THE EFFECT OF IRRADIATION ON THE FEVER OF DELAYED HYPERSENSITIVITY

BY

INGRID V. ALLEN

INTRODUCTION

The importance of circulating antibody in the delayed hypersensitive state is a fact well established although the mechanism of interaction is pyrogen is unknown. Jones and Tomlinson (1958) have postulated that during incompatible blood transfusion, antigen–antibody complexes are released which alter the cell membrane of circulating leucocytes leading to agglutination and liberation of granulocytic pyrogen. Brinetham and Chapel (1957) have reported that, during transfusions which are apparently compatible, febrile reactions may occur owing to high titre of circulating agglutinins to donor leucocytes. Both these reports stress the importance of the effect of circulating antibodies on granulocytes in the production of fever associated with haemolysis.

Though one of the prerequisites of the delayed hypersensitive state is a failure to detect circulating antibody to the specific antigen, it has been postulated that small amounts of non-specific antibody may combine with specific antigen to form a complex capable of damaging leucocytes and such a reaction might at least play a part in the fever of delayed hypersensitivity. Uhr and Scharff (1963) have shown, however, that guinea pigs irradiated before sensitisation will still develop delayed hypersensitivity and will show a febrile response to specific antigen as long as they have circulating antibody. This would suggest that the fever of delayed hypersensitivity does not depend on circulating antibody. Uhr and Schaff (1963) made the interesting observation, however, in their irradiated animals to demonstrate transferable antibodiesogenous pyrogen of the type described by Hall and Atkins (1956). It is suggested that irradiation has no effect on the delayed hypersensitive
The Effect of Irradiation on the Fever of Delayed Hypersensitivity

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Summary. The effect of total body irradiation on the development of delayed hypersensitivity and on the febrile response to specific antigen has been studied in guinea-pigs with the following results:

1. 200 R. whole body irradiation in guinea-pigs, while suppressing circulating antibody response, did not prevent the development of delayed hypersensitivity.
2. Irradiated and non-irradiated hypersensitive animals had an equal febrile response to systemic challenge with specific antigen.
3. Serum from antigen-challenged, irradiated, hypersensitive animals contained a pyrogenic factor of the endogenous serum type capable of producing fever in normal recipients.

These results support the conclusion that production of circulating specific antibody is not essential either for development of delayed hypersensitivity or for the febrile response of the hypersensitive animal to specific antigen.

INTRODUCTION

The importance of circulating antibodies in various febrile reactions is well established though the mechanism of liberation of pyrogen is unknown. Jandl and Tomlinson (1958) have postulated that, during incompatible blood transfusion, antigen–antibody complexes are released which alter the cell membrane of circulating leucocytes leading to agglutination and liberation of granulocytic pyrogen. Brittingham and Chaplin (1957) have reported that, during transfusions which are apparently compatible, febrile reactions may occur owing to high titres of circulating agglutinins to donor leucocytes. Both these reports stress the importance of the effect of circulating antibodies on granulocytes in the production of fever associated with haemolysis.

Though one of the prerequisites of the delayed hypersensitive state is a failure to detect circulating antibody to the specific antigen, it has been postulated that small amounts of specific antibody may nevertheless circulate (Cole and Favour, 1955). Alternatively, a non-specific antibody may combine with specific antigen to form a complex capable of damaging leucocytes and such a reaction might at least play a part in the fever of delayed hypersensitivity. Uhr and Scharff (1960) have shown, however, that guinea-pigs irradiated before sensitization will still develop delayed hypersensitivity and will show a febrile response to specific antigen despite their inability to form circulating antibody. This would suggest that the fever of delayed hypersensitivity does not depend on circulating antibody. Uhr and Scharff (1960) made no attempt, however, in their irradiated animals to demonstrate transferable endogenous pyrogen of the type described by Hall and Atkins (1959). If the conclusion that irradiation has no effect on the delayed hypersensitive
reaction is correct then serum from specifically-challenged, irradiated, hypersensitive animals should contain a pyrogenic factor identical to that described by Hall and Atkins (1959). In the studies reported here the febrile response of irradiated and non-irradiated, hypersensitive animals to specific antigen has been studied and the serum of each group has been tested for transferable pyrogen.

**MATERIALS AND METHODS**

**Experimental Animals**

Male guinea-pigs were used weighing 300–600 g. The animals were kept in a well-aired room maintained at a constant temperature of 24°C. They were divided into three experimental groups:

*Group A.* Irradiated, prospective hypersensitive donors.

*Group B.* Non-irradiated, prospective hypersensitive donors.

*Group C.* Normal recipients.

The experimental procedures for the three groups are summarized in Fig. 1.

![Diagram](image)

**Fig. 1.** Regime for hypersensitive, irradiated and non-irradiated donors and for recipients.

**Irradiation**

Animals were irradiated 24 hours before sensitization with antigen. They were placed in tightly fitting Perspex boxes and were subjected to 200 R. total body irradiation at a distance of 60 cm. from the tube (kilovoltage 230 kV.; filter 1.35 mm. Cu). This dose, given 24 hours before injection of antigen, was sufficient to suppress antibody formation for 15 days.
Sensitization of Donors

Antigen. A preparation of diphtheria toxoid floculated at 80 per cent neutralization point with diphtheria antitoxin was used according to the method described by Uhr, Salvin and Pappenheimer (1957).

Sensitization procedure. Each animal received a sensitizing dose of 0·6 L.F. (0·002 mg.) of antigen. This was given into the foot-pads of each animal in 0·5 ml. of Freund’s incomplete adjuvant in two divided doses at a 12 hour interval.

Skin testing for hypersensitivity. Seven to 10 days after sensitization animals were shaved over a 5 cm. diameter area and the underlying skin was injected with 1 L.F. of diphtheria toxoid. Reactions were read at 24 and 48 hours.

A few animals not used as donors but in which positive skin tests developed had sections of the inoculation site taken for histology. Tissue was fixed in Helly’s solution and stained with haematoxylin and eosin.

Detection of Circulating Antibody

This was done 10 or 11 days after sensitization and when skin tests had become positive. Since the experiment was primarily designed to detect transferable serum pyrogen the choice of tests for detecting circulating antibody was difficult: guinea-pigs could not undergo vigorous bleeding before challenge with specific antigen. The toxin neutralization test was considered a crude screening test for antibody. Active cutaneous anaphylaxis, though perhaps not quite as sensitive as the haemagglutination test, has been shown to be more sensitive than toxin neutralization or passive cutaneous anaphylaxis probably because of the ability of guinea-pig skin to bind γ-globulin. This test was therefore chosen as a second more sensitive indicator of circulating antibody.

Toxin Neutralization in Guinea-Pig Skin

Each donor was lightly anaesthetized and 5 ml. of blood was removed by cardiac puncture. Serum from animals of each group was pooled. Albino guinea-pigs were used for skin testing, the flank of each animal having been shaved the previous day.

Diphtheria toxin. The standard Schick test toxin was used. Buffer of the following composition was used for dilution: 0·30 per cent crystalline borax; 0·44 per cent boric acid; 0·76 per cent sodium chloride; pH 8·2–8·4.

Minimum reacting dose of toxin. The animals were inoculated intradermally with 0·2 ml. quantities of 1:25, 1:50 and 1:100 dilutions of toxin. Skin reactions were read at 24 and at 48 hours after injection; 0·2 ml. of 1:50 dilution was found to be the minimum reacting dose.

Toxin neutralization by test serum. The ability of the pooled serum of the two experimental groups to neutralize toxin was tested in two ways:

1. Each animal was injected intraperitoneally with 4 ml. of serum and simultaneously intradermally with 0·2 ml. of 1:10, 1:25 and 1:50 dilutions of toxin.

2. Each animal was injected intradermally with an 0·2 ml. quantity of toxin–serum mixture containing 0·1 ml. of 1:10, 1:25 or 1:50 dilution of toxin mixed with 0·1 ml. of 1:10 dilution of serum in saline. The mixture was prepared and kept at room temperature for 30 minutes before injection.

Active Cutaneous Anaphylaxis

Diphtheria toxoid (0·02 mg.) was injected intradermally in the shaved flank of each hypersensitive animal and immediately afterwards 0·25 ml. of 2 per cent Evans blue dye
was given by intra-cardiac injection. The site of intradermal injection was examined for accumulation of dye during the period 15–45 minutes after injection.

**Systemic challenge with antigen.** Hypersensitive donors were challenged systemically with antigen after skin testing and testing for circulating antibody. The challenge was 0·2 mg. of diphtheria toxoid given by intracardiac injection.

**Transfer of serum.** One hundred and twenty to 180 minutes after systemic challenge with specific antigen donors were bled by cardiac puncture and were exsanguinated. The blood was allowed to clot in a sterile glass container at room temperature for 1 hour and was stored overnight at 4°. The serum was then separated and cleared by centrifugation at 1500 rev./min. for 20 minutes at 4°. Serum from two or more donors was pooled and was stored at 4° after culturing to ensure sterility. It was tested in recipients within 4 days of preparation and incubated at 37° for 30 minutes before use. Each animal received 5 ml. by intracardiac injection.

**Bacterial pyrogen.** Purified lipopolysaccharide from *S. abortus equi* (‘Pyrexal’, Wander) was used. Hypersensitive donors were injected daily (1 µg./day) for 7 days before systemic challenge with antigen and normal recipients received the same course of injections for 7 days before injection of test serum. All animals became completely tolerant to bacterial pyrogen.

**Sterilization of glassware, needles, etc.** was by dry heat at 170° for 2 hours.

**Temperature recording.** Temperature readings were recorded in degrees centigrade using a fine rectal thermometer (sensitive to 0.1° and inserted to a distance of 4 cm.). The thermometer was left in position for 3 minutes before each reading. Baseline readings were taken 60 and 30 minutes before intracardiac injections—animals showing a variation of more than 0·5° were discarded. Readings were taken at 30 minute intervals until cardiac puncture in donors and at 15 minute intervals in recipients for 2 hours after injection of serum.

**Assessment of febrile response.** Temperature changes were plotted as degrees of fever against time. The mean febrile responses of the various experimental groups were plotted and the areas under the mean fever curves were measured using planimetry and recorded in degrees centigrade-minutes (°minutes).

In addition the fever curve of each animal was plotted and measured separately and the results for the various experimental groups were compared statistically using the ‘t’ test.

**RESULTS**

**Effects of Irradiation**

Thirty-two animals were irradiated, nine of which (28 per cent) died before completion of the experiment. Surviving animals were listless, prone to infection and showed patchy loss of body hair.

**Production of Hypersensitivity**

Twenty-three irradiated animals and fourteen non-irradiated animals were successfully sensitized to diphtheria toxoid. Injection of antigen intradermally showed a positive response within 7–10 days of sensitization. Fig. 2 shows examples of the histology of positive skin tests in irradiated and non-irradiated guinea-pigs 36 hours after intradermal injection of antigen. The histology of both groups was the same, showing diffuse dermal lymphocytic infiltration: but the cellular reaction in the irradiated animals tended to be less.
Detection of Circulating Antibody

The results of the various tests for detection of circulating antibody are summarized in the Table 1.

Irradiated Animals

Circulating antibodies could not be demonstrated by toxin neutralization or by active cutaneous anaphylaxis in any of the twenty-three surviving, irradiated animals.

Non-Irradiated Animals

The pooled serum from the non-irradiated donors neutralized the minimum reacting dose of toxin whether the serum was given intraperitoneally or as a toxin–serum mixture.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Number of animals</th>
<th>Antibody detectable by</th>
<th>Active cutaneous anaphylaxis</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Toxin neutralization</td>
<td></td>
</tr>
<tr>
<td>Irradiated</td>
<td>23</td>
<td>0.2 ml. 1:50 dilution</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2 ml. 1:25 dilution</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2 ml. 1:10 dilution</td>
<td>No</td>
</tr>
<tr>
<td>Non-irradiated</td>
<td>14</td>
<td>0.2 ml. 1:50 dilution</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2 ml. 1:25 dilution</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2 ml. 1:10 dilution</td>
<td>No</td>
</tr>
</tbody>
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* G* IMMUN.
intradermally. Since 1:25 dilutions of toxin were not neutralized the titre of the circulating antibody was low, but its undoubted presence was borne out by the fact that all fourteen non-irradiated animals reacted positively to active cutaneous anaphylaxis.

**Febrile Response to Intracardiac Injection of Diphtheria Toxoid**

(a) **Normal animals.** Sixteen normal animals were each given 0.2 mg. of diphtheria toxoid antigen into the heart. None developed fever in the 240 minutes after injection.

(b) **Non-irradiated hypersensitive animals.** All of the fourteen animals in this group developed fever when challenged systemically with specific antigen; their mean febrile response is shown in Fig. 3. The pattern of response was that associated with delayed hypersensitivity.

![Graph](image)

**Fig. 3.** Mean febrile responses of irradiated (— —) and non-irradiated (—), hypersensitive animals to injection of specific antigen; · · · ·, normal control.

Fever developed after a latent period of 60 minutes and the mean maximum response, occurring 120 minutes after injection of antigen, was 0.6°C. Defervescence was not studied since all animals were exsanguinated at the height of their fever. The area under the mean fever curve was 6.2°C minutes. Statistical comparison of each animal with the febrile reaction of the control normal animals to specific antigen was made by measuring the area under the individual fever curves. The febrile response of the hypersensitive group when compared with the controls was highly significant: \( P < 0.001. \)

(c) **Irradiated hypersensitive animals.** Thirteen of the twenty-three surviving irradiated animals were challenged systemically with antigen (ten were used for skin histology and to perfect techniques for detection of antibody). All thirteen developed fever with a response very similar to that of the non-irradiated group. Fig. 3 shows that the mean latent period for this group was longer—90 minutes as compared with 60 minutes in the non-irradiated group. The mean maximum response (0.5°C) was almost identical to that of the non-irradiated group and the area under the mean fever curve was 4.8°C minutes. Statistical comparison of this group with the normal control animals again shows a highly significant result: \( P < 0.001. \)

**Febrile Response of Recipients to Test Sera**

(a) **Normal guinea-pig serum.** Fig. 4 shows the mean febrile response of four normal guinea-pigs to intracardiac injection of 5 ml. of normal guinea-pig serum. The mean maximum response was 0.2°C and the area under the mean fever curve was 0.2°C minutes.

(b) **Serum from non-irradiated, hypersensitive, antigen-challenged donors.** Serum from these donors proved to be pyrogenic in all of the eight recipients in which it was tested. Their
mean maximum response (see Fig. 4) was 0·5° occurring 60 minutes after injection of serum with a latent period of 30 minutes. Defervescence was complete at 120 minutes after injection. The area under the mean fever curve was 4·0°minutes. Comparison of the group's response with that of the control animals to normal serum shows that the result is significant: 0·02 > P > 0·01.

(c) Serum from irradiated, hypersensitive, antigen-challenged donors. Serum from these donors was pyrogenic in the four recipients in which it was tested. Fig. 4 shows that their febrile response was almost immediate with a mean maximum response of 1·2° occurring 60 minutes after injection. The area under the mean fever curve was 18·4°minutes.

Comparison of this group with the response of the control animals to normal serum shows that the result is significant: 0·02 > P > 0·01.

DISCUSSION

The behaviour of the irradiated animals in this experiment differed only in one respect from the non-irradiated groups, namely, in their failure to develop circulating antibody. This was a very striking difference since antibody tests were negative in all irradiated animals, whereas all non-irradiated animals developed antibody. The difference in the response of the two groups agrees in general with that reported by Uhr and Scharff (1960), but is more clearly defined since these workers were able to demonstrate antibody in a few irradiated guinea-pigs. The failure of animals irradiated with 200 R. to develop any circulating antibody was also found by Salvin and Smith (1959) but a strict comparison cannot be made since they used different tests for antibody detection.

Irradiated and non-irradiated animals, however, developed delayed hypersensitivity with equal ease, and the febrile response of the irradiated animals to antigen systemically though slightly less than that of the non-irradiated group did not differ significantly. Their pattern of response corresponded to that reported by Uhr and Scharff (1960), and was characteristic of delayed hypersensitivity with a latent period of 90 minutes. The fact
that the non-irradiated group, while demonstrating delayed hypersensitivity, also had circulating antibody would support Salvin’s conclusion that this type of delayed sensitivity is an early stage in the development of the classical immune response (Salvin and Smith, 1960).

It is of interest that despite the slightly lesser response of irradiated animals to specific antigen, the recipient febrile response to their donated serum indicated the presence of endogenous serum pyrogen with a more marked recipient response than to non-irradiated donor serum. Both classes, however, produced a significant fever of the type associated with injection of endogenous serum pyrogen. In view of the fact that comparatively small groups of recipients were used in which individual variation might have considerable effect on mean response, the greater pyrogenicity of irradiated serum is probably not of biological significance. One may nevertheless conclude that 200 R. whole body irradiation in no way inhibits the production of delayed hypersensitivity nor affects the febrile response to specific antigen and liberation of an endogenous pyrogenic factor.

This experiment is not without interest since in recent years much discussion has centred around the role of circulating antibody in delayed hypersensitivity and the effect of antigen–antibody complexes on granulocytes. It has been noted, for example, that normal granulocytes when incubated with a mixture of hypersensitive serum and specific antigen are lysed (Waksman, 1953). This phenomenon is thought to be of importance in various obscure diseases, such as disseminated lupus erythematosus, in which abnormal circulating antibodies can be detected, and some workers have inferred that this same mechanism may play a part in all varieties of delayed hypersensitivity. The fact that sensitized serum is sufficient for the reaction raises the possibility that serum antibody unites with antigen, and the resulting complex predisposes to leucocyte damage. This view was upheld by Farr (1958), who attributed the reaction to non-precipitable antibody; though Vaughan and Kabat (1953) did not support this hypothesis. There is however no convincing evidence that circulating antibody plays any part in the development of delayed hypersensitivity and all attempts to reproduce the work of Zinsser and Mueller (1925) and Cole and Favour (1955) have failed. It is perhaps significant that it has been shown, contrary to earlier reports, that children with agammaglobulinaemia may develop tuberculin delayed hypersensitivity and may also reject homografts (Rosen, Gitlin and Janeway, 1962). It is therefore unlikely that serum antibody is important in these reactions.

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REFERENCES


