

10782 P

Standardization of the Anti-viral Content of Immune Globulin from Placental Extract.

GEOFFREY RAKE.

From the Squibb Institute for Medical Research, New Brunswick, N. J.

It is now accepted that preparations of globulin from human placentas can modify or prevent measles in humans.¹ In addition to its content of anti-measles antibody indicated clinically, the placental extract has been shown to contain diphtheria and scarlet fever antitoxins and antipoliomyelitis antibodies.² At the present time the diphtheria antitoxin content is widely employed as a means of determining the potency of different preparations; and, despite the fact that the antitoxic and antiviral antibodies are distributed differently in the placental extract, with the former entirely in the pseudoglobulin and the latter distributed in several fractions,³ it has been stated that the determination of the diphtheria antitoxin titer is satisfactory as a basis for determining the anti-measles potency of different preparations.

Ideally, each preparation should be standardized for its anti-measles antibody content. Since, however, the lack of a susceptible animal other than the monkey renders such standardization at present impossible, it would seem advisable to titrate the different preparations for some other antiviral antibody. Poliomyelitis virus neutralization tests have been successfully carried out³ but here again no routine standardization is possible. Antibodies for other viruses are to be found in human sera, however, and those against swine influenza are probably as widespread as those against measles. These influenza antibodies are readily titratable in the albino Swiss mouse and different preparations of placental extract can be tested for this antiviral antibody. Such tests are reported below.

Lungs of mice, sacrificed while moribund from swine influenza infection* after intranasal inoculation and showing extensive lung involvement, were used after being preserved in 50% glycerine from 1-5 days. Such lungs were ground in sufficient tyrodes to give a 1/50 dilution of the wet weight of the lung. Half cc amounts of

¹ Council on Pharmacy and Chemistry, *J. A. M. A.*, 1938, **111**, 1764.

² McKhann, C. F., and Chu, F. T., *J. Inf. Dis.*, 1933, **52**, 268.

³ McKhann, C. F., Green, A. A., and Coady, H., *J. Pediat.*, 1935, **6**, 603.

* The swine influenza virus was received from Dr. R. E. Shope to whom for this and many helpful suggestions I wish to express my thanks.

the 1/50 suspension of virus were mixed with $\frac{1}{2}$ cc amounts of the placental extract dilution to be tested and the mixture allowed to stand for 30 minutes at 37°C. As a control a saline solution of preservative at the same strength as that of the maximum present in any one of the placental extract dilutions was mixed and incubated with the virus in the same way. 0.04 cc amounts of these incubated mixtures were given intranasally to anesthetized Swiss mice, using a minimum of 4 mice for each dilution tested. The mice were observed for 8 days and then sacrificed and examined for lung lesions. Table I shows typical results. Here dilutions of these 2 placental extracts showed some protection at 1/80 and good protection at 1/20. One specimen of placental extract P¹ has been chosen as a standard to which all others may be referred. After several titrations of its potency it has been given a provisional titer of 100 units per cc.

TABLE I.

Placental preparation	1/5	1/20	1/80
243-254	0* 0 0 0	0 0 0 0	+++† ++ ++ ++
257-268	0 0 0 0	+ 0 0 0	+++ ++ ++ +
Control		4‡ 5 5 6	

‡ Mouse died with characteristic lung lesions on the 4th day after inoculation.

† Mouse killed on the 8th day and showed approximately $\frac{3}{4}$ of the lungs consolidated (++ = approximately $\frac{1}{2}$ of the lungs consolidated).

* Mouse killed on the 8th day and showed normal lungs.

This method of titration has been used to follow the distribution of antiviral antibodies during the purification and concentration of the placental extract. Table II shows the results of the testing of various fractions. It will be seen that fractions C, D, and E contain no neutralizing antibodies; A, B, and F contain varying amounts. The technic used was that given above. All survivors were sacrificed on the 8th day and examined for lung lesions.

TABLE II.

	+++	0	0	0
A	+++	0	0	0
B	+++	+++	+++	++
C	4	5	5	6
D	5	5	5	6
E	4	5	5	7
F	+++	+++	++	±
Control	4	5	5	7

It was important to show that the neutralization being obtained was due to specific antiviral antibodies. There was a possibility of a non-specific destruction of the virus by hypothetical toxic substances in the placental extract. This supposition was rendered improbable by the fact that, in following up the fractionation of the placental

extract, no activity was found in those fractions which have been shown clinically to have no anti-measles activity. As a first control of this theoretical objection it has been shown that the same placental extract preparations which protect when used in the technic given above also protect when they are given to the mouse 15 minutes before the virus suspension. Thus the mixing and standing of placental extract and virus for 30 minutes at 37°C is eliminated. Furthermore, neutralization tests were carried out with a virus, Eastern Equine Encephalomyelitis, antibodies for which are not to be expected generally distributed in the sera and placentas of normal adult females. The strain used was killing mice regularly through 10^{-8} and over 50% at 10^{-9} . Fresh mouse brain was used from a mouse moribund with the infection. Dilutions of the brain were made in broth through 10^{-11} . For testing the placental extract samples the dilution 10^{-8} was used. Half cc amounts of this were mixed with $\frac{1}{2}$ cc amounts of the different fractions and of the merthiolate control, and the mixture placed at 37°C. for 30 minutes. Then 0.04 cc of each mixture was inoculated intracerebrally in each of 4 normal Swiss mice. Control titrations of the virus alone were made at 10^{-7} and through 10^{-11} . Table III shows the results. No neutralization is to be observed.

TABLE III.

A	42,* 68, 90, 90	Virus Titration
B	43, 48, 66, 66	10^{-7} 43, 43
C	43, 43, 48, 48	10^{-8} 48, 66
D	48, 48, 66, 72	10^{-9} 66, 66, S†
E	66, 66, 72, 90	10^{-10} S, S, S
F	48, 66, 66, 66	10^{-11} S, S, S
Control	48, 66, 66, 66	

*Died in 42 hours.

†S = Survived.

Thus a method of titrating the antiviral neutralizing antibodies of placental extract for the swine influenza virus has been demonstrated. This is believed to be a specific antiviral action and not due to non-specific toxic fractions in the placental extract. The suggestion is made that standardization of the antiviral antibodies of placental extracts by this method may prove a better indication of the anti-measles content of such extracts than does the present method of titrating the diphtheria antitoxin content. The adoption of a provisional standard extract and provisional unit will allow further investigation to be carried out on a quantitative basis.