Anaphylactic Shock in Mouse II. Allergenicity of Water-in-Oil Emulsions With or Without *Mycobacterium.** (25177)

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Our previous reports(1,2) have shown successful production of severe or fatal anaphylaxis in the CFW mouse sensitized with a single dose of bovine albumin incorporated into water-in-oil emulsion containing killed *Mycobacterium butyricum*. This experimental model was employed in studying effects of whole-body x-irradiation(2) on hypersensitive state and response of mice. These earlier studies revealed that anaphylactic sensitivity was lacking in animals challenged intravenously with bovine albumin 1,3,5, or 7 days postsensitization. Challenge of mice 10, 14, 21, or 28 days after sensitization killed 15, 16, 43, and 67% respectively. Pooled antiserum secured from mice 28 days postsensitization conferred passively fatal anaphylactic sensitivity to normal mice; this antiserum contained precipitins for antigens in both bovine albumin and bovine gamma globulin(2,3). This report presents evidence that under certain conditions a water-in-oil emulsion lacking *Mycobacterium butyricum* (incomplete adjuvant) may be as effective in potentiating anaphylactogenicity of bovine albumin in mice as one containing acid-fast bacilli (complete adjuvant). We report determination of minimal challenging dose of bovine albumin in mice sensitized with protein combined with complete adjuvant. Data are included to show duration of anaphylactic sensitivity in mice injected with bovine albumin incorporated into the complete adjuvant.

Materials and methods. CFW female mice between 4 to 6 weeks of age at time of sensitization were employed. The allergen used in sensitizing and challenging procedures was bovine albumin employed similarly in earlier studies(1,2). Bacto-adjuvant, complete (Freund) used in our earlier studies(1,2) and Bacto-adjuvant, incomplete (Freund) were employed in preparing separate water-in-oil emulsions containing bovine albumin. The incomplete adjuvant had the same composition as the complete one except that the former contained no *Mycobacterium butyricum*. Method of preparation of bovine albumin-adjuvant emulsions was described previously(1, 2). Details of sensitizing and challenging procedures have been given(1,2). For sensitization each mouse received subcutaneously 0.2 ml of water-in-oil emulsion containing 0.1 mg of bovine albumin. The challenging dose of bovine albumin, contained in 0.2 ml of 0.85% NaCl solution, was injected rapidly into tail vein. Deaths were recorded at 60 minutes. Measurements of rectal temperature were made immediately before administration of challenging dose and in survivors 30 minutes following injection. Rectal temperatures were measured by thermocouple device as described previously(1,2).

Results. Table I gives results of experiment *to determine minimal challenging dose of bovine albumin* in mice sensitized with 0.1 mg of protein incorporated into *complete* adjuvant. Challenging doses (2.0, 0.2, or 0.1 mg) elicited death or severe signs of anaphylaxis in sensitized mice. Survivors failed to show signs of anaphylaxis when challenged again after 24 hours with bovine albumin. Smaller challenging doses (0.05, 0.02, or 0.01 mg) resulted in lower mortality incidences. Dose of 0.005 mg produced only minimal signs of anaphylaxis. Survivors given challenging doses less than 0.1 mg exhibited marked anaphylactic reactivity when rechallenged 24 hours later with bovine albumin.

Table II includes results of experiment concerning *duration of anaphylactic sensitivity* in mice injected with bovine albumin admixed with *complete* adjuvant. A high level of sensitivity was maintained between 35 and 84

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TABLE I. Determination of a Minimal Challenging Dose of Bovine Albumin for Sensitized Mice.*

Challenging dose, mg bovine albumin†	Challenge: Mortality	Challenge: No. of survivors at 30 min.	Challenge: $\triangle t_{30}$§	Rechallenge†: Mortality	Rechallenge†: No. of survivors at 30 min.	Rechallenge†: $\triangle t_{30}$§
2.0	8/10‡	3	−4.9	0/2	2	.0
.2	"	4	−5.2	"	2	+ .2
.1	8/15	10	−4.0	0/7	7	− .2
.05	3/10	7	−1.3	0/7	7	−3.1
.02	1/15	14	−3.1	5/13	10	−2.8
.01	2/15	14	−2.3	3/13	11	−3.0
.005	0/15	15	−1.2	5/15	12	−3.9

* Each mouse inj. with single sensitizing dose (0.2 ml) of bovine albumin-adjuvant emulsion containing 0.1 mg of bovine albumin.

† Challenging dose contained in 0.2 ml was inj. rapidly into tail vein 28 days following sensitization. Survivors were rechallenged 24 hr later with 0.2 ml of 1% bovine albumin in 0.85% NaCl solution.

‡ Numerator = No. of deaths recorded at 60 min.; denominator = No. of mice challenged.

§ $\triangle t_{30}$ = Mean change in rectal temperature (°C) of mice surviving 30 min. after challenge.

days. Level of sensitivity of mice tested after 84 days was much less than that of those challenged 35 to 84 days postsensitization. Diminished, but definite residuum of anaphylactic sensitivity was present 196 days postsensitization.

Table III contains results of experiment designed *to compare efficacy of complete and incomplete adjuvant* in enhancing allergenicity of bovine albumin in mice. Animals challenged 7 days postsensitization failed to exhibit signs of anaphylactic shock. Existence of sensitized state was evident after 10 days when either the complete or incomplete adjuvant was employed in the sensitizing mixture. When challenges were made 14, 21, 28, or 35 days postsensitization, mortality incidences in mice receiving complete adjuvant was insignificantly different from that in animals receiving incomplete one.

TABLE II. Duration of Anaphylactic Sensitivity in Mice Injected with Bovine Albumin-Adjuvant Emulsion on 9 Groups.

Days between sensitization* and challenge†	Incidence of mortality	Survivors at 30 min.	$\triangle t_{30}$§
35	10/15 (.67)‡	7	−5.0
42	25/27 (.93)	7	−5.4
49	14/15 (.93)	4	−6.1
56	11/15 (.73)	6	−6.1
84	15/36 (.42)	27	−2.8
112	0/15 (0)	15	−2.8
140	" (0)	15	−2.7
168	4/15 (.27)	11	−2.4
196	1/9 (.11)	8	−3.3

* Each mouse inj. with single sensitizing dose (0.2 ml) of bovine albumin-adjuvant emulsion containing 0.1 mg of bovine albumin.

† Challenging dose (0.2 ml) consisting of 1% bovine albumin in 0.85% NaCl solution inj. into tail vein.

‡ Numerator = No. of deaths recorded at 60 min.; denominator = No. of mice challenged; No. in parentheses is decimal fraction.

§ $\triangle t_{30}$ = Mean change in rectal temperature (°C) taken 30 min. after challenge with bovine albumin.

Discussion. In our work, no attempt was made to determine the effect of *M. butyricum* in water-in-oil emulsion on antigenicity of bovine albumin in mice; precipitin determinations were not made. Furthermore, skin tests were not done to determine development of sensitivity of the delayed type. However, our results did show that anaphylactogenicity of bovine albumin in water-in-oil emulsion was not enhanced significantly by addition of *M. butyricum*, at least under conditions of experiments employed. Consequently for studies of anaphylactic shock in mice, the incomplete adjuvant may be employed as a suitable potentiating agent of sensitization. In progress are experiments to contrast precipitin levels in sera of mice sensitized with bovine albumin admixed with complete or incomplete adjuvant.

Our experiments revealed that mice sensitized with 0.1 mg of bovine albumin emulsified with the complete adjuvant demonstrated signs of anaphylactic shock when challenged with bovine albumin as long as 196 days postsensitization.

Summary. 1) Mice sensitized with 0.1 mg

TABLE III. Comparison of Complete and Incomplete Adjuvants in Their Ability to Induce Anaphylactic Sensitivity in Mice to Bovine Albumin.

Days between sensitization* and challenge†	Adjuvant in sensitizing emulsion*	Incidence of mortality	Survivors at 30 min.	$\triangle t_{30}$§
7	Complete	0/20 (0)‡	20	+ .2
7	Incomplete	" (0)	"	+ .6
10	Complete	1/25 (.04)	24‖	−3.3
"	Incomplete	0/25 (0)	25	−1.1
14	Complete	9/26 (.35)	18	−2.1
"	Incomplete	10/18 (.55)	9	−2.8
21	Complete	11/24 (.46)	16	−4.4
"	Incomplete	13/25 (.52)	15	−5.6
28	Complete	59/89 (.66)	38	−3.5
"	Incomplete	58/89 (.65)	39	−3.4
35	Complete	22/24 (.92)	11	−7.1
"	Incomplete	19/21 (.90)	5	−7.8

* Each mouse inj. on same day with a single sensitizing dose (0.2 ml) of bovine albumin-adjuvant emulsion containing 0.1 mg of bovine albumin.

† Challenging dose (0.2 ml) consisting of 1% bovine albumin in 0.85% NaCl inj. rapidly into tail vein.

‡ Numerator = No. of deaths recorded at 60 min.; denominator = No. of mice challenged; No. in parentheses is decimal fraction.

§ $\triangle t_{30}$ = Mean change in rectal temperature (°C) of mice surviving 30 min. after challenge.

‖ Two of the 24 died within 3 hr following challenge.

of bovine albumin incorporated into water-in-oil emulsion containing *Mycobacterium butyricum* showed anaphylactic reactivity when challenged 28 days postsensitization with as little as 0.01 mg of bovine albumin. 2) Mice sensitized in a similar manner exhibited signs of anaphylaxis when challenged 196 days following sensitization. 3) Anaphylactogenicity in mice of bovine albumin in emulsions lacking *Mycobacterium butyricum* was comparable to that in emulsions containing acid-fast bacilli.

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Anorexigenic Action of Methylphenidate (Ritalin) and Pipradrol (Meratran). (25178)

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Methylphenidate (Ritalin) and pipradrol (Meratran) are recently developed piperidine derivatives with central nervous system stimulant action. Meier *et al.*(1) and Brown *et al.* (2) suggested that the piperidine stimulants differ in certain respects from excitants of the amphetamine type, but they also demonstrated that these two classes of compounds share many pharmacologic properties. However, the newer agents have not been tested for possible anorexigenic action, so characteristic clinically and pharmacologically for drugs of the amphetamine class(3). Accordingly, an antiappetite assay was set up in experimental animals in order to compare piperidine stimulants with amphetamine-like agents.