Enhanced Sensitization in Mice by Simultaneous Injection of Antigen and Specific Rabbit Antiserum.* (25342)

GERONIMO TERRES AND WILLIAM WOLINS (Introduced by D. D. Van Slyke)
Medical Dept., Brookhaven National Lab., Upton, L. I., N. Y.

In following the degradation of I^{131} labeled human serum albumin (I^{131} HSA) in mice passively sensitized with rabbit antiserum, it was noted that a *second* phase of accelerated degradation of I^{131} HSA occurred approximately 100 hours following the I^{131} HSA-antiserum injection. In contrast, control mice injected only with I^{131} HSA degraded the labeled protein at a constant rate. This suggested that as a result of injecting mice intravenously with HSA-rabbit HSA antiserum, a greater antibody response was elicited to HSA than was elicited to HSA alone. The present study demonstrates that in mice, a significantly greater antibody response is elicited to serum albumin (human or bovine) by intravenous injection of serum albumin plus specific rabbit antiserum (in serum albumin excess) than is elicited by intravenous injection of serum albumin alone. The experimental design was as follows: Mice were divided into groups and injected intravenously with (1) serum albumin mixed *in vitro* with specific rabbit antiserum (*experimental*), (2) serum albumin plus normal rabbit serum (*NS controls*), or (3) serum albumin alone (*controls*). To determine if mice had become actively sensitized, they were challenged intravenously with I^{131} labeled serum albumin. Anaphylaxis was graded and the amount of I^{131} labeled serum albumin degraded in the ensuing 24 hours was determined. (In passively sensitized mice, the *amount* of antigen degraded at the acceleratd rate is a function of amount of antibody used(1)).

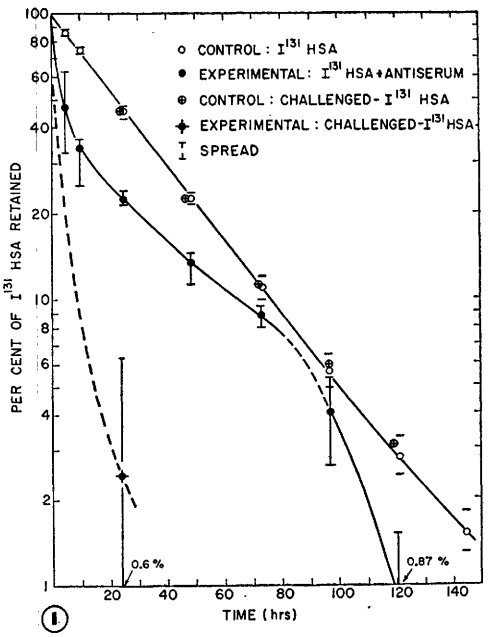
Materials and methods. Mice. Eight-week-old Swiss albino (Hale-Stoner) mice were used. I^{131} labeled human serum albumin (I^{131} HSA) was obtained commercially (Abbott Labs). Crystalline bovine serum albumin (BSA) was purchased from Armour Labs and iodinated (I^{131}) using KI_3(2). Rabbits were injected intravenously with 10 mg of serum albumin (HSA or BSA) 3 times a week for 6 weeks. One week following last injection, the rabbits were bled and sera pooled. The rabbit anti-HSA serum contained 3.2 mg of anti-HSA antibody/ml, and rabbit anti-BSA serum contained 2 mg of anti-BSA antibody/ml. *Gradation of anaphylaxis.* In mice *mild* shock is characterized by hyperexcitability to stimulation, absence of spontaneous movement, a hunched posture, dilation of blood vessels in the ear and labored breathing. *Moderate* shock is characterized by hyperexcitability, sluggishness of movement, difficulty with breathing and cyanosis of ears and feet. The hunched posture is absent. *Severe* shock is characterized by complete immobility, shallow breathing and intermittent convulsions. The degradation of I^{131} HSA and I^{131} BSA was followed by whole-body measurement of radioactivity(1). *Exp. I, HSA: Experimental group.* Eleven mice were injected intravenously with I^{131} HSA plus rabbit anti-HSA serum (0.15 ml). Rabbit anti-HSA serum diluted 1 to 4 with saline (1% NaCl) was mixed *in vitro* with equal volume of I^{131} HSA solution (0.5 mg/ml), and stored at 37°C for 2 hours. No antigen-antibody precipitate was detected in the I^{131} HSA-antiserum solution. The amount of I^{131} HSA added to antiserum was 2.5 to 3 times the amount necessary to produce maximum precipitation. *NS control group.* Eight mice were injected with a solution of normal rabbit serum plus I^{131} HSA (0.2 ml). Normal rabbit serum was diluted 1 to 4 with saline, mixed with equal volume of I^{131} HSA (0.5 mg/ml), and stored at 37°C for 2 hours prior to injection. *Control group.* Nine mice were injected intravenously with 0.025 mg of I^{131} HSA. After onset of second phase of accelerated degradation of I^{131} HSA in mice of the experimental group, all mice of the 3 groups were challenged (6th day) intravenously with 0.01 mg of I^{131} HSA. Anaphylaxis was graded and degradation of I^{131} HSA was followed. The *NS control* mice were

* Research supported by U. S. Atomic Energy Comm.

again challenged on 10th day with I^{131} HSA, anaphylaxis was graded, and degradation of I^{131} HSA was followed. *Exp. II, BSA: Experimental group.* Seventeen mice were injected intravenously with BSA plus rabbit anti-BSA serum (0.2 ml). Rabbit anti-BSA serum diluted 1 to 2 with 1% saline was mixed with an equal volume of BSA (1 mg BSA/ml). The amount of BSA used was approximately 3 times the amount necessary to produce maximum precipitation. The BSA-rabbit anti-BSA serum solution was injected immediately after mixing. *Control Group I.* Sixteen mice were injected intravenously with 0.1 mg of BSA alone. On 7th day, all mice were challenged intravenously with 0.15 mg of I^{131} BSA. Eight mice not previously exposed to BSA were also injected intravenously with 0.15 mg of I^{131} BSA (Control Group II). Anaphylaxis was graded and amount of I^{131} BSA degraded in the ensuing 24 hours was determined.

Results. Exp. I: The results obtained with *experimental* and *control* mice are presented in Fig. 1. The range in values at each measurement is designated by bars. In *experimental* mice (solid circle), the I^{131} HSA was degraded initially at an accelerated rate and later at a slower rate. Between 70 and 96 hours following administration of HSA-anti-HSA, a break in the retention curve occurred due to accelerated degradation of I^{131} HSA (2nd phase). With *control* mice a straight line was obtained throughout. On 6th day, the mice were challenged. The results are summarized in Table I. All *experimental* mice developed mild or moderate anaphylactic shock, while none of the *control* mice was anaphylactically shocked. *Experimental* mice (solid circle with a cross; Fig. 1) degraded 94 to 99% of the I^{131} HSA in the 24-hour period following challenge; control mice degraded 55% of the I^{131} HSA in the same period. The degradation of I^{131} HSA in the *control* mice was followed an additional 100 hours and the results duplicated those following the first I^{131} HSA injection.

The results obtained with *NS control* mice are presented in Fig. 2. None of these mice was anaphylactically shocked at either challenge (6th and 10th days). Following the 2 challenges, I^{131} HSA was degraded at the same rate as that observed following initial I^{131} HSA injection. The degradation of I^{131} HSA was followed a total of 13 days.



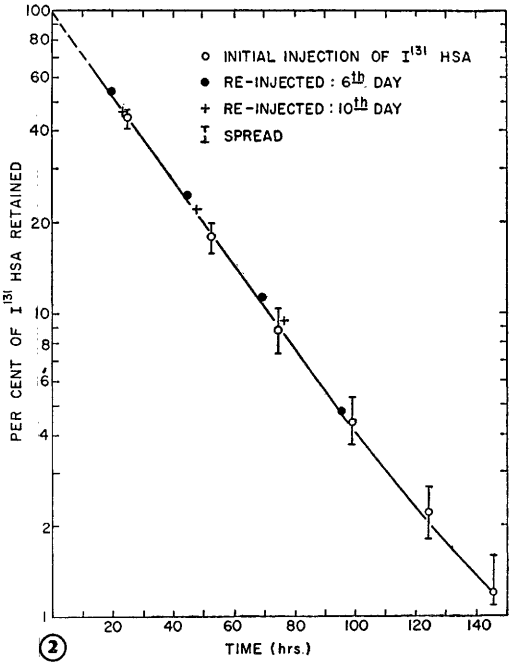


FIG. 1. The degradation of I^{131} HSA in control mice contrasted with its degradation when given with rabbit anti-HSA.

FIG. 2. The degradation of I^{131} HSA in mice inj. with I^{131} HSA and normal rabbit serum.

TABLE I. Summary of the Results of Exp. I.

Group	No. of mice	Initial inj.	Challenge* (6th day) Anaphylactic shock	Challenge* (6th day) % of I^{131} HSA retained at 24 hr
Experimental	11	I^{131} HSA + rabbit antiserum	Mild to moderate	.6 to 6.0
NRS control	8	I^{131} HSA + NRS	None	52.0 to 57.0†
Control	9	I^{131} HSA	"	40.0 to 48.0

* Intravenous: 0.01 mg of I^{131} HSA. † Determined at 19 hr.

Exp. II: The results using BSA with or without rabbit anti-BSA serum are summarized in Table II. All *experimental* mice were anaphylactically shocked and the amount of I^{131} BSA degraded in the 24-hour period following the challenge ranged from 82 to 93% of amount injected. None of the *control* mice was anaphylactically shocked and these mice degraded approximately the same amount of I^{131} BSA as did control mice on the challenge (control group II).

Discussion. These results lead to the conclusion that a significantly greater antibody response to serum albumin (human or bovine) is elicited when mice are injected intravenously with a solution of serum albumin and specific rabbit antiserum in antigen excess, than is elicited in mice injected wth serum albumin alone, or with serum albumin and normal rabbit serum. Thus the *experimental mice* given HSA and rabbit anti-HSA serum when challenged intravenously on the 6th day were anaphylactically shocked, and in the ensuing 24 hours degraded 94% or more of the I^{131} HSA used in the challenge. In contrast, none of the control mice was anaphylactically shocked and all control mice degraded I^{131} HSA at a rate identical to that following initial I^{131} HSA injection, indicating that none of these mice became actively sensitized to HSA. Similar results were obtained with the BSA system (Exp. II).

It is impossible to state unequivocally that control mice did not respond immunologically to HSA or BSA. A positive response might have been obtained with control mice, had a more sensitive method been employed to measure antibody response. With the method employed (antigen degradation), greater sensitivity could have been obtained had a smaller quantity of I^{131} labeled protein been used in the challenge, since the *per cent* of antigen degraded at an accelerated rate is a function of the amount of antigen used(1).

TABLE II. Summary of the Results of Exp. II.

Group	No. of mice	Initial inj.	Mouse	Challenge* (7th day) Anaphylactic shock	Challenge* (7th day) % of I^{131} BSA retained at 24 hr
Experimental	17	BSA + rabbit antiserum	1	Fatal	
			2	Moderate	3
			3	"	3
			4	"	4
			5	"	5
			6	"	5
			7	"	7
			8	"	7
			9	"	7
			10	"	13
			11	Mild	4
			12	"	11
			13	"	12
			14	"	13
			15	"	14
			16	"	15
			17	"	18
Control I	16	BSA		None	29.10 ± .04
" †II	8			"	26.50 ± .30

* Intravenous: 0.15 mg of I^{131} BSA. † Control mice on the challenge.

Mice can be actively sensitized to either HSA or BSA with multiple injections. Three interperitoneal injections of HSA (10 mg/injection, 7 days between injections) resulted in 50% of mice being anaphylactically shocked when challenged intravenously 21 days following the first injection(3). Mice were not anaphylactically shocked when challenged 7 or 21 days following a single intraperitoneal injection of HSA (30 mg)(3). Four subcutaneous injections of BSA (4 mg/injection) administered on an every-other-day schedule resulted in approximately 75% of mice being anaphylactically shocked when challenged 14 days following first injection(4). A single subcutaneous injection of BSA (16 mg) results in only 1 or 2% being anaphylactically shocked when challenged on 14th day. Thus in comparison, a single intravenous injection of HSA or BSA (about 0.05 mg) plus its specific antiserum is as effective in actively sensitizing mice to serum albumin as multiple (interperitoneal or subcutaneous) injection of serum albumin alone.

Several studies have been reported in which antigen and antibody were combined and active sensitization measured (*e.g.*, 5,6,7). None of these, of which we are aware, revealed the marked differences in response here described (HSA alone *vs.* HSA plus antiserum). In fact, the opposite effect has been reported(7). Thus ovalbumin-rabbit antiovalbumin precipitates were incorporated in adjuvant and injected into foot pads of guinea pigs. These precipitates were formed in both antigen and antibody excess. Other guinea pigs were similarly injected with an equal amount of "free" ovalbumin. When these animals were challenged with ovalbumin 2 to 3 weeks later, it was found that a higher degree of sensitivity had been achieved with "free" ovalbumin than with the immune precipitates.

Summary. Mice were injected intravenously with a solution of serum albumin (human and bovine) plus specific rabbit antiserum in antigen excess. The antibody response to serum albumin was tested and compared to that obtained in mice injected intravenously with serum albumin alone or with albumin and normal rabbit serum. The results show that antibody response elicited with serum albumin plus specific rabbit antiserum is significantly greater than that elicited using serum albumin alone or with normal rabbit serum.

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Received August 24, 1959. P.S.E.B.M., 1959, v102.

Level of 3-Methylcholanthrene in Mammary Glands of Rats after Intragastric Instillation of Carcinogen.* (25343)

THOMAS L. DAO, FRED G. BOCK AND SHIRLY CROUCH
(Introduced by Julian L. Ambrus)
Roswell Park Memorial Inst., Buffalo, N. Y.

Development of mammary adenocarcinoma in rats following oral administration of 3-methylcholanthrene has been reported(1,2). These observations also indicated that induced mammary cancers were hormone-dependent. Our studies have further demonstrated that, in rats, steroid hormones promote mammary carcinogenesis by 3-methylcholanthrene(3). In earlier reports by Shay *et al.*(1) and later by Huggins *et al.*(2) and by Dao *et al.*(3),

* This study was supported in part by grants from Nat. Cancer Inst., U.S.P.H.S.