vestigate the action of nicotinic acid on the isolated rabbit's heart are reported. Dilutions of from 1:100,000 to 1:2,000,000 were employed, and careful attention was paid to constancy of temperature and hydrogen-ion concentration.

It was found that the effect of nicotinic acid on well functioning hearts is insignificant. In the case of failure of the myocardium, however, this pyridine derivative causes a marked increase in amplitude of the cardiac excursion, reversal of abnormal rhythms (including partial heart block, ventricular fibrillation, and ventricular standstill), and at times a considerable augmentation of coronary flow. In no case does it appear to have an unfavorable influence.

The explanation of these effects may lie in the role played by nicotinic acid in carbohydrate metabolism. The idea is advanced that the observed disturbances of myocardial action are due to inactivation or depletion of the pyridine nucleotides by the anoxia incident to experimental technic; and that restoration of normal function probably depends on correction of this temporary and reversible deficiency by the addition of nicotinic acid to the perfusion fluid.

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Inactivation of Influenza Virus with Sulfur and Nitrogen Mustards.

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It has been demonstrated that sulfur and nitrogen mustards react chemically with a variety of proteins and enzymes of biological importance, and in so doing alter their physical properties and physiological functions. Presumably it is by such a mechanism of action that mustards abolish the infectivity of the nucleoprotein plant viruses causing tobacco mosaic and bushy stunt disease.[1] Recently, Tenbroeck and Herriott[2] have shown that the animal viruses of eastern equine encephalomyelitis, fixed rabies and hog cholera also may be rendered noninfectious by treatment with sulfur mustard without, however, altering their antigenicity. These mustard-inactivated viruses provoked a specific, protective immune response when injected into animals, suggesting the practical application of this method for the preparation of killed viral vaccines.

The present report deals with an investigation of the effects of sulfur mustard, bis-betachloroethyl sulfide (HS), and nitrogen mustards, bis- and tris-betachloroethyl amine (HN), on influenza virus. It will be shown that both types of mustard destroy the ability of this virus to infect susceptible animals, under appropriate conditions, but that high concentrations of these substances fail to alter its hemagglutinative properties.

Experimental. Effect of HS on Infectivity of Influenza Virus. The influenza virus employed was the PR8 strain in mouse lung passage. Dilutions of the virus in nutrient broth were made from 10% suspensions of infected lung, and the LD_{50} was found to be approximately .05 cc intranasally of a 10^{-6} dilution.

An approximately saturated solution of HS was prepared by adding 0.1 cc of HS to 50 cc of ice-cold 0.85% saline in a 250 cc stoppered flask, shaking vigorously by hand for one minute, and then placing the mixture in the refrigerator for 5 minutes to allow the undissolved residue of HS to separate and settle on the bottom of the flask. The assumption was made that the concentration of HS in the supernatant saline approached

1 Gilman, A., and Philips, F. S., *Science*, 1946, **103**, 409.

2 Tenbroeck, C., and Herriott, R. M., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 271.

6×10^{-3} M, according to published data.[3] The saline saturated with HS was mixed with an equal volume of virus diluted 10^{-2}; similar mixtures of virus were made with HS saturated saline diluted 1-5, and with saline alone. The final concentrations of HS were therefore half-saturated and 10th-saturated, while the final concentration of the virus in each mixture was 5×10^{-3}. The preparations were kept at 4°C for 3 hours, and then each was inoculated into 5 mice by instilling .05 cc intranasally under ether anesthesia. The animals were observed for a period of 10 days, at the end of which time the survivors were sacrificed and examined.

All of the control animals died between the 5th and 8th days after inoculation and in every instance their lungs showed complete consolidation. The mice inoculated with the virus exposed to HS at 10th-saturation also were infected, although the disease was slightly less rapid and intense than in the controls; 4 mice died from 7 to 10 days after inoculation and one mouse survived. The lungs of the survivor showed approximately 60% consolidation. In sharp contrast to these findings, all of the mice inoculated with virus exposed to half-saturated HS survived 10 days and at autopsy their lungs appeared completely normal.

Effect of HN on Infectivity of Influenza Virus. Three nitrogen mustards were tested for their ability to inactivate influenza virus: ethyl (HN1) and methyl (HN2) bis (beta chloroethyl) amine hydrochloride, and tris (beta chloroethyl) amine hydrochloride (HN3). Each was dissolved in cold 0.85% saline containing .05 molar $NaHCO_3$ (pH 8.0) to give a concentration of 1 mg per cc. Immediately after preparation, the solutions were mixed with equal volumes of 10^{-2} mouse passage influenza virus (PR8) as previously described, and a virus control was made with bicarbonate-saline alone. The mixtures were kept at 4°C for 2 hours, and .05 cc of each preparation then was inoculated intranasally into 5 etherized mice. The animals were observed for 10 days, and survivors were sacrificed and examined at the end of that period.

All of the control mice died 5 or 6 days after inoculation and presented typical lung lesions. Of the mice inoculated with virus-nitrogen mustard mixtures, all receiving HN1 and 2 receiving HN2 also died. Four of these 7 deaths occurred between the 2nd and 4th day after inoculation, before any of the controls succumbed, and the others died on the 5th, 8th, and 9th days, respectively. The lungs of 4 of the dead animals showed only slight to moderate congestive changes, while the lungs of the remaining 3 mice appeared similar to those of the controls. However, the intranasal passage of 10^{-1} suspensions of these lungs to normal mice produced neither death nor pulmonary lesions, so it would seem possible that the effects observed could be attributed to the toxic action of HN1 and HN2 rather than to influenza virus.

Three of the mice that received virus treated with HN2 and all of those that received virus treated with HN3 survived 10 days, and at autopsy their lungs appeared normal.

Effect of HS and HN on the Hemagglutinative Property of Influenza Virus. A saturated solution of HS and solutions of the 3 HN compounds containing 1 mg per cc were prepared as before. These solutions were mixed with equal volumes of PR8 influenza virus in the form of infected chick embryonic chorioallantoic fluid. After standing for 3 hours at 4°C serial doubling dilutions of the mixtures were made in tubes containing 0.5 cc of saline; 0.5 cc of a 1.5% suspension of adult chicken erythrocytes then was added to each tube. An appropriate control was included. The tubes were observed at room temperature over a period of 2 hours and the agglutinative titers were determined by the sedimentation pattern method.[4]

The results of this test are shown in Table I. It is apparent that neither HS nor HN, in the concentrations employed, affected the ability of the virus to agglutinate the erythrocytes.

HN Treatment of Mice Infected with Influenza Virus. Three series of mice were in-

[3] Herriott, R. M., Anson, M. L., and Northrop, J. H., *J. Gen. Physiol.*, 1946, **30**, 185.

[4] Salk, J. E., *J. Immunol.*, 1944, **49**, 87.

TABLE I.
Effect of HS and HN on Agglutination of Chicken Erythrocytes by Influenza Virus.

	Reciprocal of dilutions*								
	4	8	16	32	64	128	256	512	1024
Virus + HS half-saturated	++++	++++	++++	++++	++++	+++	++	+	+
Virus + HN1 .5 mg per cc	++++	++++	++++	++++	++++	+++	++	+	0
Virus + HN2 .5 mg per cc	++++	++++	++++	++++	++++	+++	+++	+	+
Virus + HN3 .5 mg per cc	++++	++++	++++	++++	++++	+++	++	+	+
Virus + saline	++++	++++	++++	++++	++++	+++	++	+	+

* 0 to ++++ = degree of sedimentation and pattern of erythrocytes after 2 hours at 25°C.

oculated intranasally with PR8 influenza virus 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}. In each series 5 mice were inoculated with each dilution of virus. Two series of these animals were treated with HN2 by the daily intraperitoneal injection of 0.5 mg per kg beginning 2 days before inoculation in one series and immediately after inoculation in the other. The third series served as controls. Therapy with HN2 did not modify the infection, and details will not be presented.

Discussion. The results of this study indicate that both sulfur and nitrogen mustards in relatively high concentration are able quickly to destroy the infectivity of influenza virus *in vitro*. Similar concentrations of these agents appear to have no effect upon the hemagglutinative properties of the virus. These findings emphasize once more the essential distinction between the power of the virus to produce infection and its ability to agglutinate erythrocytes of certain species. They suggest also that the loss of infectivity following exposure to mustards may not be accompanied by a comparable loss of antigenicity, although further work is necessary to establish more direct proof of this point.

One drawback in the use of sulfur mustard for the inactivation of viruses is its immiscibility with aqueous solutions and the consequent difficulty of predicting the amount dissolved under varying conditions of time, temperature, rate of mixing and so forth. On the other hand, the nitrogen mustards in the form of their hydrochloride salts are crystalline and are readily soluble in water, so that solutions of known concentration can be made accurately. A wider exploration of the possibilities of the nitrogen mustards as inactivating agents for viruses is indicated.

Conclusions. Influenza virus was rendered noninfectious for mice by *in vitro* exposure to relatively high concentrations of sulfur and nitrogen mustards. Concentrations of the mustards that abolished infectivity did not reduce the ability of the virus to agglutinate erythrocytes. The parenteral administration of nitrogen mustard had no effect on the course of infection with influenza virus in mice.