OBSERVATIONS ON THE FEVER CAUSED BY BACTERIAL PYROGENS

I. A Study of the Relationship between the Fever Caused by Bacterial Pyrogens and the Fever Accompanying Acute Infections*

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(Received for publication, May 3, 1948)

INTRODUCTION

Many bacteria produce substances capable of causing fever when injected into animals, and it seems possible that the fever accompanying acute infections may be attributable in some cases to a pyrogen produced by the infecting organism (1, 2). In the case of the fever following the intravenous injection of a pyrogenic substance such as typhoid vaccine, the time lag of 30 to 60 minutes before any rise in temperature suggests, as Beeson points out (3), that the pyrogen does not act directly on the hypothalamus. Since pyrogens are potent toxins, producing widespread tissue damage (4), it may be that their fever-promoting effect is secondary to injury.

The mechanism of the remarkable tolerance to the pyrogenic action of typhoid vaccine acquired by patients receiving multiple intravenous injections of this material for therapeutic purposes (5, 6) was studied by Beeson (3, 7). In 1947, he reported experiments demonstrating that rabbits acquire this tolerance readily and that an animal tolerant to one bacterial pyrogen is also insensitive in some degree to pyrogens produced by other organisms. The development of tolerance to a pyrogen was shown to be independent of the temperature rise following its injection, and mechanically induced fever failed to produce any tolerance. Beeson further showed that this tolerance is apparently dissociated from specific antibody formation and that the probable mechanism is an increase in the ability of the reticulo-endothelial system to remove the pyrogen from the circulation and thus prevent its temperature-raising effect. Tolerance is of short duration, being lost in 3 weeks.

If there exists any connection between the fever of acute infections and that produced by pyrogens of the infecting bacteria, animals convalescent from in-

* The opinions expressed in this report are to be construed as those of the author alone and do not reflect those of the Naval Medical Corps or the naval service at large.

fection might be expected to show tolerance for pyrogen. The present study was undertaken in an effort to define this relationship.

Though the amounts of pyrogen produced by bacteria of various types vary widely, it is generally true that the Gram-negative bacilli are the most potent producers of fever-promoting substances while the Gram-positive cocci are the least thermogenic (1). Therefore, the two infecting organisms chosen for this study were a Gram-negative bacillus and a Gram-positive coccus.

Materials and Methods

Animals.—Male New Zealand white rabbits weighing 2400 to 3100 gm. were used. They were caged in an air-conditioned room at 70° F. throughout the study. During tests involving

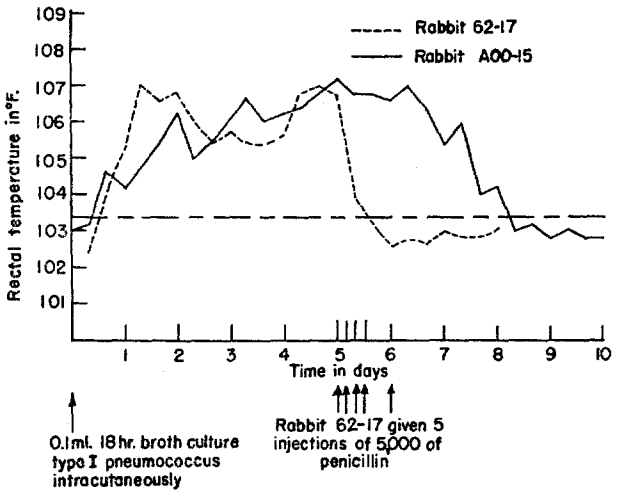

FIG. 1. Typical fever curve of an animal infected intradermally with Type I pneumococcus and allowed to recover without treatment, compared with fever curve of an animal with similar infection treated with penicillin on the 5th day of illness.

the injection of a pyrogen, they were placed in individual stalls and, after three rectal temperatures had been taken at 30 minute intervals to establish a normal level, the pyrogen was injected into the marginal ear veins. Rectal temperatures were then recorded every 30 minutes for 7 hours.

Infections.—Type I pneumococcus and *Escherichia coli* were used to produce infections in rabbits. The strain of pneumococcus was one obtained from the National Institute of Health and had been maintained in brain-heart infusion broth with weekly mouse passage. The intravenous injection of 9 billion of these organisms killed by heating at 60° C. for 30 minutes produced no significant temperature rise in rabbits. After the skin of the flank had been shaved, dermal pneumococcal infection of the type described by Goodner (8) was produced by the intracutaneous injection of 0.1 ml. of an 18 hour broth culture. The resulting infection was accompanied by high fever, and two-thirds of the infected animals died. In the surviving animals, fever ended by crisis on the 6th to 9th day. Ten animals were given 5,000 units of

penicillin intramuscularly every 4 hours on the 5th day of illness with 100 per cent recovery (Fig. 1).

Several strains of *E. coli* were tested for pathogenicity in rabbits with little success until a strain known to produce fatal peritonitis in mice was subjected to repeated passage in mice. Preliminary experimentation showed that the intraperitoneal injection of 1.0 ml. of an 8 hour broth culture of this organism in rabbits produced an illness accompanied by high fever with a duration of 7 to 8 days (Fig. 2). Approximately two-thirds of the animals thus infected survived.

Rectal temperatures of infected animals were recorded every 8 hours until a normal level (below 103.4° F.) was reached and maintained for at least 24 hours.

Pyrogenic Materials.—Two pyrogenic agents were used, *Salmonella typhi* and *E. coli* killed by heat. The dosages of these materials were adjusted so that each would cause marked pyrexia in rabbits without fatalities. The typhoid vaccine contained about 125 million *Sal-*

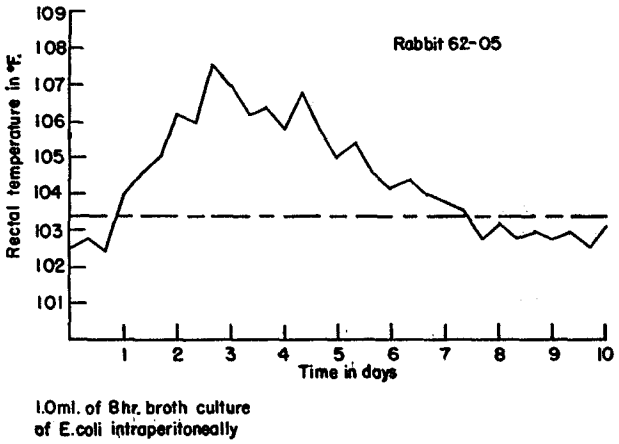

FIG. 2. Typical fever curve of an animal ill with peritonitis due to *E. coli*.

monella typhi organisms per ml. and was usually given in a dose of 1.0 ml. The comparable dose of *E. coli* was 300 million organisms in 1.0 ml. of physiologic saline.

All needles and glassware were sterilized in an oven at 170° C. for 3 hours to destroy any contaminating pyrogen. The physiologic saline used was tested frequently and was always pyrogen-free.

Method of Recording Results.—Following a method similar to that of Beeson, the temperature records after pyrogen injection were plotted on 3/16 inch graph paper and, using 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. The vernier reading of the planimeter was taken as the "fever index," an expression of the height and duration of the fever.

RESULTS

Response to Single Injection of Pyrogen.—Forty-five animals received one injection of typhoid vaccine. The fever indices ranged from 92 to 193 with a mean of 128.4. Twenty-four animals were given one injection of *E. coli* vaccine. The fever indices of these animals varied from 98 to 200 with a mean

of 153.1. The first column in Fig. 3 shows the distribution of the fevers in these animals.

Effect of Repeated Injections of Pyrogens.—Twelve animals received daily injections of typhoid vaccine for 4 weeks. Beginning on the 4th day, a marked diminution in febrile response to the injections appeared. This reduction occurred both in height and duration of the fever. At the end of 4 weeks, each

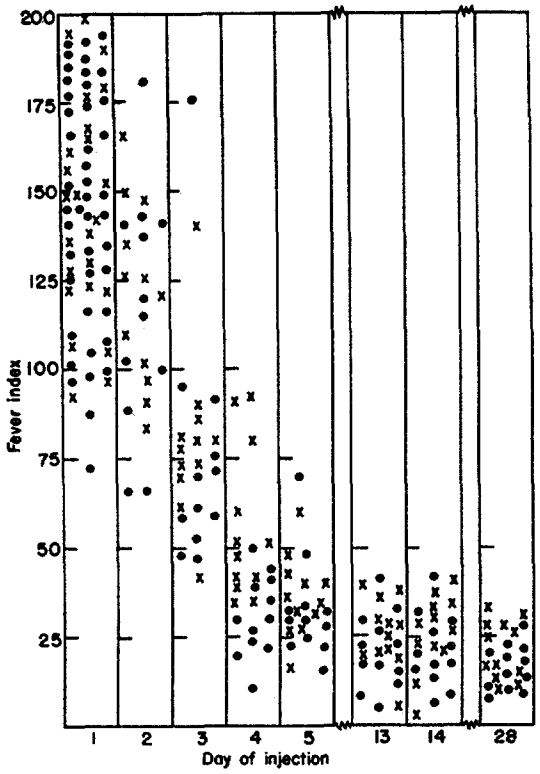

FIG. 3. Distribution of fevers recorded in animals at various times during the course of daily injections of 1.0 ml. of *S. typhi* (circles) or *E. coli* (crosses) vaccine.

of the animals tolerant to typhoid vaccine was given an injection of *E. coli* vaccine and the temperature response was recorded; they were also insensitive to the *E. coli* pyrogen, the fever indices for the group averaging 31.3. After a 3 week rest period, six animals received typhoid and six received *E. coli* vaccine; all tolerance had disappeared (Fig. 4).

Twelve animals were given daily injections of *E. coli* vaccine for 4 weeks. Again beginning on the 4th day, a marked lessening in the height and duration of the fever was apparent. At 4 weeks, these animals received typhoid

vaccine and exhibited tolerance for this material, their fever indices averaging 32.6. After a rest period of 3 weeks, six animals were given *E. coli*, and six, typhoid vaccine. All tolerance had disappeared during this interval (Fig. 5).

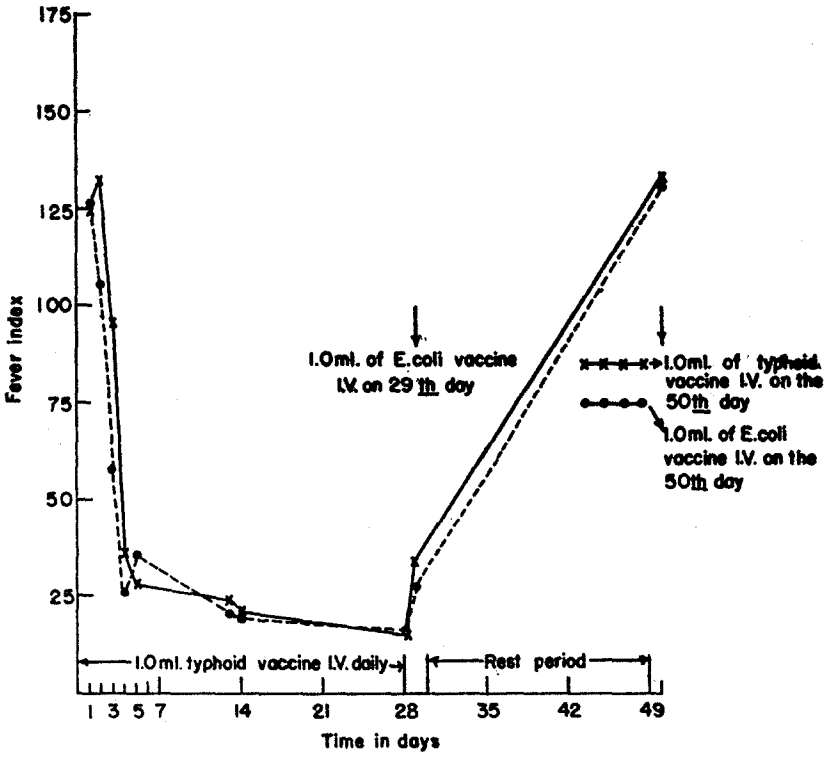

FIG. 4. Average fever indices in two groups of six rabbits given 1.0 ml. of *S. typhi* vaccine daily for 4 weeks. Note that by the 4th day the febrile responses had dropped almost to the level reached at the 28th day. On the 29th day, both groups received 1.0 ml. of *E. coli* vaccine, with only slight increase in the fever indices, indicating their tolerance for this pyrogen as well. The increased response to both vaccines after a rest period of 3 weeks indicates loss of tolerance with discontinuance of daily injections.

This study was repeated, employing smaller doses of pyrogen. One ml. of the 1:10 dilution of typhoid vaccine contained 12.5 million bacilli; the *E. coli* vaccine was also used in a dose of 1.0 ml. of a 1:10 dilution, or 30 milion bacilli. With repeated injections, animals developed tolerance to these smaller doses (Fig. 6).

Effect of Pneumococcal Infection upon Response to Pyrogens.—Ten animals surviving infection with Type I pneumococcus received 1.0 ml. of typhoid vaccine 24 hours after their temperatures had returned to normal, and a second

injection 3 weeks after infection since any tolerance resulting from infection would have disappeared by this time. Five of the animals in this group had recovered from the infection without treatment and five had received penicillin on the 5th day. There was no evidence of any tolerance to typhoid vaccine in

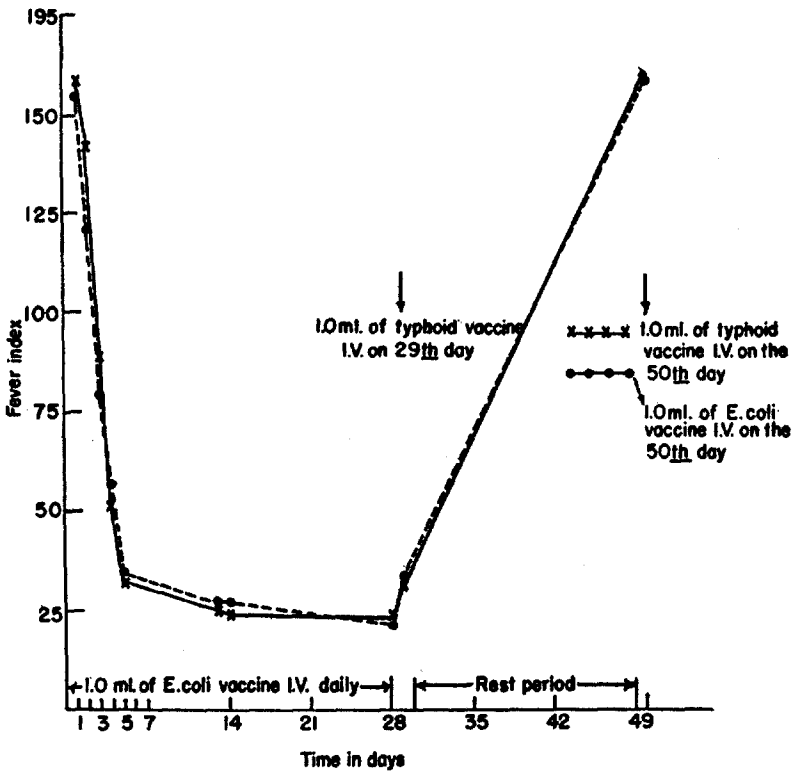

FIG. 5. Average fever indices of two groups of six animals showing development of tolerance to daily injections of *E. coli* vaccine over a period of 4 weeks, with slight rise in fever indices when *S. typhi* vaccine was substituted on the 29th day. Again the high fevers in response to both vaccines after 3 weeks indicate the disappearance of pyrogen tolerance.

any of these animals, the indices averaging 154.1 at 24 hours and 153.5 at 3 weeks (Table I).

A second group of animals surviving dermal pneumococcal infection showed no tolerance to 1.0 ml. of *E. coli* vaccine. The fever indices averaged 133.4 at 24 hours and 140.3 at 3 weeks (Table II).

Effect of E. coli Infection upon Response to Pyrogens.—Twelve animals surviving *E. coli* peritonitis were given 1.0 ml. of typhoid vaccine 24 hours and again 3 weeks after their temperatures had returned to normal. There was no

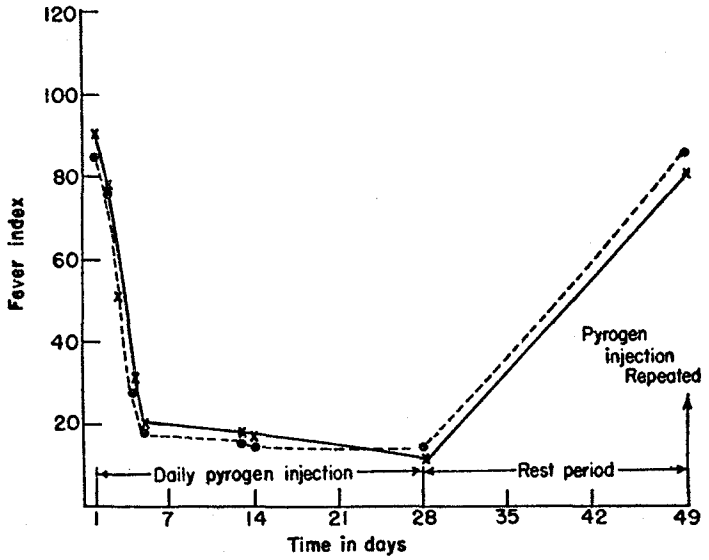

FIG. 6. Average fever indices of two groups of six animals, one receiving 0.1 ml. of *E. coli* vaccine daily for 4 weeks and the other a similar dose of *S. typhi* vaccine. Note the development of tolerance to these smaller amounts of pyrogen with loss of tolerance after discontinuance of injections for 3 weeks.

TABLE I

Response of Ten Animals, Convalescent from Dermal Infection with Type I Pneumococcus, to Injection of 1.0 Ml. of Typhoid Vaccine 24 Hours and 3 Weeks after Return of Temperatures to Normal

Animal No.	Penicillin on 5th day	Duration of infection	Fever index 24 hrs. after recovery	Fever index 3 wks. after recovery
		days		
90-27	Yes	6	168	156
90-38	Yes	6	134	153
90-47	Yes	6	213	178
90-40	Yes	6	126	133
65-26	Yes	6	128	112
62-16	No	8	140	135
A0-01	No	7	145	152
A0-02	No	8	157	163
A0-04	No	8	179	180
A0-06	No	7	151	173
Average			154.1	153.5

evidence that the infection had produced any tolerance to the *S. typhi* pyrogen, the fever indices averaging 161.3 at 24 hours and 162.5 at 3 weeks. This

experiment was repeated with twelve animals, using *E. coli* vaccine instead of typhoid. The fever indices, which averaged 166.6 at 24 hours and 168.0 at 3

TABLE II

Response of Ten Animals, Convalescent from Dermal Pneumococcal Infection, to Intravenous Injection of 1.0 Ml. of E. coli Vaccine 24 Hours and 3 Weeks after Return of Temperatures to Normal

Animal No.	Penicillin on 5th day	Duration of infection	Fever index 24 hrs. after recovery	Fever index 3 wks. after recovery
		days		
A2-32	Yes	6	98	130
A2-33	Yes	6	127	140
A2-34	Yes	6	171	154
A2-35	Yes	6	163	160
A2-36	Yes	6	128	144
A2-23	No	6	113	142
A2-25	No	8	122	138
A2-29	No	8	104	109
A2-30	No	7	161	147
A2-31	No	8	147	139
Average			133.4	140.3

TABLE III

Response of Twelve Animals to Injection of 1.0 Ml. of Typhoid Vaccine 24 Hours and 3 Weeks after Recovery from E. coli Peritonitis

Animal No.	Duration of infection	Fever index 24 hrs. after recovery	Fever index 3 wks. after recovery
	days		
45-51	7	211	194
45-52	8	114	143
45-54	9	168	198
45-55	7	127	136
45-58	7	156	163
45-59	9	147	151
45-60	9	171	160
45-61	8	187	172
45-62	9	193	147
45-66	7	138	156
45-67	9	171	142
45-68	8	153	188
Average		161.3	162.5

weeks, demonstrated that active infection with the colon bacillus had produced no tolerance to the fever-promoting effect of this dose of pyrogen derived from the same strain (Tables III and IV).

Smaller doses of pyrogen were given to animals surviving *E. coli* peritonitis. Six convalescent animals received 1.0 ml. of the 1:10 dilution of typhoid vaccine at 24 hours and 3 weeks and six animals received 1.0 ml. of the 1:10 dilution of *E. coli* vaccine. There was no evidence that the infection had produced tolerance for these smaller amounts of pyrogen (Table V).

TABLE IV

Response of Twelve Animals to Injection of 1.0 Ml. of E. coli Vaccine 24 Hours and 3 Weeks after Recovery from E. coli Peritonitis

Animal No.	Duration of infection	Fever index 24 hrs. after recovery	Fever index 3 wks. after recovery
	days		
45-71	8	197	143
45-73	9	156	203
45-77	7	188	181
45-78	8	173	184
45-79	8	162	190
45-81	9	141	128
45-82	9	157	151
45-83	7	168	172
45-86	8	214	192
45-87	8	162	198
45-88	9	130	136
45-89	7	151	138
Average		166.6	168.0

TABLE V

Response of Twelve Animals to Injections of 0.1 Ml. of Typhoid or E. coli Vaccine 24 Hours and 3 Weeks after Recovery from E. coli Peritonitis

Animal No.	Duration of infection	Pyrogenic material used	Fever index 24 hrs. after recovery	Fever index 3 wks. after recovery
	days			
90-35	8	*S. typhi*	81	76
90-36	9	" "	93	74
90-37	7	" "	88	94
90-38	8	" "	109	108
90-39	8	" "	76	83
90-40	8	" "	104	94
90-44	9	*E. coli*	110	98
90-45	9	" "	91	90
90-46	7	" "	69	79
90-47	7	" "	78	73
90-48	9	" "	87	106
90-49	8	" "	93	88
Average			89.0	88.6

Effect of Concomitant Infection upon Development of Tolerance to Repeated Injections of Pyrogens.—Twelve rabbits with *E. coli* peritonitis received daily injections of 1.0 ml. of the 1:10 dilution of typhoid vaccine throughout the course of the infection. It was necessary to use the smaller dose of pyrogen in these studies in order to obtain survivors since the combination of the peritonitis and the larger doses almost invariably caused hyperpyrexia and death. The temperatures of the seven surviving animals had returned to normal in 7 or 8 days. 24 hours and again 3 weeks after recovery, the injection of typhoid vaccine was repeated (Table VI). The fever indices at 24 hours were low (average, 23.0), indicating that the animals were capable of developing tolerance to pyrogen in the presence of infection. This tolerance had disappeared in 3 weeks (average, 90.1). In a similar experiment using *E. coli* vaccine, the fever

TABLE VI

Response of Seven Animals, Given Daily Injections of 0.1 Ml. of Typhoid Vaccine during the Course of E. coli Peritonitis, to a Repeated Dose of Vaccine 24 Hours and Again 3 Weeks after Recovery from Infection

Animal No.	Duration of infection	Fever index 24 hrs. after recovery	Fever index 3 wks. after recovery
	days		
91-26	8	21	81
91-28	9	32	106
91-29	7	17	92
91-33	6	19	73
91-34	9	27	112
91-36	8	17	68
91-37	9	28	99
Average		23.0	90.1

TABLE VII

Response of Five Animals Given Daily Injections of 0.1 Ml. of E. coli Vaccine during the Course of E. coli Peritonitis, to a Repeated Dose of Vaccine 24 Hours and Again 3 Weeks after Recovery from Infection

Animal No.	Duration of infection	Fever index 24 hrs. after recovery	Fever index 3 wks. after recovery
	days		
91-40	9	25	84
91-44	8	31	78
91-48	7	11	91
91-49	9	27	77
91-51	8	24	82
Average		23.6	82.4

indices of the five surviving animals averaged 23.6 at 24 hours and 82.4 at 3 weeks, indicating the development of tolerance to the *E. coli* pyrogen during the infection, and its subsequent loss (Table VII).

DISCUSSION

The results presented above confirm the finding of Beeson that rabbits will become tolerant to bacterial pyrogens if given a course of daily injections. Animals tolerant to one pyrogen are also insensitive in large measure to pyrogens produced by other organisms. The duration of the tolerance is less than 3 weeks.

Although fever accompanying various types of disease is attributable in a general way to some imbalance of the temperature-regulating centers, the mechanism involved is poorly understood. In cases of brain trauma or increased intracranial pressure, mechanical stimulation undoubtedly plays a part. Whether there is a common factor underlying the fever of such varied conditions as acute infections, hemolytic crises, serum sickness, rheumatic fever, pernicious anemia, neoplasms, hepatic failure, trauma, and myocardial infarction is not definitely known. The presence of tissue injury is a characteristic of all these states and it seems possible that some product of cell damage or altered cell metabolism may act on the hypothalamus to produce fever accompanying them. Menkin (9) has isolated from inflammatory exudates a substance which he considers responsible for the fever accompanying acute inflammatory states. This material he terms *pyrexin* and describes as a product of cell injury. His work has not been confirmed up to this time.

The failure of animals infected with Type I pneumococcus to show tolerance for pyrogens after recovery is not surprising, since this organism produces little or no pyrogen demonstrable by the methods used in this study. The failure of actual febrile infection with the colon bacillus to produce tolerance for pyrogens in rabbits seemed to indicate that the pyrogen produced by this organism plays little or no part in the production of the fever accompanying infection or that the conditions necessary for the development of this tolerance are not present during infection (perhaps due to some effect on the reticulo-endothelial system). Animals receiving repeated pyrogen injections during infection, however, developed tolerance in the usual fashion. Thus, if seems probable that the pyrogen produced by *E. coli* is not the primary factor responsible for the production of fever accompanying infections with this organism.

According to the hypothesis that fever of infection is caused by some product of cellular injury, the failure of *E. coli* infection to confer tolerance to the fever-promoting effect of its pyrogen may be explained by assuming that the pyrogen itself is not the primary cause of tissue injury in infection, though the introduction of a comparatively large quantity of pyrogen directly into the blood stream brings about enough cell injury to cause fever.

These findings, then, apparently represent another example of the difficulty frequently experienced in correlating the manifestations of disease with properties of specific toxic fractions of the causative organism.

SUMMARY AND CONCLUSIONS

The relationship between the fever of acute infection and that following injection of bacterial pyrogen was studied by administering pyrogens to animals convalescent from acute infections.

Rabbits surviving dermal pneumococcal infections or peritonitis due to *Escherichia coli* were given intravenous injections of typhoid or *E. coli* vaccine. They showed no evidence of tolerance to the fever-promoting effect of these pyrogenic materials.

Tolerance did develop in infected animals given daily pyrogen injections during the course of the infection.

Certain previous observations upon the ability of rabbits to develop tolerance to pyrogens, the broad nature of the tolerance, and its duration were confirmed.

It is concluded that the pyrogen produced by certain bacteria plays little or no rôle in the production of the fever of infection.

These findings are compatible with the hypothesis that there is a common factor, perhaps a product of cell injury, underlying the fever accompanying diseases of various types.

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