the rest of the brain and then from the olfactory nerves without injury to the cribriform plate and lifted out without any pull on structures in the nasal mucosa (Table I). It would appear, therefore, that there is as yet no evidence that nasally instilled viruses invade the CNS by any direct, open space; the available data seem to point rather to progression along nerve cells and their processes.

Conclusions. Different neurotropic viruses instilled into the nose of the same host (mouse) can select different nervous pathways for invading the central nervous system. The viruses of vesicular stomatitis and equine encephalomyelitis were shown to use the olfactory pathway but not the trigeminus, sympathetic, or parasympathetic pathways, while pseudorabic virus invaded along the latter three routes and not along the olfactory.

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Inhibition of Streptococcal Hemolysin by Sulfonamide Compounds.

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In vitro experiments, in which prontosil I* appeared to inhibit streptococcal hemolysin and leukocidin, have led Levaditi and Vaisman[1] to believe that certain sulfonamide compounds exert an antitoxic action.

The present report concerns the effect of sulfanilamide and other substances upon streptococcal hemolysin, and also the effect of sulfanilamide upon staphylococcal hemolysin.

Hemolysin was prepared by growing beta hemolytic streptococcus strain C 203 in 2% neopeptone beef broth of pH 7.6-7.8 for 16-18 hours and removing most of the bacteria by centrifugation.

In some experiments this hemotoxin was incubated with various amounts of sulfanilamide† (50 mg. per 100 cc. up to saturation) for 30 minutes at 37.5°C. The untreated toxin and the sulfanil-

* Prontosil I (4-sulfonamide-2',4'-diaminoazobenzol) and prontosil II (disodium salt of 4-sulfamidophenyl-2'-azo-7'acetylamino-1'hydroxynaphthalene-3',6' disulfonic acid) kindly supplied by Winthrop Chemical Co., N. Y.

1 Levaditi, C., and Vaisman, A., *Compt. rend. Soc. de biol.*, 1935, **120**, 1077.

† Kindly supplied by E. R. Squibb & Sons, New York.

amide-toxin were then titrated with washed rabbit erythrocytes. An incubation period of 45 minutes at 37.5°C. was allowed before the tubes were centrifuged and readings made.

The addition of sulfanilamide to streptococcal hemolysin up to the point of saturation did not remove the hemolytic effect of the toxin. However, a very slight inhibitory action of the sulfanilamide was demonstrable provided a careful and close titration was done (Table I).

TABLE I.
Comparison of Hemolytic Action of Untreated Streptococcal Hemolysin with That Incubated with Sulfanilamide.

Toxin A 1:10 cc.*	Toxin B 1:10 cc.*	2% Rabbit R.B.C. cc.	Saline cc.	Hemolysis	
				Normal Toxin	Toxin saturated with sulfanilamide
.3		.1	.3	+	0
.4		.1	.2	++	+
.5		.1	.1	+++	++
.6		.1	.0	++++	+++
	.1	.1	.4	0	0
	.2	.1	.3	++++	+
	.3	.1	.2	++++	++++
	.4	.1	.1	++++	++++

*C 203 Toxin.

This slight inhibitory effect of sulfanilamide was apparent in numerous titrations only when the amounts of toxin corresponded to one minimal hemolytic dose or less. It was observed that if amounts of toxin in excess of one minimal hemolytic dose were used, complete hemolysis was obtained regardless of the amount of sulfanilamide present.

The effects of prontosil I and II* as well as of other substances upon streptococcal hemolysin were similarly investigated. Toxin samples containing the following: 0.4% prontosil I, 0.4% prontosil II, 0.2% eosin, 0.2% mercuric chloride, 0.2% phenol, and 0.4% sodium carbonate were incubated for 30 minutes at 37.5°C. and then titrated along with untreated toxin using 4% rabbit erythrocytes. After 45 minutes of incubation at 37.5°C. the tubes were centrifugated and readings taken.

Marked inhibition of hemolysis occurred with prontosil II, eosin, mercuric chloride, phenol, and sodium carbonate but the hemolytic titre of toxin containing 0.4% prontosil I was identical to that of untreated toxin (Table II).

TABLE II.
Comparison of Hemolytic Action of Untreated Streptococcal Hemolysin with That Incubated with Various Chemicals.

Toxin Mixture* cc.	4% Rabbit R.B.C. cc.	Saline cc.	Hemolysis: Toxin Alone	Prontosil I 0.4%	Prontosil II 0.4%	Eosin 0.2%	$HgCl_2$ 0.2%	Phenol 0.2%	Na_2CO_3 0.4%
.03	.1	.47	0	0	0	0	0	0	0
.06	.1	.44	+	+	0	0	0	0	+
.09	.1	.41	++	++	0	0	0	0	+
.12	.1	.38	+++	+++	0	0	0	0	+
.15	.1	.35	++++	++++	+	0	0	0	+
.18	.1	.32	++++	++++	+	0	0	0	+

*To 2.0 cc. portions of C 203 toxin 0.5 cc. saline and 5 or 10 mg. of the various inhibitory substances were added to make 0.2 or 0.4% solutions as indicated. These portions were then incubated for 30 minutes at 37.5°C. prior to titration.

Normal rabbit serum was found capable of inhibiting hemolysis by streptococcal hemotoxin.[2] This inhibition was not enhanced by the addition of sulfanilamide. Identical results were obtained with sera from rabbits which had received sulfanilamide orally (1.0 to 1.7 gm. per kilo) 4 hours prior to bleeding. These sera, containing 5 to 56 mg. of sulfanilamide per 100 cc., were no more inhibitory than the sera taken from the same animals prior to the administration of the drug.

The inhibitory effect of the sera was not destroyed by heating for 30 minutes at 55 to 61°C., and was found to be more pronounced in the case of a preliminary incubation of erythrocytes with serum than in the case of a preliminary incubation of toxin with serum.

Experiments with staphylococcal hemolysin† also indicated a very slight inhibitory effect when the hemotoxin was given a preliminary incubation with sulfanilamide (300 mg. per 100 cc.). This inhibitory effect could be observed only at and below one hemolytic dose.

A number of repeated tests with hypotonic saline indicated that the fragility of rabbit erythrocytes was not affected by the presence of sulfanilamide.

The demonstration in these experiments that prontosil I does not inhibit the hemolytic activity of streptococcal hemotoxin is opposed to the findings of Levaditi and Vaisman[1] and throws some doubt upon their assumption that sulfonamide compounds are antitoxic. Although a marked inhibitory effect upon the hemolytic activity of streptococcal hemotoxin was obtained with prontosil II and a very slight one with sulfanilamide, the assumption that this effect is due

2 Zinsser, H., and Bayne-Jones, S., *A Textbook of Bacteriology*, Seventh Edition, D. Appleton-Century Co., New York, 1935, p. 319.

TABLE III.

Comparison of Inhibitory Action of Untreated Serum (on Streptococcal Hemolysin) with That of Serum Containing Various Concentrations of Sulfanilamide.

C 203 Toxin cc.	Rabbit Serum cc.	4% Rabbit R.B.C. cc.	Saline cc.	Hemolysis					
				No Serum	Serum containing sulfanilamide mg. per 100 cc.				
					0	10	100	500	Saturated
.10	—	.1	.40	++++					
.15	.05	.1	.30		+	+	+	+	+
.15	.04	.1	.31		+	+	++	+	++
.15	.03	.1	.32		++	++	+++	+++	+++
.15	.02	.1	.33		+++	++++	++++	+++	+++
.15	.01	.1	.34		++++	++++	++++	++++	++++

to antitoxic action does not appear warranted because other substances such as normal serum (heated to 61°C.), eosin, phenol, mercuric chloride, and sodium carbonate have a similar effect.

Conclusions. Sulfanilamide causes a slight, almost negligible, inhibition of the hemolytic activity of streptococcal and staphylococcal hemotoxin. More marked inhibition of streptococcal hemolysin is produced by prontosil II, normal rabbit serum, rabbit serum heated to 61°C., eosin, phenol, mercuric chloride, and sodium carbonate. The administration of sulfanilamide *in vivo* or its addition to serum *in vitro* does not enhance the inhibitory effect of rabbit serum upon streptococcal hemolysin. Prontosil I was found to have no inhibitory effect upon streptococcal hemolysin.

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Protective Effects of $CaCl_2$ Against Procaine Convulsions in Guinea Pigs.*

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In order to protect animals against the convulsions from high doses of procaine-HCl, an addition of $CaCl_2$ was found to be effective. Guinea pigs, which *rarely* succumb to procaine convulsions, are more suitable for such studies than rabbits, which were formerly used by Beutner, Prusmack, and Miller[1]; rabbits almost invariably die from procaine convulsions. Guinea pigs, however, can be injected repeatedly with convulsant doses. In the case of procaine, just as with various other previously observed convulsant drugs,[2] numerous observations are necessary to determine the average convulsant dose.

As controls, 55 guinea pigs received 159 injections of 100 mg. per kilo, as 10% solution, of procaine-HCl *alone.* This means that each of these 55 pigs was injected at least twice and some as many

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1 Beutner, Prusmack, and Miller, *J. Pharm. and Exp. Therap.*, 1936, **57**, 114.

2 Lennox, Nelson, and Beetham, *Arch. Neurol. and Psych.*, 1929, **21**, 625; *Proc. Physiol. Soc. of Philadelphia*, 1937, **12**, 10.