Production of Fever by Influenzal Viruses. IV. Tolerance to Mumps and Influenza Pyrogens. (20435)

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Intravenous injection of influenza or Newcastle disease viruses produces a characteristic febrile response in the rabbit(1). Animals are resistant to a second injection of homologous virus 24 hours later even if the initial febrile reaction is blocked by the administration of antipyrine(2). Resistance to virus toxic action can also be demonstrated for heterologous strains but the degree of tolerance is somewhat more variable under these conditions. In the earlier papers in this series, it was suggested that this nonreactive state to a second injection of virus was correlated with the receptor-destroying activity of the virus strain used for the initial injection.

Recently, French(3) has demonstrated that tolerance to the fever-producing effects of heterologous strains is dependent upon the dose of the blocking virus. The experiments reported here with the PR8 strain of influenza A and mumps virus support French's observations.

Materials and methods. Viruses. Allantoic fluid suspensions of the 151st egg passage of the PR8 strain of influenza A virus and the 161st egg passage of the Enders strain of mumps virus were used. Fluids containing the PR8 virus were aspirated from 12-day-old chick embryos which had been infected 44 hours previously with a 10^{-3} dilution of passage material. The mumps allantoic fluids were obtained from 13-day-old embryos 5 days after intra-allantoic injection with a 10^{-3} dilution of seed inoculum. The individual virus suspensions were pooled and stored in glass-sealed ampoules at −60°C for future use. The fluids were found to be free of bacteria by culture in thioglycollate broth. All glassware was baked at 170°C to prevent contamination with bacterial pyrogens. Hemagglutination titrations were done by a modification of the Salk method(4) using 0.5% chicken erythrocytes. The hemagglutinin titer of the PR8 virus preparation was 1920 and the mumps allantoic fluid, 960.

Rabbits. Male New Zealand rabbits weighing 2-3 kg were used. The experimental procedure was identical to that previously described(1). The usual duration of a single experiment was 7 hours during which time the animals were immobilized by tight-fitting neck-bands. Temperatures were recorded at ½- or 1-hour intervals for 6 hours after the intravenous injection of the virus suspension. Three temperatures were recorded prior to the injection of virus to establish a base line.

Results. Response to a single injection of mumps virus. Six animals in each of 3 groups were injected intravenously with 1, 2 or 5 ml of the undiluted mumps virus suspension. All 18 animals showed a uniform type of febrile response irrespective of the virus dose. In general, a slight elevation of temperature above the base line was noted 1½ to 2 hours after injection. The peak response of 3-4°F occurred between the 3rd and 5th hours followed by a gradual return to the preinjection base line level. Most of the rabbits had normal or only slightly elevated temperatures 24 hours after the start of the experiment. Control animals receiving normal allantoic fluid from 13-day-old chick embryos remained afebrile. The average temperature responses for each dose of virus are shown in Fig. 1. These curves are strikingly similar in appearance to those previously demonstrated for influenza viruses(1).

Tolerance to the mumps pyrogen. Twenty-four hours after the initial injection, the animals which had received 1 and 5 ml of mumps virus were challenged with 1 ml of the homologous virus. Six animals which had previously been injected with normal allantoic fluid served as controls. This last group responded in typical fashion to the mumps virus injection. On the other hand previous exposure to a 1 ml dose of the virus produced a state of partial resistance; the onset of the fever was delayed and the height of the maximal elevation was significantly lower than the controls.

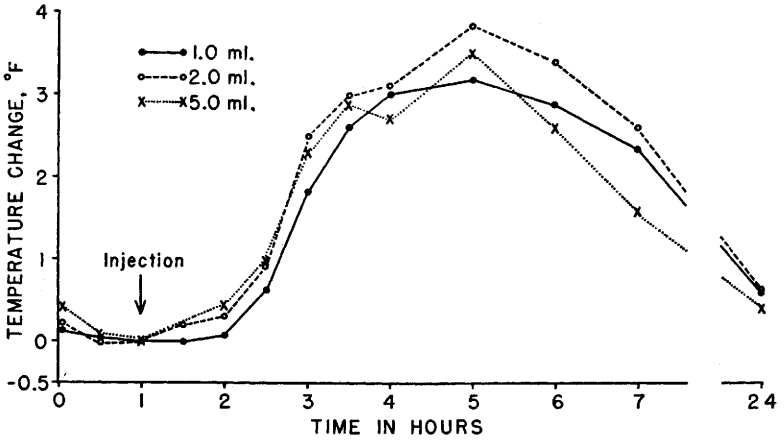

FIG. 1. Febrile responses in rabbits to various doses of a single intravenous injection of mumps allantoic fluid. Each point on the curves represents mean temperature of 6 rabbits.

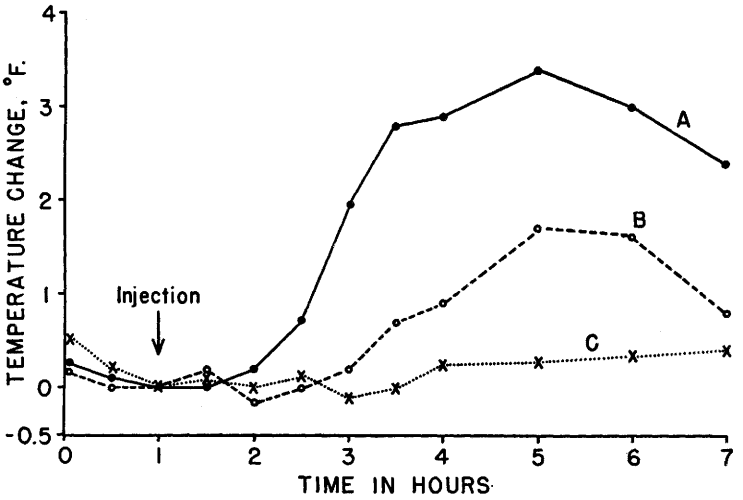

FIG. 2. Temperature responses of groups of 6 rabbits to 1 ml of mumps virus 24 hr after intravenous injection of A. normal allantoic fluid, B. 1 ml mumps virus, C. 5 ml mumps virus.

In the group pretreated with 5 ml of the mumps virus suspension the capacity to respond to a repeat injection was completely blocked (Fig. 2). It is apparent from these results that the degree of resistance induced by the first injection of virus is independent of the magnitude of the initial febrile response. As shown in Fig. 1 the temperature elevations following injection of 1 or 5 ml of virus in normal animals are in the same range. Nevertheless the 5 ml dose of the virus suspension afforded more effective protection to subsequent challenge.

Tolerance to the PR8 pyrogen. The presence of a refractory state to a repeat injection of PR8 virus(2) was readily confirmed. Six animals responded to an intravenous injection of 1 ml of PR8 virus with febrile reactions of a slightly lower order but which were otherwise quite similar to those following mumps virus injection. A second injection of the same dose of PR8 virus 24 hours later was completely without effect. The only apparent difference in the tolerance-producing action of these two agents is the more solid resistance to the homologous strain after a smaller dose of PR8 virus(2).

Cross tolerance between mumps and influenza viruses. The fever-producing capacities of mumps and PR8 viruses were studied in

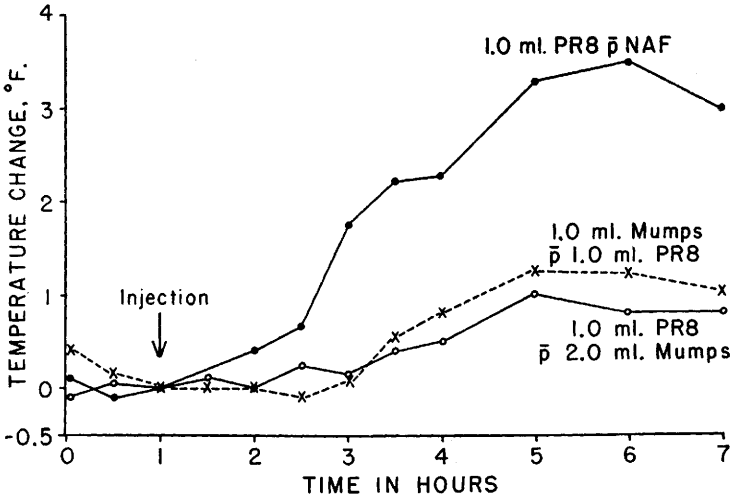

FIG. 3. Tolerance to heterologous virus challenge. Temperature records are for the second virus injection in groups of 6 animals.

animals pretreated with the heterologous strain. Six animals were prepared by intravenous injection of 2 ml of mumps virus and another group of 6 received 1 ml of PR8 virus. Each of the 12 animals, therefore, was pretreated with 7,680 hemagglutinating units of the respective virus preparations. Twenty-four hours later the mumps-treated animals were challenged with 1 ml of PR8 virus and the PR8-treated animals with 1 ml of mumps virus. The mean temperature curves for the second injection of virus are shown in Fig. 3. For comparison the average response in 6 rabbits to an injection of PR8 virus 24 hours after an injection of normal allantoic fluid is also presented in Fig. 3. It is readily apparent that a refractory state develops to heterologous virus challenge. The resistance was not complete but was of the same degree in each cross tolerance experiment.

Discussion. A property shared by all viruses of the influenza group is their capacity to destroy virus receptors on erythrocyte and other cell surfaces (influenza C is an obvious exception). However certain strains are more effective receptor-destroying agents than others. By testing the capacity of a standard dose of 1 strain to remove the erythrocyte receptors for other strains, Burnet, McCrea and Stone(5) were able to construct a virus "receptor gradient". Thus mumps virus, dose for dose, had less "enzymic activity" than swine influenza virus, and all other strains were intermediate in action.

The original observations that rabbits responded with less fever to a second injection of virus were made with the PR8 and Lee influenza strains and NDV(2). In these studies it was found that tolerance to heterologous virus challenge coincided with the position of the blocking agent in the receptor gradient. It is apparent now that this relationship is purely fortuitous. French(3) has confirmed in essence the resistance of rabbits to repeat injections of virus but he found that the degree of tolerance was dependent on dose and not receptor-destroying activity. Thus animals treated with a sufficiently large amount of mumps, Melbourne A, Lee B or swine influenza viruses were resistant to the fever-producing effects of all of these viruses. Wagner(6) has also reported that the cross tolerance in mice to the neurotoxic action of the WS and Lee influenza viruses was unrelated to the relative receptor-destroying activities of these strains. In addition, treatment of rabbits with the purified receptor-destroying principle (RDE) of *Vibrio comma* filtrates does not modify the febrile response to influenza viruses(3) although crude cholera filtrates possess antipyrogenic(3,7) and anti-neurotoxic(6) activity.

It would appear, therefore, that a property common to all strains of the influenza-mumps-

NDV group thus far tested is their capacity to produce fever in rabbits. The close relationship of these viruses is further emphasized by their ability to produce tolerance to a second injection of heterologous virus. These phenomena are correlated with hemagglutinating activity(1-3) but do not appear to be related to other biological properties.

Summary. Intravenous injection of mumps virus causes a febrile response in rabbits similar to that produced by influenza or Newcastle disease viruses. Animals are partially or completely resistant to a second injection of mumps virus 24 hours later. Pretreatment with the PR8 strain of influenza A virus also inhibits the febrile response to mumps virus. Mumps virus itself is equally effective in producing tolerance to the PR8 strain.

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Effect of Growth Hormone and Antibiotics upon Nitrogen Mustard Treated Rats.* (20436)

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The remarkable similarity in catabolic patterns resulting from treatment with either total body X-irradiation or systemic nitrogen mustard has been attested by several investigators(1-9). Selye *et al.* have reported upon the anticatabolic effect of somatotrophic hormone (STH) in rats that had received LD_{50} of whole body X-irradiation(10). There have also been gratifying results achieved with the use of antibiotics in animals exposed to ionizing rays(11-15). In contrast to this, the use of antibiotics in acute and chronic nitrogen mustard intoxication has been difficult to evaluate(9,16). It therefore seemed of interest to study the influence of STH and antibiotics, singly and in combination, on the catabolism produced in animals treated with nitrogen mustard.

Material and methods. Seventy male Sherman rats, initial body weight between 60-65 g, were divided into 7 groups of 10 animals per group. Group I served as absolute controls. Group II received penicillin combined with dihydrostreptomycin (Combiotic).‡ Group III received STH.§ Group IV received nitrogen mustard (HN-2) Methyl bis β-Chlorethyl Amine Hydrochloride‖). Group V received HN-2 and Combiotic. Group VI received HN-2 plus STH. Group VII received HN-2, Combiotic and STH.

The animals that were treated with HN-2 received only a single subcutaneous injection of 0.09 mg at the start of the experiment. The HN-2 was prepared immediately prior to administration. One hour later, all the animals were started on their respective treatment with Combiotic, STH, or the combined therapy.

The STH (Armour lot no. R-285-183) was made up freshly every second day with sterile physiological saline, and administered subcutaneously in 2 daily 8 hour intervals, each

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‖ Nitrogen mustard (Mustargen) was kindly provided by Merck and Co., Inc., Rahway, N. J.