THE PRODUCTION OF FEVER BY INFLUENZAL VIRUSES

III. Effect of Receptor-Destroying Substances

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In a previous report (1) the production of fever in rabbits by single intravenous injections of the viruses of influenza A and B and Newcastle disease (NDV) was described. The salient feature of this pyrogenic response was its remarkable uniformity with respect to dose and strain of virus. The capacity of these viruses to produce fever was found to be unrelated to infectivity but was apparently associated with hemagglutinating activity. It was shown (2) that animals given an injection of virus on the 1st day of the experiment were completely unresponsive to the fever-producing effects of the homologous strain on the 2nd day. This tolerance was also found for heterologous strains in a pattern which corresponded to the receptor gradient described by Burnet (3).

On the basis of these findings, it was postulated that union of virus particles with a receptor substance *in vivo* might play a part in the production of fever. The protective effect of prior injection of homologous or heterologous virus could be due to inactivation of supplies of this receptor. The gradual return of the animals to a responsive state, which occurs over a period of approximately 10 days with the PR8 strain and is not related to specific antibody formation, might be the result of restoration of receptors.

By the use of materials known to destroy receptors, it might be possible to obtain additional evidence relating to the hypothesis that adsorption of virus by a receptor substance plays a rôle in this febrile response. The virus receptor material found in various tissues is destroyed by oxidation and certain bacterial toxins. Hirst (4) has shown that erythrocytes treated with sodium periodate will no longer adsorb influenza virus. Exposure of red cells to *Clostridium welchii* toxin also renders them incapable of virus adsorption (3). Filtrates of certain strains of *Vibrio comma* also have the capacity to destroy the receptor material of erythrocytes (3); preinoculation of these filtrates into chick embryos (5) and mice (6) results in decreased susceptibility to infection by challenge inoculations of virus. Briody (7) has shown that cholera vibrio filtrates will elute heated influenza virus from red cells, evidence for

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the selectivity of the "enzymic" action of this bacterial product on the receptor substance. Both *Cl. welchii* and cholera filtrates are more effective in removing the receptors for strains of the mumps-Newcastle-influenza group that are higher in the receptor gradient (3). For example, a longer exposure is needed to render red cells inagglutinable by influenza B than by mumps virus.

The present report describes the effect of sodium periodate, *Cl. welchii* filtrate, and *V. comma* filtrate on the febrile responses of rabbits to influenzal viruses.

Materials and Methods

Male rabbits of mixed breed weighing 2000 to 2500 gm. were used. Experiments were conducted in an air-conditioned room in the manner described in a previous report (1), rectal temperatures being recorded at 30 minute intervals for an observation period of 6 hours.

The virus preparations used were pooled allantoic fluid suspensions of the PR8 strain of influenza A, the Lee strain of influenza B, and the B strain of NDV. Hemagglutination determinations were performed at room temperature by the method of Salk (8), the titers being read at 90 minutes for influenza strains and 45 minutes for NDV. All virus suspensions were adjusted to a hemagglutinin titer of 1:1024 by dilution with physiologic salt solution before injection. All glassware was baked at 170°C. for 2 hours as a precaution against contamination with bacterial pyrogens. Saline solutions were tested frequently in animals and were found to be non-pyrogenic.

The following receptor-destroying materials were used:

(*a*) *Sodium Periodate.*—This compound was administered by intravenous injection of a solution containing 20 mg./ml. A single injection of 50 mg./kilo caused death in two-thirds of the animals. It was found, however, that nine-tenths of the animals would survive 75 mg./kilo given in 3 divided doses of 25 mg. each at 4-hour intervals.

(*b*) *Cl. welchii Filtrate.*—The SR-12 strain of *Cl. welchii*, Type A, was obtained from the Microbiological Institute of the National Institutes of Health. The organism was grown in fresh veal infusion tryptose phosphate broth with 1 per cent glucose. After incubation at 37°C. in a Brewer anaerobic jar for 18 hours, the broth was filtered through a Seitz No. 6 pad and stored at −76°C. in sealed glass ampoules. The hyaluronidase activity of the crude filtrate was 137 viscosity reducing units as determined by the method of Meyer (9). Lecithinase activity was found to be present in a dilution of 1:256 using a serological modification of the lecithovitellin hydrolysis technique for the assay of this enzyme (10). The LD_{33} of this material was 0.75 ml.; this was the largest dose employed in this study.

(*c*) *Cholera Vibrio Filtrate.*—This material was prepared by Dr. J. J. Griffitts of the National Institutes of Health. An Inaba strain (35A3) of *V. comma* was grown in a simple nutrient broth for 18 hours at 37°C. and the organisms removed by centrifugation and Berkefeld filtration. This material rendered chicken erythrocytes inagglutinable by PR8 and Lee virus *in vitro*. The intravenous injection of 10.0 ml. of cholera filtrate resulted in death of about one-fourth of the rabbits; this was the maximum single dose of this material that was used.

The receptor-destorying materials were given intravenously in single or divided doses on the 1st day of the experiment. On the 2nd day, surviving animals were given an injection of virus in allantoic fluid and their temperature responses were recorded. In certain cases, these substances were given daily for 3 days and the animals were challenged with virus on the 4th day.

In order to facilitate comparison of febrile responses, 2 methods of representing temperature curves were used. For individual animals, temperature records after injection of virus prepa-

rations were plotted on 3/16 inch graph paper and, with the temperature at the time of injection as a base line, the area beneath the curve was measured with a Keuffel and Esser compensating planimeter No. F4236. When the temperature curve failed to return to the normal level within the 6 hour observation period, the last temperature reading and the base line were connected by a vertical line and the enclosed area was measured. The vernier reading of the planimeter was taken as the "fever index" (11, 12), an expression of both height and duration of the fever. For groups of animals, composite curves were constructed. Using the average temperature reading at the time of injection as a base line, mean temperature changes were charted at 30 minute intervals.

EXPERIMENTAL RESULTS

Twelve animals given an initial injection of 1.0 ml. of an allantoic fluid suspension of PR8 virus showed febrile responses varying from a fever index of 96 to 186, with a mean of 125. Fourteen animals given 1.0 ml. of Lee virus in allantoic fluid showed fever indices of 126 to 214, averaging 166. Eight control animals which received 4.0 ml. of NDV in allantoic fluid averaged 171, the range being 143 to 213.

Effect of Receptor-Destroying Materials on Production of Fever by PR8 Virus.—After administration of sodium periodate on the 1st day of the experiment, there appeared to be some diminution in the ability of rabbits to respond with fever to an injection of PR8 virus on the 2nd day. Six animals were given 40 mg./kilo of sodium periodate intravenously. Twenty-four hours later, after injection of 1.0 ml. of allantoic fluid containing PR8 virus (hemagglutinin titer 1:1024), these animals had what appeared to be lessened temperature elevations, the fever indices averaging 71. Each of 8 animals was given 25 mg./kilo of sodium periodate every 4 hours for 3 doses and the 6 survivors were challenged on the following day with 1.0 ml. of PR8 virus in allantoic fluid. They showed fevers similar to those of animals given 40 mg./kilo, the fever indices averaging 75.

The effect of the filtrate of *Cl. welchii* in lessening the fever after PR8 virus was more obvious. Four animals given 0.5 ml. of this material had slight elevations of temperature after virus challenge on the following day (fever indices averaging 45). When the dose of filtrate was increased to 0.75 ml., the average fever index was only 27 for a group of 4 animals.

The administration of 10.0 ml. of the cholera vibrio filtrate was most effective in altering the febrile response to PR8 virus. Six animals pretreated with this material showed practically no fever, the indices averaging 13.

Table I compares the fever indices of normal and treated animals after injection of PR8 virus. Fig. 1 shows composite temperature curves for these groups of animals.

Effect on Lee Virus.—The prior administration of sodium periodate had little influence upon fever production by Lee virus. The mean fever index for 6 animals pretreated with 40 mg./kilo of this material and challenged with

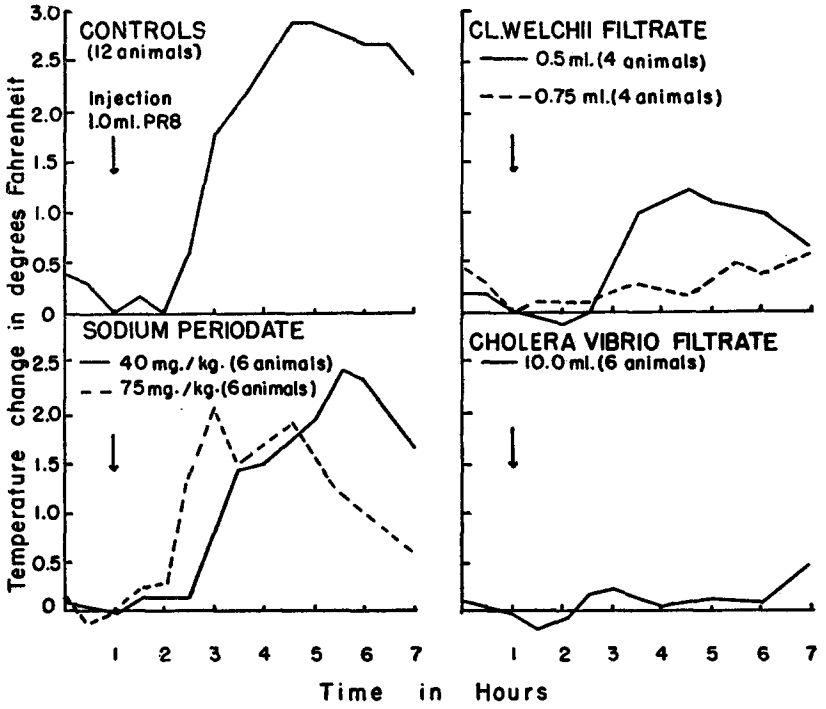

FIG. 1. Temperature curves showing the modification of the fever-producing effect of 1.0 ml. of PR8 virus in allantoic fluid (hemagglutinin titer 1:1024) by inoculation of receptor-destroying substances on the previous day.

TABLE I

Fever Indices after Intravenous Injection of 1.0 Ml. of an Allantoic Fluid Suspension of PR8 Virus (Hemagglutinin Titer 1:1024) in Normal Animals and Animals Given Various Receptor-Destroying Substances

Normal controls	Na periodate		*Cl. welchii* filtrate		Cholera vibrio filtrate
	40 mg./kg.	75 mg./kg.	0.5 ml.	0.75 ml.	10.0 ml.
118	83	77			11
186			53	34	
121	61	90			14
157			39	27	
101	67	82			8
104			44	29	
162	81	71			21
115			47	19	
100	74	63			17
96					
143	62	67			11
107					
Mean 125	71	75	45	27	13

1.0 ml. of Lee virus in allantoic fluid was 151 as compared with the mean of 166 for untreated animals. When the dose of periodate was increased to 75 mg./kilo, the fever indices averaged 146. As can be seen in Fig. 2, the main difference in the temperature responses of these animals was a tendency for the curves to return to the base line sooner in the periodate-treated groups.

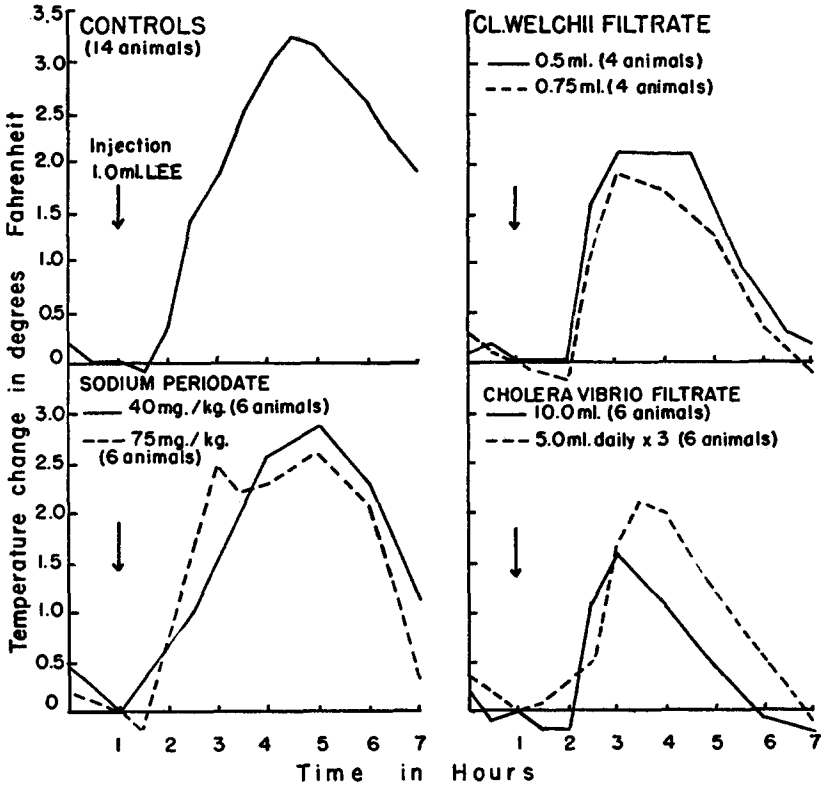

FIG. 2. Temperature responses of normal and treated animals to 1.0 ml. of Lee virus in allantoic fluid (hemagglutinin titer 1:1024).

The intravenous injection of *Cl. welchii* filtrate substantially decreased the febrile reaction following injection of Lee virus. The mean fever index for 4 animals given 0.5 ml. of this material and challenged with 1.0 ml. of virus was 89. After 0.75 ml., the fever indices averaged 55.

Two groups of 6 animals were given cholera vibrio filtrate and challenged with Lee virus. The first group received a single injection of 10.0 ml. of this material and responded with slight elevations of temperature to 1.0 ml. amounts of virus on the next day (average fever index 28). The other group was given 5.0 ml. of filtrate daily for 3 days and challenged with 1.0 ml. of virus on the 4th day. These animals had higher elevations of temperature than those given a single dose of 10.0 ml. of filtrate (average fever index 54).

Fig. 2 shows the temperature records of animals given Lee virus with or without pretreatment with these receptor-destroying substances. Table II gives the fever indices obtained for individual animals in each group.

Effect on NDV.—Six animals given 75 mg./kilo of sodium periodate on the 1st day of the experiment showed decreased febrile responses after challenge with 4.0 ml. of NDV in allantoic fluid on the 2nd day, their fever indices averaging 116 as compared with 171 for untreated controls. As was the case with

TABLE II

Fever Indices after Intravenous Injection of 1.0 Ml. of an Allantoic Fluid Suspension of Lee Virus (Hemagglutinin Titer 1:1024) in Normal Animals and Animals Given Various Receptor-Destroying Substances

Normal controls	Na periodate		*Cl. welchii* filtrate		Cholera vibrio filtrate	
	40 mg./kg.	75 mg./kg.	0.5 ml.	0.75 ml.	10.0 ml.	5.0 ml. q.d. × 3
187						
193	172	132	111	71	41	57
141						
126	211	163	83	56	18	68
202						
138	148	177	71	42	26	56
159						
171	121	128	92	51	32	62
214						
167	130	137			21	38
184						
172	127	142			33	41
143						
138						
Mean 166	151	146	89	55	28	54

Lee virus, this decrease was a reflection of the tendency of the curves to return to the normal level more rapidly than those of the untreated group (Fig. 3).

A group of 6 animals challenged with 4.0 ml. of an allantoic fluid suspension of NDV after pretreatment with 0.5 ml. of *Cl. welchii* filtrate showed reduced febrile responses (average fever index 67).

Six animals given a single injection of 10.0 ml. of cholera vibrio filtrate showed reduced febrile responses when challenged with 4.0 ml. of NDV on the next day (average fever index 46). Another group received 5.0 ml. of the filtrate daily for 3 days and was challenged on the 4th day with 4.0 ml. of virus. Their fever indices averaged 32.

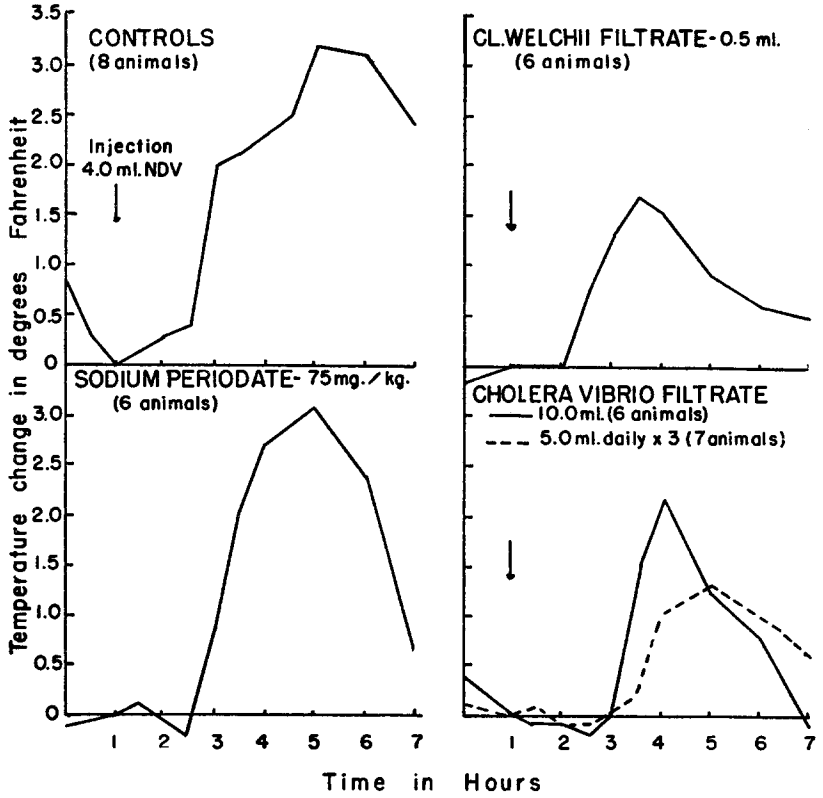

FIG. 3. Temperature records of normal and treated animals after injection of 4.0 ml. of an allantoic fluid suspension of Newcastle disease virus (hemagglutinin titer 1:1024).

TABLE III

Fever Indices after Intravenous Injection of 4.0 Ml. of an Allantoic Fluid Suspension of NDV (Hemagglutinin Titer 1:1024) in Normal Animals and Animals Given Various Receptor-Destroying Substances

Normal controls	Na periodate	*Cl. welchii* filtrate	Cholera vibrio filtrate	
	75 mg./kg.	0.5 ml.	10.0 ml.	5.0 ml. q.d. × 3
169	92	62	51	31
213	131	87	24	19
196	126	66	36	46
143	112	52	47	38
152	129	91	60	27
174	108	48	58	39
179				25
148				
Mean 171	116	67	46	32

Table III gives the fever indices for individual animals after 4.0 ml. of NDV. Fig. 3 shows the temperature records of groups of normal and treated animals after injection of this virus.

Effect of Receptor-Destroying Substances on Production of Fever by Typhoid Vaccine.—All the substances used in the foregoing experiments exerted toxic effects separate from their ability to destroy the receptor substance for virus hemagglutination. In an attempt to achieve maximal effects in reducing the febrile response to the viruses, the materials were given in amounts which caused death in numerous animals, the survivors being challenged with virus.

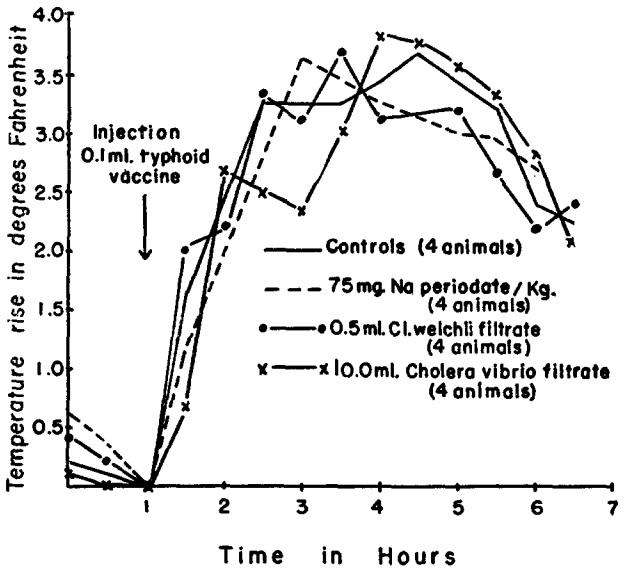

FIG. 4. Failure of receptor-destroying substances to modify pyrogenic effect of 0.1 ml. typhoid vaccine (2 billion heat-killed *Salmonella typhosa* organisms per ml.).

In order to determine whether the reduction in temperature responses following the use of receptor-destroying substances was specific and not simply a manifestation of the severe injury produced by them, the ability of rabbits to respond to the pyrogenic effects of typhoid vaccine after treatment with these materials was tested.

Fig. 4 shows the temperature curves after administration of 0.1 ml. of typhoid vaccine to a group of normal animals and groups treated with sodium periodate, *Cl. welchii* filtrate, or cholera vibrio filtrate. There was no essential difference in the height or duration of the fevers.

It has previously been shown that the prior injection of typhoid vaccine does not modify the pyrogenic effect of these viruses (2), additional evidence that this effect is specific.

DISCUSSION

It is apparent from these studies that the injection of receptor-destroying substances produces a diminution in the febrile responses of rabbits to influenzal viruses. In general, cholera vibrio filtrate was the most effective agent, *Cl. welchii* filtrate slightly less so, and it was questionable whether sodium periodate produced any significant alteration in the fever. There are difficulties in comparing the relative effectiveness of these agents. In all cases, the margin between the lethal dose and the effective fever-neutralizing dose was small. A problem encountered in evaluating the effect of periodate is the probability that the compound is reduced before its influence can be exerted. The action of the host blood and tissues in inactivating these agents would seem to be less for the active principles of bacterial filtrates than for a labile oxidizing agent. It has been shown by one of us (13) that the action of sodium periodate on the infectivity of influenza virus is blocked by normal serum and normal allantoic fluid; Hirst (4) has demonstrated that its receptor-destroying action is neutralized by glucose. If, as has been postulated (5), the receptor-destroying activity of the bacterial filtrates is the function of a specific enzyme, supplies of this enzyme should be difficult to exhaust and its action would be exerted over a prolonged period. Evidence that the "receptor-destroying enzyme" of cholera vibrio filtrate is not rapidly inactivated by body fluids is implied in the effect of this material in rendering chick embryos less susceptible to infection with influenza virus (5) and the destruction of virus receptors in the lungs of mice by this substance (14). De St. Groth (15) raises the question as to whether the receptor-destroying mechanisms of periodate and cholera filtrate are similar or whether these agents have different sites of action.

It is evident that fever production by individual virus strains is differentially affected by similar doses of these receptor-destroying substances. In general, there was a greater protective action against the pyrogenic effect of PR8 virus than against that of the Lee strain. In a previous report (2), it was shown that the resistance of rabbits to fever produced by previous injections of homologous or heterologous virus was more difficult to demonstrate for the Lee strain. Greater concentrations of filtrate and longer exposures of erythrocytes are necessary to destroy the receptors for influenza B strains than for A strains. The difference in effectiveness of *Cl. welchii* and cholera vibrio filtrates in modifying the febrile response also appears to conform to the positions of these two viruses in the receptor gradient. Evaluation of the effects of these materials on the fever following injection of NDV is more difficult due to the prolonged febrile response produced by this virus. Although fever indices indicated that inhibition of fever was great for NDV, the febrile response was not abolished as for the PR8 strain.

The question arises whether the action of these materials in impairing the febrile response of rabbits to influenzal viruses is specific or whether the

decreased reactivity is a reflection of a generalized debility produced by their toxic effects. It may be noted that near lethal doses of NDV do not appreciably diminish the response to PR8 or Lee viruses, nor does the toxic effect of typhoid vaccine in any way alter the febrile reaction to influenzal viruses (2). In addition, sodium periodate, *Cl. welchii* filtrate, and cholera vibrio filtrate do not lessen the pyrogenic reaction to typhoid vaccine.

It appears then, that certain substances which destroy cell receptors for influenza virus are also capable of inhibiting the pyrogenic action of virus in rabbits. As with influenza viruses themselves, the action of these materials appears to be directed against a specific substrate. It seems possible that their site of action is the same and that they all possess the capacity to inactivate the receptor material upon which the production of fever by these viruses depends.

SUMMARY

The effect of treating rabbits with materials which destroy the cell receptors for influenzal viruses upon the ability of these animals to respond with fever to injection of the PR8 and Lee strains of influenza virus and Newcastle disease virus (NDV) is described. In general, both cholera vibrio and *Cl. welchii* filtrates produced diminution of febrile responses. The effect of sodium periodate upon the pyrogenic reaction was not significant.

Near-lethal amounts of these materials were necessary to demonstrate their protective effects against virus challenge. In order to rule out general debility as a factor in lessening the fever, it was shown that the ability of animals to respond to the pyrogenic effect of typhoid vaccine was unimpaired by injection of receptor-destroying substances.

The substances tested were more effective in abolishing the febrile response to PR8 virus than to Lee virus or NDV. This finding is compatible with previous studies of the protective effect exerted by homologous and heterologous viruses.

These findings give support to the hypothesis that union of virus and host receptor substance plays a part in the production of fever by these viruses.

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