TOLERANCE TO BACTERIAL PYROGENS

II. ROLE OF THE RETICULO-ENDOTHELIAL SYSTEM*

BY PAUL B. BEESON, M.D.

WITH THE TECHNICAL ASSISTANCE OF ELIZABETH ROBERTS

(From the Medical Service, Grady Hospital, and the Department of Medicine, Emory University School of Medicine, Atlanta)

(Received for publication, March 19, 1947)

The preceding article (1) describes a series of observations on the tolerance which develops as a result of repeated intravenous injections of bacterial pyrogens, and which is characterized by a diminished febrile reaction to these substances. This state appears to be largely independent of serologic specificity; it is also remarkable for its short duration. The present article deals with experiments designed to elucidate the mechanism by which it develops, with special reference to the role of the reticulo-endothelial (R-E) system.

Materials and Methods

Tests were conducted under the conditions described in the preceding article. Two agents were employed for R-E blockade: 25 per cent colloidal thorium dioxide (thorotrast, Heyden Co.), and 1 per cent aqueous trypan blue. The dose of thorotrast was 9 ml.; this was given intravenously 16 hours before the test injection of pyrogen. The dose of trypan blue was 6 ml.; this was given twice, 16 hours and 1 hour previous to injection of the pyrogen. In experiments on the effect of R-E blockade the doses of pyrogens used were the same as those given in the preceding article. In tests of the speed of removal of pyrogen from the blood, the donor animal was prepared for cardiac puncture. The pyrogenic material was then injected into an ear vein, at a rate of 1 ml. in 5 seconds. Exactly 4 minutes after the conclusion of the injection, the heart was punctured, and 8 ml. of blood drawn rapidly into a syringe containing 2 ml. of 3.8 per cent sodium citrate. The citrated plasma was separated immediately by centrifugation, and was injected into a test animal within 1 hour of the time of the bleeding. The technic for asepsis was observed at all stages of the procedure. The amounts of pyrogens given to the donor animals were: *Eberthella typhosa* vaccine, 5 ml.; *Serratia marcescens* vaccine, 3 ml.; *Pseudomonas aeruginosa* filtrate, 5 ml.; purified *Eberthella typhosa* pyrogen, 0.5 mg. in 5 ml. saline; purified *Serratia marcescens* pyrogen, 1.0 mg. in 5 ml. saline.

EXPERIMENTAL

Effect of R-E Blockade on the Tolerance.—Rabbits were given daily injections of one of the pyrogen preparations until a marked diminution in febrile response had developed; i.e., for 6 to 10 days. They then received intravenous injections of a blocking agent, after which the same dose of pyrogen was administered. Under these circumstances there was a marked increase in the febrile reaction. In most instances the febrile reaction following blockade was even greater than

* Aided by a grant from the United States Public Health Service.
that which occurred after the first injection of the pyrogen. Experiments of this type were carried out on 64 rabbits, and without exception the fever was greater after R-E blockade than before. Fig. 1 shows a typical example, with each of the 5 different pyrogen preparations, using thorotrast as the blocking agent. The effect of thorotrast was usually quantitatively greater than that of trypan blue, although the latter agent invariably was responsible for a marked increase in the febrile reaction.
Speed of Disappearance of Pyrogen from the Blood.—In view of the finding that R-E blockade appeared to abolish the induced tolerance for pyrogens, the possibility was considered that tolerance might be determined by the efficiency of the mechanism for removing pyrogen from the circulating blood. Accordingly, experiments were carried out in which blood samples were taken from normal animals and those with induced tolerance a few minutes after they had received large doses of pyrogen. These samples were then tested for pyrogen content, by measuring their effect in causing temperature elevations in other rabbits. Preliminary tests on normal animals showed that, even after administration of large doses of pyrogen, little trace could be detected in the circulating blood 8 to 10 minutes later. At 4 to 6 minutes, however, pyrogenic activity was always considerable.

An animal was given increasing quantities of E. typhosa vaccine during a period of 2 weeks, up to a dose of 5 ml. daily. On the 15th day this quantity was given and the animal was tested for capacity to remove it from the blood, in comparison with a normal animal of the same breed and size. The experimental technique has already been described. Fig. 2 shows the pyrogenic potency of the blood of a rabbit treated as just described and of a normal rabbit 4 minutes after each had received 5 ml. of E. typhosa vaccine intravenously. Three normal animals were used in testing the blood of each rabbit receiving the pyrogen. It will be observed that a considerable difference in pyrogenic activity was found. With the undiluted samples and with the 1-4 dilutions there was a marked difference in febrile responses of the test rabbits, and with

![Fig. 2. Effect of previous injections of E. typhosa vaccine on speed of disappearance of the pyrogen from the blood. This shows the relative pyrogenic activity of plasma from an animal so treated, and a normal rabbit 4 minutes after each had received 5.0 ml. E. typhosa vaccine. Different dilutions of each sample were given to 3 normal rabbits.](image-url)
TOLERANCE TO BACTERIAL PYROGENS. II

Fig. 3. Effect of previous injection of E. typhosa vaccine on ability to remove different pyrogens from the blood. This shows comparative febrile reactions of normal rabbits to plasma from tolerant and normal donor rabbits. Donors had been given large doses of pyrogen 4 minutes before plasma samples were obtained.

Fig. 4. Effect of R-E blockade on ability of previously tolerant animal to remove pyrogen from the blood. This shows the pyrexial reactions of normal rabbits to plasma samples from 2 rabbits taken 4 minutes after each had received 5.0 ml E. typhosa vaccine. One of these donor animals had received thorium dioxide on the previous day.

1-8 dilutions the sample from the previously injected donor animal caused practically no temperature elevation, whereas that from the normal animal still contained sufficient pyrogen to cause a considerable pyrexial reaction.

Similar experiments were carried out, testing the speed of disappearance of other pyrogens from the blood. It was found that repeated injections of E. typhosa...
typhosa vaccine created a state such that the pyrogens of *S. marcescens* and *Ps. aeruginosa* disappeared from the circulating blood more quickly than was the case with normal animals. Fig. 3 presents examples of such experiments, using the 4 other pyrogen preparations employed in this work: *S. marcescens* vaccine, *Ps. aeruginosa* filtrate, purified *S. marcescens* pyrogen, and purified *E. typhosa* pyrogen. As is shown, the amount of pyrogenic material remaining in the circulation 4 minutes after injection was always less in treated than in normal animals.

**R-E Blockade and the Speed of Disappearance of Pyrogen from the Blood.**—A study was made of the effect of R-E blockade on the rate of disappearance of pyrogen from the circulating blood. Two rabbits were given daily injections of *E. typhosa* vaccine in increasing doses up to 5 ml., during a period of 2 weeks. One of them was then given thorotrast as a blocking agent, and on the following day both animals were tested for speed of removal of the same dose of *E. typhosa* vaccine pyrogen from the blood. It was found that the animal given R-E blockade had more circulating pyrogen 4 minutes after the injection than did the other tolerant animal. Four experiments of this kind were done, with the same result; one of them is illustrated in Fig. 4.

**DISCUSSION**

Explanation of the mechanism of tolerance for pyrogens is hampered by the inadequacy of our knowledge regarding the pathogenesis of fever. Clinical experience has demonstrated that fever is a sensitive index of many kinds of pathologic process, whose only common factor is injury of tissue. It is probable that the temperature elevation produced by bacterial pyrogens is also a reflection of injury, and not due to a direct effect on temperature-regulating centers in the brain. This view is supported by the fact that there is always a time lag between the injection of the pyrogen and the beginning of the rise in temperature, and by the fact that purified pyrogens are lethal toxins (2).

The experimental evidence which has been presented here indicates that the functional state of the R-E system is an important factor in determining the extent of the febrile reaction to pyrogens. It seems reasonable, as already stated, to assume that the febrile response to a pyrogen is a reflection of injury. If this assumption is correct the next problem is to determine the site of tissue injury. There is evidence that bacterial pyrogens can cause injury to vascular structures, as was mentioned in the preceding article. Another possible site of injury is the R-E cells themselves. The evidence obtained in this work could be interpreted, however, as indicating that the R-E cells are relatively resistant to injury, and that they serve to protect other, more susceptible cells. Evidence in support of this supposition was obtained in our studies on the effect of R-E blockade on immunity to the Shwartzman phenomenon (3). This phenomenon is advantageous as a method of studying the present problem,
because it provides a defined, observable site of injury in the skin. Some rabbits exhibit natural immunity to the Shwartzman reaction, and in all rabbits an immunity develops after repeated injections of bacterial toxin. It was found that either the natural or the acquired immunity could be abolished by blockade of the R-E system. Following an injection of thorotrast an animal that had previously given no purpuric reaction on test showed severe hemorrhagic necrosis in the skin. These findings can be interpreted as indicating that the blocking substance, by interfering with the cells which normally take up most of the bacterial toxin from the blood, allows the toxin to reach the skin in sufficient quantity to produce the hemorrhagic reaction.

On the basis of the present findings the following hypothesis can be offered regarding the development of tolerance to pyrogens: A series of injections of these causes a change in the functional capacity of the R-E system whereby the ability to remove bacterial toxins from the blood is enhanced. As a result of this increase in ability, other susceptible tissues are protected from injury, and the lessened injury is reflected by a diminution in the febrile response.

SUMMARY

In experiments designed to elucidate the mechanism by which tolerance to bacterial pyrogens is developed, the following observations were made:

1. Animals whose febrile reactions to bacterial pyrogens were markedly diminished, as a result of repeated injections, showed increases in response following R-E blockade.
2. Pyrogenic substances disappeared from the circulating blood more rapidly in rabbits rendered pyrogen-tolerant than in normal animals. Lack of specificity was shown by the fact that rabbits previously injected with *Eberthella typhosa* bacterial vaccine were able to remove the pyrogens of *Serratia marcescens* and *Pseudomonas aeruginosa* from their blood more rapidly than normal animals.
3. R-E blockade retarded the speed of disappearance of pyrogens from the circulating blood of animals which had been rendered relatively tolerant by previous injections of these substances.

A possible mechanism for the development of unresponsiveness to bacterial pyrogens is suggested.

BIBLIOGRAPHY