THE ROLE OF THE CARRIER STATE AND HYPERSENSITIVITY IN EXPERIMENTAL STAPHYLOCOCCAL INFECTIONS IN MICE

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THE development of delayed hypersensitivity in rabbits as a result of a repeated skin infection with *Staphylococcus aureus* was demonstrated by Panton and Valentine (1929) and Johnson, Cluff and Goshi (1961). In our previous paper (Modak, Banerjee and Basu Mallik, 1971) we demonstrated sensitisation in mice experimentally infected with staphylococci. Staphylococcal infection is more common among human carriers of *Staph. aureus* than in non-carriers, but very little is known regarding the role that hypersensitivity may play in association with the carrier state in the development of a staphylococcal infection. In our present studies, susceptibility to staphylococcal infection among mice that were carriers and non-carriers, with or without associated hypersensitivity, was determined by challenging with standard trauma or a pyrogenic stimulus or both, and we attempt to assess the role of these two important factors in the dissemination and localisation of staphylococci.

MATERIALS AND METHODS

Animals. A strain of albino Swiss mice has been maintained in our laboratory for the last 12 yr. Adult mice of 22–24 g were used. Inoculation of *Staphylococcus aureus* in the nose of mice by a multiple puncture method (Modak *et al.*) produced four categories of animals: (i) carrier hypersensitive, (ii) carrier non-hypersensitive, (iii) non-carrier hypersensitive, and (iv) non-carrier non-hypersensitive. Groups of mice belonging to these various categories were subjected to standard trauma or pyrogen injection singly or in combination. Blood and bone samples of the animals were examined at various intervals by bacteriological culture procedures.

Infliction of trauma. Mice were anaesthetised with ether. The shaved right hind leg was treated with tincture of iodine and thereafter with alcohol; the area was then wrapped with sterile gauze to prevent extraneous contamination and to avoid direct injury to the skin. Trauma was applied to the middle portion of the shin bone by dropping a round-bottomed metal cylinder from a measured height. The weight was suspended by means of a pulley system and was allowed to pass through a metal tube in order to prevent any aberrant movement (fig. 1).

In preliminary experiments, different weights were released from different heights to determine the impact that would break the mouse bone with minimal crushing and soft-tissue injury and without macroscopic breach of the skin surface. The optimal weight was found to be 50 g, and the optimal height 30 cm. These two factors were then kept constant.

Induction of fever. The normal mouse rectal temperature was found to range between 99°F and 99.4°F. Modified Haffkine plague vaccine was used as a pyrogenic agent. Preliminary experiments showed that doses greater than 0.1 ml produced hypothermia, hence

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FIG. 1.—Diagram of the instrument for administration of trauma.

FIG. 2.—Record of rectal temperature of mice at different periods after administration of modified Haffkine plague vaccine.

0.1 ml of the vaccine was selected as the standard dose given intraperitoneally with aseptic precautions. This dose produced a maximum temperature 20 min. after the injection that remained steady for a further period of up to 30 min. and then declined gradually (fig. 2).

TABLE I
Results of blood and bone culture at different intervals from various categories of mice receiving trauma, or pyrogen, or both

Mouse group	Category of mice*	Number tested	Nature of provocation	Material cultured	Number of mice, per group of three examined, showing positive result at the stated time (hr) after provocation							
					2	4	8	16	24	48	72	96
A	Carrier hyper-sensitive (92)	24	pyrogen trauma	blood	0	0	0	0	0	1	1	0
		24		blood	0	0	0	0	0	1	2	0
				bone	0	0	0	0	0	1	0	1
		24	pyrogen and trauma control (unprovoked)	blood	0	0	0	0	1	1	1	0
				bone	0	0	0	0	0	1	1	1
		20		blood	0	0	0	0	0	0	0	0
				bone	0	0	0	0	0	0	0	0
B	Carrier non-hyper-sensitive (51)	24	pyrogen trauma	blood	0	0	0	0	0	0	0	0
		24		blood	0	0	0	0	0	0	0	0
				bone	0	0	0	0	0	0	0	0
		3	control	blood	0	0	0	0	0	0	0	0
				bone	0	0	0	0	0	0	0	0
C	Non-carrier hyper-sensitive (52)	24	pyrogen trauma	blood	0	0	0	0	0	0	0	0
		24		blood	0	0	0	0	0	0	0	0
				bone	0	0	0	0	0	0	0	0
		4	control	blood	0	0	0	0	0	0	0	0
				bone	0	0	0	0	0	0	0	0
D	Non-carrier non-hypersensitive (85)	24	pyrogen trauma	blood	0	0	0	0	0	0	0	0
		24		blood	0	0	0	0	0	0	0	0
				bone	0	0	0	0	0	0	0	0
		24	pyrogen and trauma control	blood	0	0	0	0	0	0	0	0
				bone	0	0	0	0	0	0	0	0
		13		blood	0	0	0	0	0	0	0	0
				bone	0	0	0	0	0	0	0	0

* Total numbers indicated in brackets.

Cultural methods. *Blood culture* was done by collecting about 0.5 ml of heart blood at open operation and inoculating it into tubes containing 5 ml of nutrient broth. The tubes were incubated at 37°C and examined daily. After 4 days, or whenever turbidity indicated that there was growth, subcultures were made on blood agar and the organisms were identified. For *bone culture*, the desired portion was removed aseptically and ground up in a pestle and mortar with powdered glass and suspended in physiological saline with strict aseptic precautions. Several loopfuls of the homogenate were plated out on blood agar medium and the remainder was inoculated into tubes of nutrient broth. When there was growth, the organisms were identified.

RESULTS

Dissemination of organisms in the blood and their localisation at the injured site at various intervals in different categories of mice provoked by trauma, or pyrogen or both

A total of 280 mice were allocated to one of four categories (A–D) with regard to their carrier-state and sensitivity as follows: group A were carrier hypersensitive mice; group B were carrier non-hypersensitives; group C were non-carrier hypersensitives; and group D were non-carrier non-hypersensitives. Each group was divided into controls, animals subjected to trauma, animals given a pyrogenic injection, and those subjected to both injurious stimuli (table I).

TABLE II
Effect of controlled trauma on dissemination of staphylococci in carrier mice

Time after trauma (hr)	Result of culture of the blood or bone in mice that were					
	hypersensitive			non-hypersensitive		
	Number tested	Number with blood culture positive	Number with bone culture positive	Number tested	Number with blood culture positive	Number with bone culture positive
24	15	3	0	10	0	0
48	15	3	3	10	0	0
72	15	4	4	10	0	0
Totals	45	10	7	30	0	0

Control animals were killed at convenient times; animals subjected to injurious stimuli were killed in groups of three at different times after the stimuli; blood and bone cultures were carried out on all animals killed.

Results of culture of blood and bone samples showed that only in the carrier hypersensitive mice did the organism invade the bloodstream and localise at the site of trauma (table I). The optimum time for dissemination was found to be between 24 and 72 hr.

Since the test organism could be detected in the bloodstream within the period of 24–72 hr after the provocation but not before or after that period, it was decided to carry out cultural examinations at intervals of only 24, 48 and 72 hr in the subsequent experiments.

Effect of trauma on the dissemination of organisms in the blood and their localisation at the injured area in carrier mice

A group of 75 carrier mice, 45 hypersensitive and 30 non-hypersensitive, were subjected to standard trauma. Batches of 15 from the former subgroup and ten from the latter were killed at 24, 48 and 72 hr and blood and bone samples from these animals were cultured.

In the hypersensitive group, at 24 hr after traumatic injury (table II) bacteraemia was detected in three of 15 mice tested, but localisation of the cocci at the traumatised site was not detectable. At 48 hr, blood culture was positive in three of 15 mice tested and bone culture was also positive in an equal number of animals, two mice yielding positive results in both respects. At 72 hr, bacteraemia was observed in four of 15 mice. Two of these animals and two others also showed positive bone culture (fig. 3A). In the non-hypersensitive group, provocation by trauma uniformly failed to produce detectable bacteraemia and cultures of the damaged bones were also uniformly negative.

FIG. 3.—Positive blood or bone cultures, or both, per group of 15 carrier hypersensitive mice at different intervals: (A) after traumatic injury; (B) after combined trauma and pyrogen injection. Each square represents one mouse giving a positive or combined positive result at the stated time.

Effect of pyrogen on the dissemination of organisms in the blood in carrier mice

Seventy-five carrier mice, 45 hypersensitive and 30 non-hypersensitive, were given a dose of 0.1 ml of modified Haffkine plague vaccine intraperitoneally. At 24, 48 and 72 hr after the provocation, batches of 15 from the hypersensitive group and ten from the non-hypersensitive group were killed and blood samples were cultured.

Of 45 hypersensitive carrier mice tested in this way, 14 showed positive blood cultures, five each at 24 and 48 hr, and four at 72 hr after injection of the pyrogen (table III). Among the 30 non-hypersensitive carrier mice, bacteraemia was detectable in only three, and this was evident at 24 hr.

Effect of combined trauma and pyrogen on the dissemination of organisms in the blood and their localisation at the injured area in carrier mice

A group of 45 hypersensitive and another group of 30 non-hypersensitive mice, all nasal carriers of the test staphylococci, were given the standard trauma followed by intraperitoneal injection of 0.1 ml of modified Haffkine plague vaccine. Batches of 15 animals from the first group and ten from the second were killed at 24, 48 and 72 hr and blood and bone samples were cultured.

Twenty-four (53·3 per cent.) of the 45 carrier hypersensitive mice tested showed positive blood culture (table IV). At 24 hr, eight (53·3 per cent.) of

TABLE III
Effect of induced pyrexia on dissemination of staphylococci in carrier mice

Time after injection of pyrogen (hr)	Result of blood culture of mice in the stated category:			
	Hypersensitive group		Non-hypersensitive group	
	Number of animals tested	Number giving positive blood culture	Number of animals tested	Number giving positive blood culture
24	15	5	10	3
48	15	5	10	0
72	15	4	10	0
Totals	45	14	30	3

TABLE IV
Combined effect of trauma and induced pyrexia on dissemination of staphylococci in carrier mice

Time after provocation (hr)	Result of culture of blood or bone of mice in the stated category:					
	Hypersensitive group			Non-hypersensitive group		
	Number of animals tested	Number giving positive blood culture	Number giving positive bone culture	Number of animals tested	Number giving positive blood culture	Number giving positive bone culture
24	15	8	7	10	0	0
48	15	9	9	10	0	0
72	15	7	6	10	0	0
Totals	45	24	22	30	0	0

15 such animals had bacteraemia; five of these mice and two others showed evidence of localisation of the inoculum strain at the traumatised part of the bone (fig. 3B). At 48 hr, bacteraemia was demonstrable in nine mice and an equal number of animals showed positive bone cultures, six being positive in both respects. At 72 hr, blood culture was positive in seven (46·6 per cent.) and bone culture was positive in six (40 per cent.) in the 15 animals tested. Positive blood and bone cultures were obtained in three animals in this "carrier-hypersensitive" group. Bacteraemia or localisation of organisms at the site

of trauma could not be demonstrated in any of the non-hypersensitive group of carrier mice that received combined trauma and pyrogen.

Effect of combined trauma and pyrogen on the dissemination of organisms in the blood and their localisation at the injured area in non-carrier mice

A group of 75 non-carrier mice, 30 hypersensitive and 45 non-hypersensitive, received standard trauma followed by intraperitoneal injection of modified Haffkine plague vaccine. Batches of ten from the former and 15 from the latter subgroup were killed at 24, 48 and 72 hr. Blood and bone samples from these animals were cultured and the results are given in table V.

TABLE V
Combined effect of trauma and induced pyrexia on dissemination of staphylococci in non-carrier mice

Time after provocation (hr)	Result of culture of blood or bone of mice in the stated category:					
	Hypersensitive group			Non-hypersensitive group		
	Number of animals tested	Number with positive blood culture	Number with positive bone culture	Number of animals tested	Number with positive blood culture	Number with positive bone culture
24	10	1	0	15	0	0
48	10	1	1*	15	0	0
72	10	0	1	15	0	0
Totals	30	2	2	45	0	0

* The same animal gave a positive blood culture.

Blood and bone cultures of all of the 45 non-carrier non-hypersensitive mice killed at various intervals were negative.

In the group of 30 non-carrier hypersensitive mice, one of ten killed at 24 hr had bacteraemia in the absence of any evidence of localisation of the organism in the bone. At 48 hr, one gave a positive blood culture, and organisms could be recovered from the bone of this animal. At 72 hr, bacteraemia could not be detected in any of the animals but one gave a positive bone culture.

The results of the different experiments are summarised in table VI.

DISCUSSION

Nasal carriage of *Staphylococcus aureus* can be established in Swiss mice by a multiple puncture method (Modak *et al.*, 1971). Many of the carrier mice as well as some of those that failed to carry the organism at the site of inoculation for more than 3 wk developed a delayed type of hypersensitivity to staphylococcal antigen, detectable by intradermal injection of the antigen. Thus, four

TABLE VI
Effect of trauma, induced pyrexia, and combined trauma and induced pyrexia on the dissemination and localisation of staphylococcus in different categories of mice

Category of mice	Material cultured	Mice subjected to						Total	
		trauma		pyrogen injection		trauma and pyrogen			
		Number of animals examined	Percentage giving positive culture	Number of animals examined	Percentage giving positive culture	Number of animals examined	Percentage giving positive culture	Number of animals examined	Percentage giving positive culture
Carrier hypersensitive	blood bone	69 69	18.8 13.0	69 ...	23.1 ...	69 69	39.1 36.2	207 138	27.0 24.7
Carrier non-hypersensitive	blood bone	54 54	0 0	54 ...	5.5 ...	30 30	0 0	138 84	2.1 0
Non-carrier hypersensitive	blood bone	24 24	0 0	24 ...	0 ...	30 30	6.6 6.6	78 54	2.5 3.7
Non-carrier non-hyper-sensitive	blood bone	24 24	0 0	24 ...	0 ...	69 69	0 0	117 93	0 0

categories of mice were obtained, viz., carrier hypersensitive, carrier non-hypersensitive, non-carrier hypersensitive and non-carrier non-hypersensitive.

In the present study, the relative susceptibility of each of these four categories of mice to staphylococcal infection was assessed by studying the dissemination of organisms from the site of carriage into the bloodstream and their localisation at devitalised parts after standard provocative trauma or elevation of body temperature by the injection of modified Haffkine plague vaccine or both.

In the preliminary experiment to determine the optimal time for undertaking cultural examinations of blood and bone samples, it was found that both pyrogen and trauma administered separately or in combination were capable of producing bacteraemia in carrier hypersensitive mice only. The earliest positive blood culture was obtained at 24 hr in one animal receiving both trauma and pyrogen. The bacteraemia was transient and blood became sterile within 96 hr. Organisms could be recovered from bone samples not earlier than 48 hr and they then persisted for the period of observation of 96 hr.

As non-carrier mice were not demonstrably harbouring *Staph. aureus* at the time of experiment, the question of dissemination and localisation of organisms did not seem to arise with these mice. Hence it was surprising that organisms could be isolated from the blood of two (1 per cent.) of a group of 195 non-carrier mice tested. This suggests that some of the animals designated as non-carriers after repeated examination of nasal swabs were harbouring *Staph. aureus*. It may also be significant that both of these two mice were hypersensitive to staphylococcal antigen and were provoked by a combination of trauma and pyrogen.

Among the carrier mice, 27 per cent. of the hypersensitive animals (compared with 2.1 per cent. of the non-hypersensitive mice) showed positive blood culture. Our isolation of the test staphylococcus from the blood of three non-hypersensitive carrier mice after provocation indicates that hypersensitivity is not the only determining factor. It may be that the reactivity of these animals to staphylococcal antigen was different from that of normal mice although the difference is not detected by the skin test used. However, there is no doubt that the carrier state alone is insufficient to bring about an infective process and that the state of hypersensitivity is perhaps the most potent single factor that we have found to be associated with dissemination of infection in our studies.

It is also obvious that carriers continue to harbour organisms that remain localised at a particular site until the equilibrium is disturbed by some exciting factor or factors. There was ample evidence that, in carrier hypersensitive mice, organisms entered the bloodstream after inoculation of the pyrogen. Application of trauma also produced bacteraemia. How fever helps entry of organisms into the blood circulation is not clear. Probably peripheral hyperaemia produced during pyrexia is responsible for the process. It is well known that challenge with endotoxic material, such as the plague vaccine that we used, influences various types of body reactions including alteration of reticulo-endothelial activity. The possible significance of such an associated effect of the pyrogen in our studies should be considered. However, both the hypersensitive and non-hypersensitive groups of our animals were treated in identical

fashion in the present study. The role of trauma in the induction of bacteraemia is also obscure. Our records of the body temperature of animals that received trauma did not show any rise in temperature following application of the trauma; this indicates that the mechanism of induction of bacteraemia is not simply attributable to a related pyrogenic effect.

There are, however, many clinical illustrations of an association between trauma and osteomyelitis (Wiley and Trueta, 1959; Garcia and Grantham, 1960; Simon, 1960; Beddow and Weisl, 1961; Milone, Bianco and Ivins, 1962). It seems a reasonable hypothesis that patients developing staphylococcal osteomyelitis after trauma were probably carrying the organisms prior to the clinical manifestations of infection, and that there may have been at least a transient bacteraemia with localisation of the staphylococcus at the site of injury. It would be of great interest if the state of hypersensitivity of these patients were known, as the present experimental model in mice indicates that localisation of organisms at the site of trauma occurs only in hypersensitive animals. In the present series, positive evidence of bacteraemia could be obtained in 64.4 per cent. of the mice that gave a positive bone culture, but blood culture was negative in 14 (35.6 per cent.) of 36 mice yielding *Staph. aureus* from the bone. It is probable that a very transient bacteraemia occurred and that this had subsided when the blood samples were collected for examination. The other possibility is that our procedure could not detect very small numbers of circulating organisms in the blood.

Our present evidence seems to support the view that hypersensitivity to staphylococci associated with the nasal carrier state significantly increases the susceptibility of the animals to dissemination of staphylococci with resultant staphylococcal infection of damaged bone.

Further studies are now required to elucidate whether factors such as transient suppression of reticulo-endothelial activity by injected pyrogen or by induced shock may also be of significance in this model.

SUMMARY

Dissemination of *Staphylococcus aureus* from the site of carriage into the bloodstream and its localisation at a traumatised portion of a bone were studied in four categories of mice, viz., carrier hypersensitive, carrier non-hypersensitive, non-carrier hypersensitive, and non-carrier non-hypersensitive. The provocations used were a pyrogenic stimulus, produced by intraperitoneal injection of 0.1 ml of modified Haffkine plague vaccine, and controlled trauma to the right hind leg, either alone or in combination. It was observed that transient bacteraemia occurred during periods of induced fever or as a sequel to trauma. It is of interest that 27 per cent. of the hypersensitive carrier mice as opposed to 2 per cent. of the non-hypersensitive carriers showed positive blood culture. Among the non-carrier mice, positive blood culture was obtained from 2.5 per cent. of the hypersensitive animals only. Significantly, localisation of *Staph. aureus* at the injured bone was detectable in hypersensitive mice only.

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