TABLE VII. Summary of Experiment to Determine whether Vaccine Would Act as an Interfering Agent when Mixed with Active Virus.

Vaccine Rabbit brain	Dilution of virus in 5% vaccine					Vaccine controls
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	
Inactivated with mustard	6/6	8/8	8/9	2/8		0/7
Irradiated, Squibb	9/9	10/10	8/9	5/6		0/8
Control. Virus diluted in milk		10/10	5/10	1/10	0/10	

property. Some of these antigens have been excellent while others will not immunize if diluted. However, the method should be useful in producing antigens to some of the viruses that produce disease in man. It also seems noteworthy that highly active vaccines have been produced against rabies from starting material of not especially high titer. It may be that the titer does not necessarily measure the amount of virus protein. If active brain material is suspended in strong buffer at pH 8 and titered immediately and again after 1 or 2 hours, it is found that the second titer is appreciably higher than the first, suggesting that the virus is aggregated and that in the buffer there is some dispersion. It is also possible that part of the antigenic material is not active virus but a precursor.

Although a relatively small amount of mustard is used to inactivate viruses and from isotopic studies it was found that a large proportion of the mustard reacts with the water, a rough calculation indicates that there must be several thousand mustard particles reacting with each virus particle. However, this is a reflection of the large particle size of the virus for on a percentage basis the amount bound is small. Certain reactions of mustard with simple proteins are understood but no comparable information is available for the reactions with nucleic acids or nucleoproteins.

Summary. The above work shows that mustard gas added to brain suspensions from animals infected with fixed virus gives a vaccine which by the method of testing used is better than that produced when the same brains are inactivated with phenol, about equal to that when the inactivation is by ultraviolet light, and almost as good as active fixed virus. The vaccine is very stable at refrigerator temperatures, and the method of preparation is simple and requires no unusual laboratory equipment.

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Prevention of Street Virus Infection by Vaccine.* (18253)

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Webster(1), in a review of the literature up to 1936, concluded that there is no good experimental evidence that rabies vaccine given after the injection of street virus will prevent the development of the disease. Since that date we have not found published experiments on this phase of rabies immunization. In the preceding paper(2) the evaluation of rabies vaccines was made by challenging guinea pigs 3 weeks after the intraperitoneal injection of known dilutions of

* The painstaking and accurate technical assistance of Mrs. Laura Lee Crosby and Mr. George Quabeck greatly aided the rabies work in this laboratory.

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1. Webster, L. T., *Am. J. Hyg.*, 1939, v30, 113.

2. TenBroeck, C., and Herriott, R. M., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 523.

TABLE I. Relation of Amount of Vaccine to Development of Immunity.

Interval between vaccination and challenge, wk	Dilution of vaccine inj. and results				
	1:10	1:50	1:250	1:1250	1:6250
3	—	0/8*	0/8	4/8	6/8
2	—	1/8	0/8	2/8	—
1	2/8	1/8	4/8	—	—
Controls—8/8					

* In all tables, numerator is the No. of animals that died; denominator the No. inoculated.

TABLE II. Immunity in Relation to Time Between Injection of Vaccine and Street Virus.

No. of inj.*	Dilution of vaccine	Time of inj.	Results
1	1:5	24 hr before virus	0/10
1	1:5	2 hr after virus	4/10
1	1:5	24 hr after virus	4/10
5	1:25	2 hr, 1, 2, 3, and 4 days after virus	3/10
Controls			9/10

* 1 ml of vaccine inj. intraper.

vaccine. Experiments were made with shorter intervals between the challenge and the vaccine injection. In the experiment reported in Table I the results are somewhat irregular, but they confirm earlier experiments in showing that the shorter interval between the vaccination and the challenge, the larger the amount of vaccine necessary to protect.

Experiments were made to see whether vaccine injected after the street virus would protect guinea pigs. The methods used have been described(3). The vaccines were fixed virus inactivated by mustard(2). The challenge virus was a strain of street virus secured locally‡ and passed twice through guinea pigs by intracerebral inoculation, the second passage being used. This virus was selected rather than the one used in the test of immunizing ability because it had a somewhat longer incubation period. Guinea pigs weighing between 300 and 400 g were used. Twenty guinea pigs were inoculated intramuscularly with 1 X 10^{-3} dilution in milk of a 10% brain suspension of the second passage street virus. Three hours later half of them were given an intraperitoneal injection of 1 ml of a 1:5 dilution of 10% rabbit brain vaccine ½ saturated with mustard 3 months previously. This injection was repeated at 24 and 48 hours. Eight of the 10 control animals developed rabies while all 10 of those given vaccine were protected. This experiment has been repeated with variations and with different vaccines; and while not all of the vaccines have protected guinea pigs, there is no question but that experimental evidence has been provided that the development of rabies can be prevented. The intervals between the vaccine and the virus were varied, as shown in Table II. Five per cent mouse brain 2/10 saturated with mustard 1 month previous to the experiment was used.

The experiment shows that vaccine given before the virus is injected gives better protection, that it makes little difference whether the vaccine is given shortly after the virus or a day later, and that one injection of vaccine gives the same or better immunity than an equal quantity given over a period of days. This type of experiment has also been repeated and these three results have been confirmed.

Route of injection of vaccine. In this experiment a mouse brain vaccine prepared 6 months previously and a rabbit brain vaccine prepared 5 months before the experiment were used, and the protecting action following intraperitoneal, subcutaneous, and intramuscular injection was compared. The virus was given 1 hour after the vaccine, and in the intramuscular injections the virus was given into the left leg and the vaccine into the right.

It will be seen in Table III that the mouse

TABLE III. Effect of Route of Injection of Vaccine on Protection Against Street Virus.

Vaccine*	Route	Results
Mouse brain	Intraperitoneal	3/8
	Intramuscular	4/8
	Subcutaneous	3/8
Rabbit brain	Intraperitoneal	3/8
	Intramuscular	6/8
	Subcutaneous	8/8
Controls		6/8

* 1 ml of a 1:5 dilution injected 1 hr before the virus.

3. TenBroeck, C., and Herriott, R. M., PROC. SOC. EXP. BIOL. AND MED., 1946, v62, 271.

‡ From Dr. R. S. Edmonds, Princeton, N. J.

TABLE IV. Sensitization to Street Virus by Vaccine.

Vaccine				No. showing symptoms by day										
Brain of	Mustard	Route of inj.	Dilution	8	9	10	11	12	13	14	15	16	17	Deaths
Rabbit	4/10 saturated	Subcut.	1:5*	0	0	1	3	7	7	7	7	7	8	8/8
"	1/4 "	"	1:5*	0	0	0	3	4	6	7				7/8
Controls				0	0	1	1	2	3	3	4	6		6/8
Mouse	1/4 "	"	1:25†	0	2	4	6	7						7/8
"	1/4 "	"	1:2.5†	1	3	5	6	8						8/8
"	1/4 "	Intraper.	1:2.5†	0	4	5	6	7						7/8
Controls				0	1	3	3	3	5	5	5	5	6	6/8

* 1 ml of vaccine inj. 1 hr before virus.
† 1 ml of vaccine inj. 24 hr after virus.

brain vaccine protected to the same extent by all 3 routes, whereas the rabbit brain injected subcutaneously or intramuscularly had no protecting action. The intraperitoneal injection of rabbit brain vaccine did however have some protecting action. That vaccines differ, some having a much better protecting action when injected intraperitoneally than subcutaneously, has been repeatedly confirmed. Other vaccines give as good or better protection subcutaneously than they do after intraperitoneal inoculation. Most of these results have been obtained in tests made when the vaccine was injected 3 weeks previous to the virus injection. This difference in immunizing ability is apparently due to the physical state of the vaccine, for it is not connected with the animal brain used. Some mouse brains give better protection by intraperitoneal injection and others by subcutaneous, and the same variation is found with different rabbit brain preparations. It is a matter that requires further study before an explanation can be given.

Sensitizing action of the vaccine. In the above experiment there were more deaths in the guinea pigs given the subcutaneous injection of rabbit vaccine than in the controls, a result that has repeatedly occurred, suggesting that the vaccine had made them more sensitive to the virus. In Table IV is shown the time of appearance of symptoms in the guinea pigs injected subcutaneously with rabbit brain, taken from the experiment given in Table III with the addition of one set of animals used in the same experiment but not included in the above table and an experiment with a moderately active mouse brain preparation. It will be seen that symptoms appeared earlier and that there were more deaths in the vaccinated groups than in the controls. This is an effect that was sometimes observed in animals that were given vaccine 3 weeks before the challenge.

TABLE V. Sensitization to Street by Fixed Virus.

Vaccine	No. showing symptoms of street virus by day						Deaths
	8	9	10	11	12	13	
Fixed virus, intraper. inj., 1 ml, 10^{-4} 10% suspension 3 wk before inj. of street virus	4	8					8/8
Controls	2	3	3	5	5	6	6/8

Fixed virus not inactivated injected 3 weeks before the challenge brings out this sensitizing effect in a more striking manner, as shown in Table V. In this experiment, on the 9th day after challenge all 8 of the vaccinated animals showed symptoms, whereas in the control animals only 6 of 8 developed rabies and the longest incubation period was 13 days.

Discussion. All of these experiments have been made on guinea pigs and there may be some question as to how far the results can be applied to man, but some of the findings at least confirm the thinking of those who are concerned with human vaccination. Experimental evidence is presented that rabies can be prevented by vaccine given after the injection of the virus, provided a vaccine of high immunizing ability is used and in sufficient quantity. This is brought out in spite of the fact that the incubation period in the

guinea pig is approximately 10 days, a much shorter time than in the larger animals. If man were to develop immunity as rapidly as the guinea pig—and it is believed that this prevention is immunity and not interference —the results would encourage fewer injections of vaccine in larger amounts than is the current practice.

The findings suggest that a part, at least, of the antigen is incorporated in the immune body, an idea that is not new but one that has not been confirmed. It is desirable that more work be done on this subject.

For evaluating vaccine to be used in man the power to protect after the injection of virus has advantages that merit consideration. It is this property of a vaccine that we want to measure, and furthermore the results are known in a short time. Extensive comparative tests have not been made but it appears that the vaccines that have a high protective value when given 3 weeks before the challenge are the ones that give protection when injected at the same time as the virus.

That guinea pigs may be sensitized to virus by vaccines, with a resulting shorter incubation period and more infections than in the controls, is a disturbing fact. In the past since the use of inactivated vaccines was begun, great numbers of individuals have been injected with products that had little immunizing ability. It is not likely that it can be determined whether man was in some instances made sensitive to the virus, but the findings emphasize the need, which is generally realized, for better vaccines and should stimulate efforts to concentrate the immunizing substances in vaccines, with the elimination of unnecessary proteins. Many experiments have been made in our laboratory and elsewhere on this problem. We have succeeded in eliminating some of the extraneous proteins without loss of immunizing ability but have not succeeded in concentrating the immunizing substance. All such experiments have been made with active virus that was later treated with mustard. In the preceding paper(2) it is shown that mustard inactivated virus retains its immunizing ability over a long period of time when stored in the refrigerator, and it now seems that this is the material to attempt to purify rather than the active virus which is so unstable.

Summary. Guinea pigs inoculated intramuscularly with street virus can be protected by mustard inactivated fixed virus injected at the same time or 24 hours later, provided the virus has a high immunizing titer. These results give experimental proof that vaccine will protect after infection and also provide a test that could be used in evaluating vaccines for human use. If non-protecting amounts of vaccine or fixed virus are injected, the incubation period may be shorter and the death rate slightly higher than in the control animals receiving only street virus.

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Influence of APF and Aureomycin on the Growth of Dairy Calves.* (18254)

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Wallace and Loosli(1) have reported on a series of diets which can be used to replace successfully fluid whole milk in feeding dairy calves after they are 10 to 14 days of age. In these studies it was observed that generally the rates of growth on the milk substitutes were not as rapid during the first month as on whole milk and that the calves

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1. Wallace, H. D., Loosli, J. K., and Turk, K. L., *J. Dairy Sci.*, in press.