STUDIES ON THE ANTIPYRETIC ACTION OF CORTISONE IN PYROGEN-INDUCED FEVER*

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One of the most non-specific and often misleading effects of cortisone as a therapeutic agent is its ability to suppress fever. That it functions as a potent antipyretic has been clearly established (1, 2). The manner in which it influences the febrile response to disease, however, is unknown. The studies reported in this paper deal with its action on fever induced experimentally by means of bacterial pyrogens.

The pattern of fever resulting in any individual species from the intravenous injection of most bacterial pyrogens is surprisingly constant (3–5). A highly reproducible feature is the latent period of from 15 to 30 minutes,[1] which has led investigators to doubt that the fever is due to a direct action of the pyrogen (3). A second characteristic is the occurrence of a transient, but often pronounced, leucopenia during the period of latency. This leucocytic reaction is demonstrable only when sufficient doses of pyrogen are injected (5–8). The thesis has been advanced, but not yet proved, that the phenomena of fever and leucopenia are causally related through the release of a secondary endogenous pyrogen from leucocytes trapped or destroyed in the blood vessels as a consequence of the original injection (9). Evidence has also be presented, particularly by Grant (10), that the bacterial pyrogen itself may not initiate the febrile response, but that its effect may depend upon its combining with a factor present in normal serum. This "serum factor" has been shown to reside in the α or β globulin fraction (11, 12) and is lacking in the blood of animals made fever-tolerant by repeated injections of the pyrogen (13).[2] Finally, it is well established that the actual rise in body

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[1] In man the latent period is somewhat longer and usually varies from 45 to 90 minutes (5).

[2] This factor is hereafter referred to as the serum factor to indicate that its chemical identity is unknown and that it may involve the presence of more than a single substance. Recently Farr *et al.* (11) have shown that the apparent absence of this factor in tolerant animals may be due to serum inhibitors. The presence of augmenting factors may be demonstrated in tolerant serum if it is incubated with a large dose of pyrogen.

temperature depends upon the action of the autonomic nervous system under the control of thermoregulatory centers in the brain (14–20). Whether these centers are acted upon by the pyrogen-serum factor complex, or by a secondary endogenous pyrogen arising from injured or otherwise affected cells within the vascular system, is at present unknown.

Based on the existing knowledge of pyrogen-induced fever, there appear to be at least three possible ways in which cortisone may act in suppressing the febrile response. First, through its well known anti-inflammatory action (21–26) it may interfere with the prefever leucopenia, which is said to result from an intravascular inflammatory response involving the "sticking" of leucocytes to vascular endothelium (27–30). Secondly, it may alter the composition of the recipient's serum in such a way as to modify the effect of the serum factor upon which the action of the injected pyrogen appears to depend. As regards this possibility, cortisone treatment is known to exert an effect upon both the lipid (31) and protein (32, 33) constituents of serum. Or thirdly, cortisone may act upon neither of the two above mechanisms but rather may influence some later stage of the fever-producing sequence, involving possibly the release or activity of endogenous pyrogen from intravascular cells or the response of thermoregulatory centers to the pyrogenic stimulus. The results of the present studies appear to eliminate conclusively the first two possibilities and thus by exclusion support the third.

I. The Effect of Cortisone upon Pyrogen-Induced Fever and Leucopenia

The pyrogens used in these experimenst were: (*a*) pyromen, a highly purified polysaccharide from a *Pseudomonas* species[3] and (*b*) ***native dextran***, complex polysaccharide lot N-316, with average molecular weight of between 200,000 and 300,000 by viscosity measurement.[4] In preliminary tests with both pyrogens, doses were determined which would give a brisk fever of 1 to 2°F. in untreated rabbits but which could be blocked by prior treatment with cortisone.[5] The latter was injected intramuscularly in an amount of 25 mg. twice a day for 3 days preceding the experiment, and an additional injection of 25 mg. was given on the morning of the experiment. On this basis, the most satisfactory pyrogenic dose of pyromen was found to be 5 gamma per kg., whereas that of dextran was 200 mg. given, regardless of weight, in a concentration of 25 mg. per ml. of pyrogen-free saline.

Rabbits were of albino Flemish and New Zealand stock, males of 2.5 to 3.5 kg. and approximately 4 to 6 months of age. All studies were performed in an air-conditioned room (68–70°F.). Each rabbit was placed in a closed box with an opening for the head and was given neither food nor water during the test period. Temperatures were recorded by means of indoor-outdoor model air guide thermometers.[6] The cartridge for recording outdoor tem-

[3] Obtained from Baxter Laboratories, Inc., and supplied in vials containing 10 gamma per ml. of isotonic solution of sodium chloride.

[4] Kindly supplied by Commercial Solvents Corporation in same lot number as that used by Bennett (34) and found to be free of contaminating bacterial pyrogens.

[5] Cortone (cortisone acetate), furnished through the courtesy of Merck and Company.

[6] Manufactured by Fee and Stemwedel, Inc., Chicago. All thermometers were carefully calibrated over the range of 98 to 106°F., and it was found that they could be read accurately (with a small hand lens) to within 0.5°F.

perature was inserted, with a short adjacent length of coiled sleeve, into the rabbit's rectum. Readings were begun 1 hour after insertion of the recording cartridge and experiments were started after a variable period of 30 to 60 minutes of readings to establish a base line. Control temperatures were measured over periods of 6 or 7 hours on each of the 3 days prior to an experiment, and all rabbits exhibiting variations of more than 1°F. during the last two control periods, or initial temperatures of more than 103°F. on the day of experiment, were discarded. Only rarely under these conditions were instances of "emotional hypothermia" (35) encountered. During each experiment readings were taken at 10 to 15 minute intervals for the first 1½ hours following the injection of the pyrogen into the marginal ear vein. In several experiments readings were taken every 5 minutes to establish the duration of the latent period with greater accuracy. Thereafter, temperatures were recorded every 30 minutes for a total of 5½ to 6½ hours. Glassware and needles were sterilized by dry heat at 170°C. for 2 hours in order to inactivate contaminating pyrogens (3, 5). Physiological salt solution used as diluent was made with doubly distilled water in pyrogen-free glassware and after being autoclaved was tested at intervals for pyrogenicity.

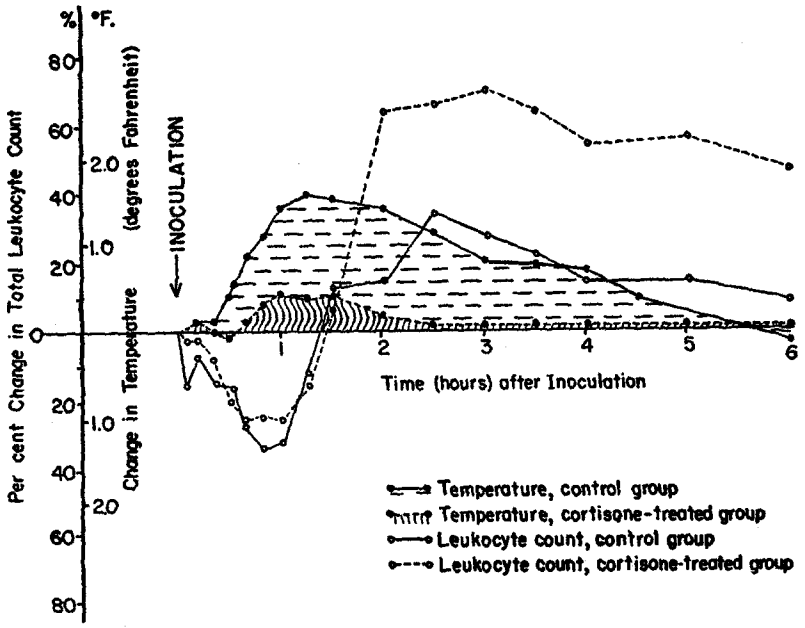
White blood cell counts were made on free flowing blood obtained from the marginal vein of the ear opposite the one used for injection of the pyrogen. Preliminary counts were made after each rabbit had been confined to its box for about 1 hour. Two successive counts obtained within a 10 minute period and exhibiting a difference of less than 2000 cells were averaged as a preinoculation figure from which subsequent variations after inoculation were calculated. Rabbits with preliminary counts of less than 5000 or more than 20,000 were eliminated. Counting was done with Trenner (N.B.S.) automatic pipettes and Spencer Bright-line chambers utilizing the four corner squares.

Experiment A.—In the experiment with pyromen, 10 rabbits were pretreated with cortisone, as described above, and 10 were left untreated as controls. The composite febrile and leucocytic responses of the two groups to the pyromen challenge are shown in Fig. 1, and the abridged data on the individual rabbits are recorded in Table I. It will be noted that the mean temperature response to pyromen was effectively blocked (maximum rise, < 0.5°F.) by the cortisone, whereas the per cent drop in white count, although averaging somewhat less in the cortisone-treated animals, did not differ by a statistically significant amount ($t = 1.24$).

Experiment B.—An even more conclusive dissociation of the febrile and leucopenic responses was observed in an analogous experiment with dextran. As shown in Fig. 2 the average temperature response of 7 untreated rabbits to dextran was somewhat greater than that caused by the pyromen and differed from the latter in that a secondary rise in temperature occurred at about 2½ hours. This double-humped pattern resembles that reported with relatively large doses of other bacterial pyrogens (*e.g.*, typhoid and coli vaccines) (3, 4, 10). In 7 rabbits pretreated with cortisone the fever was almost completely blocked (< 0.3°F.). But again the fall in white count was essentially the same in both the cortisone and control groups. (For more complete data, see Table II).

In summary, it can be stated that, whereas the febrile response in normal rabbits was regularly associated with an early leucopenia, treatment with cortisone resulted in a marked suppression of the fever without significantly

influencing the decrease in white cells[7]. It is evident, therefore, that the antipyretic effect of cortisone is not due to a blocking of the leucocytic reaction to the injected pyrogen.



TEXT-FIG. 1. Mean deviation of temperature and total leucocyte count of 10 normal and 10 cortisone-treated rabbits after inoculation with pyromen (5 gamma per kg.).

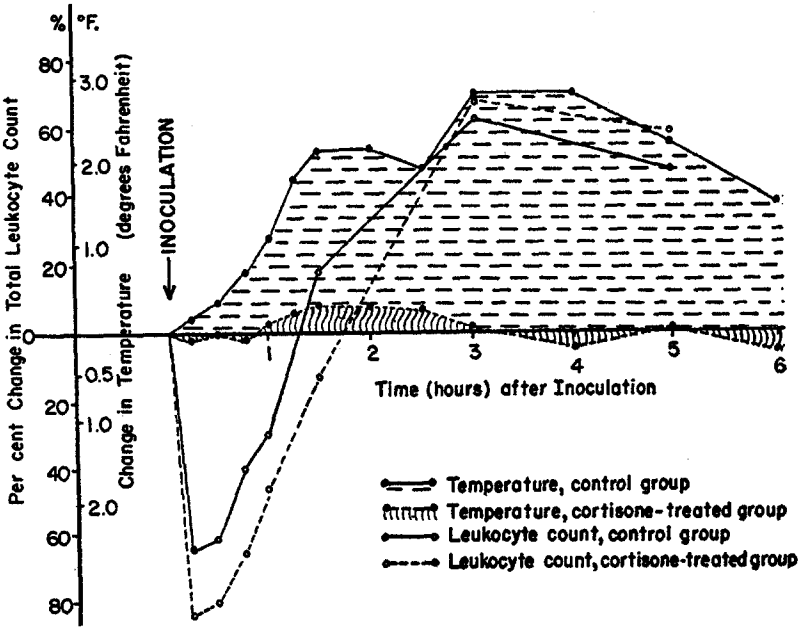
TABLE I

The Leucocytic and Febrile Responses of Normal (A) and Cortisone-Treated (B) Rabbits to Intravenous Injection of Pyromen, 5 Gamma per Kg

Animal No.		1	2	3	4	5	6	7	8	9	10	Mean values
Mean preinoculation leucocyte count*	A	8,783	6,625	5,875	11,625	11,825	9,775	19,050	16,650	10,325	11,700	11,223
	B	10,833	10,617	5,333	12,225	7,575	9,675	12,875	12,175	9,775	12,975	10,406
Maximum rise in temperature‡ (°F.)	A	1.5	1.5	2.0	1.0	2.5	1.5	2.0	2.0	1.0	2.0	1.7
	B	0	0	0	0	1.0	0.5	2.0	0.5	1.0	0.5	0.6
Maximum fall in leucocytes‡ (per cent of mean initial count)	A	43	9	0	82	82	43	81	53	30	62	49
	B	27	53	25	32	45	36	65	27	49	39	40

* Average of 2 to 3 successive counts within range of 2000 cells and 15 per cent of mean value.
‡ During the first 3 hours after injection.

[7] This same observation has recently been reported by Bennett and Beeson (36) in a study which appeared after the completion of the above experiments.



TEXT-FIG. 2. Mean deviation of temperature and total leucocyte count of 7 normal and 7 cortisone-treated rabbits after intravenous injection of crude dextran (200 mg.).

TABLE II

The Leucocytic and Febrile Responses of Normal (A) and Cortisone-Treated (B) Rabbits to Intravenous Injection of Dextran, 200 Mg.

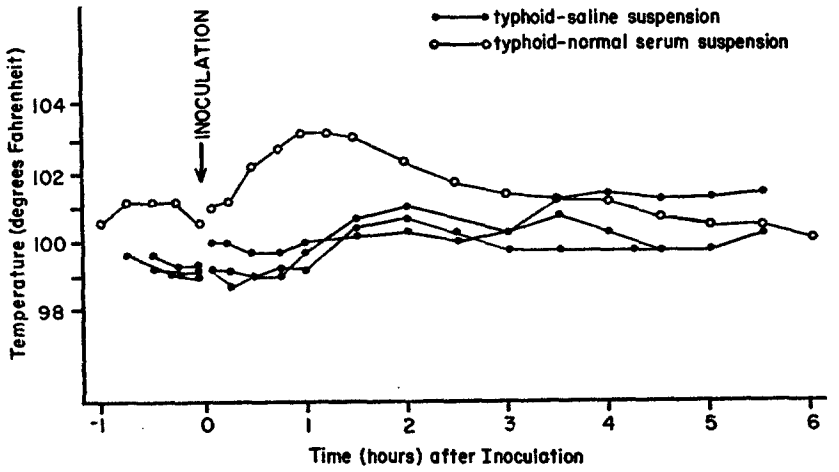
Animal No.		1	2	3	4	5	6	7	Mean values
Mean preinoculation leucocyte count*	A	7,550	8,075	10,550	7,775	10,000	7,725	17,425	9,870
	B	10,650	9,450	7,300	12,025	7,625	11,750	6,625	9,345
Maximum rise in temperature‡ (°F.)	A	4.5	3.0	3.0	3.5	2.5	2.0	2.5	3.0
	B	0.5	0	1.0	0.5	1.5	0	0.5	0.6
Maximum fall in leucocytes‡ (per cent of mean initial count)	A	64	66	83	76	63	41	93	69
	B	85	89	81	94	91	85	77	86

* Average of 2 to 3 counts within range of 2000 cells and 15 per cent of mean value.
‡ During the first 5 hours after injection.

II. The Effect of Cortisone upon the Serum Factor of Pyrogen-Induced Fever

That a serum factor is involved in pyrogen-induced fever has been conclusively demonstrated. First, it has been shown by Farr and LeQuire (8, 37–39) and by Grant

(10, 40) that previous incubation of the pyrogen in normal rabbit serum, plasma, or whole blood hastens the febrile response of recipient rabbits. According to the former workers, the height and duration of the fever is also increased. Secondly, Grant (13) has observed that pyrogen tolerance may be partially reversed by previous incubation of the challenging dose with normal serum. The response of the pyrogen-tolerant rabbit differs from that of the normal by virtue of a marked prolongation of the pre-fever latent period and a pronounced decrease in the height and duration of the fever. That this altered response of the tolerant animal involves, at least in part, a change in one or more factors normally contained in the serum, is evident from Grant's observa-



TEXT-FIG. 3. Potentiation of the febrile response of a pyrogen-tolerant rabbit by injection of 75 million typhoid bacilli incubated in 5 ml. of normal homologous serum. Responses to the same dosage of vaccine in pyrogen-free saline, given on the 3 preceding days, are shown for comparison.

tion that the prolonged latency may be completely reversed and the degree of febrile response partly restored by merely incubating the pyrogen with normal rabbit serum before injection; whereas incubation of the pyrogen with serum from a tolerant animal has no such effect. Data confirming both of these latter observations are presented in Figs. 3 and 4 (see below).

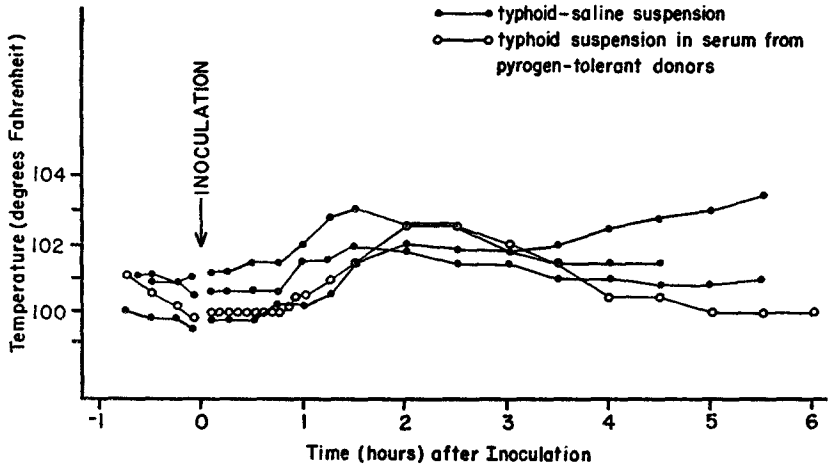
In the present studies two experiments were performed to investigate the possible effect of cortisone upon the serum factor. They were based upon the following reasoning. If cortisone acts as an antipyretic by affecting the serum factor, it should be possible to demonstrate: (1) a depression of serum factor activity in the blood of cortisone-treated rabbits and (2) a failure of cortisone to suppress fever induced by pyrogen previously exposed to the active "factor." These two possibilities were tested. In the first experiment, pyrogen, which had been incubated with serum from cortisone-treated rabbits, was injected into

pyrogen-tolerant recipients. In the second, cortisone-treated rabbits were challenged with pyrogen incubated in normal serum prior to injection.

The methods and materials used in these experiments have already been described above except for the following.

1. Pyrogen. The pyrogen used was typhoid vaccine, monovalent reference standard NRV-LS No. 1, made from *Salmonella typhosa* V-58.[8]

2. Pyrogen tolerance was established by daily intravenous injections of a uniform dose of 75 million organisms (calculated as approximately 25 million per kg.). The temperature response was recorded on the 1st day to insure that all animals included were "reactors." It was



TEXT-FIG. 4. Lack of potentiation of the febrile response of a pyrogen-tolerant rabbit inoculated with 75 million typhoid bacilli incubated with 5 ml. of serum from pyrogen-tolerant donors. Responses to the same dosage of vaccine in pyrogen-free saline, given on the 3 preceding days, are shown for comparison.

then not remeasured until after the 10th day of injections, but was recorded again on the 3 successive days preceding the experiment, in order to make certain that tolerance to the pyrogen had been established.

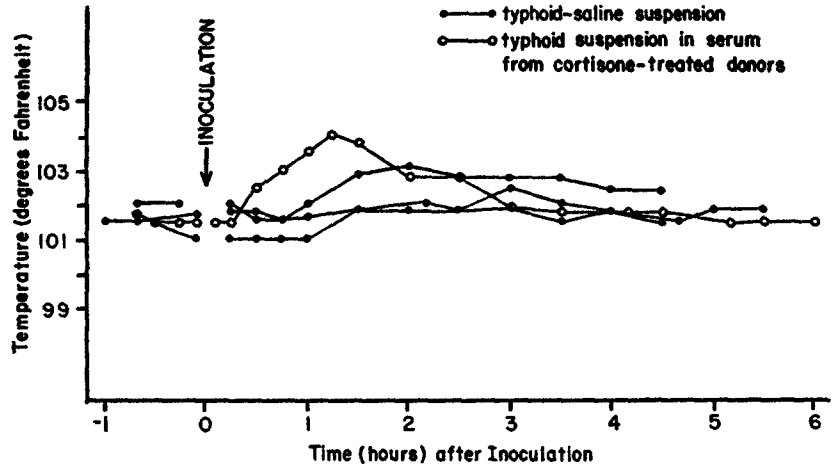
3. Collection of sera. Each sample of serum used was composed of a pool from 2 or 3 animals. Serum was obtained by cardiac puncture from (*a*) normal rabbits, (*b*) pyrogen-tolerant rabbits, and (*c*) rabbits treated for 3 successive days with cortisone as described above. The sera were stored at 4°C. and in most experiments were used within 2 or 3 days. All samples were checked for sterility.

4. Incubation of pyrogen-serum mixtures. Each typhoid vaccine-serum mixture (except as otherwise indicated in Experiment *E*) was made up to contain 75 million organisms per 5 or 10 ml. of serum and was stored in the ice box for 16 to 18 hours. Before injection the mixture

[8] Secured through the courtesy of Dr. Geoffrey Edsall of the Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington, D. C. This vaccine had a bacterial count of approximately 500 million per ml. and a nitrogen content of 0.03 mg. per ml. (±10 per cent).

was incubated at 37°C. for 1 hour during which time it was shaken frequently to insure adequate mixing. The volume injected into each rabbit (either 5 or 10 ml.) contained the standard pyrogenic dose of 75 million organisms.

Experiment C.—A preliminary experiment was first performed in which tolerant rabbits were challenged with vaccine incubated in both normal and "refractory" serum.[9] Representative results are shown in Figs 3 and 4. After approximately 10 daily injections of pyrogen in each experiment, tolerance was manifested by a lengthened latent period, elimination of the second febrile peak and a moderate reduction in the initial temperature rise. Following in-

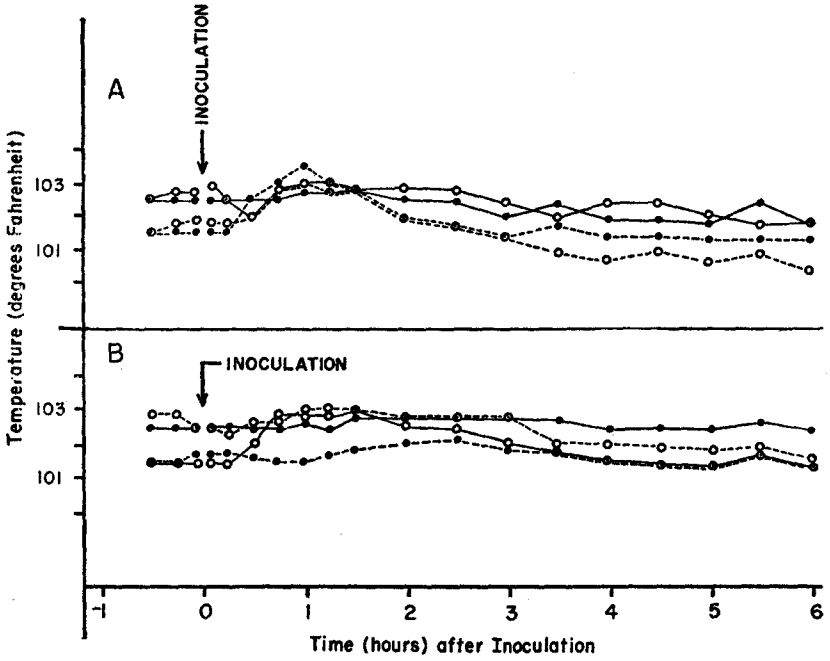


TEXT-FIG. 5. Potentiation of the febrile response of a pyrogen-tolerant rabbit by injection of 75 million typhoid bacilli incubated in 5 ml. of serum from cortisone-treated rabbits. Responses to the same dosage of vaccine in pyrogen-free saline, given on the 3 preceding days, are shown for comparison.

jection of the same dose of vaccine incubated in 5 ml. of normal ("non-refractory") serum, there was a marked and consistent shortening of the latent period. If the latent period is defined as the elapsed time between the injection and the first reading of 1°F. above the last preinoculation temperature, the latent periods in the tolerant control observations (21 instances) varied between 60 and 90 minutes, whereas those following injection of the normal serum-vaccine mixture were only 30 minutes in 5 out of 7 animals and 45 minutes in the remaining 2. In only 1, however, was the height and duration of fever increased, and in no instance was there a return of the second peak usually observed in non-refractory animals. In contrast to these results, injection of the same dose of vaccine incubated in the serum from tolerant donors produced no change in

[9] This term is used to apply to serum from animals which had received a course of pyrogen injections establishing tolerance.

the period of latency (Fig. 4). Lags in response of 75 to 90 minutes occurred in 4 out of 5 instances and the temperature of the 5th animal never rose more than 0.5°F. The febrile reaction of all 5 animals, once begun, did not differ significantly from those of the tolerant controls. Thus the observation of Grant (13) that the accelerating serum factor is present in normal but lacking or masked in refractory serum was confirmed.



TEXT-FIG. 6. Temperature responses of 4 cortisone-treated rabbits to intravenous inoculation on successive days with: (A) 15 million typhoid bacilli in pyrogen-free saline. (B) 15 million typhoid bacilli incubated in normal homologous serum.

Experiment D.—To test for a possible decrease of serum factor activity in the blood during cortisone therapy, 7 rabbits previously made tolerant by daily injections of vaccine were challenged with the same standard dose of typhoid vaccine incubated in the serum of cortisone-treated donors. The results as regards shortening of the latent period were found to be exactly the same as when the vaccine was incubated with normal (non-refractory) serum. A representative response is shown in Fig. 5. The latent period was shortened from between 60 and 90 minutes during tolerance to 30 minutes in 6 out of 7 rabbits and to 45 minutes in the 7th. Again as with normal serum, there was no significant change noted in the height or duration of the fever.

Experiment E.—The possible effect of serum factor upon the antipyretic

action of cortisone was also studied in 4 rabbits pretreated with a full course of the drug. Each rabbit was challenged first with 15 million typhoid bacilli suspended in 5 ml. of pyrogen-free saline and on the next day was given the same dose previously incubated in either 5 or 10 ml. of normal serum. This amount of vaccine (approximately 5 million bacilli per kg.) had previously been shown to cause a brisk fever in normal controls. As indicated by Fig. 6 the fever was just as effectively suppressed by cortisone on the day that the vaccine was incubated in serum as it was on the preceding day when the vaccine was suspended only in saline.

The results of these experiments (*D* and *E*) indicate: (*a*) that cortisone, when given in amounts sufficient to cause a marked antipyretic effect, does not significantly alter the serum factor activity of the blood and (*b*) that previous incubation of the pyrogen with serum factor fails to influence the antipyretic effect of the cortisone. It is concluded, therefore, that cortisone does not exert its antipyretic effect by acting upon serum factor.

DISCUSSION

Although fever is one of the most familiar manifestations of disease surprisingly little is known concerning its pathogenesis (20, 41, 42). Current knowledge of the subject is based primarily upon studies of experimental fever induced by the intravenous injection of bacterial pyrogens (5). That the resulting fever may involve mechanisms other than those that operate in febrile diseases of man is well recognized (43, 44). The pyrogen method was, nevertheless, used in the present experiments for three reasons. First, it provides a convenient laboratory model for investigating the action of an antipyretic; secondly, when given in sufficient dosage,[10] cortisone is known to suppress pyrogen-induced fever (1, 2); and thirdly, knowledge of the experimental febrile response is considerably greater than that of any other form of fever.

The following points concerning pyrogen fever are pertinent to the present study.

1. A sudden and often marked leucopenia involving primarily polymorphonuclear cells and eventually followed by leucocytosis, regularly precedes the onset of fever (5, 8). Its causal relationship to the fever is at present unproven.

2. Pyrogen previously incubated with normal serum causes an accelerated febrile response in normal recipients (8). The accelerated response is thought to result from the formation of a pyrogen-serum factor complex which functions as a fast acting pyrogen (10).

3. The accelerating factor, present in normal serum, is not apparent in the

[10] Duffy and Morgan (45) have reported that pyrogen-induced fever may actually be enhanced by relatively small doses of cortisone. The importance of dosage and time of administration in determining the effect of cortisone on pyrogen fever has also been emphasized by Bennett and Beeson (36).

blood of rabbits made pyrogen-tolerant by repeated daily injections (13, 46). Its absence, presumed to be due to removal or binding by the repeatedly injected pyrogen, has been offered as an explanation for the reduced responsiveness of pyrogen-tolerant animals.[11]

The reasons for studying the effects of cortisone on the leucopenic response to injected pyrogen and for investigating the drug's possible action on the accelerating serum factor have already been discussed. It was suggested *a priori* that cortisone might exert its antipyretic effect either by blocking the leucopenic reaction which precedes the febrile response or by affecting the serum factor with which the injected substance is thought to combine. The results of the present experiments indicate that neither of these processes is involved. They suggest that cortisone acts upon a later and as yet undefined stage of the fever-producing mechanism. Whether the drug interferes with the release or activity of endogenous pyrogen, possibly derived from intravascular cells stimulated or injured by the injected pyrogen, or whether it merely depresses the responsiveness of the central nervous system to the pyrogenic stimulus, remains to be determined.

The regularity with which leucopenia precedes pyrogen fever, the extraction of a pyrogenic substance from inflammatory exudates (44, 48–50), and the demonstration of an active pyrogen in polymorphonuclear leucocytes (51, 52), all suggest that the leucocytic reaction to the pyrogen may indeed be related to the fever produced (9). There is evidence that the leucopenia is merely a reflection of sudden sequestration of leucocytes at a variety of intravascular sites (27, 53). In fact, a number of workers (28, 29) have observed by direct microscopy that, following the injection of certain pyrogens, leucocytes are immobilized in small vessels in which they appear to "stick" to the endothelium.[12] It is possible that leucocytes thus immobilized may provide the principal source of the endogenous pyrogen, which in turn may be responsible for the fever (9).

In the present experiments cortisone has been shown to cause a marked depression of pyrogen-induced fever without significantly influencing the leucopenic reaction. This dissociation of the febrile and leucopenic responses should not be interpreted to indicate that the two phenomena are necessarily unrelated. On the contrary, it is still possible that the leucopenic reaction constitutes an important step in the pyrogenic process and that the cortisone, as already suggested, exerts its antipyretic effect by influencing a later stage of the reaction.

[11] As previously noted, there is evidence that other factors may be involved in pyrogen tolerance, including the development of plasma inhibitors (11) and an accelerated removal of pyrogen from the circulation by cells of the reticulo-endothelial system (47). The relative importance of these various factors is at present a matter of controversy (13).

[12] This "sticking" phenomenon is similar to that observed during experimental bacteriemia (30). Further evidence that circulating leucocytes are affected by injected pyrogen has recently been demonstrated by the *in vitro* studies of Berthrong and Cluff (54).

From these considerations it is obvious that more definite information is needed concerning the possible role of the leucopenic response in the pathogenesis of pyrogen-induced fever. Demonstration that inflammatory cells serve as an important source of endogenous pyrogen in this form of experimental fever would provide indirect evidence that similar cells may be involved in the fever of human disease (9).

SUMMARY

The mode of action of cortisone as an antipyretic has been studied in rabbits challenged with intravenous injections of bacterial pyrogens. The fever induced by pyromen or dextran was found to be markedly suppressed when cortisone was administered in liberal amounts (25 mg. twice daily) for 3 days prior to the challenge. Although the cortisone effectively blocked the febrile response to both pyrogens, it failed to influence the transient but marked leucopenia which characteristically precedes the onset of fever.

The antipyretic action of the drug also was shown to bear no relation to the activity of the serum factor recently demonstrated by Farr, Grant, and others to be involved in the production of pyrogen-induced fever. In preliminary experiments with typhoid vaccine as the inciting pyrogen, the presence of serum factor activity in normal blood and its absence in the blood of pyrogen-tolerant rabbits was confirmed. Subsequently the blood of rabbits treated with antipyretically effective doses of cortisone was shown to contain just as much serum factor activity as that of normal rabbits. In addition, previous incubation of the pyrogen with serum factor failed to influence the antipyretic effect of the drug.

It is concluded from these findings that in suppressing pyrogen fever, cortisone acts neither upon the leucopenic reaction nor upon the fever-accelerating factor of the serum. By exclusion it would appear that the drug must influence some later stage of the fever-producing process. The mechanisms involved in the later stages of the response to exogenous pyrogen remain undefined, and the need for determining whether they are related to the prefebrile leucopenia is emphasized.

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