

Globulin Lot No.	Amt of virus in neutralization tests (in LD_{50})	50% neutralizing titer of globulin
113-1	21	$10^{-3.0}$
116-4	21	$10^{-3.5}$
120R-3	21	$10^{-3.65}$
121-1	21	$10^{-3.27}$
127-2	21	$10^{-4.0}$
128-2	21	$10^{-3.0}$
129-2	21	$10^{-2.87}$

5. Morgan, I. M., *Am. J. Hyg.*, 1947, v45, 372.

TABLE III. Passive Protection Experiment in Mice.

Inoculum administered (0.3 ml peritoneally 24 hr before virus)	50% neutralizing titer of material inoculated	Mortality in mice challenged cerebrally with following doses of virus	
		25 LD_{50}	5 LD_{50}
Placental gamma globulin	$10^{-3.5}$	8/20 (40%)	4/19 (21.2%)
Immune monkey serum	$10^{-2.5}$	12/20 (60%)	7/20 (35 %)
Immune horse serum	$10^{-2.5}$	18/19 (95%)	13/20 (65 %)
Normal monkey serum	Nil	19/19 (100%)	15/19 (79 %)
Normal horse serum	Nil	19/19 (100%)	19/19 (100 %)

TABLE IV. Passive Protection Experiment in Mice.

Inoculum administered (0.3 ml peritoneally 24 hr before virus)	50% neutralizing titer of material inoculated	Mortality in mice challenged cerebrally with following doses of virus	
		25 LD_{50}	5 LD_{50}
Plasma gamma globulin	$10^{-2.63}$	10/18 (55.6%)	5/19 (26.3%)
Placental gamma globulin	$10^{-3.5}$	8/18 (44.5%)	5/20 (25 %)
Saline		19/20 (95 %)	17/19 (89.5%)

donors. The 50% neutralizing capacities are shown in Table II, the titers ranging from $10^{-2.87}$ to $10^{-4.0}$. It is not considered that the observed differences in titer are significant. The antibody content would appear to be similar to that of placental globulin.

Passive protection experiments. Following some preliminary experiments, two rather detailed experiments have been performed in which the protective action of various sera and globulins containing Lansing antibody has been demonstrated. These preparations were inoculated in groups of twenty 14-16 g male mice by the peritoneal route (0.3 ml), and 24 hours later the animals were challenged with an estimated 25 or 5 LD_{50} of Lansing virus. The results of the first experiment are shown in Table III, from which it will be seen that when compared with controls, placental gamma globulin exerted a significant sparing effect against challenge with 5 LD_{50} and even 25 LD_{50} of virus. These results are similar to those recorded by Kramer(6) using human convalescent serum. It will also be noted that some sparing effect was shown by an immune monkey serum.

In the second experiment, plasma gamma globulin (lot No. 116) was included and compared with placental gamma globulin. The results are shown in Table IV from which it is evident that both products give significant protection against death in mice inoculated with 25 and 5 LD_{50} of virus.

Conclusions. Both placental and plasma gamma globulins contain Lansing antibody. The 50% neutralizing titer of these products is approximately $10^{-3.5}$, as determined in mice. It would seem that a more potent gamma globulin could be obtained if prospective donors were first tested for serum Lansing antibody level. Then only those with high levels would be used to furnish plasma for the pools to be fractionated. Placental and plasma gamma globulins have a significant sparing effect when inoculated in mice 24 hours before a cerebral challenge of Lansing virus.

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6. Kramer, S. D., *J. Immunol.*, 1943, v47, 67.

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