THE EFFECT OF CORTISONE ON THE FEVER OF DELAYED HYPERSENSITIVITY

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The ability of cortisone to suppress fever in various hypersensitive diatheses is well recognised but, though its antipyretic effect has also been noted in experimental delayed hypersensitivity, its mode of action is uncertain.

The effect of cortisone in fever resulting from injection of bacterial endotoxin has, however, been studied. Atkins, Allison, Smith and Wood (1955) have found that though the febrile response is suppressed there is no evidence of suppression of formation of endogenous serum pyrogen, and serum from cortisone-treated donors is as pyrogenic for recipients as serum from untreated donors. Atkins and his co-workers have concluded therefore that in fever due to bacterial endotoxin the antipyretic action of cortisone occurs at a late stage, that is, after the liberation of endogenous serum pyrogen.

There is now good evidence that the fever of delayed hypersensitivity is also associated with the liberation of an endogenous pyrogenic factor (Hall and Atkins, 1959), and the essential criterion for the release of this endogenous pyrogen appears to be interaction of hypersensitive cell and specific antigen (Johanovsky, 1960; Allen, 1965). It would seem possible, therefore, that the effectiveness of cortisone as an antipyretic in delayed hypersensitivity might depend not on a late central action but rather on the ability to suppress antigen-antibody interaction with consequent prevention of liberation of endogenous serum pyrogen.

The present investigation was therefore designed to compare the febrile response to specific antigen in hypersensitive animals with and without the administration of cortisone. In addition the pyrogenicity of serum from each group was tested in normal recipients and in recipients treated with cortisone.

MATERIALS AND METHODS

Animals. Male adult rabbits weighing 2·5–3·0 kg. were used. The animals were kept in individual cages in a well aired room maintained at a constant temperature of 20°C and were divided into 4 experimental groups. Group A consisted of 15 hypersensitive donors that were challenged with specific antigen but not treated with cortisone. Group B consisted of 23 hypersensitive, cortisone-treated donors that were challenged with specific antigen. Group C comprised 39 normal recipients in which hypersensitive serum was tested. Group D contained 7 recipients that were treated with cortisone before injection of hypersensitive serum.

Sensitisation of prospective hypersensitive donors. BCG vaccine was obtained from the Statens Seruminstitut, Copenhagen. Animals were sensitised by intraperitoneal injection of 9·0 mg. of the standard strength vaccine (17–18 X 10⁶ viable organisms per 0·75 mg. vaccine).

Skin testing. Three weeks after the animals had received the injection of BCG, 2 µg. Purified Protein Derivative (PPD) of Mycobacterium tuberculosis (in
0·1 ml. of isotonic sterile saline) was injected intradermally in a 4 cm. diameter area shaved on the flank of each animal. The reaction was read at 24 and 48 hr.

*Cortisone.* Two preparations were used. (1) Cortisone acetate (25 mg. per ml.) was used for intramuscular injection; (2) hydrocortisone in sterile ethanol was diluted to a strength of 10 mg. per ml. and used for intravenous injection.

*Bacterial pyrogen.* Purified lipopolysaccharide from *Salmonella abortus-equ*i (“Pyrexal”, Wander) was used.

**Experimental regimens in the four groups of animals**

*Group A.* Eighteen days after the inoculation of BCG each prospective hypersensitive donor was started on daily injections of Pyrexal (2 µg. per kg. body weight per day). These were continued until the 27th day and the animals were subjected to systemic challenge with PPD antigen on the 28th day. Skin tests were done 21 days after BCG inoculation.

*Group B* was treated as group A except that cortisone (50 mg. per day by intramuscular injection) was given for 5 days before systemic antigen challenge. In addition 10 mg. of hydrocortisone was given intravenously on the day of antigen challenge shortly before the injection of PPD.

*Group C.* These animals, which had not been given BCG, received Pyrexal (2 µg. per kg. per day) for 10 days before injection of test serum.

*Group D.* These animals, which had not been given BCG, received Pyrexal (2 µg. per kg. per day) for 10 days before injection of test serum and 50 mg. cortisone by intramuscular injection for 5 days before serum was tested.

**Further examinations**

*Systemic challenge of hypersensitive donors with specific antigen.* Each animal was given 5 µg. PPD (in sterile isotonic saline) by intravenous injection.

*Transfer of serum.* Each donor was given an intraperitoneal injection of pentobarbitone sodium (30 mg. per kg. body weight). The thorax was then shaved, the skin carefully cleansed, and the animal was exsanguinated by cardiac puncture 150–180 min. after injection of PPD. The blood was allowed to clot in a sealed sterile glass container for 1 hr at room temperature. The serum was removed and cleared by centrifugation at 4°C and serum from several donors was pooled. All serum was stored at 4°C and was used within 3 days of preparation, after it had been cultured to confirm its sterility. Recipients were given intravenous injections of 10–15 ml. quantities of serum that had been incubated at 37°C for 30 min. prior to injection.

*Temperature readings.* The temperature was recorded at 60 and 30 min. before injection of PPD or serum. Where a difference of more than 0·5°C occurred the animal was discarded. Thereafter, readings were taken at 30 min. intervals until cardiac puncture in the donors of serum, and at 10 min. intervals for 2 hr in the recipients of serum. A thermometer (sensitive to 0·1°C) was inserted to a distance of 6 cm. into the rectum and left in position for 5 min. before each reading. The range of normal (pre-injection) temperature readings in the series of animals was 38·7–40·3°C.

*Assessment of febrile response.* Temperature readings were recorded in degrees centigrade and were plotted as degrees of fever against time. The degrees of fever were calculated as the difference between the temperature reading and the average of the two pre-injection temperature readings (made at 60 and 30 min.) in the same animal. Mean fever curves for the different experimental groups were plotted. The areas under the curves were compared by the use of planimetry and the results were recorded in degrees-minutes (°C-min.). In addition a statistical comparison was carried out using individual fever curve areas and the results were compared by means of the “t” test.
RESULTS

Skin testing. All the animals that had received an injection of BCG were shown by the skin tests to have become hypersensitive to PPD tuberculin.

Response of normal animals to PPD. Eleven normal animals were given an intravenous injection of 5 μg. PPD. None developed fever.

Response of hypersensitive animals to specific antigen without cortisone treatment. The 15 animals in this group all responded to intravenous injection of PPD with fever. Body temperature started to rise after a latent period of 30-60 min. and reached a maximum at about 180 min.; the mean of the maximum responses of the animals was 1.4°C (fig. 1). The area under the mean fever curve was 3.6°C-min. Statistical comparison of the fever curve areas of these animals with those of normal animals given PPD shows a highly significant difference: P < 0.001.

Response of cortisone-treated hypersensitive animals to specific antigen. Ten of the 23 animals in this group had a slight rise in temperature after injection of PPD. The mean maximum response of the group, however, was only 0.3°C; it was present 180 min. after injection of PPD (fig. 1). The area under the mean fever curve was 0.3°C-min.

Response of normal recipients to intravenous injection of serum from normal animals. Six recipients not used for testing hypersensitive serum received an injection of 10 ml. of normal rabbit serum. None developed fever (fig. 2 (c)).

Response of normal recipients to intravenous injection of serum from hypersensitive donors. Twelve recipients were given serum from hypersensitive donors that had been subjected to systemic challenge with
antigen; all developed fever. Maximum responses were present after 10 min. and their mean was 0.7°C; fever persisted for 90 min.

![Graph showing fever responses](image)

**Fig. 2.**—Febrile responses of normal recipient animals to intravenous injection of serum. Average results are given for (a) 12 recipients of serum from hypersensitive donors challenged with specific antigen (— — —), (b) 27 recipients of serum from hypersensitive donors treated with cortisone before challenge with specific antigen (———), and (c) 6 recipients of normal serum (………..).

![Graph showing comparison of fever responses](image)

**Fig. 3.**—Comparison of the mean febrile responses of (a) 27 normal, untreated animals, and (b) 7 cortisone-treated animals to intravenous injection of serum from hypersensitive donors treated with cortisone before challenge with specific antigen.

(fig. 2 (a)). The area under the mean fever curve was 3.0°C-min. Statistical comparison of the fever curve areas of these animals with those of recipients given normal serum shows a highly significant difference: P<0.001.
Response of normal recipients to intravenous injection of serum from hypersensitive, cortisone-treated donors. Twenty-seven recipients were given serum from hypersensitive donors that had been treated with cortisone before systemic challenge with antigen; 21 developed fever. Body temperature began to rise after a latent period of about 10 min. Maximum responses were present after 45 min. and their mean was 0.6°C (fig. 2(b)). Fever persisted for 120 min. in a few animals although most members of the group had regained pre-injection temperature levels within 90 min. The area under the mean fever curve was 4.6°C-min. Statistical comparison of the fever curve areas of these animals with those of recipients given normal serum shows a significant difference: 0.05 > P > 0.02.

Response of cortisone-treated recipients to intravenous injection of serum from hypersensitive, cortisone-treated donors. Seven recipients were given cortisone for 6 days before injection of serum. Three developed slight fever but the mean maximum response of the group (fig. 3) was only 0.1°C and the areas under the mean fever curve (0.2°C-min.) did not differ from those of normal animals given normal serum (0.80 > P > 0.70).

The experimental findings are summarised in the table.

**DISCUSSION**

The significant fact emerging from these experiments is the apparent inability of cortisone to prevent the liberation of endogenous pyrogen in the systemic reaction of delayed hypersensitivity. In the first place,
although the mean maximum response of the cortisone-treated hypersensitive animals to specific antigen was negligible, 48 per cent. of the animals did develop very slight fever, and this response occurred in spite of very adequate cortisone therapy; the dose used was more than double that used by Atkins et al. (1955) to suppress bacterial pyrogen fever. This finding would appear to support the current concept of the quantitative anti-inflammatory effect of cortisone. It would seem that complete suppression of the systemically provoked hypersensitive reaction necessitates very intensive therapy.

Despite this slight febrile response in some of the donors, cortisone was very effective in reducing the extent of the response in the group as a whole. It is of considerable interest, therefore, that serum from cortisone-treated and non-cortisone-treated donors was found to be equally pyrogenic in normal recipients. The response to donor serum could however be prevented by giving the recipients cortisone, and this suggests that the latter, whilst not inhibiting the liberation of endogenous serum pyrogen, does in some way interfere with its action.

The source of the endogenous serum pyrogen in the cortisone-treated donors can be assumed to be the hypersensitive cells of the lymphoid series, since earlier work (Johanovsky, 1960; Allen, 1965) has established the pyrogenic potential of these cells in the presence of specific antigen. It is significant, however, that serum from both groups of donors was equally pyrogenic in recipients, for it is well-recognised that lymphocytolysis occurs after administration of cortisone (Dougherty, 1953) and this phenomenon might be expected to reduce available stores of endogenous pyrogen before the time of challenge with specific antigen. It may be argued, however, that the reticulo-endothelial reserve of the hypersensitive animal is so considerable that even intensive cortisone therapy will in no way reduce its ability to respond to specific antigen with liberation of endogenous pyrogen. In these experiments donors were not bled prior to challenge with antigen, but it would be of interest to know whether endogenous pyrogen is liberated during cortisone therapy, as a result of lymphocytolysis before systemic challenge with antigen.

This inability of cortisone to prevent formation of endogenous serum pyrogen is of interest because of the considerable therapeutic importance of cortisone in inflammatory disease. Evidence concerning the mechanism of the anti-inflammatory effect is conflicting. It is well recognised that cellular exudation in local inflammatory lesions is decreased by cortisone therapy and this decrease is accompanied by a decrease in capillary permeability (Benditt et al., 1950). These effects, however, are probably only the end-results of an anti-inflammatory action, and the primary site and mode of action remain obscure. Great emphasis has recently been placed on the cellular protective effects of cortisone. Menkin (1960), for example, suggests that cortisone suppresses cell activity at the site of inflammation and consequently diminishes the liberation of biochemical inflammatory mediators.
Weissmann and Thomas (1962) are of the opinion that it has the ability to stabilise lysosomes against a variety of traumatic stimuli. These conclusions are based on experimental evidence largely acquired in in-vitro studies. For example, Leahy and Morgan (1952) showed that cortisone will inhibit the cytotoxic action of PPD on macrophages from tuberculous guinea-pigs.

The evidence provided by my experiments, however, would suggest that not all the effects of cortisone can be explained on the basis of cell protection. As already stated, the fact that serum from all hypersensitive donors was pyrogenic for normal recipients whether the donors were given cortisone or not, suggests that administration of cortisone does not interfere with the interaction of antigen and hypersensitive cell and the consequent liberation of endogenous serum pyrogen. It is worthy of note that Bennett and Beeson in 1953 showed that administration of cortisone did not prevent formation of granulocytic pyrogen in rabbit peritoneal exudates. Atkins et al. were also able to show that although cortisone could suppress bacterial pyrogen fever it in no way affected the concentration of endogenous serum pyrogen in transferred serum. It would seem, therefore, that cortisone exerts its specific antipyretic effect not by inhibiting the formation of endogenous serum pyrogen but by interfering with its action.

**SUMMARY**

The effect of cortisone on the fever of delayed hypersensitivity induced in rabbits by inoculation of BCG vaccine and subsequent challenge by intravenous injection of PPD antigen has been studied. Administration of cortisone was generally effective in preventing the febrile response of the hypersensitive animal to the specific antigen. Administration of cortisone did not, however, prevent the liberation of endogenous serum pyrogen by the hypersensitive animal on challenge with antigen since normal recipient animals showed a febrile response to transferred serum.

This febrile response of recipient animals to intravenous injection of serum from cortisone-treated, antigen-challenged, hypersensitive donors could be abolished by prior treatment of the recipients with cortisone. It would seem therefore that, in the fever of delayed hypersensitivity, cortisone does not exert its antipyretic effect by inhibiting the formation of endogenous serum pyrogen, but has the ability to abolish the febrile response to endogenous pyrogen.

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