Hemodynamic Changes in Systemic Tuberculin Reaction, Active Anaphylaxis and Shock Caused by Histamine–Serotonin or Endotoxin (36460)

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(Introduced by H. Bloch)
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The immunological nature of systemic tuberculin reactivity in man and in laboratory animals infected with mycobacteria is still a controversial subject. The paucity of information about the pathophysiological mechanisms underlying tuberculin shock prompted us to attempt a more precise definition of the conditions under which systemic sensitivity to tuberculin occurs in mice. In the present communication, the symptomatology and the behavior of some hemodynamic parameters during systemic tuberculin reaction are compared with those of other conditions, such as anaphylactic shock, anaphylaxis-like reactions following a histamine–serotonin injection (1) and endotoxin shock in normal or BCG-pretreated mice.

Materials and Methods. Mice. Six- to 8-week old colony-bred male Swiss albino mice (CD-1, Charles River France S.A.) weighing 20 to 22 g were kept on commercial feed and water *ad libitum*.

Mycobacteria. Mycobacterium bovis strain BCG (BCG, Phipps strain, obtained through the courtesy of Dr. J. Nuesch, Ciba-Geigy) was grown in a modified Tween–albumin medium according to the method described by Bloch and Nuesch (2). One milligram (wet wt) of mycobacteria contained 0.96 to 1.20×10^8 viable units.

Induction and elicitation of systemic reactivity to tuberculin. Mice received two injections of 0.5 ml BCG washed and suspended in Tween–albumin medium (0.1 mg wet wt ip and 0.3 mg iv on days 0 and 14, respectively). Eight to 10 days after the second injection, they were challenged (iv) with 2 mg purified protein derivative [(PPD) State Serum Institute, Copenhagen, Denmark] dissolved in 0.5 ml phosphate buffered saline (0.14 *M* NaCl, 0.01 *M* $NaH_2PO_4 \cdot H_2O$, adjusted to pH 7.2 with 10 *M* NaOH) (PBS). This amount of PPD had no effect when injected into normal mice.

Anaphylactic shock. Mice were sensitized with 0.1 mg human gamma globulin (Laboratories of the Swiss Red Cross, Berne) (HGG) incorporated into complete Freund's adjuvant and challenged with 2 mg HGG (iv) 20 to 30 days thereafter (1).

Anaphylaxis-like reactions were produced by simultaneous administration (iv) of histamine and serotonin (80 and 60 mg/kg, respectively) as described elsewhere (1).

Systemic reactivity to endotoxin. Lipopolysaccharide from *Escherichia coli* 0111: B_4 (Difco) was dissolved in PBS and administered (iv) either to normal mice or to mice which had been pretreated twice with BCG as described above. The amount of endotoxin given was 80 or 0.01 mg/kg, respectively, and corresponded in either case to an $LD_{80\text{-}100}$.

Blood pressure in nonanesthetized mice. Cannulas were implanted in the carotid artery under pentobarbital anesthesia 1 to 3 days before challenge. The cannulas consisted of heat-sealed sections of PE 10 and PE 50 polyethylene tubing (i.d. 0.28 and 0.58 mm, respectively), formed into a "U" bend and filled with saline. The thin section was trimmed to the appropriate length and inserted into the left carotid artery, which was ligated over it. The thicker section was led subcutaneously to the nape of the neck, exteriorized and closed with a metal stopper. On the day of challenge the cannulas were flushed with saline, filled with heparin solu-

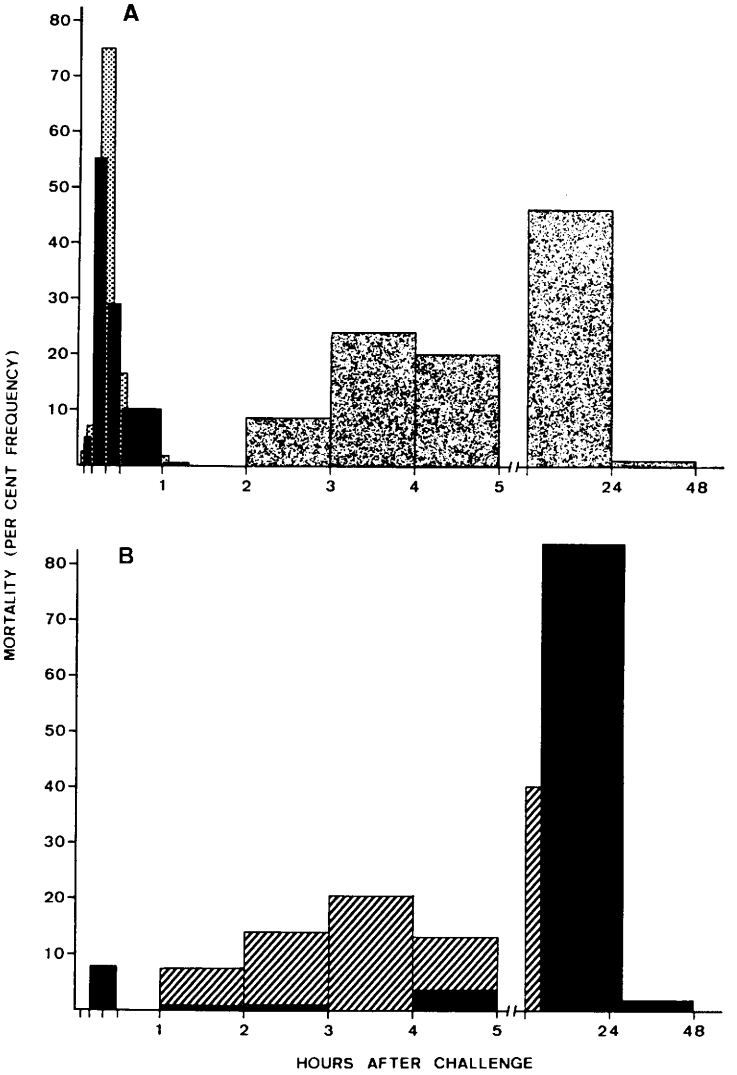

FIG. 1. The occurrence of lethal shock as a function of time after challenge: (A) ■ anaphylactic shock; ▩ histamine–serotonin shock; ▤ PPD shock; (B) ■ endotoxin shock in normal mice; ▨ endotoxin shock in BCG-pretreated mice. The various histograms comprise data obtained from 150 to more than 1500 mice/type of shock.

tion and connected to a transducer with low volume displacement (Beckman 8503/4M8). Blood pressure in the lightly restrained, nonanesthetized mouse was recorded continuously with a Sanborn oscillograph from 30 min before until 6 hr after the challenge injection. Control mice were injected with saline, and blood pressure was measured in the same manner.

Blood volume. ^{125}I- or ^{131}I-labeled human serum albumin (Swiss Institute for Reactor Research, Würenlingen) (I*-HSA, 0.1 ml, 1 μg, approx 1 μCi) was injected iv. Samples of 0.2 to 0.25 ml of blood were collected by retro-orbital or cardiac puncture 1 to 2 min after the injection of the tracer. The total volume of circulating blood was estimated from the radioactivity obtained in the blood samples and the total amount of labeled protein injected.

Hematocrit. Blood was collected in heparinized capillary tubes by retro-orbital punc-

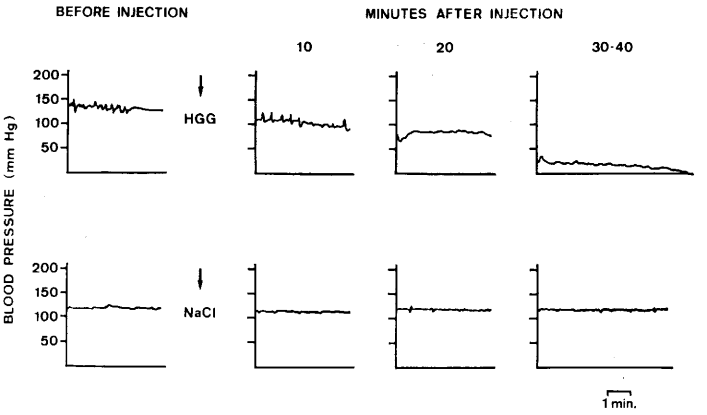

FIG. 2. Representative example of blood pressure in the nonanesthetized mouse. (upper panel) Lethal anaphylactic shock following injection of human gamma globulin. Similar results were obtained in 3 further mice; (lower panel) control mouse after injection of an equivalent volume of saline.

ture and the packed cell volume was determined using a Clay Adams Autocrit centrifuge.

Results. 1. Symptomatology. Lethal anaphylaxis and anphylaxis-like reactions following the injection of histamine and serotonin were symptomatologically similar. As is shown in Fig. 1A, they occurred as early as 5 min after the challenge, but were most commonly seen between 10 and 30 min and only exceptionally more than 1 hr thereafter. Both types of shock were characterized by a sluggish, wobbling gait at the onset. The mice huddled in the corners of the cage and exhibited cyanosis and pilo-erection. They only moved when disturbed and eventually became paretic. At irregular intervals, convulsions occurred.

In contrast, PPD shock had a delayed onset. Systemic tuberculin reactions were seen as early as 2 hr after challenge; approximately 50% of the mice were dead within 5 hr and the remainder within 24 hr. Occasionally, a mouse survived for more than 24 hr. The symptomatology is best described as extreme hyperreactivity or a general convulsive condition: the mice jumped, flicked their tails and rolled about on the floor of the cage. In addition, paroxysmal exophthalmus and ocular vasospasms were frequently observed. Death generally ensued within a few minutes of the onset of severe symptoms. The time of onset of endotoxin shock is shown in Fig. 1B. The administration of lipopolysaccharide to normal mice clearly triggered biphasic events. Shortly after the injection, 20 to 30% of the mice were severely shocked, and displayed symptoms closely resembling those seen in anaphylaxis. However, most of the animals quickly recovered and remained symptomless until 12 to 26 hr after the injection. Then signs of shock suddenly reappeared which also resembled those of anaphylactic shock. On the other hand, endotoxin injected into mice pretreated twice with BCG elicited the same symptoms (and the same shock timing) as PPD. Death occurred 1 to 24 hr after challenge.

2. Blood pressure in nonanesthetized mice. In anaphylactic shock, the challenging injection was followed immediately by a gradual decrease of blood pressure. The fall in pressure progressed steadily until the time of death (Fig. 2). In contrast to this immediate but gradual response in anaphylactic shock, mice in PPD-induced shock maintained normal blood-pressure levels for a period of several hours, until a few minutes before death. Occasionally, death was preceded by a rise in blood pressure, associated with an increase in motor activity (Fig. 3). The same pattern was observed in all animals tested, irrespective of the time of death. Blood pressure in control mice remained reasonably

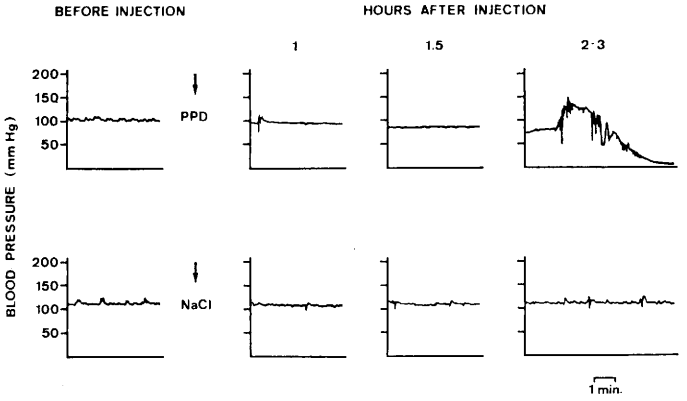

FIG. 3. Representative example of blood pressure in the nonanesthetized mouse. (upper panel) Lethal delayed shock reaction following injection of PPD. Similar results were obtained in 6 further mice; (lower panel) control mouse after injection of an equivalent volume of saline.

stable for periods of up to 6 hr (Figs. 2, 3). In one group of four control mice, blood pressure decreased by 6 ± 7 mm Hg within 2 hr and by 18 ± 10 mm Hg within 5 hr (mean ± SE). Tuberculous (*i.e.*, BCG-pretreated) mice survived, for unknown reasons, a challenge injection of endotoxin for 16 to 20 hr, during which time the blood pressure did not change.

3. Hematocrit and blood volume. As is shown in Fig. 4, hematocrit readings were greatly increased in anaphylaxis and in anaphylaxis-like histamine–serotonin reactivity. BCG-pretreated mice displayed somewhat reduced hematocrit values, which were barely changed in systemic reactions following an intravenous challenge with PPD. Correspondingly, blood volume was greatly diminished in anaphylaxis and histamine–serotonin shock, whereas in the systemic tuberculin reaction it was not changed (Fig. 5). In the case of endotoxin shock in normal mice, the biphasic reaction is also reflected by the hematocrit readings shown in Fig. 6. In the transitory, low-mortality-shock phase shortly after injection, as well as in the late lethal phase, the hematocrits were almost as high as in anaphylaxis or in histamine–serotonin shock. In the intermediary phase, in which the animals showed no signs of shock, the hematocrits were comparable to those of the controls. On the other hand, endotoxin injected into BCG-pretreated mice did not cause any change in the hematocrits. Again, the blood volumes mirrored the hematocrit results (Fig. 5).

Discussion. Systemic tuberculin reaction, commonly known as tuberculin shock, was first described some 80 years ago (3) and has since been observed repeatedly in different animal species and under various experimental conditions (4–10). Generalized tuberculin reactions have also been reported to occur in man, both in patients receiving the now obsolete tuberculin therapy and in highly sensitized laboratory personnel after accidental inhalation of volatile mycobacterial components (11–13).

Delayed tuberculin shock is held by several investigators to be a manifestation of delayed-type hypersensitivity. This view is based on the claim that systemic tuberculin reactivity can be passively transferred by mononuclear cells (14–17). In addition, reactions similar to tuberculin shock have been observed in states of delayed hypersensitivity to a variety of infectious agents (14).

On the other hand, a close relationship between systemic tuberculin reactivity and cellular immunity is not consonant with the finding that shock reactions cannot be elicited in animals exhibiting delayed hypersensitivity to heterologous proteins (18). Moreover, it has been noted that there are considerable discrepancies between the degree of delayed skin reactivity to PPD and the occurrence of fatal shock reactions [cf. (14)]. In this context, the fact that tuberculous ani-

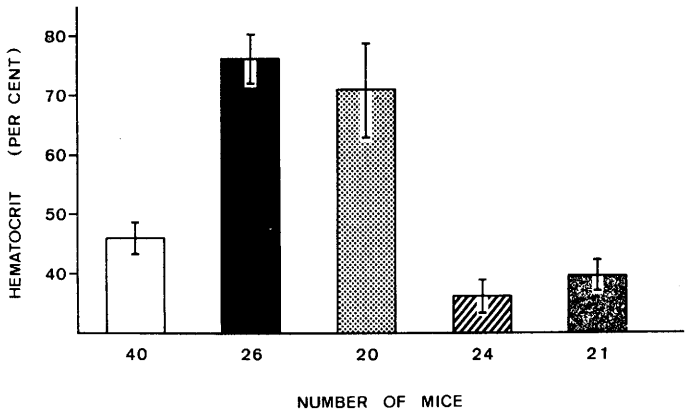

FIG. 4. Hematocrit: ☐ normal mice; ■ anaphylactic shock; ▩ histamine-serotonin shock; ▨ BCG-pretreated mice (without challenge with PPD); ▤ PPD shock. Vertical bars denote the standard deviations. Mice in the different experimental groups were bled shortly before death.

mals display greatly reduced resistance to bacterial lipopolysaccharides (19, 20), together with the fact that tuberculin is frequently contaminated by gram-negative bacterial endotoxins (21), would appear to lend weight to the view that systemic tuberculin reactivity may be a fundamentally nonimmunological phenomenon. In the present study, a striking similarity was found between the reaction of tuberculous mice to a challenge injection of PPD and their reaction to endotoxin. Both conditions were characterized by extreme motor hyperreactivity and general convulsions. The mice did not show any signs of cardiovascular failure. There was no change in the hematocrit or in blood volume, and in the case of PPD shock, the blood pressure remained unaltered until a few minutes before death. Thus, the results presented suggest that a nonimmunological mechanism may underlie systemic tuberculin reactivity.

Systemic reactivity to PPD or endotoxin in tuberculous mice stands in sharp contrast to anaphylaxis, to the anaphylaxis-like reaction

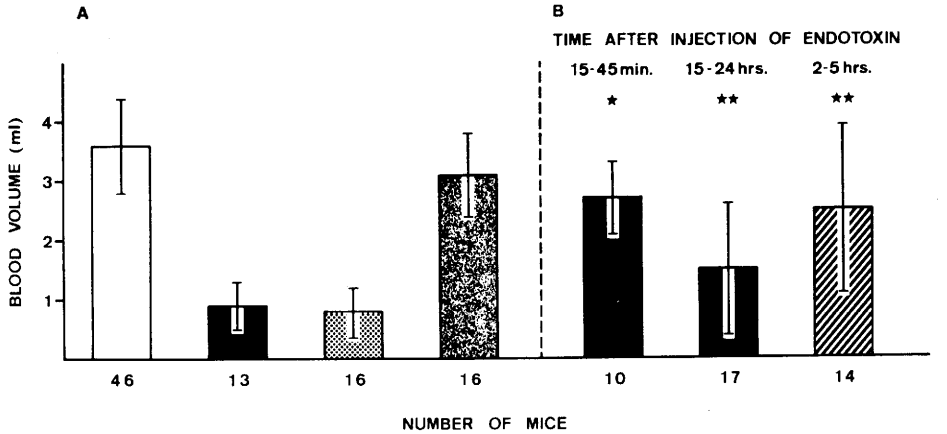

FIG. 5. Blood volume: (A) ☐ normal mice; ■ anaphylactic shock; ▩ histamine-serotonin shock; ▤ PPD-shock; Determinations in the different experimental groups were made shortly before death. (B) ■ endotoxin shock in normal, nontuberculous mice; ▨ endotoxin shock in BCG-pretreated mice; * transitory shock with low mortality; ** severe shock without recovery. Vertical bars in both panels denote the standard deviations.

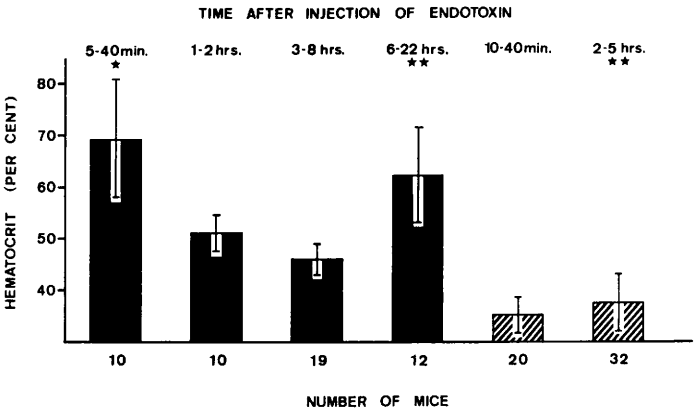

FIG. 6. Hematocrit: ■ endotoxin injected into normal, nontuberculous mice; ▨ endotoxin injected into mice pretreated with BCG; * transitory shock with low mortality; ** severe shock without recovery. For normal controls see Fig. 4. Vertical bars denote the standard deviations.

following histamine–serotonin and to endotoxin shock in normal mice. The symptomatology, the time of occurrence after challenge or after injection, as well as cardiovascular parameters clearly separate the reactions studied into different groups.

The identity of the reactions seen in anaphylaxis and in anaphylaxis-like histamine–serotonin shock further corroborate the view that murine anaphylaxis is mediated by a combination of these agents (1, 22). Moreover, the results presented confirm earlier observations of quantitative differences in the endotoxin reactivity of normal and tuberculous mice (23) and clearly demonstrate a qualitative dissimilarity.

Summary. Systemic hypersensitivity to tuberculin in mice is compared with anaphylaxis and shock reactions following the injection of histamine–serotonin or endotoxin. Time of occurrence, symptomatology and cardiovascular parameters demonstrate a striking similarity between tuberculin shock and reactivity to bacterial endotoxin in tuberculous mice and clearly differentiate these reactions from anaphylaxis and from shock caused by histamine–serotonin or by endotoxin administered to nontuberculous mice. The findings are compatible with the view that nonimmunological mechanisms may underlie the pathogenesis of systemic tuberculin reactivity. A greatly increased susceptibility to bacterial lipopolysaccharides in mycobacterial infections and the fact that endotoxins are common contaminants of tuberculin preparations may play a decisive role.

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