STUDIES ON THE PATHOGENESIS OF FEVER WITH INFLUENZAL VIRUSES

III. The Relation of Tolerance to the Production of Endogenous Pyrogen*

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A distinguishing characteristic of experimental fever is the development of tolerance to repeated injections of a pyrogenic agent. Tolerance to endotoxins has been the subject of extensive study (1–5). This phenomenon may be clearly dissociated from the classical immune response by its rapid onset, relatively short duration, non-specificity and lack of correlation with serum antibody levels. Crucial evidence for the difference is the observation that patients with agammaglobulinemia acquire tolerance to serial injections of endotoxin in a normal fashion (6).

In several respects, tolerance to the pyrogenic effects of the various viruses in the influenza group resembles that occurring with endotoxins. It is also relatively non-specific, with the injection of one virus conferring a certain degree of cross-tolerance to other viruses in the same group. Virus tolerance likewise cannot be correlated with serum antibody levels and is of brief duration: approximately 11 days as compared with 3 weeks in the case of endotoxins (7).

However, certain striking differences in these two types of tolerance have been described. Tolerance to a constant dose of endotoxin is never complete despite repeated injections; with certain viruses, on the other hand, tolerance may be complete on the 2nd or 3rd day of injection. In addition, cross-tolerance between endotoxins and virus has not been demonstrated. Finally, tolerance to virus is not reversible by injection of thorotrast. These differences have led to the conclusion that tolerance to endotoxin and viruses are produced by different mechanisms (7).

The first paper in this series (8) has described the presence of a circulating endogenous pyrogen in rabbits made febrile by intravenous injection of either PR8 strain of influenza A or Newcastle disease virus (NDV). It has been postulated that the release of an endogenous pyrogen is an essential step in the pathogenesis of fever with these two viruses. Evidence to be presented here indi-

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cates that circulating endogenous pyrogen also plays an important role in the development of virus-tolerance. The demonstration of cross-tolerance between virus and endotoxin suggests further that endogenous pyrogen occurring in these two forms of experimental fever may be similar.

Methods

General.—The preparation and titration of Newcastle disease virus (NDV) and PR8 strain of influenza virus have been described (8). Methods of determining circulating endogenous pyrogen after the inoculation of virus into rabbits were performed as outlined in the preceding papers of this series (8, 9).

Infectivity Titration.—10- or 11-day-old embryos were inoculated with serial tenfold dilutions of each serum sample as outlined previously (8). After incubation of embryos at 35°C. for 44 to 48 hours, the allantoic fluids were harvested and the presence of hemagglutinin was determined. The LD_{50} was calculated according to the method of Reed and Muench (10).

Immune Serum.—Techniques both for immunizing rabbits with NDV and for the determination of serum antibody were performed as previously described (9).

Typhoid Vaccine.—A single lot was used, monovalent reference standard NRV-LS No. 1, made from *Salmonella typhosa* V-58.[1] Pyrogen tolerance was induced by a series of at least seven daily injections of 1.5 ml. of a 1:10 dilution of the stock vaccine (11).

RESULTS

Correlation of Tolerance and Production of Endogenous Pyrogen.—The development of tolerance to a course of injections with NDV (either 4 or 1 ml.) is characterized by a progressive decrease in the height and duration of fever. The second peak generally disappears on the 2nd day and there is a progressive diminution of the first peak with subsequent injections. Complete tolerance, *e.g.* the absence of a febrile response, has not been demonstrated, however, with a course of 1 ml. NDV over a period of 1 week or more. Characteristically, a minimal response develops by about the 4th day which persists throughout the period of observation.

It was of interest to determine whether these characteristic features of developing tolerance were accompanied by comparable changes in the level of circulating transferable endogenous pyrogen.

Fig. 1 demonstrates the average fever curves of a group of rabbits on the 1st, 2nd, and 3rd day of daily injections with 4 ml. NDV. Beneath each donor curve, levels of transferable endogenous pyrogen are plotted at various intervals after inoculation of the donors with virus. The amount of circulating endogenous pyrogen was determined for each interval by calculation of the mean fever index induced by the particular serum in a group of normal recipients.

It is apparent that the progressive decline in the febrile response to virus from the 1st to the 3rd day was associated with a similar diminution in transferable endogenous pyrogen. As early as the 2nd day, a significant amount of endog-

[1] Obtained from the Army Medical Service Graduate School, Walter Reed Medical Center. This vaccine had a bacterial count of approximately 500 million per ml. (11).

enous pyrogen was only obtainable at the 3rd hour, consistent with the considerable but brief fever occurring in the donors. By the 3rd day, there was virtually no detectable endogenous pyrogen at any of the intervals tested.

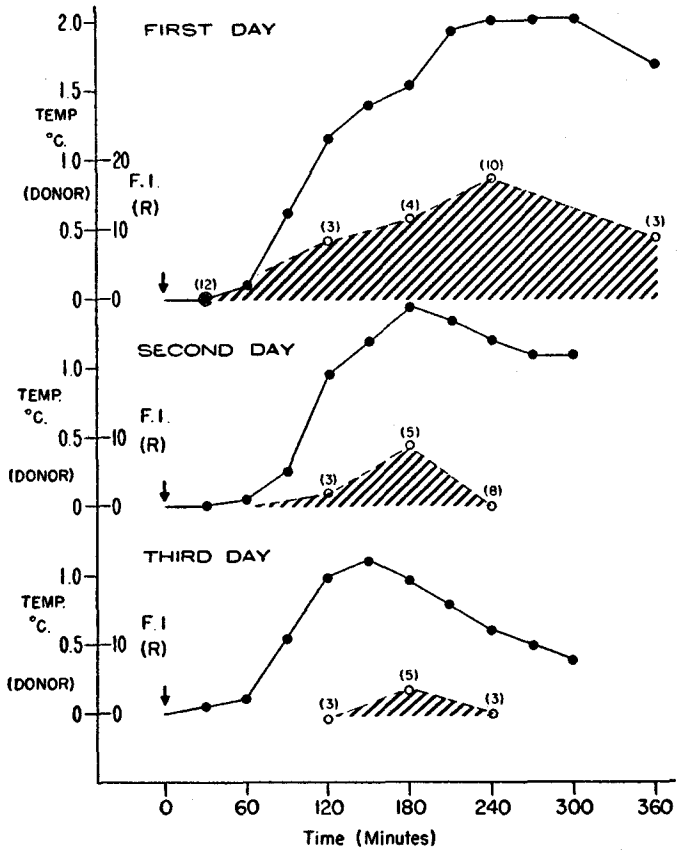

FIG. 1. Mean febrile responses of 4 animals given successive daily injections of 4 ml. NDV. The titer of circulating endogenous pyrogen at various intervals after inoculation on each day was calculated by passive transfer of 15 ml. sera to normal recipients (indicated by numbers in parentheses). F.I., fever index; R, recipients.

A similar experiment was designed with the PR8 virus. As contrasted with NDV, tolerance is complete by the 2nd day with this virus. Donors reinjected with PR8 on the 2nd day remained afebrile and were bled at 4 hours. There was no transferable endogenous pyrogen (8).

These two experiments indicate that the development of tolerance to reinjection of either PR8 or NDV is closely correlated with corresponding changes in the circulating levels of endogenous pyrogen.

Modification of Virus Fever by in Vitro Incubation of NDV with Serum from Normal, Tolerant, and Immune Animals.—Fever resulting from injections of endotoxins can be markedly modified by *in vitro* incubation of the pyrogens with sera from either normal or tolerant animals (4, 5, 12–15). Serum factors have been implicated in the development of tolerance to these agents. It was of interest, therefore, to determine whether the pyrogenicity of NDV would be similarly altered after incubation *in vitro* with sera from animals tolerant to virus.

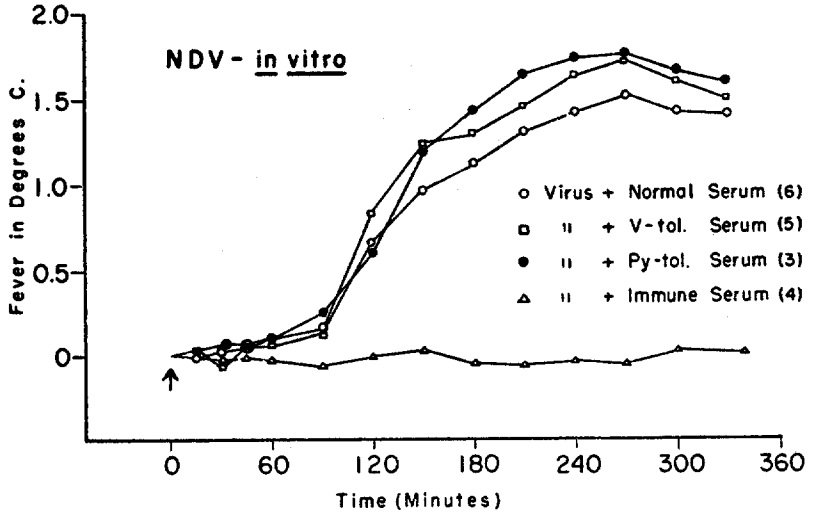

FIG. 2. Febrile responses of normal recipients given an injection of 1 ml. NDV after incubation with various sera. Average values for 3 to 6 recipients are shown.

One ml. of NDV was added to each 5 ml. of pooled sera from the following sources: (*a*) Rabbits given 4 ml. of NDV 24 hours earlier and hence designated as "virus-tolerant", (*b*) Rabbits given an 8 day course of injections of 1.5 ml. 1:10 dilution of typhoid vaccine and thus rendered "pyrogen-tolerant" (see Methods); (*c*) Rabbits immunized with a course of injections of NDV (see Methods); (*d*) Normal rabbits which had received no injections of any kind. After 1 hour incubation at 37°C., the various NDV-sera combinations were given in individual injections of 6 ml., containing 1 ml. of NDV, to groups of 3 to 6 recipients.

The complete inhibition of the pyrogenic effect of the virus by *in vitro* incubation with homologous immune sera was confirmed (16). However, neither the normal nor the tolerant sera modified the pyrogenicity of the virus (see Fig. 2). The decreased production of endogenous pyrogen occurring with reinjection of virus cannot be attributed to the development of serum inhibitors to virus *per se*.

Clearance of Viremia Following Injection of PR8 Virus.—The development of tolerance to endotoxins has been correlated with the accelerated clearance of

these substances from the circulation by cells of the reticuloendothelial system (2). An experiment was designed to determine whether tolerance to virus was similarly associated with an enhanced removal of circulating virus.

Two normal animals were injected with 1 ml. PR8 virus. The animals were then bled by cardiac puncture at 5 and 15 minutes following the inoculation. Ten ml. blood were obtained at each interval with the use of a syringe moistened with heparin. Tenfold dilutions of each

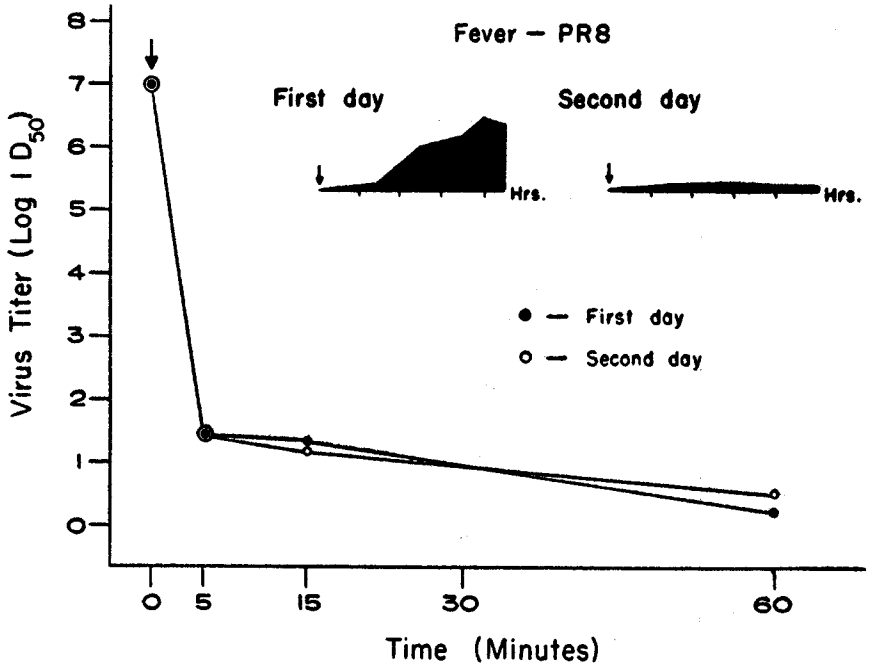

FIG. 3. Titer of circulating virus at various intervals on 1st and 2nd day of inoculation with 1 ml. PR8. Each point represents an average of two determinations. Mean febrile responses to the virus on the 2 days are shown in shadowgraphs.

sample were immediately made in broth and 0.1 ml. of each dilution was inoculated into 10- or 11-day-old chick embryos. Infectivity titrations were then performed (see Methods).

On the 2nd day, the animals were reinjected with 1 ml. PR8 and the procedure repeated.

In order to obtain comparable data at a later interval, a second experiment was performed with another pair of normal rabbits. Each animal was given a single injection of PR8 on 2 successive days and bled at 60 minutes. The titer of the virus in the blood on the 2 days of the experiment was determined as in the first experiment.

The results of these two experiments are shown in Fig. 3 and Table I. Despite the development of complete tolerance to the pyrogenic effects of PR8 on the

2nd day (see shadowgraph in Fig. 3), the clearance of virus from the blood stream was unchanged.

TABLE I

Titer of PR8 Virus (Log ID_{50}) in Whole Blood of Rabbits at Various Intervals after Intravenous Inoculation

Rabbit No.	5 min.	1st day 15 min.	60 min.	5 min.	2nd day 15 min.	60 min.
9-20	1.22	1.50	—	1.23	1.00	—
9-26	1.33	1.00	—	1.33	1.38	—
9-49	—	—	0.42	—	—	0.66
9-59	—	—	0.00	—	—	0.23

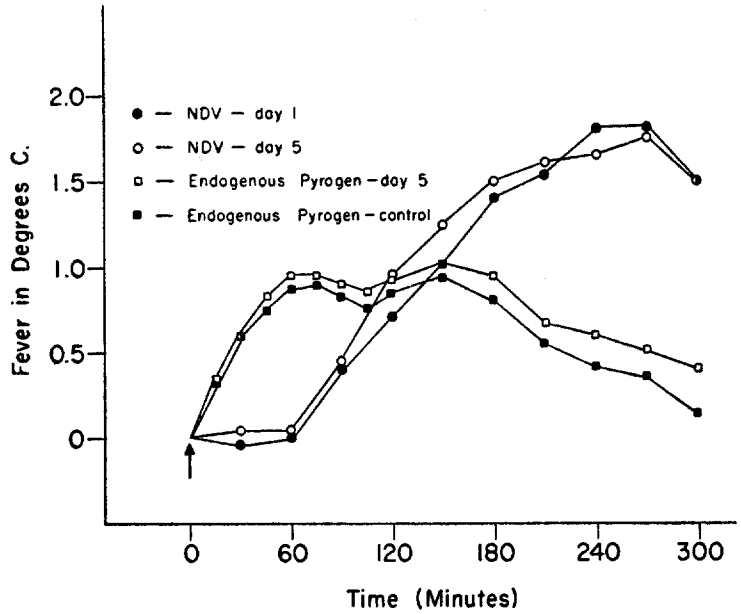

FIG. 4. Mean fevers of 3 normal rabbits to injections of 1 ml. NDV on days 1 and 5. Average pyrogenic response of 3 recipients to 15 ml. endogenous pyrogen on day 5 after preceding virus injection is compared with the mean of 4 normal recipients to same dosage of endogenous pyrogen. Note appearance of second fever peak after injection of endogenous pyrogen and normal response to virus on day 5 indicating lapse of tolerance.

Recovery from Tolerance to Virus and to Endogenous Pyrogen.—Tolerance to the pyrogenic effect of PR8 virus is lost after a comparatively brief interval of approximately 11 days. Despite high levels of circulating antibody conferred by the initial injection, the fever response at this time is indistinguishable from that seen with the first injection (7).

An experiment was performed to determine at what time tolerance would disappear to a small dose of NDV. Six normal animals were given initial injections of 1 ml. NDV and their temperatures recorded. On the 5th day, the injection was repeated in 3 animals, at which time they responded with identical fevers indicating that tolerance had lapsed. On the same day, the other 3 recipients received injections of 15 ml. endogenous pyrogen.

All of these animals had normal biphasic febrile responses indicating a similar loss of tolerance to endogenous pyrogen (Fig. 4).[2] Since the normal febrile response to virus and to injected endogenous pyrogen both require the production of endogenous pyrogen, there is an obvious correlation between resumption of the ability to produce endogenous pyrogen to an injection of endogenous

TABLE II

Febrile Responses of Various Types of Recipients to Injection of 15 Ml. Endogenous Pyrogen from NDV-Injected Donors

Monophasic	Biphasic
A. Single injection on preceding day	
Homologous virus (NDV)	Normal
Heterologous virus (PR8)	Rested for 4 days Immune
Typhoid vaccine	Pyrogen-tolerant (course of injections of typhoid vaccine)
Virus or typhoid vaccine plus antipyrine	Antipyrine only (previous day)
Endogenous pyrogen	Epinephrine (previous day)
B. Course of daily injections	
Virus	
Endogenous pyrogen	

pyrogen, as evidenced by the return of the second fever peak, and recovery from tolerance to virus.

Three recipients immunized to NDV by a course of injections (see Methods) and rested for 2 weeks were also found to have normal pyrogenic responses to reinjection of either virus or endogenous pyrogen (see Table II). This observation supplies further evidence for the correlation between the normal response to virus and to endogenous pyrogen and confirms the dissociation previously noted between tolerance to virus and classical antibody levels (7).

Cross-Tolerance to Endogenous Pyrogen after Injection of Heterologous Virus or Endotoxin.—There is a considerable degree of cross-tolerance to the pyrogenic effects of the various viruses in the influenza group (7). Recipients given PR8 virus have characteristically depressed febrile responses to injection of NDV on the following day (see Fig. 5).

[2] Tolerance to endogenous pyrogen is characterized by a monophasic fever only, indicating the absence of activation of endogenous pyrogen in the recipient (9).

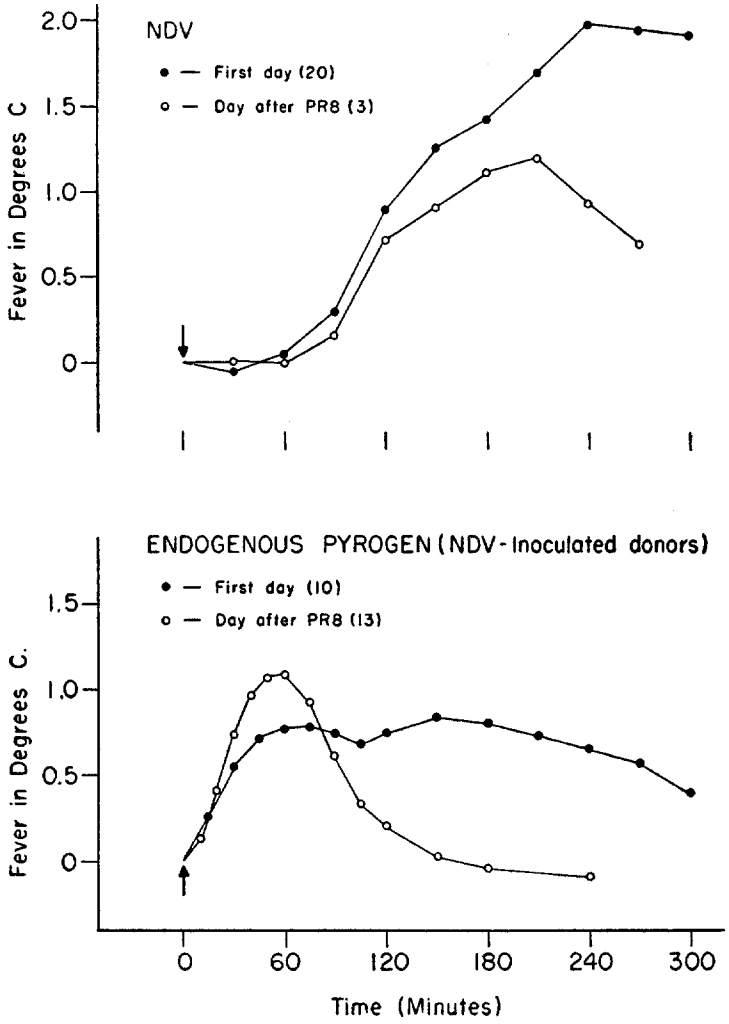

FIG. 5. Mean febrile responses to 4 ml. NDV and 15 ml. endogenous pyrogen before and after challenge of recipients with 1 or 2 ml. PR8 virus. Note the loss of the second peak of fever indicating tolerance in animals given heterologous virus on the preceding day. Number of recipients in each group is shown in parentheses.

It was of interest to determine, therefore, whether an injection of heterologous virus would likewise confer "tolerance," *i.e.*, the disappearance of the second fever peak, to an injection of endogenous pyrogen from NDV-inoculated donors (9).

Thirteen rabbits were given initial injections of 1 or 2 ml. PR8. On the following day these animals were challenged with 15 ml. of NDV-induced endogenous pyrogen. They all responded

with brief monophasic fevers indicating that heterologous virus had rendered the recipients tolerant to the injection of endogenous pyrogen (see Fig. 5).

Another group of 3 recipients was given injections of 2 ml. undiluted typhoid vaccine on the day preceding challenge with endogenous pyrogen. The response of these animals was also monophasic showing that cross-tolerance to the endogenous pyrogen had similarly been established by a single injection of endotoxin (see Fig. 6). The experiment was then reversed and 50 ml. endogenous pyrogen from vaccine-injected donors were given to two groups of recipients: one normal and the other injected on the preceding day with 4 ml. NDV. The second fever peak, produced by this dose of endotoxin-induced endogenous pyrogen in the

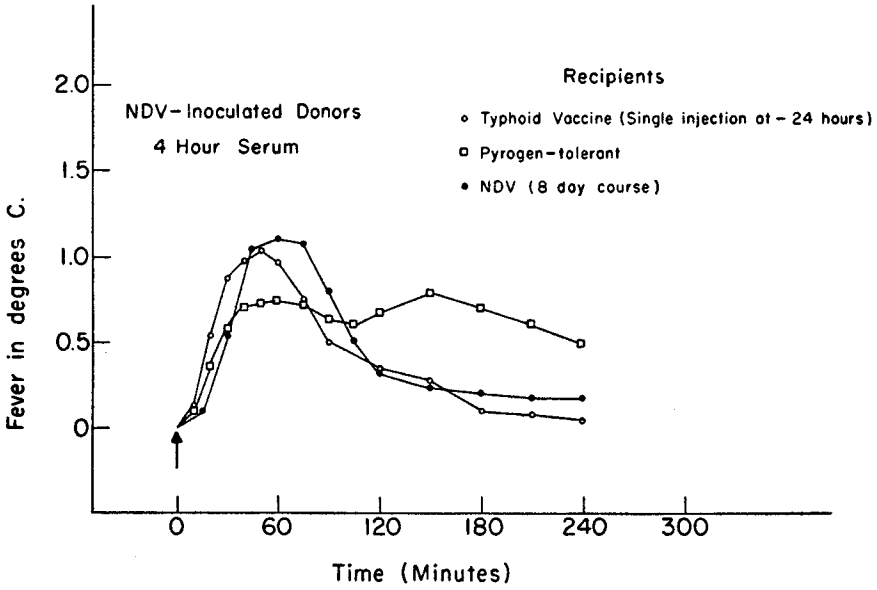

FIG. 6. Comparison of mean pyrogenic responses of recipients to 15 ml. endogenous pyrogen. Rabbits were given either single inoculation of typhoid vaccine or course of injections with vaccine or NDV. Results are averaged from 3, 10, and 4 individual responses, respectively.

normal recipients,[3] was suppressed in the recipients given NDV indicating that the injection of virus had established a similar type of cross-tolerance (see Fig. 7).

In a further experiment, viral-induced endogenous pyrogen was given to a group of 10 recipients which had been made tolerant to bacterial pyrogens by a course of injections of typhoid vaccine (see Methods). These recipients had normal biphasic responses when challenged on the day following the last of the daily injections of vaccine (see Fig. 6 and Table II).

The *endotoxin-tolerant* recipient, unlike the recipient given a *single previous injection of virus or endotoxin*, shows no cross-tolerance to endogenous pyrogen.

Cross-Tolerance to Exogenous Pyrogens.—It has been reported that there is no cross-tolerance to the fever due to virus and endotoxin (7). However, as

[3] In dosages less than 40 ml., endotoxin-induced endogenous pyrogen produced a monophasic fever in normal recipients.

shown in the preceding paragraph, a single injection of typhoid vaccine or virus conferred tolerance (suppression of endogenous pyrogen release and the second fever peak) to an injection of endogenous pyrogen induced by the other agent. Since the febrile response to both virus and endotoxin appears to depend primarily upon the production of endogenous pyrogen, the injection of either one of these exogenous agents should modify the release of endogenous pyrogen and hence the febrile response to a subsequent injection of the other. Cross-tolerance, therefore, should be demonstrable between virus and endotoxin.

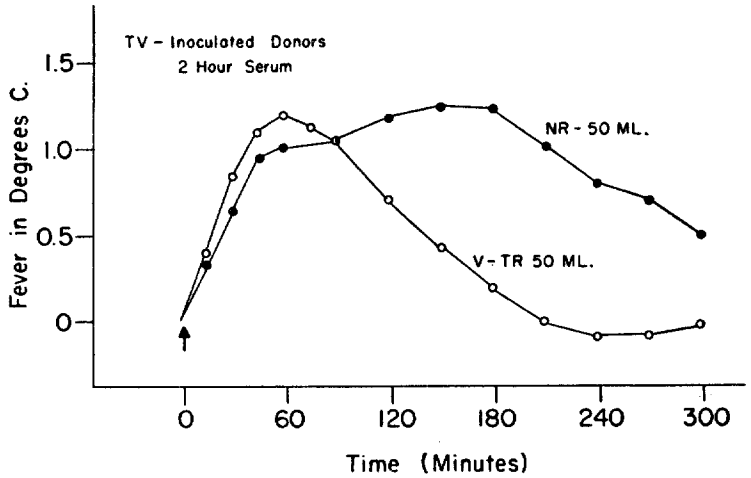

FIG. 7. Mean pyrogenic responses of 5 normal and 4 virus-tolerant recipients to 50 ml. endogenous pyrogen pooled from 8 donors which were inoculated with 5 ml. typhoid vaccine and bled at 2 hours. NR, normal recipient; V-TR, virus-tolerant recipient; TV, typhoid vaccine.

A group of recipients was given a 3 day course of daily injections of 4 ml. NDV. A second group was given a similar course of daily injections with 2 ml. undiluted typhoid vaccine. On the 4th day, each group was injected with the opposite agent. The recipients receiving the course of NDV were inoculated with 0.05 ml. typhoid vaccine (0.5 ml. of a 1:10 dilution); the typhoid vaccine-injected group, in turn, received 0.5 ml. NDV.

Two groups of normal recipients were given a single injection of the same dose of either vaccine or virus on the 4th day.

The composite fever curves of each group are plotted in Fig. 8. The recipients receiving daily injections of typhoid vaccine were almost completely tolerant to NDV.[4] The daily course of NDV was not as effective in conferring tolerance to the final injection of typhoid vaccine. However, there was a definite suppres-

[4] In a similar experiment, a single injection of 2 ml. undiluted typhoid vaccine induced an almost complete tolerance to 1 ml. PR8 virus on the following day. (Mean fever in 4 vaccine-inoculated rabbits: 0.2°C. at 5 hours as opposed to 1.2°C. in 2 controls given virus only.)

sion of the second peak in these animals as compared with the controls. This has been shown to be an early feature in the development of tolerance to this pyrogen (5).

Cross-Tolerance to Exogenous Pyrogen after Injection of Endogenous Pyrogen.— The evidence presented so far has shown that exogenous pyrogens produce circulating endogenous pyrogen and, in addition, confer tolerance to an injection of either exogenous or endogenous pyrogen. The possibility, therefore, was

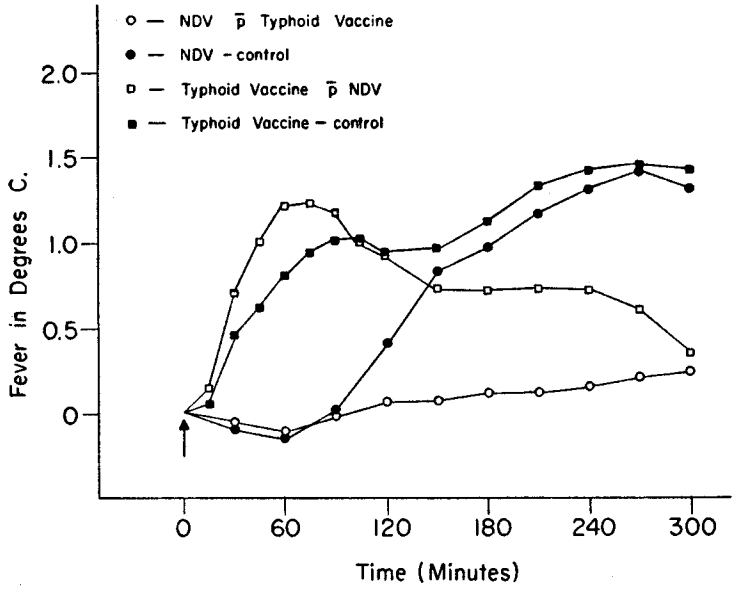

FIG. 8. Mean responses of recipients to injection of either typhoid vaccine or NDV after three daily injections of virus or vaccine, respectively. Control fevers to the same dosages are shown for comparison. Each curve (including controls) represents average of 5 animals to NDV and of 7 animals to vaccine. Difference between experimental and control groups is significant ($p = <0.02$).

considered that these effects were mediated by endogenous pyrogen itself and that the injection of a large amount of endogenous pyrogen might, conversely, induce tolerance to a small dose of virus.

One group of 7 normal rabbits was given individual 50 ml. injections of NDV-induced endogenous pyrogen. The following day these animals were challenged with 0.1 ml. PR8 virus (1.0 ml. of a 1:10 dilution). A control group of 3 rabbits received a 50 ml. injection of normal serum on the day preceding the inoculation of virus.

The mean fever curves of these two groups to the virus are shown in Fig. 9. The prior injection of a large amount of endogenous pyrogen conferred complete tolerance to this dosage of PR8 virus.

In a second experiment of similar design, 9 rabbits were given 50 ml. injections of endogenous pyrogen followed on the next day with 0.5 ml. NDV. Eleven control recipients were given the same dosage of virus after a preliminary injection of 50 ml. non-pyrogenic sera from one of the following sources: (*a*) normal donors (6 recipients), (*b*) donors given 4 ml. NDV and bled at 30 minutes during the latent period (2 recipients), (*c*) donors injected for the 3rd successive day with 4 ml. NDV and bled at 4 hours (3 recipients).

The mean febrile responses of both the experimental and control groups to the injection of virus on the 2nd day of the experiment are also shown in Fig. 9.

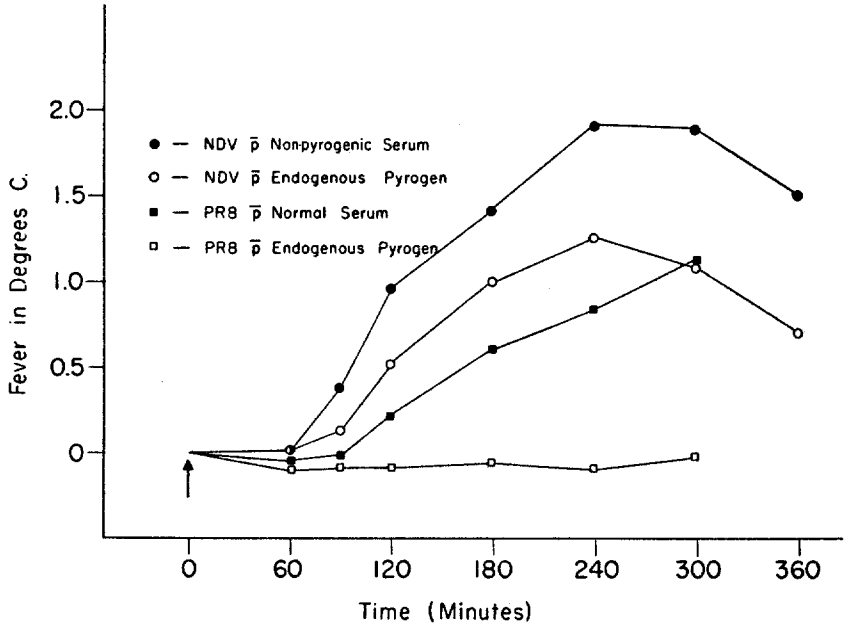

FIG. 9. Mean fevers produced by injection of either 0.5 ml. NDV or 0.1 ml. PR8 in animals receiving preceding injection of 50 ml. endogenous pyrogen. Controls were given similar dosage of non-pyrogenic sera before inoculation of virus. Difference between experimental and control groups at 5 hours with both viruses is significant ($p = <0.01$).

Since the controls had a remarkably uniform response to virus, regardless of the type of sera injected on the preceding day, a single curve is shown. The group which had received endogenous pyrogen, on the other hand, had a depressed febrile response to NDV simulating the tolerance which develops to this virus on the 2nd day of injection (see Figs. 1 and 10).

The Development of Tolerance to Endogenous Pyrogen with Successive Injections.—In order to determine whether there was any development of tolerance to repeated injections of endogenous pyrogen, a group of recipients was given a series of eight daily inoculations of 15 ml. endogenous pyrogen.

Since the responses of the group to this procedure were remarkably similar,

a representative recipient has been selected. The responses of this animal on alternate days are plotted in Fig. 10 (lower shadowgraphs).

The following features of developing tolerance to this dosage of endogenous pyrogen are particularly noteworthy: (*a*) The absence of any consistent change in the onset, height or duration of the first peak; (*b*) The progressive depression, delay, and eventual disappearance of the second fever peak with a marked decrease occurring as early as the 2nd day.

These characteristics of tolerance to endogenous pyrogen permit the following conclusions: (*a*) No tolerance exists to the initial effect of injected endoge-

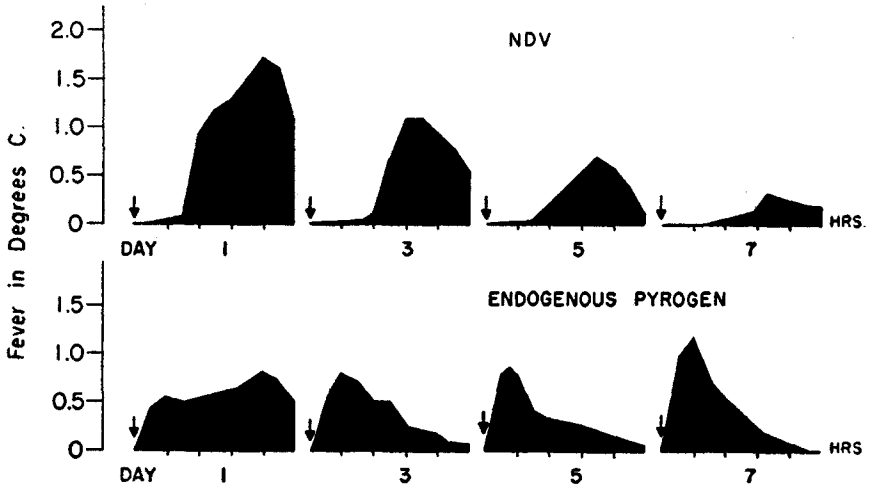

FIG. 10. Febrile responses of representative normal recipients given daily injections of either 1 ml. NDV or 15 ml. endogenous pyrogen.

nous pyrogen. (*b*) Tolerance develops to the production of endogenous pyrogen as evidenced by the disappearance of the second peak.

The Relation of Tolerance to Dosage of either Virus or Endogenous Pyrogen.— French (17) and Wagner (18) have shown that tolerance to an injection of virus is closely related to the dosage of either homologous or heterologous virus given on the preceding day. A large dose of virus, though producing no greater fever, may completely block the pyrogenic response to a small dose the following day. Tolerance developing to daily injections of endogenous pyrogen, however, has been shown to affect only the second peak which, like fever due to virus, appears to be due to the release of endogenous pyrogen (9). The presumably direct action of endogenous pyrogen which causes the first peak should be unaffected, in contrast to the pyrogenic action of virus, by an abrupt reduction in the dosage of injected endogenous pyrogen.

In the upper half of Fig. 11 are shown the mean febrile responses of two

groups of rabbits to an initial injection of either 1 ml. or 4 ml. of NDV followed on the next day by a second injection of 4 ml. and 1 ml. of NDV, respectively. Tolerance to the smaller dosage was complete when it followed the larger. Although the initial fevers produced by the 1 ml. and 4 ml. dosages were indis-

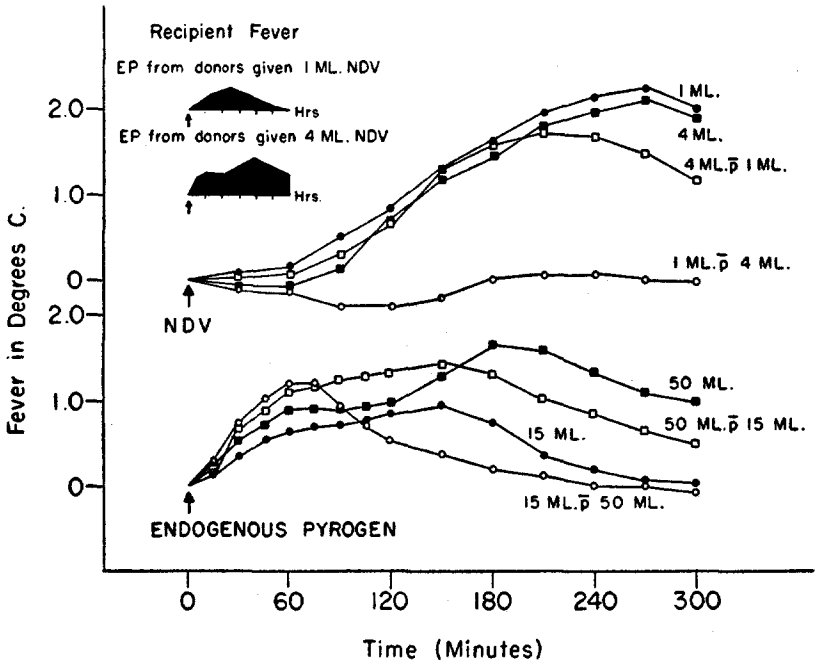

FIG. 11. Pyrogenic effect of change in dosage of either virus or endogenous pyrogen. In upper chart are mean fevers in 4 recipients given either 1 ml. or 4 ml. NDV followed by reversal in dosage the next day. Shadowgraphs show average pyrogenic responses induced in 4 normal recipients by endogenous pyrogen from donors receiving either 1 or 4 ml. virus. Lower chart records similar experiment with endogenous pyrogen using 15 and 50 ml. dosages. Virus fevers represent average of 4 individual curves; mean fevers of 3 recipients are shown with endogenous pyrogen. Ordinate is reduced to one-half scale shown in previous figures. EP, endogenous pyrogen.

tinguishable, the shadowgraphs indicate that there was a greater amount of transferable endogenous pyrogen in the donors receiving the 4 ml. injection.

The same experiment was then carried out with endogenous pyrogen. Three normal rabbits were first injected with 15 ml. endogenous pyrogen and their temperatures recorded. The following day the dosage was increased to 50 ml. In another group of 3 recipients the order of injection was reversed, the larger dose being followed by the smaller.

The results are plotted in the lower half of Fig. 11. In contrast to the results with virus, the order of injection had no effect on the first peak, although the

second peak was abolished in the recipients receiving the smaller dose of endogenous pyrogen after the larger.

From these two experiments the following conclusions can be drawn:

(*a*) The first peak of fever produced by endogenous pyrogen, unlike that following the injection of virus, was not influenced by an abrupt decrease in dosage. This supports the concept that endogenous pyrogen has an initial direct action on the thermoregulatory center.

(*b*) Tolerance occurred only when the febrile response normally required the *production* of endogenous pyrogen. This applies both to fever with virus and to the second fever peak induced by endogenous pyrogen. Tolerance in both instances, therefore, indicates the absence of endogenous pyrogen production.

(*c*) The degree of tolerance is directly related to relative size of the initial and the second doses of either virus or endogenous pyrogen. These dosages, in turn, are directly proportional to the *amount of endogenous pyrogen released in the recipients.* (See shadowgraphs and febrile responses to endogenous pyrogen in Fig. 11.)

In summary, the evidence suggests that tolerance, or the absence of endogenous pyrogen production, bears a direct relationship to the amount of endogenous pyrogen mobilized, either by virus or by endogenous pyrogen itself, on the preceding day.

Maintenance of Tolerance after Course of NDV or Endogenous Pyrogen.—As shown above, animals given daily injection of virus respond with progressively less fever and endogenous pyrogen production (Fig. 1). Two possibilities were considered: the refractory state results from a depletion of endogenous pyrogen due to its prior release or, conversely, that circulating endogenous pyrogen itself suppresses the further mobilization of this substance.

A group of recipients was given a course of eight daily injections of 15 ml. endogenous pyrogen from NDV-inoculated donors. The responses of a representative recipient have been shown in Fig. 10. It will be noted that the second peak, indicating activation of the recipient's endogenous pyrogen, did not persist beyond the 2nd day. However, when the 4 recipients were challenged with a large dose of endogenous pyrogen (50 ml.) on the 9th day, they all responded with monophasic fevers characteristic of tolerance (see Figs. 5 and 10). For comparison, the mean response of a group of normal recipients is shown to 50 ml. endogenous pyrogen after a single injection of 15 ml. endogenous pyrogen (Fig. 11). The presence in this latter group of a marked second peak indicating the release of endogenous pyrogen to the larger dosage is evident.

A course of injections of endogenous pyrogen thus appears more effective than a single injection in blocking the further activation of this substance, although there seems to be no more endogenous pyrogen released during a course than with a single injection (note absence of second fever peak from 3rd through 7th day, Fig. 10). This finding suggests that tolerance is not due to depletion of

available endogenous pyrogen but that circulating endogenous pyrogen itself may actually suppress the release of this substance to a subsequent pyrogenic stimulus. The evidence, however, at present does not permit a definite conclusion on the mechanism by which endogenous pyrogen release induces tolerance.

In an additional experiment, 4 recipients were given a course of eight daily injections of 1 ml. NDV. The development of tolerance is shown in the shadowgraph (Fig. 10). At the conclusion of the series, the 4 recipients were given 15 ml. endogenous pyrogen. All the animals had monophasic responses (see Fig. 6).

The persistence of tolerance to endogenous pyrogen after daily injections of virus contrasts with the response of recipients rendered endotoxin-tolerant by a course of injections of typhoid vaccine. These recipients, when challenged with endogenous pyrogen, had normal biphasic fever curves indicating that tolerance to endogenous pyrogen had lapsed during the acquisition of tolerance to bacterial pyrogens (see Fig. 6).

DISCUSSION

These experiments have tended to indicate a close relationship between the production of endogenous pyrogen and virus tolerance. The decrease in fever to reinjected virus, characteristic of tolerance, is associated with a comparable reduction in the level of circulating transferable endogenous pyrogen.

Correlated with the change which occurs in production of endogenous pyrogen to reinjected virus is the altered response of the virus-tolerant recipient to an injection of endogenous pyrogen itself.

As shown in the preceding paper in this series, injected endogenous pyrogen seems to cause the release of further endogenous pyrogen in the normal recipient with the development of a characteristic second peak of fever (9). However, the recipient given virus on the preceding day (and hence designated virus-tolerant) responds only to the direct pyrogenic action of injected endogenous pyrogen as evidenced by a monophasic rather than a biphasic fever.

There appear to be two basic febrile responses to the injection of endogenous pyrogen: monophasic and biphasic. All recipients which react with a biphasic fever curve also react normally to an injection of virus. These include the various types listed in Table II. None of these recipients has received an immediately preceding injection of a pyrogenic agent which releases a transferable amount of circulating endogenous pyrogen.[5] The endotoxin-tolerant recipient deserves special mention. Although daily injections of endotoxin are given up to the time of the experiment, clearance of exogenous pyrogen is so rapid that no detectable endogenous pyrogen is present in the sera of this group (2, 19, 20). On the other hand, all of the recipients which have a monophasic response to endogenous pyrogen have had an injection of a pyrogenic agent on the preceding day and

[5] Fever accompanying the intramuscular injection of epinephrine is unassociated with a detectable level of endogenous pyrogen (8).

also show some degree of tolerance to virus. The presence of a second fever peak to injected endogenous pyrogen appears, therefore, to be a measure of the recipient's ability to produce endogenous pyrogen which also seems to be essential for the normal febrile response to virus. Similarly, tolerance to endogenous pyrogen, characterized by absence of a second fever peak, may be equated with tolerance to virus since in both instances there is a reduction or absence of endogenous pyrogen production. From the observed differences in the response of normal and virus-tolerant recipients to an injection of endogenous pyrogen, *it is postulated that the circulation of endogenous pyrogen on the previous day has rendered the recipient unable to mobilize its own endogenous pyrogen and hence tolerant to an injection of either endogenous pyrogen or virus on the following day.*

The normal recipient also develops tolerance to daily injections of endogenous pyrogen as indicated by the gradual disappearance of the second fever peak. However, there is no change in either onset or height of the first fever peak with continued injections of endogenous pyrogen (Fig. 10).[6] The absence of tolerance to the *direct* pyrogenic effect of endogenous pyrogen would appear to rule out the participation of the thermoregulatory center in the development of tolerance.

Virus, on the other hand, seems to cause fever only indirectly by the liberation of endogenous pyrogen in the recipient, as indicated by the long latent period. Accordingly, with successive daily injections of virus, there is a progressive reduction in the height of the *first* as well as the second peak consistent with a demonstrable decrease in the production of endogenous pyrogen (Fig. 10). Tolerance to the pyrogenic effect of virus, therefore, involves *both* fever peaks and apparently results from mechanisms which interfere with the release of endogenous pyrogen. The mechanisms seem to be similar to those operative in the development of tolerance to endogenous pyrogen itself.

The foregoing experiments have defined four distinct categories of tolerance:

(*a*) Cross-tolerance between two different exogenous pyrogens: virus and endotoxin;[7] (*b*) Tolerance to endogenous pyrogen (absence of second fever peak) after preceding injection of homologous or heterologous exogenous pyrogen (virus or endotoxin); (*c*) Tolerance to exogenous pyrogen (virus) after a large injection of endogenous pyrogen; (*d*) Tolerance to endogenous pyrogen (loss of second fever peak only) developing with daily injections.

[6] A similar type of tolerance seems to develop with the daily injection of canine granulocytic exudates in dogs (21, see shadowgraphs).

[7] In retrospect, it seems not unlikely that the effectiveness of crude *Cholera vibrio* extract in conferring tolerance to the pyrogenic effects of a subsequent injection of influenzal viruses (22) may have been due to an endotoxin present in this material. It is not stated whether fevers were obtained with the extract, although its toxicity is evident by the fact that approximately one-fourth of the animals died. In this regard, it has been shown recently that the intravenous injection of endotoxin is capable of inducing tolerance to the toxic effects of influenza virus in mice (23).

It is postulated that all of the changes described above may be actually due to the last mechanism. The injection of a number of pyrogenic agents including endogenous pyrogen itself, seems to be followed by the release of a detectable amount of endogenous pyrogen in the recipient. This latter reaction then appears to induce a temporary and relative refractoriness to further endogenous pyrogen release which is characteristic of tolerance to both virus and endogenous pyrogen. The similar duration of tolerance to small amounts of virus and endogenous pyrogen is also suggestive of a common underlying process. Of interest in this regard are the results with antipyrine. Abolition of virus fever with antipyrine does not prevent the development of tolerance either to virus (7) or to injected endogenous pyrogen (9). The hypothesis that endogenous pyrogen is itself important in the development of tolerance to these agents is strengthened by the additional findings that antipyrine does not modify the appearance of circulating endogenous pyrogen following an injection of virus (8). Conversely, procedures which render virus non-pyrogenic (*e.g.* heating or incubation with homologous immune serum) also make virus incapable of inducing tolerance (7, 17) suggesting again that tolerance is dependent upon the prior release of endogenous pyrogen.

Certain differences should be pointed out between the type of tolerance described here and that which develops with successive injections of endotoxins. Studies of the "clearance" of intravenously injected PR8 virus in normal and virus-tolerant rabbits show identical values despite the absence of transferable endogenous pyrogen in the circulation of the tolerant animal.

This finding contrasts sharply with the situation existing in tolerance to bacterial pyrogens, in which the development of an accelerated clearance of injected endotoxin appears to play a major role in the pathogenesis of tolerance (2, 19, 20, 24–26).[8] Furthermore, the reversal of tolerance to endotoxins but not to PR8 virus with the so called reticuloendothelial blocking agent, thorotrast (7), is consistent with the concept that pyrogen tolerance is largely due to an enhanced removal of endotoxins by cells of the reticuloendothelial system.

It should be emphasized, however, that there is a decrease in the detectable level of endogenous pyrogen in both these forms of tolerance. With endotoxins, the acceleration of clearance apparently results in a correspondingly diminished *stimulus* to endogenous pyrogen production. Since relatively little endogenous pyrogen has been mobilized, the pyrogen-tolerant recipient has available supplies and therefore responds like a "rested" or normal recipient to the injection of either virus (7) or endogenous pyrogen. The virus-tolerant recipient, on the other hand, seems to be unable to respond normally either to endogenous pyrogen or reinjection of virus because the previous production of endogenous

[8] With certain endotoxins, however, the onset of tolerance occurs as early as the 2nd day with disappearance of the second fever peak (5). Since the phagocytic function of the reticuloendothelial system is actually depressed at this time (25, 27, 28), this type of early tolerance may be analogous to that seen with NDV (see Fig. 1).

pyrogen has resulted in a temporary impairment of its ability to release further amounts of this material.

The diminution in the febrile response, characteristic of both forms of tolerance, appears to be a result of the decrease in endogenous pyrogen production although the mechanism of this decrease is different in the two situations.

Although the source and intermediate events in the liberation of endogenous pyrogen remain unknown, the particular types of cross-tolerance demonstrated here between virus and endotoxin suggest that endogenous pyrogen circulating in these two types of fever may be similar. Furthermore, the fact that both viral and vaccine-induced endogenous pyrogen produce an equal degree of fever in normal and endotoxin-tolerant animals (20, 29, 30) indicates a similarity to leukocytic pyrogen (31) and, conversely, would seem additional evidence that these substances are not identical with known pyrogenic polysaccharides of either bacterial or tissue origin (32).

SUMMARY

Tolerance to the pyrogenic action of intravenously injected virus has been studied in rabbits given either PR8 strain of influenza A virus or Newcastle disease virus (NDV). The following findings suggest that the capacity of the animal to release an endogenous pyrogen is a critical factor.

(*a*) Viruses produce fever by the release of endogenous pyrogen.

(*b*) Tolerance to the pyrogenic effect of reinjected virus is associated with a decrease or absence of endogenous pyrogen production.

(*c*) Tolerance to virus fever may be induced by the prior injection of endogenous pyrogen.

(*d*) Cross-tolerance has been demonstrated between virus and bacterial endotoxin (typhoid vaccine), both of which cause the release of endogenous pyrogen.

A difference has been noted in the mechanism of tolerance to successive injections of virus and of bacterial endotoxin. Repeated injections of endotoxins bring about an accelerated clearance of these substances from the blood stream with the result that there is a diminished stimulus to endogenous pyrogen production. Tolerance to virus, on the other hand, is unaccompanied by changes in the rate at which virus disappears from the circulation. It is postulated that circulating endogenous pyrogen, induced by the injection of a number of pyrogenic agents including viruses, temporarily suppresses the further release of endogenous pyrogen and thereby contributes to virus tolerance.

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