Effect of BCG Infection on Sensitivity of Mice to Histamine, Serotonin and Passively Induced Anaphylaxis.* (28628)

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Hyperreactivity to endotoxin readily develops in mice infected with BCG(1). Experimental analysis indicated that criteria distinctive for hypersensitivity reactions of the delayed type were not applicable to this phenomenon, and it was concluded that delayed type hypersensitivity could not be made responsible for the change of sensitivity to endotoxin as observed in numerous experimental infections. This conclusion was based on the relative code of specificity and observations that transfer of hyperreactivity from a sensitive donor to a sensitive recipient could not be accomplished, and that reactions characteristic for delayed hypersensitivity such as fever, skin response, and weight loss were not exaggerated in hyperreactive animals(2). The high degree of sensitivity to endotoxin of mice infected with BCG is comparable to that reported in mice injected with *Bordetella pertussis* prior to administration of endotoxin (3). In addition, mice prepared by intraperitoneal administration of pertussis vaccine are highly sensitive to active and passive anaphylaxis, to histamine, serotonin and the various other stresses(4). The mechanism or mechanisms which mediate these reactions are unknown. It was therefore of interest to explore whether infection with BCG would be as effective as is pertussis vaccine in inducing sensitivity to anaphylaxis, to histamine and to serotonin. The effect of heparin upon hyperreactivity to endotoxin was also studied to obtain information as to the similarity of this phenomenon to the generalized Shwartzman reaction which is known to be suppressed in the rabbit by administration of heparin(5).

Material and methods. Mice and BCG infection. Swiss albino mice of the CFW strain raised at the Rocky Mountain Laboratory and of the ICR strain supplied by a dealer to University of Florida were used. Groups of 10 animals were kept in stainless steel cages and fed pellets and water *ad libitum*. Hyperreactive state to endotoxin in mice was induced by inoculating them intravenously with 0.1 or 0.2 ml of an undiluted 6-10 day old culture of BCG grown in a liquid medium containing "Tween 80" and albumin. The animals were used 10 days after injection of BCG.

Passive anaphylaxis. The antibody used for sensitization was a pool of mouse peritoneal fluid prepared as previously described (6). It contained 1800 μg anti-egg albumin N per ml. The antigen employed was 2x recrystalized egg albumin (purchased from Nutritional Biochemicals Corp., Cleveland, Ohio). When passively sensitized mice were challenged with 0.5 mg of egg albumin 4-5 hours after i.p. injection of mouse anti-egg albumin antibody the 50% sensitizing dose (SD_{50}) was found to be 26 μg of antibody N(7). Consequently, mice were sensitized by intraperitoneal injection of either 20 or 40 μg antibody N in 0.2 ml physiological saline, and 4-5 hours later challenged i.v. with 0.5 mg egg albumin in 0.2 ml of physiological saline.

Histamine, serotonin and heparin. Histamine diphosphate and heparin were purchased from Fisher Scientific Co. and 5-hydroxy tryptamine creatinine sulfate (serotonin creatinine sulfate) from Nutritional Biochemicals Labs. Appropriate solutions were prepared in physiologic saline. Serotonin and histamine were administered intraperitoneally. BCG infected mice received 1 or 2 injections of 1000 μg of heparin in a volume of 0.2 ml. The first injection was given 20 minutes prior to challenge with endotoxin and the second, if given, 1 hour after.

Endotoxin. Endotoxin prepared from *Salmonella enteritidis* by aqueous ether ex-

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TABLE I. Mortality from Passive Anaphylaxis in Normal and BCG Infected Mice.

Ab N per mouse (μg)	CFW strain				ICR strain			
	Normal		BCG		Normal		BCG	
	D/T*	%	D/T	%	D/T	%	D/T	%
20	11/15	73	1/15	7	14/20	70	6/20	30
40	10/15	67	4/15	27	6/15	40	2/15	13
Total	21/30	70	5/30	17	20/35	57	8/35	29

* D/T = No. deaths/No. tested.

traction procedure(8) was kindly supplied by Dr. E. Ribi.

Results. Passive anaphylaxis. The results recorded in Table I show that BCG infected animals proved more resistant to passive anaphylaxis than were uninfected animals. The experiments done in the two laboratories with different strains of mice agree well. Desensitization with endotoxin of BCG infected mice had no effect on their response to anaphylaxis.

Sensitivity to histamine and serotonin. As is true for many mouse strains, the ICR strain of mice proved to be very resistant to histamine and serotonin and large quantities had to be injected to cause death. BCG infected and control mice were equally resistant to histamine or serotonin given intraperitoneally (Table II).

Effect of heparin upon hyperreactivity to endotoxin. Heparin injected either before or before and after challenge with endotoxin had no influence on the incidence of deaths due to endotoxin, in spite of the fact that the animals exhibited the anticoagulant effect of the heparin, as evidenced by prolonged bleeding from the injection site on the tail (Table III).

Discussion. The object of these experiments was to obtain information regarding the similarity between hyperreactivity to endotoxin induced by BCG(1) and pertussis vaccine(4) and its relation to the Shwartzman phenomenon. The results presented indicate clearly that BCG-infected mice do not resemble *B. pertussis*-treated animals with regard to their sensitivity to passive anaphylaxis, to histamine, or to serotonin. The recent suggestion that endotoxin action is mediated by circulating antibody and resembles an anaphylactic reaction(9) does not agree with the experiments on passive anaphylaxis in BCG-infected mice. If hyperreactivity to endotoxin were dependent upon increased sensitivity of BCG-vaccinated mice to antigen-antibody reactions, the threshold of sensitivity to passive anaphylaxis should also be lowered in these animals, this was not observed. In addition, involvement of histamine or serotonin can be excluded as major participants in endotoxin shock in hyperreactive animals.

In spite of the absence of typical pathologic findings, the endotoxin shock of the hyperreactive animals has been compared with the generalized Shwartzman reaction. Intravascular fibrin deposition has been implicated as one of the major factors in the Shwartzman reaction, an occurrence which can be prevented by heparinization of the animal during the provocative injection of endo-

TABLE II. Toxicity of Histamine and Serotonin for Normal and BCG Infected Mice of the ICR Strain.*

Dose (mg)†	Histamine		Serotonin	
	Normal	BCG	Normal	BCG
100	4/5‡	5/5	2/6	6/6
50	3/5	4/5	2/6	3/6
25	0/5	0/5	0/6	0/6
12	0/5	0/5	—	—

* LD_{50} of endotoxin in BCG infected mice was approximately 0.5 μg.

† Histamine diphosphate or serotonin creatinine sulfate.

‡ Deaths/Total.

TABLE III. Lack of Effect of Heparin on Hyperreactivity to Endotoxin of BCG Infected Mice.

Heparin injections		Challenge with endotoxin (μg)		
No.	Relation to endotoxin	1	5	10
0	—	8/10*	7/10	10/10
1	Before	7/10	9/10	10/10
2	Before & after	10/10	10/10	—

* Deaths within 48 hr/Total No. inj.

toxin. Therefore, the lack of effect of this procedure in the experiments presented above suggests that hyperreactivity to endotoxin induced by infection with BCG is a phenomenon which must be considered an entity distinguishable from the Shwartzman reaction. The results presented here and those mentioned in the introduction also indicate that hyperreactivity to endotoxin induced by BCG is not related either to anaphylactic or delayed type of hypersensitivity.

Summary. BCG infection, although previously shown to increase the sensitivity of mice to endotoxin, does not increase the sensitivity of mice to passively induced anaphylaxis to histamine or to serotonin. Heparin, which inhibits the Shwartzman reaction in rabbits, failed to prevent endotoxin hyperreactivity in BCG-inoculated mice.

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Bactericidal Activity of Fixed Phagocytes in Irradiated and Unirradiated Mice Treated with RNA.* (28629)

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Hammond, Anderle and Miller(1) reported the intravenous or intraperitoneal injection of RNA or yeast autolysate into mice before or after irradiation with 475 r reduced the mortality from experimental infection with *Pseudomonas aeruginosa* initiated by intraperitoneal inoculation on the 5th day post-irradiation. The experiments to be reported here were undertaken to determine whether or not the lower mortality among irradiated mice injected with RNA might not be due, at least in part, to an increased ability of phagocytes in liver and spleen to kill ingested bacteria. This explanation seemed feasible since Gordon, Cooper and Miller(2) had shown that irradiated rabbits were able to clear their blood streams of intravenously injected *Klebsiella pneumoniae* equally as well as unirradiated rabbits. In most of the irradiated rabbits, however, the microorganisms reappeared in the blood stream 8 hours later where they multiplied until the animal died. These investigators concluded irradiation had not damaged the ability of the phagocytes to ingest bacteria but had damaged their ability to destroy ingested microorganisms.

Materials and methods. Mice. CF1 females 9-10 weeks old were housed in stainless steel cages, supplied with food and water *ad libitum,* and kept in an air conditioned, humidity controlled room. *Irradiation.* 550 r were delivered by a Picker X-ray machine using 220 kv, 20 ma and no filtration. The target distance was 94.5 cm and the dose was delivered at approximately 15.4 r per minute. The mice were placed in individual compartments of a lucite box which revolved slowly in the field during exposure. *Experimental procedure.* Thirty minutes before irradiation or on 4th day after irradiation 4 mice were injected intraperitoneally with 1.0 ml of a 2% solution of RNA. RNA was dissolved in N/20 NaOH and adjusted to pH 7.2. At the same time 4 irradiated mice were injected intraperitoneally with buffered saline to serve as controls. Unirradiated mice were

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