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Evidence for the involvement of the IgE-basophil system in acute serum sickness

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SUMMARY

The role of the basophils in acute serum sickness of rabbits was examined by monitoring daily the absolute number of basophils before, during and after the disease period. After antigen (bovine serum albumin, BSA) elimination, levels of serum IgE and *in vitro* basophil degranulation in the presence of BSA were determined. The results showed that the onset of glomerular lesions depends upon the simultaneous occurrence of circulating immune complexes greater than 19 S and of an *in vivo* basophil depletion—probably equivalent to degranulation—reaching 70% of the pre-disease number. Post-disease antigen-dependent *in vitro* degranulation of the basophils and levels of serum IgE anti BSA did not prove to be good indexes of basophil sensitization. Our data suggest that basophils are instrumental at early stages of the deposition of immune complexes, most probably through their sensitization by membrane-bound IgE antibodies.

INTRODUCTION

The deposition of immune complexes (IC) in the blood-vessel walls in rabbit acute serum sickness involves an increase in vascular permeability induced by the release of vasoactive amines from the platelets (Kniker & Cochrane, 1968). Complement depletion does not inhibit IC deposition (Henson & Cochrane, 1971; Rhyne & Germuth, 1961 which suggests that a leucocyte-dependent mechanism (leucocyte-dependent histamine release, LDHR) is responsible for the release of platelet vasoactive amines. LDHR was described in 1966 (Barbaro & Zvaifler, 1966) and later shown to require leucocytes and act independently of complement (Schoenbechler & Barbaro, 1968; Siraganian & Osler, 1969, Henson, 1970). Our study concerning the LDHR reaction (Benveniste, Henson & Cochrane, 1972) indicated (1) that the antibody responsible was of the IgE class (2) the leucocyte involved was the basophil, as shown by electron microscopy, and (3) upon addition of the specific antigen, IgE-sensitized basophils degranulated and released histamine and a platelet-activating factor (PAF); PAF in turn caused platelets to aggregate and release their histamine content. A positive correlation was found between occurrence of the lesions of acute serum sickness and presence of the LDHR reaction (Henson & Cochrane, 1971). However, direct evidence for IgE-basophil-PAF involvement in acute serum sickness is lacking.

Because of the presence of circulating antigen BSA in great excess, it is not possible to assess basophil sensitization by a direct method during the disease period. However, we postulated that, should basophil sensitization occur before or during the onset of lesions, it would result in *in vivo* degranulation, detectable by monitoring daily the absolute number of granulated circulating basophils. We therefore developed a one-step simple technique for staining specifically the basophils in whole blood. We also applied this technique to the detection of *in vitro* basophil degranulation in the presence of BSA (Benveniste *et al.*, 1976), after its elimination from the circulation.

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In this paper, we will show that reduction in basophil counts (BCR) occurs 48 hr before the onset of proteinuria in acute serum sickness. This reduction, most probably reflecting *in vivo* degranulation, appears to be a prerequisite for the occurence of kidney lesions

MATERIALS AND METHODS

Induction of serum sickness. New Zealand white rabbits weighing 1·5–2·5 kg were employed. Serum sickness was produced by a single i.v. injection of BSA, labelled with ^{125}I (McConahey & Dixon, 1966), at a dose of 250 mg/kg body weight (Germuth, 1955). To enhance the incidence of disease, four immunization schedules were used. In schedules A and B, respectively, 10 mg/kg of BSA in Freund's complete adjuvant (P. H. Lambert, personal communication) and 1 mg/kg of BSA in aluminium hydroxyde gel (Levine & Vaz, 1970) were injected s.c. 3 days before the large dose of BSA. In schedule C, rabbit anti-BSA antiserum (5 mg of antibody N/kg) was administered i.v. 24 hr before the antigen injection (Kniker & Cochrane, 1965). In schedule D, 5·50 mg/kg of heat-aggregated BSA were injected simultaneously with the large dose of BSA. Circulating BSA levels were monitored by performing daily determinations of the plasmatic radioactivity in a well-type scintillation counter.

Study of the circulating immune complexes (IC). Circulating IC were assayed by measuring the amount of [^{125}I]BSA precipitated from the plasma by 50% ammonium sulphate at 4°C. The precipitates were washed twice with 50% ammonium sulphate, counted, and the results were expressed as per cent of the total daily radioactivity. The size of the circulating complexes was estimated by ultra-centrifugation at 280·000*g* for 14 hr in a 10–37% sucrose gradient (Cochrane & Hawkins, 1968).

Staining and counting of basophils. Basophils were counted on the basis of metachromatic staining of their cytoplasmic granules by toluidine blue. The staining solution was prepared as described elsewhere (Benveniste *et al.*, 1976, *J. of Allergy*, in press).

Basophil counts were performed as follows: 10 μl of heparinized (10 u heparin/ml) blood obtained from the central ear artery were mixed with 90 μl of staining solution. After gentle shaking, leucocytes and basophils were counted in a Malassez chamber. With this technique, the only cells with stained cytoplasm were the basophils, which appeared red-brown against a clear background. The nuclei of the other leucocytes stained light blue; thus, these cells could be easily identified and counted. The total number of leucocytes and the absolute number of basophils were measured daily beginning 7 days before the induction of serum sickness. Rabbits with grossly irregular blood-cell counts were discarded from the study. All the chambers were counted 'blindly' since tape was placed over the etched labels.

For each rabbit, the daily total leucocyte and basophil counts were compared to the mean level determined for the 7 days preceding the injection of the large dose of BSA. Percentage of RBC was calculated using the formula:

$$\frac{\text{mean counts before antigen injection} - \text{daily counts}}{\text{mean counts before antigen injection}} \times 100.$$

In vitro basophil degranulation (IVD). Heparinized blood was obtained at intervals before, during, and several times after the occurrence of serum sickness. Antigen, usually 200 μg BSA in saline, but in some experiments doses ranging from 20–500 μg, was added to 0·5 mg of blood. A control tube containing an unrelated antigen (peroxidase) was always used at the same weight concentration as the BSA. The tubes were covered, placed on a rotating mixer for 1 min at room temperature, and incubated in a 37°C water bath for 15 min. After incubation, 25 μl of 0·2 M ethylene diamine-tetra-acetic-acid (disodium and tetrasodium salt) pH 7·4 were added to stop the reaction, and the cells were resuspended on the rotating mixer until they appeared evenly dispersed at the bottom of the tubes. All samples were counted twice by the method described above. The percentage of basophil degranulation was calculated using the following formula:

$$\frac{\text{Basophil n° in control sample} - \text{basophil n° in test sample}}{\text{Basophil n° in control sample}} \times 100.$$

Only degranulation above 30% was considered as positive.

Homologous passive cutaneous anaphylaxis (HoPCA). Serum IgE specific for BSA was determined as published by Ovary (1964). Sera were obtained at various intervals during the course of serum sickness. One-tenth ml of undiluted and two-fold diluted sera were injected i.d. in 2·0–3·0 kg rabbits. Seventy-two hr after sensitization, 1·0 ml of 2·5% Evans blue/kg and 20 mg BSA in 1 ml of saline were injected i.v. The HoPCA reactions were observed from 5–30 min thereafter, and the blueing areas which measured 5 mm or more in diameter were considered as a positive reaction. One rabbit was injected i.d. with undiluted sera but challenged with 20 mg of peroxidase as a control. The size and intensity of the blueing areas were recorded and the positive results expressed from + to +++.

Measurement of proteinuria. Following the induction of sickness, 24-hr urine samples were collected and their protein concentration assessed by the Biuret method.

RESULTS

Comparison between schedules of immunization

Schedules A and B resulted in higher incidence of proteinuria compared to schedules C and D

TABLE 1. Comparison between immunization schedules in rabbits with proteinuria

Schedules*	Rabbits with proteinuria		Maximal BCR†	Duration of BCR in days	Maximal levels of circulating IC‡
	No/total	Percentage			
A	6/8	75	67·5±6·6¶	1·7±0·5	46·7±2·7
B	10/13	77	72·5±2·9	3·2±0·4	42·3±4·6
C	2/5	40	70·0±5·0	1·0±0·0	33·0±5·2
D	1/4	25	44·0	1	29·0

* See the Materials and Methods section.
† BCR: expressed as percent of the mean individual values during the pre-disease period.
‡ Expressed as percent of total daily plasmatic radioactivity.
¶ Mean±s.e.m.

(Table 1). In rabbits with proteinuria, the BCR were the same for schedules A, B and C; however, the duration of the BCR was longer in rabbits immunized by schedule B. The levels of circulating IC were about the same for all the schedules.

In the case of rabbits without proteinuria (Table 2), high BCR were noted in one animal from group A and group B. The other three rabbits in groups A and B had no BCR. The levels of IC were high in the five rabbits in groups A and B, but the IC were smaller than 19 S for three animals. No BCR could be detected in two-thirds of the animals in group C, and the third animal displayed a moderate BCR; in this group the levels of IC were widely dispersed. Schedule D resulted in moderate BCR and very low levels of IC.

Relationship between basophil counts, amount and size of immune complexes and proteinuria

The results obtained from all the experimental animals, irrespective of the immunization schedule, could be arranged into 4 groups (Table 3). Group I comprised the animals that showed glomerulonephritis. In this group, it was noticeable that the BCR and the amount and size of IC were high. Groups II,

TABLE 2. Comparison between immunization schedules in rabbits without proteinuria

Schedules*	Rabbits without proteinuria		Maximal BCR†	Duration of BCR in days	Maximal levels of circulating IC‡
	No/total	Percentage			
A	2/8	25	28¶	0	52·4§
			100	3	37·0§
B	3/13	23	0	0	57·6
			28	0	53·4
			78	4	40·9§
C	3/5	60	16	0	41·0
			24	0	27·5
			39	1	12·9
D	3/4	75	38	2	5·0
			48	2	6·9
			61	1	23·6

* See the Materials and Methods section.
† BCR: expressed as percent of the mean individual values in the predisease period
‡ Expressed as percent of total daily plasmatic radioactivity.
¶ Individual values.
§ Immune complexes < 19 S.

TABLE 3. BCR, amount and size of IC in rabbits with or without proteinuria

Groups	No of rabbits	Proteinuria	Maximal BCR*	Circulating IC	
				Maximal levels†	Size
I	18	+	70·4±2·7	42·9±2·7	>19 S
			$P<0·001$‡	n.s.	
II	5	–	20·0±3·0	44·2±4·7	>19 S
			$P<0·001$	n.s.	
III	3	–	77·7±11·6	38·9±3·6	<19 S
			$P<0·05$	$P<0·01$	
IV	4	–	29·7±10·4	7·7±2·7	n.a.

n.s. = Not significant; n.a. = not applicable.

* Expressed as percent of the mean individual values in the predisease period. Mean ± s.e.m.

† Expressed as percent of total daily plasmatic radioactivity. Mean±s.e.m.

‡ Student's *t*-test.

III and IV included rabbits without disease. Group II had as much IC, which were heavier than 19 S, but no BCR. Group III had high BCR and high amount of IC, but the size of the complexes was smaller than 19 S. Group IV showed moderate BCR and virtually no circulating IC.

Time course of reduction in basophil counts in rabbits with and without proteinuria

The onset of the BCR was detected on the 7th day after injection of the large dose of BSA in groups I, III and IV. The BCR reached its maximum immediately and its duration was about the same in groups I and III (2·5±0·4 and 2·7±0·9 days) but shorter in group IV (1·7±0·3 days).

There was a moderate drop in total leucocyte counts, reaching 35% beginning the 7th day and lasting 4 days. There was no statistical differences between the 4 groups with respect to this total leucocyte drop.

Relationship between maximal BCR and the onset and amount of proteinuria

The mean starting day of proteinuria for group I was 10·0±0·6; the mean interval between the beginning of BCR and subsequent occurrence of proteinura, calculated for individual rabbits was 2·2±0·3 days (mean±s.e.m.). No statistical correlation was found between maximal BCR and amount of proteinuria for the rabbits in group I.

IVD (Table 4)

No IVD was obtained as long as [^{125}I]BSA was detectable in the circulation. Fourteen of the twenty-one rabbits with BCR (groups I+III) showed *in vitro* degranulation within 20 days following the elimination of more than 99% of the circulating BSA. Two types of responses were noted: for six rabbits, the maximal *in vitro* degranulation (between 30 and 67%) was found 1–4 days after antigen elimination; the responses disappeared after a few days. In the other type of response the maximal degranulation was obtained later, on the 20th day, with most values over 85%. Of the seven rabbits in groups I+III which showed no IVD, three were followed until the 20th day, whereas the other four were followed until the 4th day only. One of the nine rabbits with no BCR (groups II+IV) showed *in vitro* degranulation which reached 50% on the 20th day.

Serum IgE anti BSA

The levels of serum IgE anti BSA antibodies (sIgE) determined by the HoPCA test, were very erratic from one animal to the other. The sera from 8/18 rabbits in group I were positive. None of the five rabbits in group II, 1/2 and 0/1 tested from groups III and IV, respectively, were positive.

TABLE 4. BCR during the disease period and IVD in the presence of BSA after the disease period

Groups*	No. of rabbits	Maximal BCR‡	Maximal IVD§
I	18	70·4±2·7 (18)‖ $P<0·001$¶	46·3±7·3 (12) $P<0·2$
II	5	20·0±3·0 (0) $P<0·001$	23·4±8·3 (1) n.s.
III	3	77·7±11·6 (3) $P<0·05$	34·7±6·2 (2) $P<0·001$
IV	4	29·7±10·4 (3)	0 (0)
I+III	21	71·5±2·7 (21) $P<0·001$	44·6±6·3 (14) $P<0·01$
II+IV	9	24·3±4·9 (3)	13·0±6·0 (1)

* As defined in Table 3.

‡ Expressed as percent of the mean individual values in the predisease period.

§ Expressed as percent basophils degranulation after exposure to BSA. See material and methods.

‖ Mean±standard error of the mean (number of rabbits above 30%)

¶ Student's *t* test. n.s., not significant.

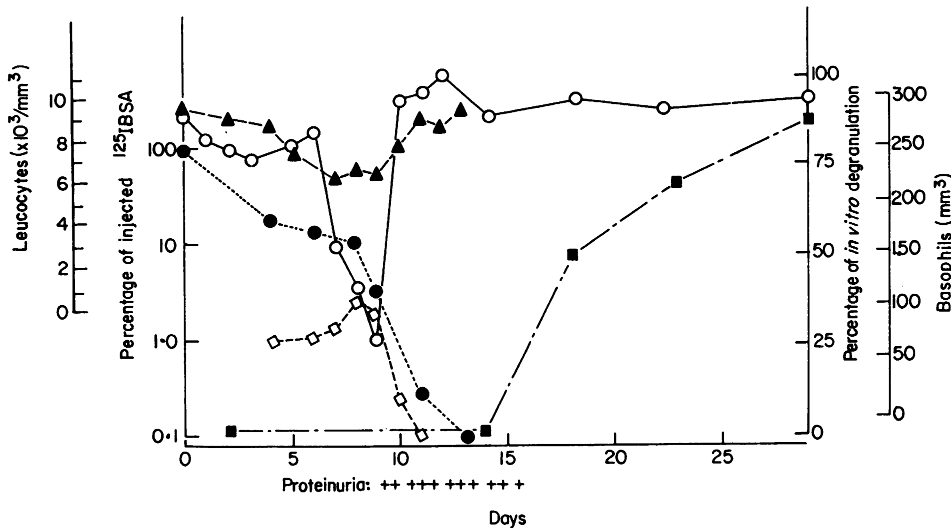

FIG. 1. Features of acute serum sickness in a typical rabbit. A large dose of ^{125}I BSA was injected at day 0. Total leucocyte number (▲– – –▲); ^{125}I BSA total (●· · ·●); ^{125}I BSA immune complexes (□– – –□); *in vitro* a degranulation (■– – –■); number of basophils (○———○).

Lack of correlation between RBC, IVD, and sIgE

No correlations were noted between BCR and IVD, BCR and sIgE, and IVD and sIgE in individual rabbits. However, the mean IVD was lower in rabbits with no or moderate BCR (groups II+IV) when compared to rabbits with high BCR (groups I+III) (Table 4). The same result was noted for the circulating IgE anti BSA antibodies: positive rabbits belonged to the BCR positive groups.

Fig. 1 shows the features for a typical rabbit undergoing acute serum sickness.

DISCUSSION

The data presented herein support the hypothesis that basophils are involved in the deposition of IC in acute serum sickness of rabbits. It appears that at least three conditions are required for the onset of glomerular injury in this model. (1) the presence of circulating IC, reaching about 40% of total plasmatic radioactivity between the 7th and the 10th days after the injection of BSA. (2) IC bigger than 19 S in size. These results are in agreement with previously published observations (Cochrane & Hawkins, 1968). (3) The degranulation of basophils 2 days before the onset of proteinuria. Rabbits showing any two of these conditions but not the third do not exhibit glomerular injury. Several questions can be raised in regard to our results:

Is the reduction in basophil counts equivalent to in vivo degranulation? The technique which we used to detect basophils is based on the staining of their cytoplasmic granules. This does not give any information on the presence or absence of degranulated basophils. However disappearance of the basophils from the peripheral blood could result from the sequestration of either degranulated or undegranulated cells. Therefore, this question may not receive a simple definitive answer. Other alternatives to degranulation could be either reduced basophil production by the bone marrow, which is unlikely or a peripheral destruction of basophils. The fact that total leucocyte numbers do not vary from one group to another reinforces the concept that the basophils are involved specifically and not merely as a component of the inflammatory cell group. When rabbits which have produced IgE antibody against a given antigen are injected with small amounts of the same antigen, there is a very great reduction in basophil counts that reaches 100% within a few minutes (Halonen & Pinckard, 1975, and our unpublished observations). It is very likely that we are dealing with the same phenomenon in the rabbits undergoing serum sickness; in other words, these animals could be in chronic anaphylactic shock during the onset of the disease.

What is the mechanism of basophil degranulation? Two immunological mechanisms have been described that are able to trigger *in vitro* basophil/mastocyte degranulation: the reaction of antigen with membrane-bound IgE antibody (Osler, Lichtenstein & Levy, 1968; Benveniste, Henson & Cochrane 1972) and the action of anaphylatoxins C3a and C5a (Mota, 1959; Lepow *et al.*, 1970). It must be noted that the generation of anaphylatoxins in rabbit serum has not been described. In our experiments, the presence of IC in the circulation could activate the complement sequence and generate C3a and C5a. This does not seem likely for several reasons. (1) During the course of the disease, small soluble complexes in antigen excess are produced and these are relatively inefficient in triggering complement activation (Henson & Cochrane, 1969). (2) Once deposited, IC may undergo redistribution of their antigen-antibody ratios towards equivalence or antibody excess. Anaphylatoxin production may then be triggered. This hypothesis is not compatible with previous findings which showed that the deposition of IC occurs as well in decomplemented rabbits as in intact animals (Henson & Cochrane, 1971; Rhyne & Germuth, 1961). (3) All experiments showing an effect of anaphylatoxins on basophils or mast cells have been made on washed cells or by intradermal injections (Mota, 1959; Lepow *et al.*, 1970; Grant *et al.*, 1975). If the anaphylatoxin inactivator described in human plasma (Bokisch & Müller-Eberhard, 1970) exists in rabbits, activation of basophils by anaphylatoxin should not take place in the vascular space. The activation of human basophils by a factor generated in whole human serum by 10 mg/ml IC at equivalence has been recently reported (Grant *et al.*, 1975). However, these conditions cannot be considered likely to occur in physiological or pathological states. (4) In our experiments, the five rabbits belonging to group II (Table 3), with no proteinuria, have the same level and size of circulating IC as the rabbits in group I. Therefore, the presence of circulating IC is not a sufficient condition for triggering basophil degranulation. This is in agreement with the well-documented inability of passively administered IC and macromolecules to deposit unless an increase in vascular permeability is experimentally induced (Benacerraf, McCluskey & Patras, 1959; Cochrane, 1963). From this discussion, it seems likely that the rabbits undergo basophil degranulation through mechanisms involving the production of IgE antibodies, their fixation onto basophils and subsequent degranulation of the cells in the presence of the antigen. But, as yet, we have no direct proof that this occurs.

Is basophil degranulation instrumental in IC deposition and glomerular injury? Our observations show that basophil degranulation is well correlated with the occurrence of glomerular injury, exhibited by the proteinuria. This result is in accord with previously published experiments on the role of vasoactive mediators on IC deposition in vessel walls (Kniker & Cochrane, 1968). The possibility that basophil degranulation and the resulting increase in permeability might create proteinuria, is ruled out by the existence of rabbits showing basophil degranulation without proteinuria (group III). However, it is impossible to exclude totally that basophil degranulation and IC deposition are both the result of an undescribed phenomenon and have no causal relationship with each other.

Relationship between serum IgE and in vivo and in vitro basophil degranulation. In individual rabbits, there is no relationship between the level of serum IgE, the degranulation during the disease period (BCR) and the post-disease IVD in the presence of the antigen. This is only an apparent paradox if one takes into account the variability of the mechanisms which lead to basophil sensitization and degranulation. First, the presence of detectable serum IgE is not necessary for full sensitization of the basophils (Benveniste *et al.*, 1972; Benveniste *et al.*, 1976). Secondly, some animals probably start producing IgE antibodies during the disease period, but this production may be quickly shut off when IgG antibodies are produced (Okumura & Tada, 1971); therefore, the basophils may be sensitized only weakly or even not at all after antigen elimination. However, in spite of the absence of correlation in individual rabbits between BCR, IVD and serum IgE, there were more rabbits with serum IgE and IVD in the BCR positive than in the BCR negative groups.

Conditions for in vivo activation of the IgE-basophil system. If the IgE-basophil system plays a role in the deposition of IC, there remain many questions on how it could operate *in vivo*. Among them at least two are of particular interest: what are the mediators released by the degranulating basophils, and the haemodynamic conditions that favour the pathological manifestations of this degranulation? Basophils sensitized with IgE antibodies release numerous mediators upon exposure to antigen *in vitro* at 37°C in the presence of calcium. These mediators are generally described in the literature as instrumental in the onset of local and general manifestations of immediate hypersensitivity (Becker, 1971). The existence of a soluble factor capable of releasing histamine from rabbit platelets was reported in 1969, and was regarded to derive from mononuclear leucocytes (Henson, 1970). This factor was described to be 'considerably labile' which makes its identity with the newly described platelet activating factor (PAF) rather unlikely. Evidence for the implication of basophils in allergic release from platelets and the existence of a soluble factor released by the degranulating basophils was published later (Siraganian & Osler, 1971a; Siraganian & Osler, 1971b; Benveniste, Henson & Cochrane, 1972); some characteristics of the release and recovery of PAF were described by us (Benveniste *et al.*, 1972). We have subsequently described the nature of PAF, a phospholipid, its presence in human leucocytes (Benveniste, 1974), and its probable involvement in immunopathology in humans (Benveniste, Le Couedic & Kamoun, 1975; Benveniste, 1975). However, to date, there is no direct experimental proof of a role for this factor in immunological diseases. Nevertheless, PAF represents a possible link between the allergic degranulation of basophils and the well noted, but rather poorly explained, involvement of platelets in a number of immunological diseases with severe structural injury.

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REFERENCES

Barbaro, J.F. & Zvaifler, N.J. (1966) Antigen-induced histamine release from platelets of rabbits producing homologous PCA antibodies. *Proc. Soc. exp. Biol.* (*N.Y.*) **122**, 1245.

Becker, E.L. (1971) Nature and classification of immediate-type allergic reactions. *Advanc. Immunol.* **13**, 267.

Benacerraf, B., McCluskey, R.T. & Patras, D. (1959) Localization of colloidal substances in vascular endothelium. A mechanism of tissue damage. I. Factors causing the pathogenic deposition of colloidal carbon. *Amer. J. Path.* **35**, 75.

Benveniste, J. (1974) Platelet-activating factor, a new mediator of anaphylaxis and immune complex deposition from rabbit and human basophils. *Nature* (*Lond.*), **249**, 581.

Benveniste, J. (1975) Passage of immune complexes through vascular walls. Evidence for the role of an immediate hypersensitivity mechanism and its mediator: platelet

activating factor. *Immunological Aspects of Rheumatoid Arthritis.* Karger, Basel.

BENVENISTE, J., HENSON, P.M. & COCHRANE, C.G. (1972) Leukocyte-dependent histamine release from rabbit platelets: the role of IgE, basophils and platelet-activating factor. *J. exp. Med.* **136**, 1356.

BENVENISTE, J., LE COUEDIC, J.P. & KAMOUN, P. (1975) Aggregation of human platelets by platelet-activating-factor. *Lancet*, **i**, 344.

BENVENISTE *et al.* (1976) *J. Allergy*, (In press.)

BOKISCH, V.A. & MÜLLER-EBERHARD, H.J. (1970) Anaphylatoxin activator of human plasma: its isolation and characterization as a carboxypeptidase. *J. clin. Invest.* **49**, 2427.

COCHRANE, C.G. (1963) Studies on the localization of circulating antigen-antibody complexes and other macromolecules in vessels. I. Structural studies. *J. exp. Med.* **118**, 503.

COCHRANE, C.G. & HAWKINS, D. (1968) Studies on circulating immune complexes. III. Factors governing the ability of circulating complexes to localize in blood vessels. *J. exp. Med.* **127**, 137.

GERMUTH, F.G., Jr (1955) A comparative histologic and immunologic study in rabbits of induced hypersensitivity of the serum sickness type. *J. exp. Med.* **97**, 257.

GRANT, J.A., DUPREE, E., GOLDMAN, A.S., SCHULTZ, D.R. & JACKSON, A.L. (1975) Complement-mediated release of histamine from human leukocytes. *J. Immunol.* **114**, 1101.

HALONEN, M. & PINCKARD, R.N. (1975) Intravascular effects of IgE antibody upon basophils, neutrophils, platelets and blood coagulation in the rabbit. *J. Immunol.* **115**, 519.

HENSON, P.M. (1970) Release of vasoactive amines from rabbit platelets induced by sensitized mononuclear leukocytes and antigen. *J. exp. Med.* **131**, 287.

HENSON, P.M. & COCHRANE, C.G. (1969) Immunological induction of increased vascular permeability. II. Two mechanisms of histamine release from rabbit platelets involving complement. *J. exp. Med.* **129**, 167.

HENSON, P.M. & COCHRANE, C.G (1971). Immune complex disease in rabbits. The role of complement and of a leukocyte-dependent release of vasoactive amines from platelets. *J. exp. Med.* **133**, 554.

KNIKER, W.T. & COCHRANE, C.G. (1965) Pathogenic factors in vascular lesions of experimental serum sickness. *J. exp. Med.* **122**, 83.

KNIKER, W.T. & COCHRANE, C.G. (1968) The localization of circulating immune complexes in experimental serum sickness. The role of vasoactive amines and hydrodynamic forces. *J. exp. Med.* **127**, 119.

LEPOW, I.H., WILLMS-KRETSCHNER, K., PATRICK, R.A. & ROSEN, F.S. (1970) Cross and ultrastructural observations on lesions produced by intradermal injection of human C3a in man. *Amer. J. Path.* **61**, 13.

LEVINE, B.B. & VAZ, N.M. (1970) Effect of combinations of imbred stain, antigen, and antigen dose on immune responsiveness and reagin production in the mouse. *Int. Arch. Allergy*, **39**, 156.

MCCONAHEY, P.J. & DIXON, F.J. (1966) A method of trace iodination of proteins for immunologic studies. *Int. Arch. Allergy*, **29**, 185.

MOTA, I. (1959) The mechanism of action of anaphylatoxin. Its effect on guinea pig mast cells. *Immunology*, **2**, 403.

OKUMURA, K. & TADA, T. (1971) Regulation of homocytotropic-antibody formations in the rat. VI. Inhibitory effect of thymocytes on the homocytotropic antibody response. *J. Immunol.* **107**, 1682.

OSLER, A.G., LICHTENSTEIN, L.M. & LEVY, D.A. (1968) *In vitro* studies of human reaginic allergy. *Advanc. Immunol.* **8**, 183.

OVARY, Z. (1964) *Immunological methods CIOMS Sym. posium* (ed. by J.F. Ackroyd), p. 259, Blackwell Scientific Publications, Oxford.

RHYNE, M.B. & GERMUTH, F.G. (1961) The relationship between serum complement activity and the development of allergic lesions in rabbits. *J. exp. Med.* **114**, 633.

SCHOENBECHLER, M.J. & BARBARO, J.F. (1968) The requirement for sensitized lymphocytes in one form of antigen-induced histamine release from rabbit platelets. *Proc. nat. Acad. Sci.* (*Wash.*), **60**, 1247.

SIRAGANIAN, R.P. & OSLER, A.G. (1969) Histamine release from sensitized rabbit leukocytes and associated platelet involvement. *J. Allergy clin. Immunol.* **43**, 167.

SIRAGANIAN, R.P. & OSLER, A.G. (1971a) Destruction of rabbit platelets in the allergic response of sensitized leukocytes. I. Demonstration of a fluid phase intermediate. *J. Immunol.* **106**, 1244.

SIRAGANIAN, R.P. & OSLER, A.G. (1971b) Destruction of rabbit platelets in the allergic response of sensitized leukocytes. II. Evidence for basophil involvement. *J. Immunol.* **106**, 1252.