THE ROLE OF CHEMICAL MEDIATORS IN THE INFLAMMATORY RESPONSE INDUCED BY FOREIGN BODIES: COMPARISON WITH THE SCHISTOSOME EGG GRANULOMA*, ‡

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(Received for publication 1 August 1969)

A granuloma is a circumscribed inflammatory reaction, often but not always forming around a nidus consisting of either an infectious agent or a foreign body. Thus these lesions are frequently classified into two main categories, infectious or hypersensitivity granuloma and foreign body granuloma (1–3). Characteristically, the cellular accumulation in a hypersensitivity granuloma includes lymphocytes, epitheloid cells, and multinucleated giant cells (3, 4); this develops slowly, but upon reexposure there is an anamnestic response. The inflammatory response to foreign bodies usually develops quite rapidly, often beginning within an hour, but this is rarely accelerated by reexposure to the same agent (1, 2, 5, 6). Initially in the foreign body response, polymorphonuclear leukocytes accompanied by relatively few mononuclear cells are seen; this is followed within hours by an increase in the latter (6). After a few days, the cellular response is composed more of monocytes than of neutrophils and within a week fibroblasts are seen (6). Not all foreign substances produce a marked inflammatory response after implantation in a tissue (7); ordinary dust or carbon soot produce minimal inflammation (8). Substances that produce a foreign body granuloma include silica, quartz, asbestos (magnesium silicate), kaolin (aluminum silicate), talc (magnesium trisilicate), and beryllium oxide (1, 9).

The prototype of the infectious granuloma is found in tuberculosis, although the specific etiology has never been established. Recently, studies of the infectious granuloma utilizing schistosome eggs injected into the pulmonary microvasculature of mice suggest that this type of lesion is a form of delayed hypersensitivity (10). The development of this lesion is suppressed by measures that characteristically inhibit delayed hypersensitivity (11–13). These immunosuppressive measures have virtually no effect on the reaction developing around divinyl benzene copolymer beads (plastic beads) injected into the microvasculature of the mouse lung (11–13). Furthermore, the plastic bead

* Supported in part by Grants AM07841-05, 5K03-AM096560, AI31814-02, AI08163 from the National Institutes of Health, and in part by a grant from the Frackelton Memorial Fund. Dr. Kellermeyer is also a John and Mary R. Markle Scholar in Academic Medicine.

‡ Presented in part at the Annual Meeting of the American Society for Clinical Investigation, Atlantic City, New Jersey, May 1968.

reaction resembles the foreign body granuloma in that a maximum polymorphonuclear and mononuclear cellular response occurs within 48 hr (11, 14). The data in this report indicate that the inflammatory response initiated by these plastic beads and perhaps by other substances inducing foreign body reactions is a nonimmunologic response that appears to be largely dependent on chemical mediators of inflammation associated with the Hageman factor-PF/dil-kallikrein-kinin reaction sequence (15).

Materials and Methods

In Vitro Studies.—

Divinyl benzene copolymer beads (Bio Rad Laboratories, Richmond, Calif., Bio-Beads, S-X8, 200–400 mesh, No. control 3874) were further sized by passage through a size 70 steel mesh sieve, thrice washed with 0.9% saline, and stored in suspension at 5°C. The mean diameter of these beads as measured with an AEI Image-Splitting eyepiece (Frank Cooke, North Brookfield, Mass.) is given in the tables.

Schistosome eggs were obtained from the livers of Swiss albino female mice infected 8 wk previously with a Puerto Rican strain of Schistosoma mansoni (16). The eggs averaged 62 by 134 μ; their mean diameter as measured in the tissue sections is given in the tables.

Magnesium trisilicate (talc) (Pfaltz and Bauer, Inc., N. Y.) was suspended in 0.145 N sodium chloride at a concentration of 2500 particles per ml. The average particle size was 30.23 $\pm$ SE 0.79 μ.

Reagents: Barbital-saline buffer (pH 7.4, isotonic) and de Jalon solution were prepared as previously described (17, 18). Soy bean trypsin inhibitor (SBTI) (Worthington Biochemical Corp., Freehold, N. J.) was prepared fresh in barbital-saline buffer. *o*-Phenanthroline (Fisher Scientific Co., Pittsburgh, Pa.) and ellagic acid (K & K Laboratories, Inc., Plainview, N. Y.) were made fresh daily by solution in barbital saline buffer. Sodium warfarin (Coumadin, Injectable, Endo Laboratories, Inc., Richmond Hill, N. Y.) was made fresh daily at a concentration of 200 μg per ml of 0.145 M sodium chloride.

Platelet-deficient plasma (human) was prepared from venous blood in equipment coated with silicone so that the plasma did not come into contact with glass (17).

Mouse plasma: Blood was drawn from the inferior vena cava into silicone-coated 1 cc plastic syringes after ether anesthesia and surgical exposure. The blood was anticoagulated with citrate (1 part 0.13 M citrate buffer pH 5.0 + 9 parts blood) in silicone coated tubes, and the plasma separated by centrifugation at 2000 *g* for 10 min (4°C). The plasma was transferred into silicone-coated tubes and used the same day. For use in clotting times, the plasma was mixed with 0.1 volume of 0.55% aluminum hydroxide gel, incubated at 22°C for 10 min, and then separated from the gel by centrifugation (4°C); this treatment depletes all known clotting factors except plasma thromboplastin antecedent (PTA) and Hageman factor when done to human plasma (17).

Clotting studies: Recalcified clotting times were performed in duplicate as follows: 0.1 ml of the test substance (see Tables I and II), 0.1 ml of the substrate plasma, and 0.1 ml of 0.025 M $CaCl_2$ were mixed in 10 $\times$ 75 mm polystyrene tubes (Falcon Plastics, Los Angeles, Calif.) in an ice water bath. The tubes were transferred to a 25°C or 37°C water bath, a clock was started, and then tilted at 1 min intervals; the clotting time was measured as that time when the fibrin first appeared. Prothrombin times were determined by the method of Quick et al. (19).

Kinin formation: The induction of kinin-like activity in normal human plasma was studied by the following procedure. 0.2 ml of platelet deficient plasma, 0.1 ml of the induction test substance, and 0.05 ml of *o*-phenanthroline (0.01 M) were mixed in polystyrene tubes (Falcon

Plastics, 10 × 75 mm). The final concentration of beads or eggs (test substance) was 1375/0.1 ml and 1750/0.1 ml, respectively. The tubes were allowed to stand at room temperature (22°C) for 5 min and then a 0.15 ml sample was tested in an isolated rat uterus preparation (20). The response was measured by using a linear transducer (Brush Instruments Div., Clevite Corp., Cleveland, Ohio, Model 33-03-981) and a Grass polygraph (Grass Instrument Co., Quincy, Mass., Model 5D). The de Jalon solution was heated to 29°C before entering the muscle bath (8 ml volume). The muscle was washed three times between contractions, allowing 5 min after the last wash until addition of the next test sample. Only a contraction occurring within 1 min after addition of the sample was considered a valid response. The uterus was isolated from virgin albino female rats (Sprague-Dawley strain), treated with a 1.0 ml subcutaneous injection of diethylstilbestrol (Eli Lilly and Co., Indianapolis, Ind., 100 μg/ml in olive oil) 18–24 hr prior to sacrifice. The uterus was stored less than 24 hr at 4°C in de Jalon solution.

Vascular permeability-enhancing activity: This activity was assayed by determining the average dimeter (mm) of the blue spot that developed within 15–30 min after the intradermal injection of 0.1 ml of the test substance into each of four guinea pigs previously injected intravenously with Pontamine Sky Blue (21).

In Vivo Studies.—

Granuloma formation in Swiss albino mice: Female mice (18–22 g, Carworth Farms, N. Y.) were injected via the tail vein with 5000 beads or 1000 eggs suspended in 0.5 cc of 0.9% sodium chloride. Fewer eggs were used since they were isolated in smaller numbers, yet similar results were obtained with 5000 eggs per mouse. At various time intervals after injection, groups of mice were anesthetized and 1 ml of 10% buffered formalin (pH 6.9–7.1, Fisher Scientific) was injected intratracheally into the lungs prior to removal. After fixation, histologic sections of the lungs were prepared (12). The diameter of each egg or bead examined and the associated inflammatory reaction were determined by measuring two perpendicular diameters for each with the image-splitting eyepiece. The mean diameter of the eggs alone, as measured in the tissue sections, remained constant, necessitating measurement of only the diameter of the lesion (egg and inflammatory reaction). The mean diameter of 100 such lesions, bead or egg, was calculated for each period.

After the mean granuloma diameter for each experimental group was determined, the sections were searched for a lesion with the same diameter as the mean for the group. This granuloma was photographed and presented as objectively representative of an average lesion. The mean lesion of the beads and of the beads plus host granulomatous reactions were calculated from their diameters, and the former was subtracted from the latter in order to determine the mean volume of host reactions for each time period.

Granuloma formation in white Carneau pigeons: Adult pigeons were injected with a suspension of 20,000 plastic beads or schistosome eggs via the wing vein. The pigeons were sacrificed by an intravenous overdose of pentobarbital and portions of the lungs were removed for histological examination.

RESULTS

In Vitro.—

The effect of divinyl benzene copolymer beads and schistosome eggs on the clotting time of human and mouse plasma: The beads diminished the recalcified clotting time of normal human plasma but had no detectable influence on the recalcified time of plasma from a patient with Hageman trait (Table I). Both the beads and ellagic acid enhanced the procoagulant effect of mouse plasma (treated

with $Al(OH)_3$) on the recalcified clotting time of Hageman-deficient plasma (Table II).

The minimal effect of the schistosome eggs on the recalcified clotting time of normal plasma compared to their more marked effect on Hageman-deficient plasma suggested that their effect on Hageman factor was minimal (Table I). The addition of schistosome eggs to mouse plasma previously incubated with $Al(OH)_3$ produced only a slight procoagulant effect on Hageman-deficient

TABLE I

Effect of Divinyl Benzene Copolymer Beads and Schistosome Eggs on Recalcified Clotting Time of Normal and Hageman Trait Platelet Deficient Human Plasma

Reagents	Clotting time*
	min
Beads:	
Normal human plasma + buffer	14.0, 12.0
Normal human plasma + beads‡	6.0, 6.0
Hageman trait plasma + buffer	>45, >45
Hageman trait plasma + beads	>45, >45
Eggs:	
Normal human plasma + buffer	14.5, 16.5
Normal human plasma + eggs§	11.0, 11.0
Normal human plasma + supernatant fluid of egg suspension	15.0, 16.5
Hageman trait plasma + buffer	24.0, 26.5
Hageman trait plasma + eggs§	15.5, 17.5
Hageman trait plasma + supernatant	38.0, 39.5

* Clotting times determined at 37°C in silicone coated tubes as described in text.

‡ The stock solution of beads was the same as that injected into the mice. Final concentrations of beads in the reaction mixture was 850/0.1 ml.

§ The final concentration of eggs was 3300/mm^3.

plasma (Table II) The supernatant barbital-saline buffer from the eggs prolonged the clotting time of Hageman-deficient plasma (Table I). This anticoagulant effect was also noted when the supernatant buffer was added to mouse plasma that had been incubated with $Al(OH)_3$ and then added to recalcified Hageman-deficient plasma (Table II).

The induction of kinin-like activity in plasma incubated with plastic beads or schistosome eggs: The addition of plastic beads to either normal human plasma or mouse plasma in the presence of *o*-phenanthroline induced kinin-like activity that could be eliminated by carboxypeptidase-B. Moreover, kinin-like activity did not develop (*a*) after the addition of beads to plasma from a patient with Hageman trait, (*b*) after the addition of eggs to normal human plasma, or (*c*)

after the separate addition of normal human plasma, normal mouse plasma, beads, or eggs with appropriate volume of buffer.

The induction of vascular permeability enhancing activity in plasma by divinyl benzene copolymer beads and schistosome eggs: Vascular permeability enhancing activity developed in mouse plasma and normal human plasma but not in

TABLE II

*Activation of Hageman Factor in Treated Mouse Plasma with Plastic Beads and Schistosome Eggs**

Reagents	Substrate plasma	Clotting time
		min
Beads:		
Plasma (treated with $Al(OH)_3$ + heat) + buffer	Hageman trait plasma	22.0, 23.5
Plasma (treated with $Al(OH)_3$ + heat) + beads‡	Hageman trait plasma	10.0, 11.5
Plasma (treated with $Al(OH)_3$ + heat) + ellagic acid§	Hageman trait plasma	5.0, 5.0
Ellagic acid + buffer	Hageman trait plasma	29.5, 29.5
Eggs:		
Plasma (treated with $Al(OH)_3$ + heat) + buffer	Hageman trait plasma	13.5, 15.0
Plasma (treated with $Al(OH)_3$ + heat) + eggs‖	Hageman trait plasma	12.0, 13.5
Plasma (treated with $Al(OH)_3$ + heat) + supernatant of eggs	Hageman trait plasma	19.0, 21.0

* After the mouse plasma was treated with $Al(OH)_3$ and heat, it was mixed with either buffer, beads, schistosome eggs, or supernatant fluid of the schistosome eggs and incubated at 37°C for 8 min. After this incubation 0.1 cc of Hageman trait plasma and 0.1 cc of .025 M $CaCl_2$ were added, mixed, and the clotting time determined at 37°C. The plasma used in the experiment recorded under "Beads" was not the same as that used in the experiments recorded under "Eggs"; this accounts for the different plasma plus buffer control values.

‡ The final concentration of the beads was 1250/0.1 cc.

§ The final concentration of ellagic acid was 3.3×10^{-5} M.

‖ The final concentration of eggs was 3300/ml.

Hageman factor-deficient human plasma incubated with either plastic beads or ellagic acid (Table III). The development of this activity was inhibited by soy bean trypsin inhibitor (SBTI). The incubation of schistosome eggs with normal human plasma may have resulted in the development of a small amount of permeability enhancing activity, but the supernatant fluid of the eggs alone produced increased vascular permeability in guinea pig skin that was not inhibited by SBTI; it was not possible, therefore, to determine if schistosome

eggs would activate Hageman factor-dependent permeability enhancing factors in human plasma.

In Vivo.—

Quantitative estimate of granulomatous response to the plastic beads in mouse lung: The average diameter of the granulomatous process associated with 100 plastic beads lodged in the pulmonary capillary bed was determined by sub-

TABLE III

Attempt to Induce Permeability Enhancing Activity in Mouse Plasma, Normal Human Plasma, and Hageman Trait Human Plasma

Reaction mixture*	Permeability enhancing activity (avg diameter of blue spot)
	mm
Mouse:	
Plasma + buffer	2.7
Plasma + beads	5.7
Plasma + ellagic acid	8.7
Normal human:	
Plasma + buffer	1.7
Plasma + beads	6.7
Plasma + ellagic acid	8.0
Plasma + eggs	3.4
Hageman trait:	
Plasma + buffer	1.0
Plasma + beads	1.0
Plasma + ellagic acid	1.7
Plasma + eggs	1.9

* The final dilution was 1:100. The final concentration of beads and ellagic acid was 75,000/ml and 2.5×10^{-5} M, respectively. The final concentration of eggs was 30,000/ml.

tracting the average diameter of the bead from the average diameter of the inflammatory lesion including the bead (Table IV). A significant inflammatory response, composed primarily of neutrophils, was detectable around the bead at 6 hr. The inflammatory reaction reached a maximum size at 48 hr (Table IV), at which time the volume was frequently more than 14 times the volume of the bead. The cellular reaction at 48 hr was composed primarily of neutrophils with a few scattered large mononuclear cells. Photographs representative of this reaction at 6 hr, 12 hr, 24 hr, and 2, 4, 8, and 32 days are shown in Fig. 2. During the 94 days after the peak reaction at 48 hr, the cellular reaction diminished in size until the volume was only about twice the volume of the bead (Fig. 2).

Failure to produce sensitization in mice to plastic beads: A preliminary intraperitoneal injection of plastic beads 2 wk prior to the intravenous deposition of beads in mouse lung did not enhance the inflammatory process (Fig. 1).

The effect of ellagic acid on the inflammatory granulomatous process associated with plastic beads: The intraperitoneal administration of ellagic acid to the mice beginning 1 hr before the injection of the beads and continuing every 6 hr until the time of sacrifice produced a marked reduction in the size of the inflammatory response (Table V). At 48 hr, when the reaction associated with the bead is ordinarily maximal (Table IV), the administration of ellagic acid

TABLE IV

Diameter and Volume of Granuloma Associated with Divinyl Benzene Copolymer Beads

Time	Granuloma + bead (mean diameter)	Bead* (mean diameter)	Granuloma (mean volume)
	μ	μ	$mm^3 \times 10^{-4}$
0	60.68 ± 1.59‡	57.52 ± 1.31‡	0.17
3 hr	61.62 ± 1.36	56.71 ± 1.05	0.23
6 hr	79.07 ± 1.87	55.91 ± 1.39	1.67
12 hr	82.18 ± 2.23	55.97 ± 1.14	1.99
24 hr	88.25 ± 3.30	50.80 ± 1.36	2.92
48 hr	127.95 ± 4.06	51.63 ± 1.26	10.25
4 days	119.71 ± 3.49	51.75 ± 0.94	8.26
8 days	102.21 ± 2.81	51.61 ± 1.16	4.87
16 days	92.23 ± 2.13	55.68 ± 1.41	3.20
32 days	91.12 ± 2.21	55.40 ± 1.28	3.07
64 days	86.48 ± 2.33	55.28 ± 1.19	2.51
96 days	79.29 ± 1.99	51.78 ± 1.10	1.88

* The mean volume of each mean bead diameter was calculated and varied from 0.72 to 1.0×10^{-4} mm^3.

‡ One standard error.

reduced the volume of this granuloma to less than 10% of what was expected (Fig. 2), a change that is highly significant statistically. In two groups of mice sacrificed 5 and 7 days after the beads were injected, the ellagic acid was administered for only the first 72 hr after the beads were injected, yet the volume of the granuloma did not increase after the drug was discontinued (Table V). Ellagic acid had no effect on the peripheral blood leukocyte count.

Effect of other inhibitors of inflammation on the inflammatory process associated with divinyl benzene copolymer beads: An antihistamine (Actidil) had no effect on the inflammatory process associated with plastic beads in mice (Table VI). Trasylol (bovine kallikrein inhibitor) and Liquoid (polyanetholsulfonate), a heparin-like reagent, produced a decrease in the inflammatory process (Table VI) nearly as striking as that associated with ellagic acid administration. These drugs also did not affect the total peripheral blood leukocyte count.

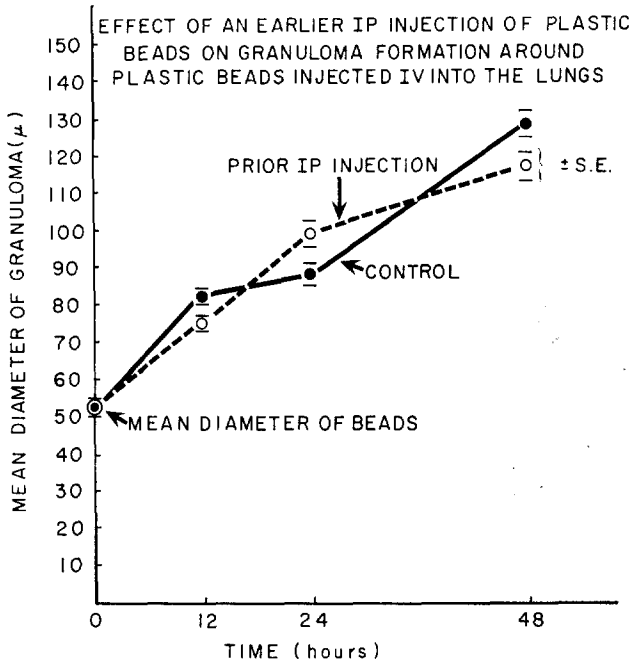

FIG. 1. The diameter of the granuloma that develops around plastic beads deposited in lungs of mice that had been given a single intraperitoneal injection of plastic beads 2 wk earlier is compared to the size of the granuloma that develops in mice not exposed to the beads previously.

TABLE V

*Effect of Ellagic Acid on the Diameter and Volume of the Granuloma Associated with Divinyl Benzene Copolymer Beads**

Time of sacrifice	Time duration ellagic acid administered	Granuloma + bead (mean diameter)	Bead‡ (mean diameter)	Granuloma (mean volume)
	hr	*μ*	*μ*	*mm³ × 10⁻⁴*
24 hr	24	73.76 ± 1.87§	58.61 ± 1.10§	1.05
48 hr	48	71.72 ± 1.81	50.24 ± 1.05	1.27
72 hr	72	83.57 ± 2.07	56.91 ± 1.47	2.09
5 days	72	82.17 ± 2.20	54.23 ± 1.40	2.07
7 days	72	78.01 ± 2.07	55.16 ± 1.42	1.61

* 100 granulomatous lesions and the enclosed bead were measured in each group of 6 mice.

‡ The mean volume of each mean bead diameter was calculated and varied from 0.66 to 1.05×10^{-4} mm³.

§ One standard error.

Cobra venom factor[1] (which deletes C3 in vivo) did not appear to influence the inflammatory process associated with plastic beads. At 48 hr the ratio of the volume of the mean granuloma size to the mean volume of the bead was

TABLE VI

*Effect of Actidil, Liquoid, and Trasylol on the Granulomatous Process Associated with Divinyl Benzene Copolymer Beads in Mouse Lung**

Concentration	Drug dosage	Granuloma + bead (mean diameter)	Bead (mean diameter)	Granuloma (mean volume)
		μ	μ	$mm^3 \times 10^{-4}$
Untreated	None	127.95 ± 4.06‡	51.63 ± 1.26‡	10.25
Actidil	0.1 ml i.v. 1 hr prior to beads and twice daily i.p. until sacrifice	136.45 ± 4.28	57.63 ± 1.21	12.30
Trasylol (5000 kallikrein units/ml	0.05 ml i.v. and i.p. 2 hr prior to beads and then 0.1 ml i.p. three times daily until sacrifice	87.66 ± 2.58	55.71 ± 1.31	2.63
Liquoid (0.8 mg/ml)	"	115.37 ± 3.26	54.34 ± 1.26	7.20
Liquoid (8 mg/ml)	"	77.02 ± 2.19	50.02 ± 1.41	1.74
Ellagic acid (2×10^{-4} M)	0.5 cc i.v. 2 hr prior to beads and twice daily until sacrifice	71.72 ± 1.81	50.24 ± 1.05	1.29

* All mice were sacrificed 48 hr after the plastic beads were injected and a total of 100 granulomatous lesions were measured in the lungs of each group of 6 mice to obtain the diameter and volume determinations.

‡ One standard error.

5.53 in six untreated mice and 5.82 in six mice treated with cobra venom factor prior to the injection of beads. The deletion of C3 was confirmed by detecting the altered C3 in the mouse serum obtained at the time of sacrifice. Further, the plastic bead granuloma that developed in mice congenitally deficient in

[1] The cobra venom factor used in these experiments was generously supplied by Dr. C. Cochrane of Scripps Institute.

TABLE VII

Inflammatory Response to Plastic Beads and Schistosome Eggs Injected into Pigeon Lung

Time of sacrifice	Number of lesions measured	Granuloma + bead or egg (mean diameter)	Bead or egg (mean diameter)	Granuloma (mean volume)
		μ	μ	$mm^3 \times 10^{-4}$
Beads:				
0	33	59.27 ± 2.28*	56.45 ± 1.96*	0.15
24 hr	13	61.88 ± 3.94	54.2 ± 3.43	0.41
48 hr	35	76.02 ± 2.88	56.53 ± 2.03	1.35
4 days	31	74.78 ± 3.04	58.04 ± 2.05	1.17
Eggs:				
8 days	100	164.94 ± 12.39	57.00 ± 1.00	23.50
8 days‡	100	211.77 ± 9.44	57.00 ± 1.00	49.73

* One standard error.

‡ 20,000 eggs had been injected intraperitoneally into each pigeon 10 days prior to the intravenous dose.

TABLE VIII

*Effect of Ellagic Acid, Liquoid, and Coumadin on the Schistosome Egg Granuloma in Mice**

Drug (concentration)	Dosage	Granuloma‡ + egg (mean diameter)	Granuloma + egg (mean volume)	Granuloma§ (mean volume)
		μ	$mm^3 \times 10^{-4}$	$mm^3 \times 10^{-4}$
None	—	139.27 ± 13.37	14.14 ± 4.07	13.49
Liquoid (8 mg/ml)	0.1 ml i.p. 1 hr prior to eggs and twice daily until sacrifice	99.17 ± 5.64	5.11 ± 0.87	4.26
None	—	148.36 ± 8.39	17.10 ± 2.90	16.15
Ellagic acid (2×10^{-4} M) pH 7.4 in PO_4 buffer	0.5 ml i.v. 2 hr prior to eggs and then four times daily i.p. until sacrifice	106.4 ± 5.63	6.31 ± 1.01	5.26
None	—	110.94 ± 5.44	7.15 ± 1.05	6.30
Coumadin (200 g/ml)	0.25–0.5 cc i.p. daily depending on prothrombin time (varied from 25 sec to 2½ min; control 17–18 sec	87.91 ± 4.20	3.56 ± 0.51	2.70

* 1000 eggs were injected intravenously via the tail vein and the mice were sacrificed 8 days later.

‡ The mean diameter was determined from the measurement of 100 eggs and 100 granuloma lesions in sections taken from each of 6 mice that formed a standard experimental group except in the first control experiment where only 14 lesions were measured.

§ Mean volume of schistosome egg was 0.95 $mm^3 \times 10^{-4} \pm 0.068$ (SE).

the fifth component of complement (C5) was comparable to that found in normal mice.

Anticoagulation with Coumadin did not influence the inflammatory response to the plastic bead. 48 hr after the plastic beads were intravenously administered to the mouse, the mean volume of the granuloma was 2.64×10^{-4} mm^3; the mean volume of the granuloma at 48 hr in mice anticoagulated with Coumadin was 2.50×10^{-4} mm^3. The mean volumes in both the experimental and the concomitant control group of mice were smaller than those usually obtained; the reason for this is not known, although this had been noted previously.

Inflammatory reaction associated with plastic beads or schistosome eggs in the lungs of pigeons: The inflammatory process around beads in the lungs of pigeons was very small (Table VII, Fig. 3). The maximal mean volume of the granuloma (48 hr) was smaller than that observed in mice treated with ellagic acid (Table V).

A definite inflammatory reaction had developed around the schistosome egg 8 days after the eggs were deposited in the lungs of pigeons (Table VII, Fig. 3); this reaction was enhanced by preliminary intraperitoneal inoculation of the pigeons with schistosome eggs (Table VII). In nonsensitized mice the inflammatory response to the schistosome egg usually reaches a peak value at 16 days.

Effect of ellagic acid, polyanethol sulfonic acid (Liquoid), and warfarin on the schistosome egg granuloma: Ellagic acid, polyanethol sulfonate, and warfarin diminished the inflammatory response associated with the schistosome egg to a volume about ⅓ of the control (Table VIII). The degree of suppression was not as great as that produced by ellagic acid toward the plastic bead granuloma (Table V) at a time when the maximum granuloma in response to the egg would be expected for each.

DISCUSSION

The inflammatory reaction associated with either the plastic bead or the schistosome egg in mouse lung varies with time. This report confirms the more rapid development of the plastic bead reaction compared to the egg (14) and, in addition, demonstrates the cellular inflammatory process to the bead well under way as early as 6 hr. The inflammatory response to the schistosome egg, however, is delayed and is still undetectable at 24 hr. The peak reaction associated with the bead occurs at 2 days, while the reaction associated with the schistosome egg does not reach a peak until 16 days (10, 14). Near the peak of the cellular accumulation associated with each, the cellular composition can be characterized as follows: the bead (2 days) is surrounded by neutrophils, and fewer mononuclear cells (15); the egg (16 days) is surrounded by round cells, mononuclear cells, epithelioid, giant cells, but fewer neutrophils than the plastic bead (10, 14). Thus the bead reaction reaches a peak size earlier, recedes more quickly, and contains relatively more neutrophils and fewer epithelioid

and giant cells than the schistosome egg granuloma. On this basis, the inflammatory response associated with the schistosome egg is comparable to a delayed hypersensitivity reaction, while the response to the plastic beads in mouse lung is comparable to a foreign body reaction.

Like other types of foreign body reactions, the reaction to the plastic bead is not enhanced by prior exposure to the beads. Furthermore, the reaction to the plastic bead is not influenced by manipulations that diminish delayed hypersensitivity (11–13). In contradistinction, both rapidity of development and the size of the schistosome egg granuloma are greatly enhanced by prior exposure of the animal to the egg (10), and the inflammatory response is markedly suppressed by neonatal thymectomy (12) or administration of anti-lymphocyte serum (11). The egg granuloma appears to be dependent on an immune mechanism that is cell mediated (10), but not on circulating antibodies (13).

Since immune mechanisms are not responsible for the inflammatory reaction associated with the plastic bead, consideration has been given to the direct activation of chemical mediators of inflammation, including histamines and kinins, by the surfaces of the foreign body or by direct tissue damage by the beads. Although all foreign substances deposited in the body induce an inflammatory reaction, the reaction varies with the substance (3, 7, 22, 23). Carbon dust produces little inflammation in experimental animals, in contrast to such substances as silicon dioxide and magnesium trisilicate (8, 23). These and other studies indicated the importance of the surface characteristics, the composition of the foreign material, and the size of the particle to the development of a foreign body reaction (22).

The addition of certain particulate substances to mammalian plasma will result in the formation of kinins and anaphylatoxin (24–27). The biological effects of anaphylatoxin include enhancement of vascular permeability in guinea pig skin and the release of histamine from rat peritoneal mast cell suspensions; both of these actions are effectively inhibited by antihistamines (28). Antihistamines, however, had no effect on the plastic bead reaction. Kinin formation, dependent on the activation of Hageman factor, can be initiated in human plasma by numerous surfaces, including glass, kaolin, magnesium trisilicate, and monosodium urate crystals (27, 29). Kinins are potent pharmacologic agents capable of influencing smooth muscle contraction, inducing hypotension, vasodilation, increased vascular permeability, and pain (24, 30–34). These polypeptides have been implicated in several acute inflammatory processes (29). The activation of kinins in plasma by certain particulate substances is dependent on the activation of Hageman factor, which in turn enzymatically initiates the activation of PF/dil and kallikrein; kallikrein enzymatically cleaves kinin from its precursor protein, kininogen (35, 36).

Plastic beads, like glass, magnesium trisilicate, urate crystals, and other substances, were capable of activating Hageman factor and initiating kinin-

like activity in human and mouse plasma. The plastic beads also appeared to activate Hageman factor in mouse plasma. In contradistinction, the addition of schistosome eggs to normal human plasma did not induce the formation of kinin-like activity. Although it appeared that the eggs failed to have a procoagulant effect on a mixture of heated and adsorbed mouse plasma (containing Hageman factor) and Hageman-deficient plasma, the results were not interpretable, since the supernatant wash fluid from the eggs had an anticoagulant effect.

Plastic beads deposited in the lungs of pigeons did not develop a significant infiltrate around the bead, whereas the schistosome egg induced a reaction similar to that seen in mammals. Pigeons, like other birds, lack Hageman factor (37), suggesting that Hageman factor is important in the development of the inflammatory process associated with the plastic bead.

In an attempt to define the molecular mechanism leading to the initiation of these two granulomatous processes, the effect of several inhibitors were tested against these inflammatory processes. The administration of ellagic acid to mice before and after the deposition of plastic beads in their lungs markedly diminished the inflammatory response without altering the peripheral leukocyte count. Recently, it has been demonstrated that the concentration of the parent molecule (kininogen), from which kinins are derived, can be diminished in vivo by the administration of ellagic acid or cellulose sulfate (38, 39). Both ellagic acid and cellulose sulfate activate the Hageman factor-kinin sequence (40–42), thereby resulting in a consumptive depletion of the kininogen. At a time when the kininogen is depressed, the animal is less susceptible to hypotension by substances that activate Hageman factor and induce kinin formation (38, 39).

Ellagic acid suppressed the size of the granuloma associated with the schistosome egg (as measured at 8 days), but not to the same marked degree as that associated with the plastic bead reaction (as measured at 48 hr). Since the schistosome egg granuloma has the pertinent characteristics of a hypersensitivity granuloma, the suppression by ellagic acid was unexpected. This observation suggests that kinins or components of the kinin generating system play a role in the development of inflammation initiated by an immunologic process, or that ellagic acid suppresses an inflammatory process in a nonspecific manner. Carageenan (polygalactose sulfate) is known to suppress the tuberculin reaction (47); various mechanisms have been proposed to explain this observation, including anticoagulation (48) and a toxic effect on monocytes (47).[2] Recently, cellulose sulfate, a polyglucose sulfate similar to polygalactose sulfate, has been demonstrated to activate Hageman factor in vitro (41) and reduce kininogen levels in vivo in experimental animals (39). Carageenan also activates Hageman factor and induces the formation of permeability-enhancing factors and kinins

[2] Cantanzaro, P., R. C. Graham, Jr., and H. J. Schwartz. Unpublished observations.

in normal and human plasma, but not in Hageman factor-deficient plasma (49). These parallels between compounds that activate Hageman factor, induce consumptive depletion of kininogen, and cause suppression of inflammatory processes associated with hypersensitivity states are of interest and further suggest that kinins may play some role in these processes.

The plastic bead granuloma was also diminished by the administration of Trasylol, an inhibitor of kallikrein derived from bovine parotid gland (43). Trasylol is not a specific inhibitor of the Hageman factor-kallikrein-kinin system since it also inhibits trypsin, chymotrypsin, and plasmin (44). Liquoid, an even less specific inhibitor, since it inhibits both the Hageman factor-kallikrein-kinin sequence and complement (42, 45), depressed the inflammatory reaction associated with both the plastic bead and the schistosome egg granuloma. Although the data obtained with these inhibitors are consistent with a possible role of kinins in either of the inflammatory reactions, the inhibitors are too nonspecific. More specific inhibitors would be of considerable value in assessing the role of these inflammatory mediators.

The third component of complement (C3) did not appear to play a role in the plastic bead granuloma, since the concurrent administration of purified cobra venom factor, which deletes this component in vivo (46), did not influence the inflammatory reaction. Further, the reaction to the plastic bead developed equally well in mice deficient in C5.

Anticoagulation (Coumadin) has been shown to suppress delayed hypersensitivity reactions (48). Coumadin did suppress the inflammatory response to the schistosome egg at 8 days (Table VIII), but it had no effect on the inflammatory response to the plastic bead at 48 hr.

None of the inhibitors currently available specifically suppress one or another biochemical pathway leading to the development of an inflammatory mediator. It is unlikely, however, that only one biochemical sequence of reactions supports the development of an inflammatory process. It is possible that numerous interdigitating pathways result in several mediators, each of which participates in the induction of an inflammatory process. Moreover, it may be possible to block one pathway for the formation of a chemical mediator only to have the same mediator formed by another pathway.

In an attempt to relate the information obtained in the study of the plastic bead foreign body response to a human disease process, preliminary investigations were initiated to determine if the time course, presence or absence of hypersensitivity, and the influence of ellagic acid in experimental silicosis (silica) and "blue velvet disease" (magnesium trisilicate) were similar to the plastic bead granuloma. It has been proposed previously that silica and magnesium trisilicate might induce the inflammatory process in silicosis and "blue velvet disease" by activating Hageman factor and initiating the formation of kinins (27). It has also been determined previously that both of these particulate

substances can induce kinin formation that is dependent on the activation of Hageman factor (27).

Preliminary data indicate that the deposition of silica particles in the alveoli or magnesium trisilicate in the microvasculature of the lung result in an inflammatory response similar to the plastic bead that is maximal at 48 hr.[3] Ellagic acid also produces a marked suppression of these inflammatory responses.[3] These data support the idea that both silicosis and blue velvet disease are nonimmunologic foreign body reactions and suggest a biochemical mechanism similar to the one proposed for the initiation of the plastic bead granuloma.

SUMMARY

Both divinyl benzene copolymer (plastic) beads and schistosome eggs produce inflammatory reactions after intravenous deposition into the lung of a mouse. As reported previously, the schistosome egg granuloma is an immunologic reaction of the delayed hypersensitivity type; this inflammatory process is prevented by immunosuppressive measures, and characteristically demonstrates an anamnestic response. In contradistinction, the plastic bead granuloma appears to be characteristic of a foreign body reaction; it is unaffected by immunosuppressive measures and does not demonstrate an anamnestic response with repeated exposure. The data in this report suggest that the granuloma formation around plastic beads is a nonimmunologic reaction induced by chemical mediators of inflammation. This proposal is supported by the following findings: the plastic beads activate Hageman factor in normal human and mouse plasma; the plastic beads induce vascular permeability-enhancing activity as measured in guinea pig skin and kinin-like activity in normal human and mouse plasma that is dependent on Hageman factor; ellagic acid, an agent that activates Hageman factor in vivo and is reported to diminish kininogen by consumptive depletion, markedly depresses the plastic bead granuloma. These data are consistent with the idea that the plastic bead granuloma and perhaps other foreign body inflammatory reactions are in major part dependent on kinin formation.

Ellagic acid also suppressed the schistosome egg granuloma, but not to the same degree as the plastic bead granuloma. The implications of this observation are discussed in the text.

Silicosis and "blue velvet disease", pathologic processes associated with the deposition of silica and magnesium trisilicate, respectively, in the lung, and the induction of a foreign body reaction may also be dependent on the activation of chemical mediators of inflammation by the silica and magnesium trisilicate particles with immunologic mechanisms participating in only a minor way, if at all. The marked suppression of experimental silicosis and blue velvet disease in mice by ellagic acid supports this idea.

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[3] Kellermeyer, W. F., Jr., R. W. Kellermeyer, and K. S. Warren. Unpublished observations.

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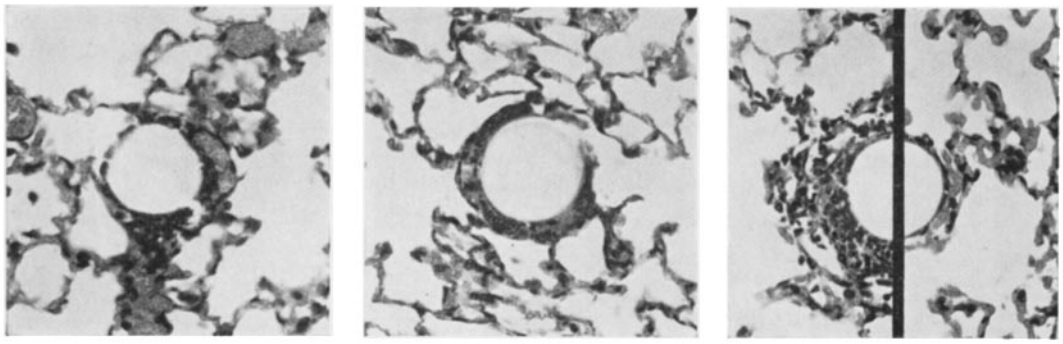

FIG. 2a

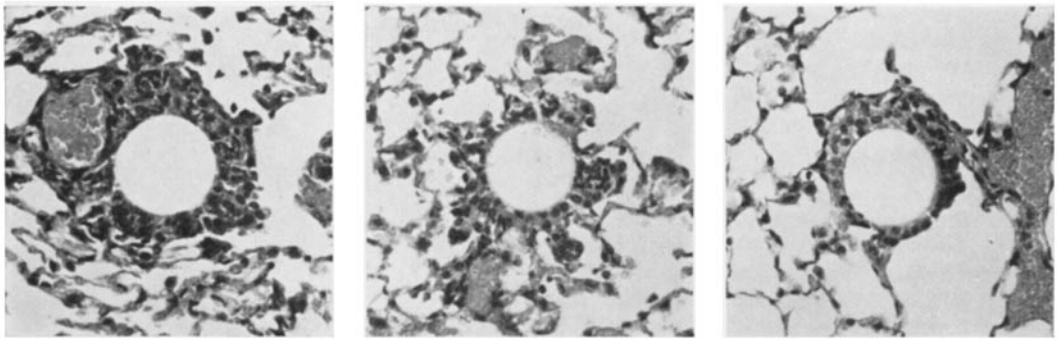

FIG. 2b

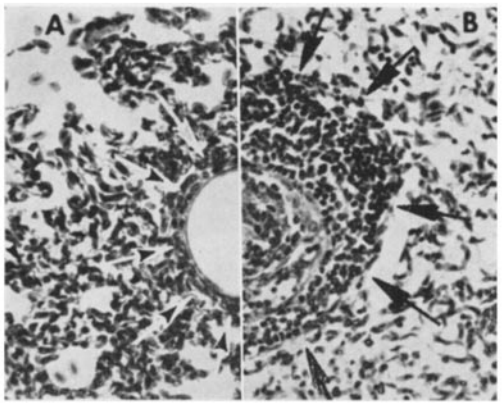

FIG. 3

FIG. 2. These are representative photographs of the granuloma associated with the plastic bead deposited in lungs of mice at 6, 24, and 48 hr and 4, 8, and 32 days. Representation of the peak granuloma size at 48 hr is shown on the left hand side of the split composite in the upper right of this figure and is labeled control, 48 hr; the right hand side of this composite is representative of the markedly diminished inflammatory response to the bead in mice treated with ellagic acid. × 250.

FIG. 3. This figure shows a representative composite photograph of the inflammatory reaction associated with the plastic beads (A) injected into the lungs of pigeons at 48 hr and the reactions around the schistosome eggs at 8 days (B). The reaction to the bead has reached a maximum and is negligible compared to the reaction to the egg which is not expected to reach its maximal size until 16 days. The limits of the reaction to the bead are indicated by the white and black arrows, and the limits of the reaction to the schistosome egg by the black arrows. × 250.