STUDIES ON THE MECHANISM OF PENICILLIN-INDUCED FEVER*

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Fever is one of the most common manifestations of allergy to penicillin (1). In unsensitized individuals, the febrile response typically appears on the 7th–10th day of drug administration, but occurs more rapidly in previously sensitized individuals (2). Despite the frequency and clinical importance of this phenomenon, the factors which produce penicillin (PCN)[1]-induced fever do not appear to have been investigated.

In a search for possible allergens in the normal metabolism of benzylpenicillin G in the host, Levine (3–5) has demonstrated that an initial breakdown product, benzylpenicillenic acid, may be metabolized to the benzylpenicilloyl (BPO) hapten which produces cutaneous penicillin hypersensitivity in rabbits. Further, rabbits sensitized to benzylpenicillin develop serum antibodies specific to BPO as demonstrated by precipitin techniques (5, 6), passive cutaneous anaphylaxis (5, 7, 8), and hemagglutination inhibition (9).

Certain immediate reactions of hypersensitivity in man, such as anaphylaxis, have been shown to be caused primarily by minor penicillin determinants such as benzylpenicilloate, benzylpenicillamine, and penicilloate (7, 10, 11); reactions such as rashes, arthritis, and fever which appear later (12) are thought to be due to the BPO hapten. The nature of the antibody responsible for the allergic reaction that produces fever has not been identified, although the delay in onset of this reaction in sensitized individuals suggests an antibody of the IgG class.

The purpose of the experiments described here was to determine whether the penicilloyl hapten, in the form of a penicillin-rabbit serum protein conjugate, would produce fevers in rabbits sensitized to penicillin and, if so, to determine what immunologic factors might be responsible for the release of the endogenous pyrogen (EP) that presumably mediates this as well as other forms of hypersensitivity fever (13).

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[1] *Abbreviations used in this paper*: Ag, antigen; BPO, benzylpenicilloyl; BSA, bovine serum albumin; EP, endogenous pyrogen; HSA, human serum albumin; HSS, penicillin-hypersensitive serum; KRP, Krebs-Ringer phosphate; NS, normal (rabbit) serum; PCMB, p-chloromercuribenzoate; PCN, penicillin.

Materials and Methods

Equipment, Reagents, and Laboratory Utensils.—All glassware were rendered pyrogen-free by heating in a dry air oven for 2 hr at 170°C. Water and normal saline were commercially obtained "pyrogen-free" (The Cutter Laboratories, Berkeley, Calif.). All solutions were periodically tested in rabbits for pyrogenicity and cultured in thioglycollate broth (14).

Animals.—Female New Zealand white rabbits (3.5–5.5 kg) obtained from several commercial sources were used in all experiments. None was known to have been exposed to penicillin, though the breeders could not insure such a status.

Penicillin Immunization.—A benzylpenicillin G-complete adjuvant emulsion was prepared by thorough mixing of 100 mg (167,000 units) of potassium benzylpenicillin G (E. R. Squibb and Sons, Princeton, N. J.) in 1 ml of water with 1 ml of complete Freund's adjuvant (Difco Labs., Detroit, Mich.). 0.2 ml of this emulsion was injected intradermally into each of 10 dorsal skin sites of rabbits. 10 days later, this procedure was repeated except that incomplete Freund's adjuvant (Difco Laboratories) was substituted for complete adjuvant in the emulsion.

8 days after the second inoculation, each animal received intravenously 100 mg of potassium benzylpenicillin G in 2 ml of water and 500,000 units of procaine penicillin (Squibb) intramuscularly. These paired injections were repeated four more times on every other day. Rabbits were used for experimentation 7–10 days after the last pair of injections (33–36 days after the initial immunization). This immunization regimen was modified from Levine and Ovary (5).

Determination of Anti-Benzylpenicillin Hemagglutinating Antibody Titer.—

Serum collection: Before the start of immunization and before injection of penicillin on immunization days, 1–3 ml of blood was obtained from the marginal ear vein of each rabbit. Immunized animals and nonpenicillin-immunized control animals were also bled before use. The blood was collected in centrifuge tubes, refrigerated for 1 hr at 4°C, and centrifuged at 1500 rpm for 15 min. The serum was removed and stored at −10°C.

Preparation of penicillin-coated red blood cells: (Technique adapted from Thiel and Parker.) Alsever's solution, phosphate saline, and barbital buffer were prepared as described previously (15). 4 ml of blood from a rabbit was collected in a heparinized test tube. The red cells were then washed with a double volume of phosphate saline and centrifuged in the cold at 1500 rpm for 15 min. This procedure was repeated three times. One volume of a 16% suspension of these cells in phosphate saline was then added to 2.5 volumes of barbital buffer. To 3.5 volumes of this mixture was added 0.5 volumes of a 20 mg/ml solution of potassium benzylpenicillin G in phosphate saline. For control cells, 0.5 volumes of phosphate saline were added instead. Finally, both the control cells and the penicillin-treated cells were incubated at 37°C in a water bath for 1 hr, and then at 4°C for 24 hr. After 24 hr, both pencillin-coated and control cells were washed with phosphate saline and centrifuged at 1500 rpm for 15 min. This procedure was repeated (generally three times) until the supernatant was clear of hemoglobin.

Hemagglutination procedure: Two 0.4-ml aliquots of serum were serially diluted with phosphate saline in plastic hemagglutination trays to produce two series of twofold serum dilutions up to 1:2048. To one series was added 0.2 ml of 0.5% freshly washed pencillin-coated red blood cells in phosphate saline, prepared as above, and to the other was added control cells. The trays were shaken, allowed to rest at room temperature for 6–12 hr, and then scored for hemagglutination, 0 to 4+.

Preparation of Penicillin-Protein Conjugates.—(Technique adapted from Parker [16].) Sterile techniques and materials were used for the conjugation procedure. A normal rabbit was exsanguinated by cardiac puncture and the serum obtained. This serum was lyophilized, and 4 g dissolved in a few milliliters of sterile water. The concentrated serum solution was then adjusted to pH 7 with 5 N NaOH or dilute HCl using a pH meter as a monitor. 40 ml of 1 M K_2CO_3 (pH 10.4) buffer was then added followed by 15.3 g of powdered sodium benzylpenicillin G (Squibb). The pH was finally adjusted to 9.6 with 5 N NaOH or dilute HCl, and the solution incubated at 37°C in a shaking water bath for 18 hr. 7.6 g of sodium benzyl-

penicillin G were then added, the pH was readjusted to 11.0 using 5 N NaOH, and the solution was returned to the shaking water bath for 6 hr. The addition of sodium benzylpenicillin G (7.6 g), pH readjustment, and incubation was repeated once more.

Dialysis of Penicillin-Protein Conjugate.—The penicillin-rabbit serum solution was poured into several 27 × 32 inch diameter cellulose dialysis bags (Arthur H. Thomas Co., Philadelphia, Pa.) which had been previously rinsed well with sterile penicillin-protein water. These bags were then suspended in a 4 liter beaker filled with 0.002 M tris(hydroxymethyl)aminomethane (Tris) buffer, pH 7.9, to which had been added 3 g of Amberlite, 20–50 mesh. The Amberlite was previously prepared by soaking with 3 M NaOH, washing with sterile water, and equilibrating with 0.2 M Tris, pH 8.5. The dialysis bath was slowly stirred at 4°C with a magnetic mixer for 4 days, and fresh Tris buffer and Amberlite were used daily. A final 24 hr dialysis was carried out against 0.005 M Na₂HPO₄ buffer, pH 7.5–8.0. The dialyzed material was then lyophilized, suspended in normal saline (7 g/100 cc saline), and stored at −10°C (17).

Spectrophotometric Assay of Penicilloyl-Protein Conjugate.—0.1 ml of penicillin-serum protein conjugate was added to 100 ml of 0.05 M K₂CO₃ (pH 9.2) buffer, and the optical density of this solution at 285 mμ measured with a Coleman 111 Spectrophotometer (Coleman Instruments Division, Perkin-Elmer Corporation, Maywood, Ill.). To a 5 ml aliquot was added 0.1 cc of a 1.5 × 10⁻² M p-chloromercuribenzoate (PCMB) solution, and after 10 min of thorough shaking, the optical density was again measured. The first value, as well as the value for the PCMB solution (0.060 OD units), was subtracted from this reading, and the result corrected for dilution by division by 1.02. The penicilloyl concentration in moles/liter was determined by dividing the corrected optical density by the molar extinction coefficient *E* of penicilloyl-protein, 23,800 (17). The penicilloyl-rabbit protein conjugated used in these experiments varied in penicilloyl concentration from 1.2 × 10⁻² M to 4.7 × 10⁻² M.

Preparation of Rabbit Tissues.—

Blood leukocytes and plasma: Donor rabbits previously sensitized to penicillin were anesthetized with 100 mg of intravenous sodium pentobarbital and anti-coagulated with 10,000 units of USP sodium heparin. The animals were then exsanguinated by cardiac puncture. The total number of white cells was calculated by hemacytometer, and the blood centrifuged at 1500 rpm for 15 min. The plasma was removed and saved for passive transfer experiments, while the cells were resuspended in sufficient modified Krebs-Ringer phosphate (KRP) buffer (18) containing 150% glucose to give a concentration of 1 × 10⁷ WBC/ml.

Spleen: After exsanguination of the animal, the spleen was removed and placed in iced saline. It was then divided into several small segments and forced through a No. 40 wire screen with a spatula and known volume of iced saline. A leukocyte count was made using a hemacytometer, the suspension was spun at 1500 rpm for 15 min, the supernate removed, and the cells suspended in KRP buffer to a concentration of 2 × 10⁸ WBC/ml.

Challenge of Penicillin-Sensitized Rabbits.—Penicillin-sensitized rabbits were trained in wooden boxes during their immunization period. During experiments, they were placed in open wooden stalls and temperatures were taken every 15 min on a Foxboro rabbit scanning switch (The Foxboro Company, Foxboro, Mass.) and fever recorder equipped with thermistor probes (14). In general, rabbits were not challenged until their temperature was stable (within 0.2°C) for a period of at least 1 hr. No animals were used whose temperatures were higher than 40.5°C. At the time of challenge, the material used, usually 1–4 ml, was injected intravenously by marginal ear vein, and the subsequent temperatures recorded at 15-min intervals for the next 3–4 hr.

RESULTS

Development of Circulating Hemagglutinating Anti-Benzylpenicillin Antibodies in Penicillin-Immunized Rabbits.—Rabbits were immunized to penicillin according to the regimen outlined in Materials and Methods. Serum samples

were obtained initially, and at approximately weekly intervals throughout and
after immunization were tested for hemagglutinating anti-benzylpenicillin anti-
bodies. Initial samples showed no detectable antibody in any of 27 rabbits. 1 of
14 control animals had an initial titer (1/256). After immunization, 26 of 27
rabbits developed hemagglutinating anti-benzylpenicillin antibodies. These
antibodies typically appeared about day 15 in the immunization schedule, 5
days after the second injection of penicillin in Freund's adjuvant. Hemag-
glutination titers tended to increase during the immunization process until day
22 when they started to decline slightly. Fig. 1 shows the distribution of the
maximum anti-penicillin antibody titers developed in each animal during im-
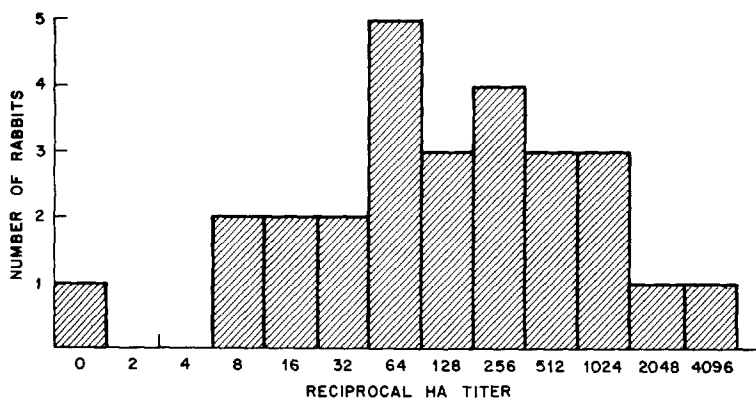munization.

Fig. 1. Maximum hemagglutinating (HA) anti-benzylpenicillin antibody titer developed in
each of 27 penicillin-immunized animals.

Since almost all rabbits (40/41) had no hemagglutinating anti-benzylpenicil-
lin antibodies before penicillin immunization, the development of such anti-
bodies in the experimental group was evidence of penicillin sensitization. It was
next determined whether such sensitization would be accompanied by febrile
response to antigen.

*Febrile Response of Penicillin-Immunized Rabbits to Aqueous Benzylpenicil-
lin.*—17 rabbits immunized to benzylpenicillin G were challenged with intra-
venous sodium benzylpenicillin G 15–20 days after receiving their last immuniz-
ing dose of aqueous and intramuscular penicillin. Challenge doses varied from
100,000 to 1,000,000 units. No rabbit developed a fever greater than 0.25°C,
and none developed any sign of immediate allergic reaction to penicillin. Thus,
animals sensitized by this immunization regimen did not develop immediate
allergic reactions to parenterally administered aqueous penicillin, nor did they
respond with significant fevers within 3 hr of such challenge.

Febrile Response of Penicillin-Immunized Rabbits to Challenge with Intra-

venous Penicillin-Serum Protein Conjugates.—Since aqueous PCN alone did not produce fever in specifically immunized animals, a penicillin-rabbit serum protein conjugate (BPO) was prepared (see Materials and Methods). Immunized and normal rabbits were then challenged intravenously with varying amounts of this conjugate. As seen in Fig. 2, the penicillin-immunized animals responded with significant fevers, while the control animals remained afebrile. The fever appeared to be biphasic, with a small rapid peak at 15–30 min followed by a much larger one starting at about 75 min after challenge. As shown in Fig. 3,
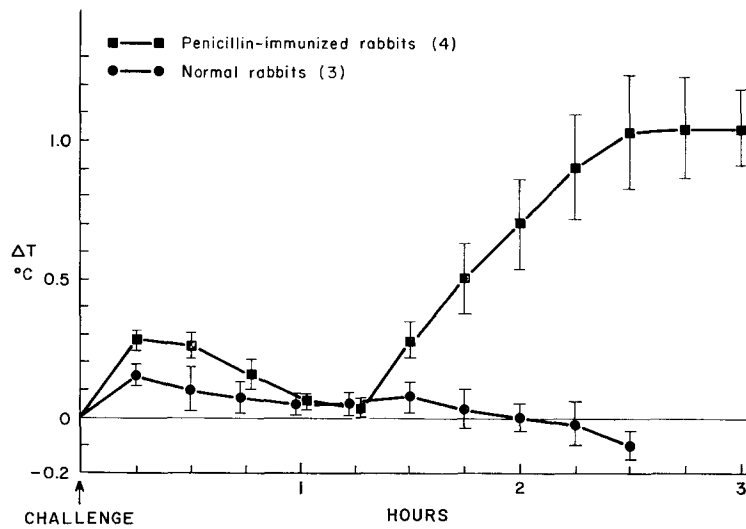

FIG. 2. Mean febrile responses (±SEM) of penicillin-immunized and unimmunized rabbit to intravenous challenge with 48 μmoles of pencillin-rabbit serum protein conjugate are compared. Numbers in parentheses in this and subsequent figures indicate the number of rabbits.

there was a direct relationship between the amount of conjugate injected and the height of the fever.

During these experiments, three immunized animals died after challenge with the conjugate. Approximately 30 sec after intravenous injection, these animals developed lateral nystagmus which was quickly followed by stridorous breathing, cyanosis of the mucous membranes, and screaming. Death ensued within 2 min. Necropsy of these animals revealed livers congested with blood and relatively bloodless lungs.

In order to determine whether human serum conjugate would also elicit fever in rabbits, 100 μm of penicillin-human serum protein conjugate was injected into two penicillin-sensitized animals never previously injected with human materials. Two control animals without prior exposure to penicillin or human products were also challenged. The penicillin-immunized rabbits responded with

fevers similar to those seen with challenge by penicillin-rabbit serum protein conjugate, whereas control animals remained afebrile.

From these experiments, it seems apparent that animals immunized to penicillin, unlike normal rabbits, develop fever after challenge with penicillin-serum protein conjugates, although not after challenge with simple intravenous aqueous penicillin. The main component of these fevers begins between 60 and
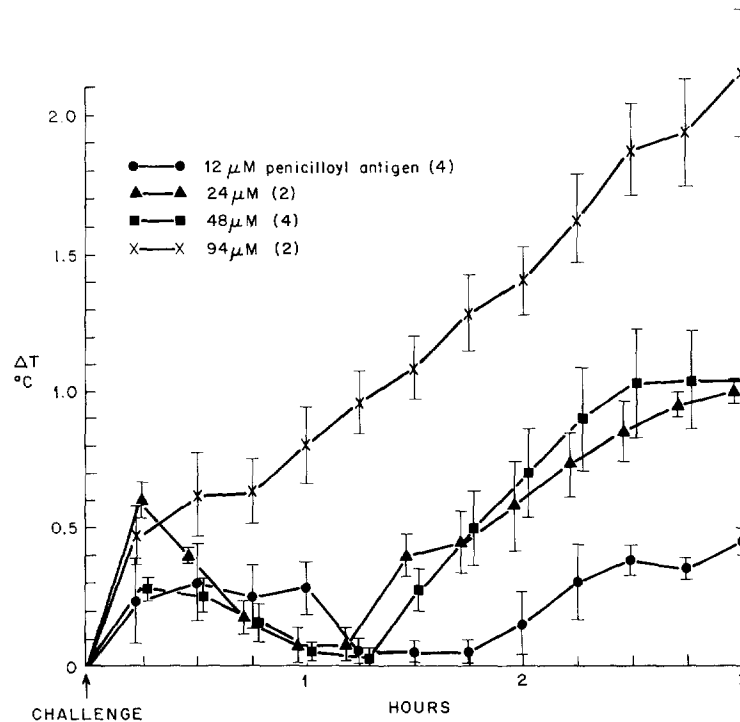

FIG. 3. Comparison of febrile responses ($\pm$SEM) of four groups of penicillin-immunized rabbits to intravenous challenge with four different doses of penicillin-rabbit serum protein conjugate.

90 min after injection, and the height of fever varies directly with the dose of conjugate. Immunized animals appear to respond similarly to both human and rabbit serum penicillin conjugates.

Development of Febrile Tolerance in Penicillin-Immunized Rabbits after Challenge with Penicillin-Rabbit Serum Protein Conjugate.—When penicillin-hypersensitive rabbits received a second injection of penicillin-protein conjugate, the second febrile response was much smaller than the first. Fig. 4 shows the mean febrile responses of eight penicillin-immunized animals to a first injection of penicillin-rabbit serum protein conjugate, and to a second challenge 3 days later. It is evident that an almost complete febrile tolerance developed after

the first injection. Repeated testing in some animals indicated that tolerance usually lasted at least 2 wk. In 10 animals during the period of tolerance, one intramuscular injection of 600,000 units of procaine penicillin was given, and a challenge dose of conjugate 3 days later. Febrile responsiveness was partially restored by this boosting procedure in six rabbits. Thus, tolerance appeared to be partially reversible by reimmunization with intramuscular procaine penicillin in some animals.

Passive Transfer of Penicillin Hypersensitivity with Plasma.—To determine whether febrile reactivity to penicillin could be passively transferred, unpooled
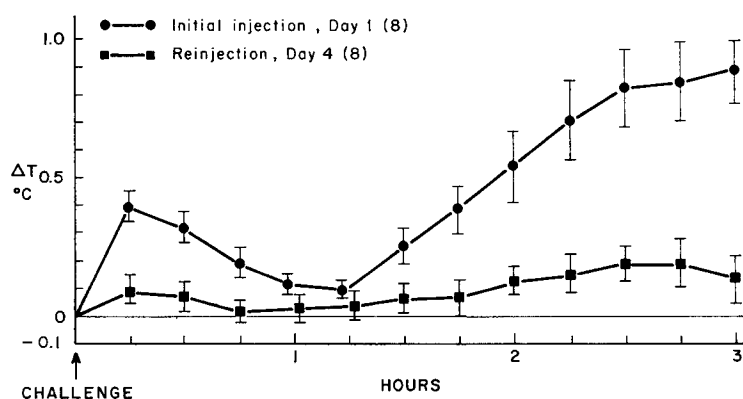

Fig. 4. Initial mean febrile responses (±SEM) of eight penicillin-immunized rabbits to challenge with an average of 30 μmoles penicillin-rabbit serum protein conjugate. Also shown is the mean febrile response (±SEM) of these same eight rabbits challenged 3 days later with a similar dose of conjugate.

plasma obtained from three immunized rabbits was intravenously administered in doses of 20 ml to each of seven control animals (two or three recipients per donor). After plasma infusion and temperature stabilization for at least 2 hr, the recipient animals were challenged intravenously with penicillin-rabbit serum protein conjugate and their febrile responses recorded. Recipients of the hypersensitive plasma from two of the donor rabbits showed suggestive febrile responses (0.3–0.4°C) to challenge with 47 μm of penicillin-rabbit serum protein conjugate. The recipients of the third donor's plasma were given twice the dosage of antigen and showed a more definite febrile response (Fig. 5). Although fevers were small, perhaps because both the volume of plasma and the dosage of antigen given were relatively small, the responses were all characteristic of antigen-induced fever.

In Vitro Release of Endogenous Pyrogen from Tissues of Penicillin-Immunized Rabbits.—In order to examine the ability of cells from sensitized animals to release EP in vitro in response to antigenic stimulation, blood and spleen cells from sensitized rabbits were incubated with penicillin-protein conjugate and

the supernatants assayed for EP. Blood leukocytes from penicillin-immunized animals, prepared as described in Materials and Methods, were suspended in buffer with serum from either hypersensitive (HSS) or normal (NS) rabbits. 100 μm of penicillin-rabbit serum protein conjugate or 1 ml of saline was added to each suspension of cells. A dose of supernatant equivalent to about 1×10^8
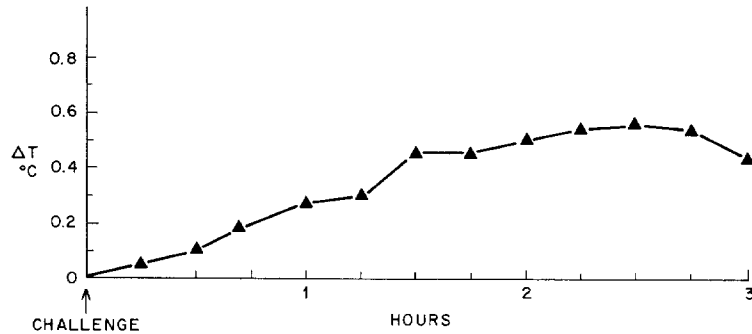

Fig. 5. Mean febrile response of two unsensitized rabbits to intravenous challenge with 94 μmoles of penicillin-rabbit serum protein conjugate given after infusion of 20 ml of penicillin-hypersensitive plasma from a sensitized donor animal.
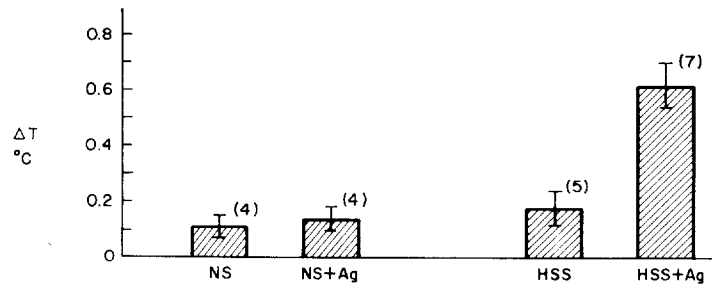

Fig. 6. Mean maximal febrile responses ($\pm$SEM) of animals injected intravenously with supernates of blood cells from penicillin-immunized rabbits. Cells were incubated with normal rabbit serum (NS) or penicillin-hypersensitive serum (HSS) and with (plus antigen Ag) and without the penicillin-rabbit serum protein conjugate. Doses were equivalent to 1×10^8 white blood cells/dose (10 ml). The numbers in parentheses represent the number of animals injected.

white blood cells was injected for endogenous pyrogen assay into normal rabbits.

As shown in Fig. 6, incubation of blood cells from penicillin-immunized rabbits with NS or HSS alone (1st and 3rd bars) did not produce endogenous pyrogen release. Similarly, no pyrogen was released by the same blood cells incubated with normal serum (NS) and penicillin-protein antigen (2nd bar). However, endogenous pyrogen was released when blood cells were incubated with hypersensitive serum (HSS) and penicillin-protein antigen (last bar). Injection of this

supernate produced a rapid monophasic fever typical of endogenous pyrogen and unlike the previously observed delayed responses typical of antigen in a sensitized host (Fig. 7). Thus, sensitized blood cells released pyrogen in vitro when incubated with both HSS and the penicillin-protein conjugate.

Analogous experiments were carried out with spleen cells obtained from a penicillin-immunized rabbit. Unlike the blood cells of penicillin-immunized rabbits, the spleen cells from these same animals did not release pyrogen when incubated with penicillin-protein antigen and either hypersensitive or normal
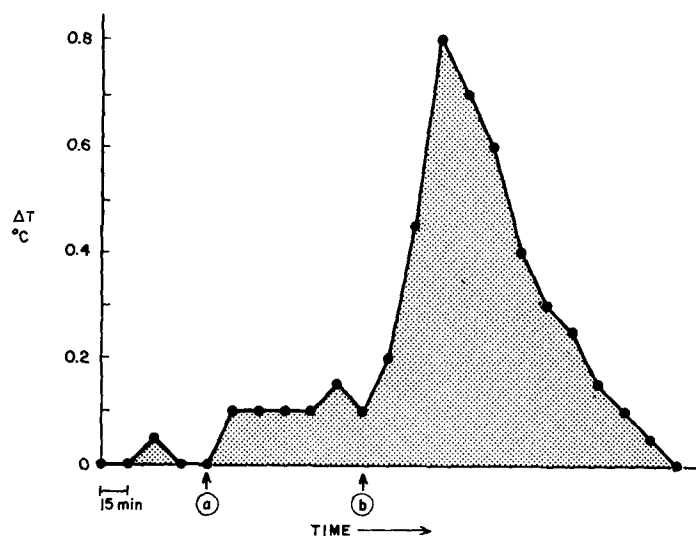

FIG. 7. Characteristic febrile responses of a single rabbit to supernates of white blood cells from a penicillin-immunized rabbit incubated with and without antigen. At (a) 10 ml of a control supernate of white blood cells incubated with sensitized serum, buffer, and saline was given intravenously. At (b) 10 ml of experimental supernate of the same cells incubated with sensitized serum, buffer, and penicillin-protein antigen was injected. Both doses were equivalent to 1×10^8 white blood cells.

serum. Supernatants from 2×10^8 cells were assayed for pyrogen in each experiment.

DISCUSSION

These studies demonstrate that rabbits immunized to penicillin (PCN), according to a regimen adapted from Levine and Ovary (5), develop circulating antibody to benzylpenicillin, but respond with fever and anaphylaxis only to penicillin-protein conjugates rather than to PCN itself. Substances with a molecular weight of <1000 are believed to become immunogenic by binding irreversibly with tissue macromolecules, presumably proteins (19). Since temperatures were not measured for more than 4 hr, later febrile responses that

might have resulted from combination of penicillin with various proteins would not have been observed.

Rarely, sensitized humans respond with rapid anaphylaxis to parenteral administration of penicillin. The difference between such clinical situations and the experimental one described here may be due to differences in the rate of conjugation of penicillin with human and rabbit protein, the type or relative concentration of anti-penicillin antibodies in sensitized humans and rabbits, or a greater vulnerability of humans to anaphylaxis. No data were obtained to support any of these alternatives.

Because aqueous penicillin did not elicit fever or anaphylaxis in specifically immunized animals, a penicillin-rabbit serum protein conjugate was prepared as the challenge antigen. This conjugate, prepared at high pH, according to a modification of the method of Parker and Thiel (16), was composed of benzyl-penicillin molecules joined to the lysine ε-amino groups of various rabbit serum proteins via benzylpenicilloyl (BPO) linkages. Intravenous injection of the conjugate produced significant febrile responses in penicillin-immunized animals. The fevers were typically biphasic, with a small rapid peak evident 15-30 min after challenge, and a larger febrile response beginning at 60-90 min after challenge. There appeared to be a rough correlation between the degree (height and duration) of the second (major) fever peak and the concentration of BPO injected. This later temperature rise closely resembled the febrile responses seen in hypersensitivity fevers, such as those induced in sensitized rabbits by staphylococcal filtrate (20), old tuberculin (21), bovine serum albumin (BSA) (22), and human serum albumin (HSA) (23), and thus appeared to represent a typical hypersensitivity fever. The significance of the smaller, transient first peak is unclear. Since all three penicillin-immunized rabbits which died of anaphylaxis shortly after penicillin-protein challenge had rapidly rising temperatures just before death, it may represent abortive anaphylactic response. Because there is no known humoral mediator that acts on the thermoregulatory center to produce a fever peak this rapidly (less than 30 min after challenge), such a response may result from a direct effect on the peripheral blood vessels or their nervous supply. This immediate, brief fever has not been described in other hypersensitivity fevers.

Penicillin-immunized animals also responded with fevers when challenged with penicillin-human serum protein conjugate, indicating either a lack of carrier specificity as opposed to hapten specificity, or cross-reactivity between rabbit and human carrier protein.

Since normal animals did not respond with fever to the PCN-protein conjugate, and immunized rabbits rendered endotoxin-tolerant by injections of typhoid vaccine had undiminished responses to the antigen (data not included), the pyrogenicity of the conjugate was clearly not due to contamination with endotoxin.

In several other aspects, the responses of penicillin-immunized animals to

penicillin-protein conjugate resembled those occurring in other hypersensitiv-ity states associated with fever. For example, as in hypersensitivity to BSA (22–24) and HSA (25), only animals with detectable titers of specific humoral antibodies had fever when challenged with antigen, and the height of fever correlated roughly with the antibody titer. Further, as described in BSA-immu-nized rabbits (26), passive transfer of small amounts of plasma from penicillin-immunized animals enabled normal rabbits to respond with fever when chal-lenged with the penicillin-protein antigen. Also, as reported in hypersensitivity to BSA (23), HSA (25), or ovalbumin in sensitized guinea pigs (27), febrile tolerance appeared with reinjection of antigen. Unlike the tolerance that develops to BSA hypersensitivity, however, febrile reactivity to antigen did not reappear after a rest of 3 or 4 days, and only one or two injections of the penicillin-protein conjugate antigen induced a long-lasting tolerant state. Variations in the tolerance-inducing dose of antigen or in the degree of hyper-sensitivity developed by immunization may explain these differences.

In summary, the evidence presented here indicates that the BPO hapten-protein conjugate induces significant, dose-related fevers and, occasionally, anaphylaxis in penicillin-immunized animals, while the PCN alone was inac-tive. These findings suggest that the hapten-protein complex may be the active agent in producing penicillin drug fever in man.

In vitro experiments confirmed the ability of this conjugate to induce EP release from sensitized blood cells in vitro. Since such cells failed to release EP when normal rabbit serum was substituted for sensitized serum, humoral anti-bodies seem clearly required for activation of leukocytes in this system. Ex-periments with normal blood leukocytes in this model were not done.

In these experiments with PCN, like others using old tuberculin (14, 28) and para-influenza virus (29), incubation of spleen cells with the pyrogenic agent did not result in detectable EP release in vitro. However, recent studies in rabbits with delayed hypersensitivity to bovine gamma globulin (30), indi-cate that some antigens may activate specifically sensitized spleen cells to pro-duce EP in vitro when these cells are in a fully supportive medium (Eagle's minimal essential medium). The mechanism appears to involve the release of a "lymphokine" activator from the lymphocytes in the cell suspensions.

The sequence of events leading to the release of EP into the circulation after an intravenous dose of antigen in PCN-sensitized rabbits remains to be investi-gated. From these studies, it seems likely that anti-benzylpenicillin antibodies, probably anti-benzylpenicilloyl in specificity, are initially important. Pre-sumably, these antibodies form antigen-antibody complexes with penicillin-protein antigen which are then phagocytized by host cells including blood leu-kocytes. Root and Wolff (31) have demonstrated that HSA-anti-HSA antigen-antibody complexes, whether produced in vivo after injection of antigen Ag, or created in vitro in the zone of Ag excess and injected intravenously, are pyrogenic and mobilize detectable amounts of circulating EP. Penicillin anti-

gen-antibody complexes also appear to be pyrogenic, since both antigen and antibody are required to induce fever or to release EP from sensitized blood leukocytes in vitro. The exact steps by which these complexes activate cells to synthesize and release this substance is uncertain, though antigen-antibody complexes are chemotactic (32), rapidly phagocytized (33, 34), and produce degranulation and membrane permeability changes in polymorphonuclear leukocytes in vitro (35).

SUMMARY

Rabbits immunized to benzylpenicillin G responded with fever when challenged with a penicillin-serum protein conjugate, but not with penicillin itself. After one or two challenges with conjugate, the rabbits became unresponsive (tolerant) to further injections. This form of hypersensitivity was transferable with plasma of immunized donors to normal rabbits. Blood leukocytes of immunized rabbits incubated with penicillin-protein conjugate and hypersensitive serum released endogenous pyrogen in vitro. Spleen cells from the same animals, on the other hand, were inactive when incubated with this antigen in vitro. These experiments appear to be the first to demonstrate in vitro a possible mechanism of drug-induced fever.

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