

the loss of the hypotensive factor indicating specific neutralization of the factor (Table II, A).

To prove further that the hypotensive factor was a fraction of the virus particles, the concentrated and purified preparation was mixed directly with the immune serum. Injection of the mixture into rats showed a significant reduction of the hypotensive effect in comparison with the control material to which normal ferret serum was added (Table II, B, C).

6. To elicit the hypotensive response a threshold concentration of virus with hemagglutination titer of 1:8000 was required. Concentrations of virus with titer between 1:16,000 and 1:64,000 elicited about the same response (Fig. 2).

Conclusion. These observations indicate that a fraction of the PR8 strain of influenza virus possesses the toxic property of lowering arterial blood pressure in rats.

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Effect of Congo Red on the "MM" Virus. (17405)

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Conflicting reports on the effect of trypan red on the "MM"¹ neurotropic virus have appeared in the literature. Wood and Rusoff² reported this compound along with two other acid dyes, Congo red and brilliant vital red, to be definitely effective against the virus. Using the intraperitoneal injection route followed by intraperitoneal inoculation of the virus, these workers found an apparent protection which was overcome by inoculation of the virus in too high concentration.

Hammon *et al.*³ have reported the failure of trypan red to protect against the "MM" virus when the dye was injected subcutaneously and the virus inoculated by intraperitoneal route. These authors interpreted the results of Wood and Rusoff as being due to a non specific protection such as afforded by various inert substances when injected previous to the inoculation of infectious agents by the same route.

Since the effect of Congo red had been reported for only one concentration of "MM"

virus it was thought desirable to determine the activity over a range of dilutions.

In one series of experiments Congo red was injected intraperitoneally into 12 g mice and in a second series the dye was administered by stomach tube. As a matter of interest trypan red was included in 2 of the experiments. All virus inoculations were by the intraperitoneal route, serial dilutions being made from frozen stock material. A range (10-fold) of infecting dilutions was employed so as to permit calculation of LD₅₀ values by the method of Reed and Muench.⁴ Unless otherwise specified, dilutions of 10⁻⁶ through 10⁻⁹ were used for controls and 10⁻⁵ through 10⁻⁸ for test groups, 6 mice being used per dilution.

In all experiments calling for the injection of dye the schedule employed was that used by Wood and Rusoff. A 1% solution was injected in 0.1 cc amounts on each of 3 days and the virus was administered one day after the last injection.

For the experiments in which the dyes were given by stomach tube the schedule called for the feeding of 0.5 ml of a 1% solution twice daily for 9 feedings with the virus inoculation following the fourth feed-

¹ Jungeblut, C. W., and Dalldorf, G., *Am. J. Pub. Health*, 1943, **33**, 169.

² Wood, H. G., and Rusoff, I. I., *J. Exp. Med.*, 1945, **82**, 297.

³ Hammon, W. McD., Aird, R. B., and Sather, Gladys, *Proc. Soc. Exp. Biol. and Med.*, 1948, **69**, 511.

⁴ Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

TABLE I.
Effect of Intraperitoneally Injected Dye on "MM" Virus.

	Total No. of mice	Control, LD ₅₀	Test, LD ₅₀	LD ₅₀ Difference
Congo red	{ 54	8.2	6.0*	-2.2
	{ 100	7.0†	7.1‡	+0.1
	{ 48	7.5	>5.0	>-2.5
Trypan red	48	7.0	7.7	+0.7

* Dilution range 10⁻⁴ through 10⁻⁸.

† Dilution range 10⁻⁵ through 10⁻⁹, 10 mice per dilution.

‡ Dilution range 10⁻⁴ through 10⁻⁸, 10 mice per dilution.

TABLE II.
Effect of Orally Administered Dye on "MM" Virus.

	Total No. of mice	Control, LD ₅₀	Test, LD ₅₀	LD ₅₀ Difference
Congo red	{ 48	7.5	5.2	-2.3
	{ 48	8.2	5.8	-2.4
Trypan red	48	7.0	5.6	-1.4

ing. It was felt that two daily feedings would roughly approximate the amounts administered in drug diets.

The results of experiments in which the dye and the virus were administered by intraperitoneal injection are shown in Table I. It can readily be seen that any protection index based on the LD₅₀ differences recorded for Congo red will have a wide range and the validity of a claim for protective action would be open to serious question. The erratic results presented are typical of other experiments and it is not felt that a truly protective compound would be subject to such variable results.

Table II shows the effect of dye administered orally, the virus being given by intraperitoneal injection. While the apparent protection may be due to non specific factors, such as handling of the mice, it is of interest to note that such apparent protection may be

evoked by administration of the dye through a route other than that used for the virus injection.

Calculation of the LD₅₀ standard errors would undoubtedly show that little confidence could be placed in the LD₅₀ differences recorded and it is not felt that any of the differences are indicative of significant protection. In considering the work of Wood and Rusoff it would not seem that a really effective compound would be active against one concentration of virus only and we feel that the expression "apparent protection" is more accurate.

Conclusions. Congo red was found to stimulate an apparent protection against the "MM" virus when administered by a route other than that used for virus inoculation. It is not felt that the degree of protection is significant.

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