In Vitro Histamine Release from Blood Cellular Elements of Rabbits Infected with *Schistosoma mansoni* (32752)

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Recently Zvaifler *et al.*(1) described a homocytotropic antibody in rabbits infected with *Schistosoma mansoni* which produced a 72-hour passive cutaneous anaphylaxis (PCA) in normal rabbits challenged with cercarial antigen. This anaphylactic antibody was inactivated by heating at 56°C and by dimercaptoethanol, migrated as fast gamma globulin electrophoretically and had a sedimentation coefficient close to 7*S*. Human reaginic antibody is capable of sensitizing leukocytes so that by the addition of antigen, histamine is released(2). Barbaro and Zvaifler(3) using dinitrophenyl–bovine serum albumin (DNP-BSA) in Freund complete adjuvant suggested that rabbit PCA antibody is capable of sensitizing rabbit platelets *in vivo* and demonstrated antigen-induced histamine release *in vitro* in the absence of free plasma factors and free antibody.

The present studies were designed to determine if *S. mansoni* infections in rabbits "sensitize" platelets and/or leukocytes for *in vitro* histamine release following the addition of *S. mansoni* cercarial antigen in the absence of added plasma. The results of these studies were compared with those obtained by PCA and slide flocculation (SF) tests. Further more, attempts were made to determine whether this *in vitro* reaction system could be utilized for a quantitative study of immediate hypersensitivity in rabbits infected with *S. mansoni*.

Materials and Methods. Animals. Albino rabbits weighing 2500–3000 gm were used in these experiments. All animals were fed a standard diet. The principles of animal care as promulgated by the National Society for Medical Research were observed. Rabbits were exposed percutaneously to either 5000 or 25,000 cercariae each and were bled for histamine and serologic studies every other week beginning the day of exposure. At the end of each experiment, the rabbits were killed and the visceral organs were examined for the presence of gross pathologic changes resulting from infection with *S. mansoni*. Each animal was perfused by the Perf-O-Suction technique(4) and examined carefully for completeness of blood clearance and possible retention of adult worms. Immediately after perfusion, the worms were separated by sex, counted, and observed microscopically.

Antigen. S. mansoni cercariae were recovered from the laboratory colony of *Australorbis glabratus (Biomphilaria glabrata)* snails, each infected with 5–10 miracida. A lipid free somatic antigen was prepared according to the method of Chaffee *et al.*(5). After lyophilization, the antigen was reconstituted with distilled water to a concentration of 0.43 mg of nitrogen protein per ml, determined by the micro-Kjeldahl method. From this standard preparation, 0.5 ml of a 1:100 dilution in Tyrode's solution was found to be minimal for 100% histamine release.

Rabbit antiserum. The globulins from appropriate serum pools were precipitated with 50% saturated ammonium sulfate. The precipitate was reconstituted to one-half the original serum volume with phosphate buffered saline (PBS), pH 7.2, and dialyzed against the same buffer for 48 hours at 4°C. The globulins were then fractionated by column chromatography utilizing Sephadex G-200(6) and diethylaminoethyl (DEAE) cellulose; the samples were eluted from the G-200 column with PBS and the DEAE columns were eluted sequentially using selected phosphate buffers with slight modifications of the method described by Askonas *et al.*(7), as reported previously(8).

At the same time, another aliquot of blood was collected separately and allowed to clot for serum separation. After the blood had clotted, the serum was separated by centrifugation and stored at —70°C. It was tested for PCA and SF activity with adherence to the methods published previously(1).

Histamine determinations. Appropriate amounts of blood were collected from the medial artery of the ear in a siliconized syringe and transferred to cold polypropylene tubes containing sufficient amounts of sodium heparin to yield a final concentration of 7 μg of heparin per ml of whole blood. The blood was then centrifuged at 1600*g* for 15 min at 3°C. The supernatant plasma was discarded and the packed cells were resuspended and washed 3 times with Tyrode's solution before reconstituting to the original blood volume. These suspensions contained red and white cells as well as the platelets. Samples of 0.5 ml of these washed cells were used in a total assay volume of 1.5 ml and tested in duplicate for total, antigen-induced and spontaneous histamine release. A pure suspension of platelets was obtained from blood collected as described by centrifuging for 50 min at 30*g* at 3°C. The platelet rich plasma supernatant mixture was then washed with Tyrode's solution and divided into aliquots.

Histamine was extracted according to the technique described by Shore *et al.*(9) and modified by Barbaro and Zvaifler(3). Fluorescence was read on a Turner model 111 Fluorometer activated at 360 mμ and emitted at 450 mμ.

Results. The first experiment was to determine whether histamine release could be induced in rabbits infected with *S. mansoni* and to compare the results with the production of PCA antibody. Eight rabbits were exposed to 25,000 cercariae each and bled every other week for 38 weeks. The results (Table I) indicate that 5 of the 8 infected rabbits produced detectable PCA antibody.

Seven of the 8 rabbits showed significant levels of antigen-induced histamine release. This occurred as early as 3 weeks after exposure to infection in 1 animal and 4–7 weeks after exposure in most of the others. Histamine release was detected both in rabbits in which PCA antibody had been demonstrated and in those which showed no detectable levels of PCA antibody. With the single exception of rabbit No. 7, antigen-induced histamine release occurred throughout the experiment. A considerable variability in

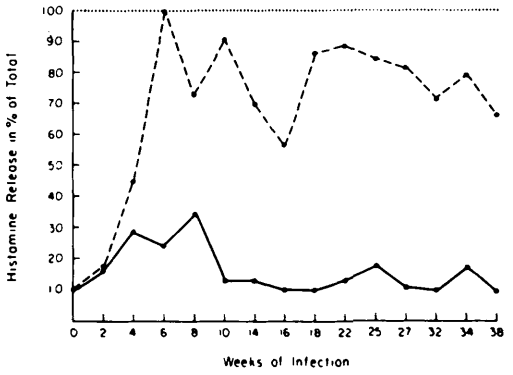

FIG. 1. Typical histamine release pattern in a PCA negative rabbit infected with *S. mansoni*. (• • •) Total; (— — —) Antigen induced; (—) Spontaneous.

worm burdens was observed in the animals at necropsy (Table I). No obvious correlation between the presence of anaphylactic antibodies and worm burden was observed. All of the animals exposed to infection gave positive results with the slide flocculation test. As indicated in Fig. 1, antigen-induced histamine release was detectable in the absence of demonstrable circulating PCA antibody but at levels lower than in PCA positive rabbits (Fig. 2). However, it was not possible to obtain PCA antibody production without accompanying histamine release. The time course of the development of PCA antibody activity and of *in vitro* antigen-induced

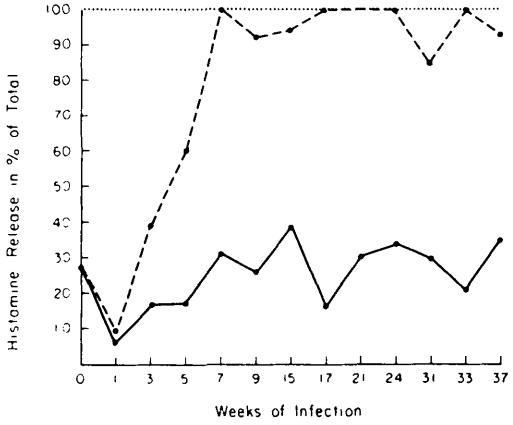

FIG. 2. Typical histamine release pattern in a PCA positive rabbit infected with *S. mansoni*. (• • •) Total; (— — —) Antigen induced; (–) Spontaneous.

TABLE I. Cellular, Serologic, and Parasitologic Findings in Rabbits Infected with *S. mansoni*.[a]

Rabbit:	No. 1			No. 2			No. 3			No. 4			No. 5			No. 6			No. 7			No. 8		
Week	HR	PCA	SF	HR	PCA	SF	HR	PCA	SF	HR	PCA	SF	HR	PCA	SF	HR	PCA	SF	HR	PCA	SF	HR	PCA	SF
										Exposed to 25,000 Cer.														
0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—		—
1	—	—	—	—	—	—	—	—	—	—	—	—												
2													—	—	—	—	—	—	—	—	—	—		—
3	—	—	—	—	—	—	—	—	—	+	—	—												
4													+	—	—	+	—	—	—	—	—	—		—
5	—	—	+	+	—	+	+	+	+	+	+	+												
6													+	—	—	+	—	—	—	—	—	+		—
7	+	—	+	+	+	+	+	+	+	+	+	+												
8													+	+	—	+	—	—	—	—	+	+	+	+
9	+	—	+	+	+	+	+	+	+	+	+	+												
10													+	+	+	+	—	+	+	—	+	+	—	+
14	+	—	+	+	—	+	+	+	+	+	+	+												
15													+	+	+	+	—	+	—	—	+	+	—	+
16	+	—	+	+	+	+	+	+	+	+	+	+												
17													+	+	+	+	—	+	—	—	+	+	—	+
18	+	—	+	+	—	+	+	+	+	+	+	+												
21													+	+	+	+	—	+	—	—	+	+	—	+
24	+	—	+	+	—	+	+	+	+	+	+	+												
25													+	+	+	+	—	+	—	—	+	+	—	+
27	+	—	+	+	—	+	+	+	+	+	+	+												
										Exposed to 20,000 Cer.														
31													+	+	+	+	—	+		Died		+	—	+
32	+	—	+	+	+	+	+	+	+	+	+	+												
33													+	+	+	+	—	+				+	—	+
34	+	—	+	+	+	+	+	+	+	+	+	+												
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36	+	—	+	+	+	+	+	+	+	+	+	+												
37													+	+	+	+	—	+				+	—	+
38	+	—	+	+	+	+	+	+	+	+	+	+												
Worm recovery at necropsy		1500			952			11			1150			718			1176			ND			863	

[a] Abbr.: HR, histamine release; PCA, passive cutaneous anaphylaxis; and SF, slide flocculation.

TABLE II. Histamine Release (μg).

Cellular contents	Spontaneous	Antigen-induced	Total
Normal platelets	.245	.243	1.170
Sensitized platelets and cells	.122	1.350	1.528
Sensitized platelets and cells normal platelets	.622	2.603	2.700

histamine release indicated that, in general, *in vitro* histamine release was present in more animals, was detected earlier and persisted longer than PCA antibody activity in the serum of infected animals. This incomplete correlation between the time course of the two activities suggests that more than one antibody may be responsible for the sensitization of platelets and for the observed histamine release, or may indicate that *in vitro* histamine release is a more sensitive method of measuring immediate hypersensitivity.

Additional experiments were designed to determine whether platelets are directly sensitized following *S. mansoni* infections or whether substances released from sensitized leukocytes following an antigenic stimulus trigger the release of histamine from platelets. Platelets in supernatant plasma from the uninfected rabbits were washed and divided into aliquots. Washed cells from an infected rabbit still containing platelets but in lesser quantity (usually 70–80% less as measured by total histamine) were added to the aliquot of platelets from an uninfected animal. As indicated in Table II, the amount of histamine in this mixture was significantly greater than that obtained when antigen was added to the sensitized cells only. Conversely, there was no discernible antigen-induced histamine release from the washed platelets, obtained from infected and noninfected animals, in the absence of leukocytes. Similarly, when supernatant fluids from sensitized cells which had been exposed to the antigen for 30 min at 37°C were added to platelets from normal rabbits they did not release histamine.

In an attempt to determine whether a quantitative *in vitro* system could be established, the relationship between antigen concentration and the percentage of histamine release was studied. As shown in Fig. 3, a 50% histamine release was observed at different antigenic dilutions in different animals. Although no obvious correlation between the amount of antigen required to permit a 50% histamine release and PCA titers was detected, the animal in which no demonstrable PCA reaction was present required a higher antigenic concentration for a 50% histamine release.

The antigenic concentration required for a 50% histamine release at various intervals following exposure to infection was studied in a series of 5 rabbits, 2 of which were exposed to 25,000 *S. mansoni* cercariae and 3 to 5000 cercariae each. As shown in Table III, increasingly lower concentrations of antigen were required in both groups of animals from the third to the ninth week following exposure to infection. During this time, it seemed that the cells from animals exposed to 25,000 cercariae demonstrated 50% histamine release with less antigen than those exposed to only 5000 cercariae.

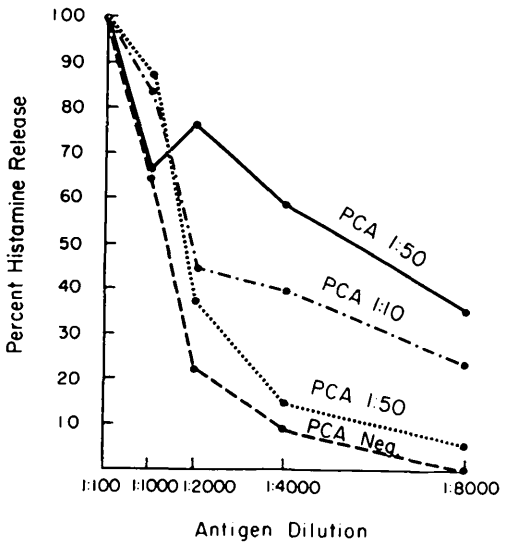

FIG. 3. Relationship between antigen concentration and percentage histamine release.

TABLE III. Antigen Dilution at Which 50% Histamine Release Occurs.

No. of cercariae	Rabbit no.	Weeks after exposure				
		0	3	5	7	9
25,000	93	0	1:800	1:3000	1:4000	1:4000
25,000	94	0	1:400	ND	1:4000	1:4000
5,000	1	0	1:1000	1:1000	1:2000	1:4000
5,000	2	0	<1:100	1:1000	1:2000	1:1000
5,000	3	0	<1:100	1:1000	1:2000	1:4000

Molecular sieving and ion exchange chromatography were employed to separate some of the antibodies present in the rabbit antiserum. The globulin in the combined sera from 4 rabbits exposed to 25,000 cercariae, all of which had high titers of PCA antibodies, was precipitated with 50% saturated ammonium sulfate. Passage of the reconstituted precipitate through Sephadex G-200 chromatography column yielded 4 fractions as shown in Fig. 4. Ion exchange chromatography was then employed to separate some of the antibodies present in the fraction which contained most of the PCA activity. Aliquots of each of the 4 fractions obtained were analyzed for their ability to produce PCA reactions and for their flocculating activity. As reported with fluorescent antibody tests on sera from rabbits infected with *Dirofilaria uniformis*(8), the fractions containing most of the IgG immunoglobulin had all of the detectable flocculating activity whereas electrophoretically faster immunoglobulins contained PCA activity but no detectable flocculating activity.

Discussion. The finding that only some of the infected rabbits produced a demonstrable amount of PCA antibody is in agreement with previous results obtained with infectious or artificial immunization(1,3,10,11).

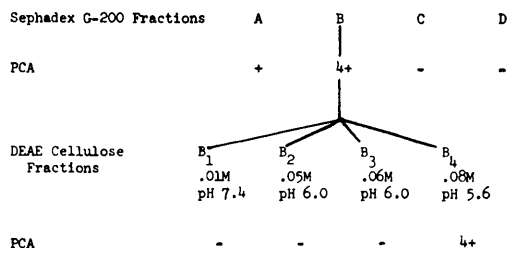

FIG. 4. Fractionation of PCA positive serum from rabbits infected with *S. mansoni*.

In all the rabbits with circulating homologous PCA antibody, it was possible to obtain antigen-induced *in vitro* histamine release. Conversely, *in vitro* histamine release was observed in a number of rabbits with no detectable PCA antibody activity. This consideration, added to the fact that antigen-induced histamine release appeared earlier and persisted longer than PCA activity, suggests that the release of *in vitro* histamine from the platelets is a more sensitive reaction and/or that more than one antibody is responsible for the former phenomenon.

Washed platelets from uninfected rabbits are capable of histamine release only when mixed with washed sensitized cells challenged with antigen. This suggests that some mechanism exists for histamine release from platelets obtained from unsensitized rabbits. Determination of a 50% histamine release by serial dilution of antigens indicated that this reaction is very sensitive and that titers are directly related to the length of infection. As shown in previous experiments conducted with rabbits infected with filarial parasites(8), the flocculating and the PCA activities are found in different immunoglobulin classes.

Studies are in progress to detemine the possible relationship between histamine release, PCA antibodies, immunity and intensity of infection of rabbits to *S. mansoni*.

Summary. In vitro antigen-induced histamine release from blood platelets of rabbits infected with *Schistosoma mansoni* is described. Histamine release was detectable both in the presence and absence of passive cutaneous anaphylactic antibodies. Ion exchange chromatography, used to separate some of the antibodies in the rabbit antisera, revealed that fractions containing most of the IgG immunoglobulin had all the detectable floc-

culating activity. Electrophoretically faster immunoglobulins contained passive cutaneous anaphylactic activity but no detectable flocculating titer. A pure platelet suspension from sensitized rabbits did not release histamine when challenged with antigen. Histamine release did occur in the presence of whole washed blood when similarly challenged. A significant increase in histamine release was noted when normal blood platelets in a suspension of washed sensitized cells were added to antigen.

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The Series Elasticity of Cardiac Muscle in Hyperthyroidism, Ventricular Hypertrophy, and Heart Failure (32753)

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The three-component model for muscle proposed by A. V. Hill (1) has been useful in describing the contractile activity of cardiac muscle *in vitro* (2) and *in vivo* (3). The contractile element (CE) of this model is assumed to be freely extensible at rest, but with activation it shortens according to a characteristic inverse relation between the velocity of shortening and the load (4). The CE is in series with an elastic element (SE) so that during isometric contraction, the activated CE shortens and stretches the SE, the rate of tension development (dP/dt) being determined by the CE velocity and the stress strain relation of the SE (1). Thus the external manifestations of CE activity depend to a large extent on the properties of the SE.

Although the stiffness of the SE is unaffected by inotropic interventions, or the course of active state (5,6), it does become somewhat stiffer following damage from segmental compression (7). With the recent application of cardiac muscle mechanics to the study of pathologic states such as hyperthyroidism, cardiac hyperthophy and failure (8–10), a quantitative knowledge of the SE compliance in these conditions is required if CE velocity and work are to be evaluated. Accordingly the present study was undertaken to measure the series elasticity of papillary muscles from cats with hyperthyroidism, cats with cardiac hypertrophy, and those with cardiac hypertrophy and heart failure.

Methods. Right ventricular papillary muscles from three groups of cats (1.5–2.5 kg) were used. Hyperthyroidism was induced in six cats by the intraperitoneal injection of l-thyroxine (1 mg per kg per day) for 10–14 days (10). Serum protein bound iodine (PBI) and cholesterol determinations were made at the time of sacrifice. In a second group of 15 cats, right ventricular hypertrophy with or without heart failure was produced

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