sensitivity of the hypophysectomized animal to regular insulin is well known. This possibility was investigated in 2 groups of animals, one of which was injected intraperitoneally with 0.02 unit of insulin daily for 4 days, whereas the second group received the same dose of insulin together with 100 μg of glucagon per day. The results of these experiments given in lines 3 and 4 of Table II, demonstrate complete lack of growth activity on the part of insulin alone in this dosage, or of insulin together with glucagon. Insofar as toxicity is concerned, one animal from each of these 2 groups died after the first injection, and all the other animals appeared in poor condition. The practice was therefore instituted of injecting one ml of a 20% glucose solution intraperitoneally one-half hour after the hormone injection. Under this regime, only one animal, from the combined hormone group, died in the following 3 days, and the general appearance of all animals appeared greatly improved. Although the desire to obtain more information about toxicity had to be relinquished in favor of obtaining a sufficient number of surviving animals for growth assay, it appeared, nonetheless, that insulin, at 0.02 unit per day, could be quite toxic to the hypophysectomized animal, and thus could possibly explain the toxicity of cruder glucagon preparations.

Summary. A highly purified preparation of glucagon in doses as high as 0.25 mg/day, has no effect on the width of the epiphyseal cartilage of the hypophysectomized rat as determined by the tibia test for growth hormone. Such a preparation possesses no toxicity when injected into these test animals. Possible reasons for the previously claimed growth promoting activity and toxicity of glucagon fractions are discussed.

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Effects of Interfering Virus and RDE upon Development of Toxic Reactions.* (20605)

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The occurrence of toxic reactions following intracerebral, intraperitoneal or intravenous injection of influenza viruses into certain species of animals was first described by Henle and Henle(1,2,3) and subsequently confirmed and elaborated upon by various other workers (4-7). Despite the approximately 9 years which have elapsed since the first description of the phenomenon, however, little is known about the nature of the toxins or the mechanisms of their action. Earlier reports(1-5) based on passage and cultivation experiments have shown that viral multiplication was not an essential factor in the induction of toxic episodes. These findings might possibly pose a question concerning the necessity for intimate contact between virus and susceptible cells in animals which exhibit toxic symptoms.

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It seemed of interest, therefore, to determine the effects of prior injections of interfering virus and receptor-destroying enzyme upon the development of toxic reactions. The present paper describes the results of these studies.

Materials and methods. Virus. The strain of virus used in these studies was an egg-adapted strain of influenza A (PR8). Virus was prepared by suballantoic inoculation of 10- or 11-day embryonated eggs with 0.05 ml of a 10^{-5} or 10^{-6} dilution of virus in beef-heart infusion broth. Inoculated eggs were incubated at 36°C for 40-48 hours. After overnight chilling in the refrigerator, the allantoic fluids were harvested, pooled, and stored in ampoules in a dry ice cabinet. This was used as a source of toxic virus. *Preparation of interfering virus.* Virus prepared in the above manner was concentrated by the method of red cell adsorption and elution. A final concentration of 5% chicken red cells was added to virus; after 45 minutes in an ice water bath, the supernatant fluid was removed and the agglutinated cells resuspended in a small volume of 0.2 M phosphate buffer, pH 7.4. Elution was carried out in a 37°C water bath for 4 hours. The red cells were sedimented by light centrifugation and the supernatant fluid removed and adjusted to an hemagglutinin titer of 2560 (titer expressed as reciprocal of dilution of virus in last tube showing complete agglutination when read by pattern method). The viral concentrate was then exposed to 5×10^{-3} M sulfur mustard for 3 hours at room temperature. Virus inactivated in this manner gave rise to no hemagglutinins in each of 6 eggs inoculated allantoically with 0.05 ml of undiluted material or with 0.05 ml of serial 10-fold dilutions of virus. As reported previously, such preparations were markedly interfering(8). *Receptor-destroying enzyme. Vibrio cholerae* (Inaba), Culture 35A3 (National Institute of Health, Bethesda, Md.) was grown in beef-heart infusion broth for 18 hours at 37°C. The culture was passed through a Berkefeld N filter and the filtrate used as a source of RDE. The activity of this preparation was adequate to destroy all hemagglutinin-inhibitors in mouse brain extracts after 1 hour at 37°C. *Experiments with interfering virus.* Injection of inactivated influenza A (PR8) virus for determination of its effect upon development of toxic symptoms consisted of 0.03 ml of undiluted interfering suspension introduced into the right cerebral hemisphere of 3-week-old mice. (Swiss, Webster strain, random sex) under light ether anesthesia. This was followed 2 hours later by inoculation of 0.03 ml of active (toxic) influenza A virus into the same general area. Control mice received normal allantoic fluid in the first injection instead of interfering virus. All mice were observed daily for a period of 7 days. Convulsions upon spinning of animals or deaths occurring after 24 hours were used as criteria for virus-induced toxic reactions. *Experiments with RDE.* Intracerebral inoculation of RDE and toxic virus in mice and their subsequent observation followed the same general procedure described in the preceding section. Control animals were given broth instead of RDE in the first injection.

Results. Effect of prior injections of inactivated virus or RDE upon development of toxic reactions. The results of toxicity tests in mice receiving prior inoculations of either homologous interfering virus or RDE are shown in Table I. The data of this table represent the combined results of 2 to 3 separate experiments.

The suspension of active virus used in these experiments was toxic to slightly more than 50% of experimental animals when 0.03 ml of a 1:2 dilution was inoculated (Animal Group 3). The data for Animal Group 1 indicate that intracerebral inoculation of 0.03 ml of inactivated interfering virus 2 hours before administration of a similar volume of undiluted toxic virus resulted in a marked reduction in the incidence of toxic reactions. It may be seen that the ratio of toxic reactions in mice receiving prior injections of inactivated virus and in control animals (Group 2) is approximately 1:2.5. An even greater reduction in the number of toxic reactions was evident when 0.03 ml of RDE was administered prior to intracerebral inoculation of toxic virus. Comparison of the data shown for animal groups 4 and 5 reveals that the ratio of toxic reactions in the two groups is about 1:7. The results shown for Animal Group 6 emphasized the necessity for administration of RDE before virus if effective sup-

TABLE I. Effects of Prior Injections of Interfering Virus and RDE upon Development of Toxic Symptoms.

Animal group	Nature of 1st inoc.	Nature of 2nd inoc.	Toxicity results*	% positive reactions
1	Inactivated PR8	Undil. active PR8	9/24	37.5
2	Allantoic fluid	" " "	23/24	95.8
3	" "	½ dil. active PR8	13/24	54.2
4	RDE	Undil. active PR8	4/32	12.5
5	Broth	" " "	20/23	86.9
6	Undil. active PR8	RDE	22/23	95.7
7	RDE	Undil. active PR8†	24/24	100.

Time between inoc. = 2 hr.
* No. of animals dead or showing convulsions/total No. of animals in test.
† Second inj. consisted of 1 ml of virus intrav. (All other inj. intracer.)

pression of toxic reactions is to be established. *Vibrio cholerae* filtrates given 2 hours after active virus failed to influence the subsequent course of events in mouse brain.

It is apparent from the data shown in Animal Group 7 that intracerebral inoculation of 0.03 ml of RDE 2 hours earlier failed to prevent the development of toxic symptoms upon intravenous injection of 1 ml of active influenza A virus. The action following intracerebral injection of RDE was apparently a local effect, presumably because the amount of RDE introduced into the host was so small. However, the activity of RDE within the mouse brain was not a unilateral one confined to the side of inoculation, for other tests (not shown) employing injections of RDE and virus into opposite hemispheres led to an equally effective suppression of toxic reactions, provided the receptor-destroying enzyme was given prior to virus.

Discussion and summary. The results reported in this paper have shown that prior injections of inactivated interfering virus or RDE into mouse brain resulted in a marked reduction in the incidence of toxic episodes usually associated with intracerebral administrations of concentrated suspensions of influenza viruses. Intracerebral injections of RDE failed to prevent toxic manifestations, however, when virus was introduced by an intravenous route. The somewhat greater efficiency of RDE in suppression of toxic manifestations, as compared with inactivated virus, may possibly be a reflection of its position in the receptor gradient. The fact that both inactivated interfering virus and RDE proved effective in protection of animals against toxic doses of virus introduced into the central nervous system might appear to suggest that intimate contact between virus and susceptible cell was essential for development of toxic reactions. If union of virus and cell is indispensable, it is apparently a fairly rapid process, for injection of RDE two hours after toxic virus failed to modify the incidence of toxic reactions.

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