Effect of Sodium Salicylate on Antibodies Produced in Guinea Pigs.* (17900)

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Many investigations have dealt with the effect of salicylates on immunological phenomena. These studies may be divided into 3 groups, namely, the *in vitro* inhibition of antigen-antibody reactions, the *in vivo* inhibition of the production of antibodies, and the *in vivo* inhibition of circulating antibodies. A recent review by Smith(1) dealing with the pharmacology of salicylates makes note of the most important contributions in this field. The only experiment which deals with the *in vivo* inhibition of circulating antibodies by salicylates was carried out by McLennan, Jager and Matson (2) in women whose pregnancy was complicated with erythroblastosis fetalis. Eight to 10 g of acetylsalicylic acid (aspirin) per day for 20 weeks failed to alter the course of the disease. The present experiment was designed to evaluate under more controlled conditions the effect of sodium salicylate in guinea pigs on the antibodies formed in response to the antigens in Rh^+ type O human red blood cells. The immunized animals were divided into 2 groups, one group for the determination of control antibody titers and the other for observation of the effect of injected sodium salicylate on antibody titers.

Methods. Hybrid guinea pigs weighing 250-400 g were used. Under sterile conditions and without anesthesia 2-3 ml of blood was taken from the heart. The syringe contained 1-2 mg of dry heparin. The blood was centrifuged for an hour and 0.65 ml of plasma was used for the antibody assay. The remaining portion was divided for duplicate salicylate determinations. Sterile 3-day-old Rh^+ and Rh^- type O human blood was obtained from the Albert Merrit Billings Hospital Blood Bank. The red blood cells were washed 4 times by centrifuging and resuspending in 0.9% saline under sterile conditions. A hematocrit was done in the usual fashion after bringing the volume of the cells in the suspension to approximately 50%. This preparation of cells was injected intraperitoneally into guinea pigs at 48-hour intervals on the following schedule: initial dose, 1 ml of packed cell equivalent; 2nd dose, 1.5 ml; and 3rd, 4th, 5th, and 6th doses 2 ml. The booster dose given on the 44th day was 2 ml. The method described by Burrows *et al.*(3) was used for determining antibody titers. Two percent suspensions of washed Rh^+ or Rh^- type O cells were used as the antigen in the antibody assay. Plasma salicylate concentration was determined by the method of Brodie *et al.*(4). It was found in preliminary experiments that normal guinea pig plasma did not interfere with this colorimetric determination of salicylate and that after the highest dosage used in these experiments no salicylate could be detected 12 hours after injection. The dosage schedule for the salicylated animals is given in the accompanying table.

Results and discussion. The data are summarized in the table. Before immunization the average blood plasma Rh^+ and Rh^- titer was respectively, 1:38 and 1:30. The Student t test(5) indicated that the average Rh^+ titer did not differ from the average Rh^- titer. A p value of 0.05 or less is considered significant. Eight Rh^+ and 7 Rh^- antibody assays were done at various times after immunization on the plasma of non-

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3. Burrows, W., Mather, A. N., McGann, V. G., and Wagner, S. M., *J. Inf. Dis.*, 1946, v79, 168.

4. Brodie, B. B., Undenfriend, S., and Coburn, A. F., *J. Pharm. and Exp. Therap.*, 1944, v38, 114.

5. Fisher, R. A., Statistical Methods for Research Workers, 1948, Oliver-Boyd, London.

TABLE I. Rh+ and Rh- Antibody Titers to Human Rh+ Type O Red Blood Cells in Guinea Pigs With and Without Injected Sodium Salicylate.

Days after immunization	No. of animals		Control plasma antibody titer				Salicylated plasma antibody titer				Avg salicylate plasma conc.,† mg %	Sodium salicylate dosage schedule,‡ (g/kg)
	Control	Salicylated	Avg Rh+ titer	S.D.*	Avg Rh- titer	S.D.	Avg Rh+ titer	S.D.	Avg Rh- titer	S.D.		
Before	55	—	1:38	1:41	1:30	1:32	—	—	—	—	—	—
4	16	3	1:778	1:477	1:819	1:439	1:300	1:110	1:300	1:110	40. (29-45)	.32 q.4h.
12	12	2	1:312	1:340	1:136	1:539	1:50	—	1:20	—	15. (10-21)	.16 q.3h.
20	5	9	1:38	1:16	1:38	1:16	1:42	1:50	1:46	1:42	15.2 (6-28)	.16 q.3h.
28	6	1	1:50	—	1:50	—	1:50	—	1:50	—	15.2	.16 q.3h.
36	5	8	1:50	—	1:50	—	1:53	1:35	1:53	1:35	37. (28-42)	.3 q.4h.
44	13	—	1:69	1:48	1:65	1:29	—	—	—	—	—	—
44						Booster						
48	3	6	1:1500	1:866	1:1500	1:866	1:1750	1:612	1:1500	1:548	49.6	.24 q.3h.
74	6	—	1:5333	1:2479	—	—	—	—	—	—	—	—

* Stand. dev. $\sqrt{\frac{\Sigma d^2}{n-1}}$.

† 3 hours after the last injection of sodium salicylate.

‡ Given intraperitoneally during the 24 hr immediately preceding the withdrawal of blood samples.

salicylated immunized guinea pigs. Although antibodies were formed against the Rh+ type O human red blood cells, none of the antibody assays done showed significant antibody formation against the Rh+ antigen. Hence, the data indicated that the Rh+ antigen added little to the antigenicity of the type O cells. On the 12h day after completion of immunization, plasmas from 7 different guinea pigs were incubated for 2 hours with Rh- type O cells in order to absorb the type O antibody. Antibody titers were determined on these plasma preparations; three of the 7 plasmas showed a partial but definite agglutination of Rh+ cells.

The average Rh+ and Rh- circulating antibody level rose on the 4th day after immunization to 1:778 and 1:819, respectively, fell to 1:312 and 1:136, respectively, on the 12th day and by the 20th day had fallen to the baseline level. The Student t test was used to compare the average Rh+ and Rh- antibody titers before immunization with the average Rh+ and Rh- antibody titers of the 20th, 28th, 36th, and 44th day. No significant difference was noted. On the 44th day a booster injection of Rh+ type O cells was given. Four days after the booster injection the average Rh+ and Rh- titer rose to 1:1500. The average Rh+ titer was 1:5333 30 days after the booster dose. The results of the immunization with Rh+ type O cells indicate then, that antibodies are formed to the antigens in the Rh+ type O cells. These circulating antibodies disappear by the 20th day after immunization. A booster dose 44 days after initial immunization produces a greater and more lasting circulating antibody titer.

On the 3rd, 11th, 19th, 27th, 35th, and 47th day following immunization several of the guinea pigs were given different doses of sodium salicylate and bled 3 hours after the last injection. On the 4th day after immunization the average salicylated plasma Rh+ and Rh- titers were approximately 37% of the control. The student t test indicated that the average salicylated plasma Rh+ and Rh- titers on the 4th day after immunization differed significantly from the average control Rh+ and Rh- titers. On the 12th day after immunization the average salicylated plasma

Rh^+ and Rh^- titers were approximately 15% of the control, a significant difference. The average Rh^+ and Rh^- titers on the 20th, 28th, and 36th days, which did not differ from the baseline anti-human hemoagglutinins were not depressed by sodium salicylate. This was not an unexpected finding. On the 48th day after immunization or 4 days after the booster injection it was found that the average Rh^+ or Rh^- titer was not depressed by salicylate. These data on the 48th day after immunization are of interest in view of the findings on the 4th and 12th day after immunization. The average plasma salicylate concentration on the 4th day after immunization was 40 mg %; whereas the plasma salicylate level on the 48th day after immunization was 50 mg %, an increase of 25%. A plasma salicylate level of 50 mg % is toxic. The average Rh^+ and Rh^- titers on the 4th day after immunization were 1:778 and 1:819, respectively; whereas the average Rh^+ and Rh^- titers on the 48th day after immunization were 1:1500, an increase of approximately 93% and 83%, respectively. Comparing the average plasma salicylate levels and the average plasma antibody titers on the 12th and 48th day after immunization, it was found that plasma salicylate level on the 48th day was 236% higher and the Rh^+ and Rh^- titers were 381% and 1000% higher, respectively. Since the increase in plasma salicylate concentration was not equal to the increase in the antibody titer on the 48th day as compared to the 4th and 12th day, it is postulated that there was not enough salicylate present to inhibit the antibodies on the 48th day. This theory is supported by several facts. In the range of Rh^+ or Rh^- titers between 1:136 and 1:819 the antibody titer was depressed by non-toxic plasma concentrations of salicylates. However, if the antibody titer was 1:1500, it was not depressed even by toxic plasma concentrations of salicylates. The *in vitro* inhibition of antibodies has been shown to be proportional to the concentration of salicylates(6,7).

Summary. Antibody titers produced in guinea pigs receiving Rh^+ type O human red blood cells intraperitoneally were compared each week after immunization with those produced in similarly treated guinea pigs which also received varying doses of sodium salicylate for one day before drawing blood for the measurement of antibody titers and salicylate plasma levels. Rh^+ type O human red blood cells were antigenic in the guinea pig. The Rh^+ antigen added little to the antigenicity of the type O cells. The average Rh^+ or Rh^- antibody titers rose to 1:778 or 1:819 on the 4th day after immunization, fell to 1:313 or 1:136 on the 12th day and by the 20th day had fallen to 1:50, the baseline level. A more lasting circulating antibody titer was produced after a booster dose of Rh^+ type O cells. It is concluded that the natural anti-human hemoagglutinins cannot be depressed by sodium salicylate. The data indicated that the depressant action of sodium salicylate on the Rh^+ type O human antigen-antibody reaction in guinea pigs was limited by the toxicity of salicylates. Rh^+ or Rh^- titers between 1:136 and 1:819 were depressed significantly by non-toxic plasma levels of sodium salicylate (15-40 mg %); whereas Rh^+ or Rh^- titers of 1:1500 were not depressed even by toxic plasma levels (approximately 50 mg %).

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