Immunology, 1968, **14**, 637.

The Role of PMN-Leucocyte Lysosomes in Tissue Injury, Inflammation and Hypersensitivity

VI. THE PARTICIPATION OF THE PMN-LEUCOCYTE AND THE BLOOD PLATELET IN SYSTEMIC AGGREGATE ANAPHYLAXIS

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(*Received* 13*th August* 1967)

Summary. Anaphylaxis due to intravascular interaction of hyperimmune antibody with antigen was studied in rabbits, swine and rats. Obstruction of the pulmonary vessels by the immune precipitates was found to initiate the process. This is followed by aggregation of PMN-leucocytes and platelets in pulmonary vessels and phagocytosis of the precipitates by these blood elements. During this process degranulation of the cells takes place with release of lysosomal contents. As a concomitant a rise in plasma acid protease and other hydrolases was demonstrated, presumably derived from the degranulating PMN-leucocytes and platelets. Unlike leukopaenic animals, normal ones showed a more marked hypotension, a greater tendency to protracted shock and developed focal and confluent haemorrhagic pulmonary lesions. It is suggested that anaphylaxis due to intravascular antigen–antibody interaction or aggregate anaphylaxis is a systemic or pulmonary Arthus reaction, rather than a 'true' anaphylaxis.

INTRODUCTION

Anaphylaxis due to the intravascular precipitation of antigen and antibody requires a high level of circulating antibody and occurs primarily in the rabbit (Austen and Humphrey, 1963). It can be induced also in other species. This is best done by challenging with antigen: (a) actively hyperimmunized animals, (b) animals that had received a large dose of antibody, or (c) by injecting preformed antigen–antibody complexes (McKinnon, Andrews, Heptinstall and Germuth, 1957; Weigle, Cochrane and Dixon, 1960). This has recently been referred to as 'aggregate anaphylaxis' to differentiate it from 'cytotropic anaphylaxis' (Becker and Austen, 1967).

The role of polymorphonuclear (PMN) leucocytes and platelets in intravascular aggregate anaphylaxis has been a subject of controversy. During *in vitro* 'anaphylaxis', when antigen was added to the blood of sensitized rabbits a shift of histamine from cells

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to plasma was observed (Katz, 1940; Rose and Browne, 1941). In *in vivo* anaphylaxis in rabbits and other animals a decrease in circulating platelets and white cells was found to parallel a fall in total blood histamine (Rose and Weil, 1939; Kopeloff and Kopeloff, 1941; Rose, 1941). A fall in leucocytes also occurred when antigen and antibody were added to the blood during perfusion of rabbit lungs (Dragstedt, Arellano, Lawton and Youmans, 1940). Based on this circumstantial evidence Rocha e Silva (1955) concluded that '. . . besides histamine which is liberated from white cells, the mechanical plugging of capillaries with microthrombi formed by agglutinated blood elements might constitute an aggravating factor' in systemic anaphylaxis.

These earlier observations were further extended by Waalkes and co-workers (Waalkes, Weisbach, Bozicevich and Udenfriend, 1957; Waalkes and Coburn, 1959), who showed that the decrease in circulating leucocytes and platelets during anaphylaxis is associated with a decrease of histamine in whole blood and an increase of these amines in plasma and in the lungs. Based on the *in vitro* studies of Humphrey and Jaques (1955), Waalkes *et al.* demonstrated that there was also a release of 5-hydroxytryptamine from blood elements during anaphylaxis.

Despite these detailed studies histamine and serotonin seem to play but a secondary role in systemic anaphylaxis, at least in the rabbit. Administration of an antihistamine had no ameliorating effect on anaphylaxis of the rabbit (Reuse, 1956) and depleting animals of 5-hydroxytryptamine likewise had little effect (Fischer and Lecomte, 1956). What is the role of PMN-leucocytes and platelets in systemic aggregate anaphylaxis? Except for the early studies of Vaubel (1932), Sato (1934) and Gregory and Rich (1946) showing amorphous masses in pulmonary vessels, and those of Dixon (1953) and McKinnon *et al.* (1957) demonstrating antigen in these eosinophilic masses, there are no detailed morphological and no ultrastructural studies on the lung in systemic aggregate anaphylaxis. In fact, the presence of platelets and leucocytes in pulmonary vessels has not been demonstrated. There are, however, some *in vivo* observations describing leucocyte and platelet emboli in the ear chambers of rabbits given repeated injections of foreign protein (Abell and Schenk, 1938; Ebert and Wissler, 1951).

With these facts in mind and the knowledge that PMN-leucocytes ingest antigen–antibody complexes both *in vivo* (Cochrane, Weigle and Dixon, 1959; Movat, Fernando, Uriuhara and Weiser, 1963; Uriuhara and Movat, 1966) and *in vitro* (Movat, Uriuhara, Macmorine and Burke, 1964), we studied the events that occur in the pulmonary vessels during intravascular aggregate anaphylaxis. Preliminary observations on acid protease activity in the serum (Wasi, Uriuhara, Taichman, Murray and Movat, 1966b) and on changes in platelets (Movat, Mustard, Tiachman and Uriuhara, 1965) were reported recently.

MATERIALS AND METHODS

Systemic anaphylaxis was induced by: (1) the intravenous injection of antigen in actively immunized rabbits, (2) by the intravenous injection of homologous hyperimmune antibody into swine, followed by antigen, (3) by injection of hyperimmune rabbit antibody into rats, followed by antigen, and (4) by the intravenous infusion of preformed antigen–antibody (Ag–Ab) precipitates into rabbits.

Active anaphylaxis was studied clinically, i.e. observed for signs of anaphylaxis, in twenty-one normal and thirteen leukopaenic rabbits. Passive anaphylaxis was investigated

clinically in nine swine and eight rats and anaphylaxis due to injection of preformed Ag–Ab precipitates was examined in seven normal and in four leukopaenic rabbits. Blood counts were done on all the rabbits observed for signs of anaphylaxis. The proteolytic activity of the serum during anaphylaxis was measured at pH 4·0 in thirteen normal and in seven leukopaenic rabbits. The proteolytic activity of the serum was also studied over a wide pH range in three rabbits. Serum protease activity was determined at pH 3 simultaneously with β-glucuronidase and acid phosphatase activity in eight additional rabbits. Not included in the above groups were ten rabbits in which the blood pressure was examined during anaphylaxis and five swine in whom blood pressure, heart rate and electrocardiogram (ECG) were examined. Blood counts in swine were done on the five anaesthetized animals used for studies of the cardiovascular function.

Production of antibody

Rabbits were immunized with bovine serum albumin (BSA) or with horse ferritin (Pentex, Kankakee, Illinois), 15 mg/kg body weight. The proteins were incorporated into 2–3 ml of complete Freund's adjuvant (Difco, Detroit, Michigan) and administered into the footpads, intramuscularly and at multiple subcutaneous sites. The animals were bled 6–8 weeks later and the antibody titre was estimated by the quantitative precipitin and Kjeldahl procedures (Campbell, Garvey, Cremer and Sussdorf, 1964). The serum antibody N levels ranged between 0·4 and 2·0 mg/ml. These rabbits were either utilized for experiments in active anaphylaxis (see below) or their sera were pooled, heated at 56° for 30 minutes and stored at −20° for use in experiments in passive anaphylaxis.

Swine were immunized with egg albumin (Nutritional Biochemicals, Cleveland, Ohio) or horse ferritin 5 mg/kg, incorporated in complete Freund's adjuvant and injected intramuscularly each week for 3 months. Antibody levels were estimated as above and ranged from 0·15 to 1·1 mg N/ml serum. Animals with the highest levels served as a source of antibody for subsequent experiments and were repeatedly immunized and bled over a period of several months.

Active anaphylaxis

Rabbits were challenged intravenously via the marginal ear vein. The amount of antigen used varied with the serum antibody level and was based on the amount of antigen needed for *in vitro* precipitation at equivalence and the estimated blood volume of the animal. The challenging dose was administered over a 5–15-minute period.

Passive anaphylaxis

Swine (20–25 kg) were passively sensitized by an intravenous injection of swine anti-egg albumin (2·2 mg N/kg body weight) and challenged immediately thereafter by an intravenous injection of egg albumin (0·3 mg N/kg body weight).

Rats were sensitized by an intraperitoneal inoculation of rabbit anti-ferritin (5 mg N/100 g body weight) and were challenged intravenously 1 hour later with antigen (1 mg N/100 g body weight). In both swine and rats the amount of antigen used for challenge was calculated so as to produce maximum *in vivo* precipitation (see above).

Passive anaphylaxis with preformed antigen–antibody precipitates

Washed precipitates of ferritin–anti-ferritin (60 mg N) were injected intravenously into rabbits. The precipitates were dispersed in 15 ml of phosphate buffered saline (pH 7·4) and were given slowly over a period of 5–15 minutes.

Blood counts

Total and differential white blood cell and platelet counts were performed in rabbits and swine. The counts were made prior to anaphylaxis and for varying periods after challenge.

Leucocyte depletion

Anaphylaxis was compared in normal and leukopaenic rabbits. Leukopaenia was induced by an intravenous injection of nitrogen mustard (Mustargen; Merck, Sharp and Dohme), 1·75 mg/kg followed in 2 days by a second injection, 1·0 mg/kg. These procedures resulted in a drop in the total leucocyte count to less than 1000 cells/mm^3, of which less than 5 per cent were neutrophils, many rabbits having an absolute neutropaenia. Platelets were also depressed by 40–60 per cent.

Anaphylaxis was induced in these animals as in normal rabbits, i.e. actively immunized animals were challenged with antigen and non-immunized rabbits were injected with preformed antigen–antibody precipitates (see above).

Cardiovascular function studies

In swine polyethylene canulae were placed in a femoral and carotid artery and in a jugular vein. Sodium barbital was used for general anaesthesia. The femoral artery and jugular venous pressure, the respiration and electrocardiogram were recorded with a four-channel recorder (Grass-polygraph, Quincy, Massachusetts) before and during the actual experiment.

Attempts to assess the difference in degree of anaphylaxis between normal and leukopaenic rabbits were made by measuring their blood pressure. For this purpose normal and leukopaenic rabbits with approximately the same antibody N level were paired and the fall in their blood pressure during anaphylaxis recorded and compared. A polyethylene canula was introduced into the right carotid artery during general anaesthesia, using sodium barbital. The canula was connected to a manometer and the blood pressure was recorded before anaphylaxis and up to 1 hour after challenge with antigen.

Fluorescent tracer studies

Fluorescein isothiocyanate (Nutritional Biochemicals, Cleveland, Ohio) labelled BSA and egg albumin were injected into rabbits and swine in the same dose and manner as unlabelled antigens. Tissue samples of lung, heart, kidney, liver mesentery, and small and large bowel from animals dying of anaphylaxis or from animals killed 1 hour after challenge with the antigen were fixed in phosphate buffered (pH 7·0) 10 per cent formaldehyde solution, dehydrated in ethanol and embedded in butyl methacrylate or paraffin. These were examined in a Leitz fluorescent (ultraviolet) microscope. After photography the sections were stained with Azure II and the same areas were examined by light microscopy and again photographed. In this fashion the fluorescent micrographs could be compared with those obtained by light microscopy.

Electron microscope studies

Fragments of lung tissue were fixed in osmium tetroxide buffered with Tyrode solution (pH 7·4). After dehydration in ethanol the tissue was embedded in Epon and examined in a Phillips EM-200 or RCA-EMU-3F electron microscope.

Serum protease, β-glucuronidase and acid phosphatase activity

Proteolytic enzyme activity in the serum of both normal and leukopaenic rabbits was

determined as described previously (Wasi *et al.*, 1966b). Acid phosphatase activity of the serum was determined by the method of Bessey, Lowry and Brock (1946) and the β-glucuronidase activity by that of Fishman (1950). All the enzyme determinations were performed both before and during anaphylaxis.

Injection of PMN-leucocyte components and derivations

In a previous study it was shown that leucocytes phagocytosing Ag–Ab precipitates *in vitro* release their granule contents into the ambient fluid (Movat *et al.*, 1964; Burke, Uriuhara, Macmorine and Movat, 1964). Therefore, released granule content (10 mg protein) was injected intravenously into three leukopaenic rabbits which had been challenged with preformed Ag–Ab precipitates. One leukopaenic rabbit was injected with 10 mg of lysed leucocyte lysosomes (Cohn and Hirsch, 1960), immediately after the injection of immune precipitates. Two control leukopaenic rabbits were given immune precipitates alone. Material released *in vitro* from phagocytosing leucocytes was injected also into two additional control rabbits, not receiving any immune aggregates. All animals were observed for signs of shock and were autopsied 1 hour after challenge.

RESULTS

CLINICAL FINDINGS

Anaphylactic shock in both rabbits and swine could be divided into two phases: the first or acute phase was evident within a few moments following challenge. The animal developed varying degrees of weakness, tachypnoea and cyanosis. Rabbits often developed nystagmus and weaving of the head. In severe reactions the animal would drop to one side, have a few convulsive movements and die. If the animal survived the initial 5–10 minutes of anaphylaxis it entered the second or protracted stage of the syndrome. This state lasted from one to several hours and was characterized by laboured breathing and weakness. The animals remained uninterested in their surroundings. With time they regained their strength and were able to stand up.

The acute symptoms in leukopaenic rabbits were identical to those in normal animals. However, if leukopaenic animals survived the initial phase, they had less protracted anaphylaxis. Attempts were made to quantify the clinical symptoms, in order to compare the normal and leukopaenic group, but this proved to be difficult and was, therefore, abandoned.

Normal and leukopaenic rabbits challenged with preformed immune aggregates behaved like the actively immunized animals challenged with antigen.

Anaphylaxis in swine was similar to that in rabbits. However, in these animals there was in addition marked cyanosis and petechial discolouration of the skin.

Anaphylactic shock in rats was relatively undramatic. They showed signs of weakness, dyspnea and cyanosis but rarely died during the period of observation.

HAEMATOLOGICAL STUDIES

Blood counts in rabbits (Fig. 1) and swine (Fig. 2) revealed that there was a profound fall in the white cell count during anaphylaxis, with neutrophils showing the most marked depression. Platelet counts were likewise lowered during anaphylaxis.

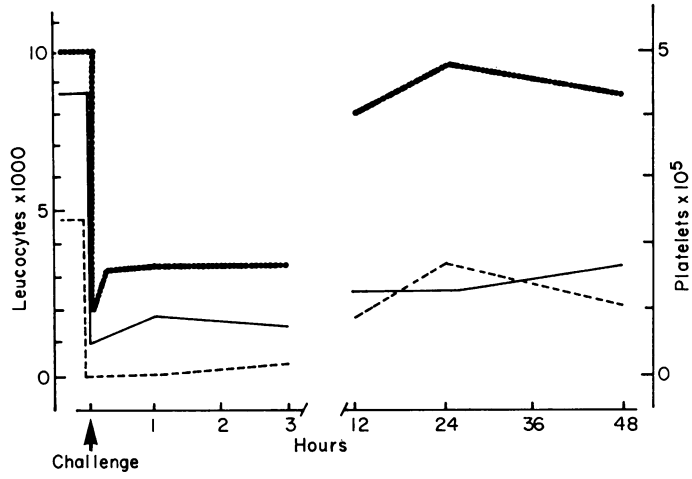

FIG. 1. Total WBC and platelet counts and differential counts in rabbits before and during anaphylaxis. —— Total leucocytes; - - -, PMN leucocytes; —, platelets.

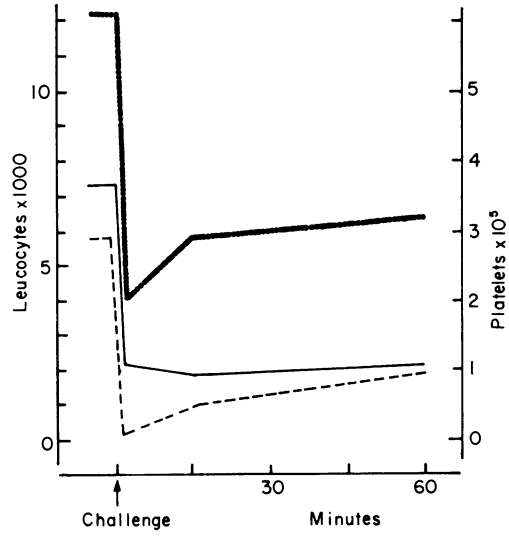

FIG. 2. Total WBC and platelet counts and differential counts in swine before and during 1st hour of anaphylaxis. Key as in Fig. 1.

FUNCTIONAL STUDIES

Changes in the polygraph tracings were detected within 2 minutes after challenge. Three marked alterations were observed in these experiments, a fall in the systemic blood pressure, a rise in venous pressure and apnea. A slight depression of the ST-segment in the electrocardiogram was also noted.

The blood pressure of rabbits before and during anaphylaxis is shown in Table 1. The percentage fall in the normal rabbits was about twice that of leukopaenic animals.

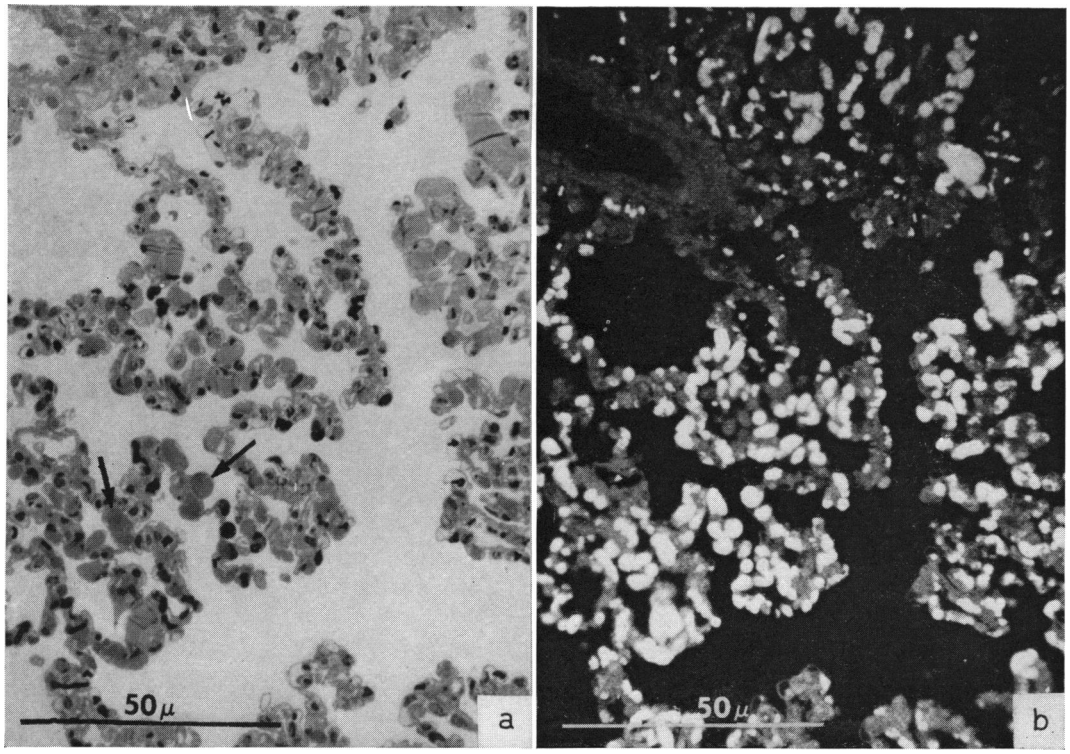

FIG. 3. Conventional and fluorescent microscopic sections of same area in lung. Actively immunized rabbit challenged with antigen and killed 15 minutes later.

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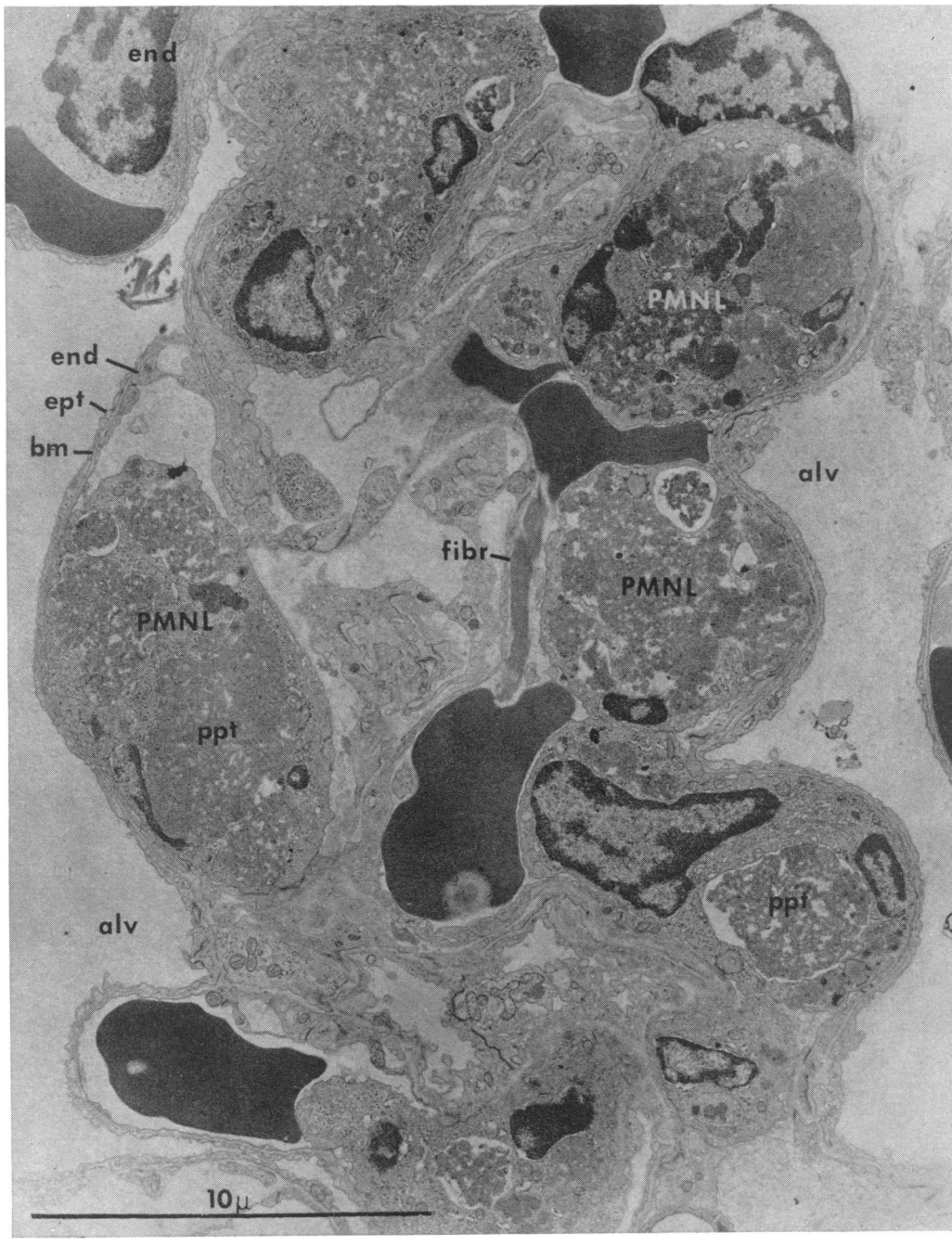

FIG. 4. Several capillary loops from rat during passive systemic anaphylaxis. Precipitates (ppt) of ferritin-anti-ferritin are seen free in the lumina and PMN-leucocytes. alv, Alveolus; bm, basement membrane; end, endothelium; ept, epithelium; fibr, fibrin; PMNL, polymorphonuclear leucocyte.

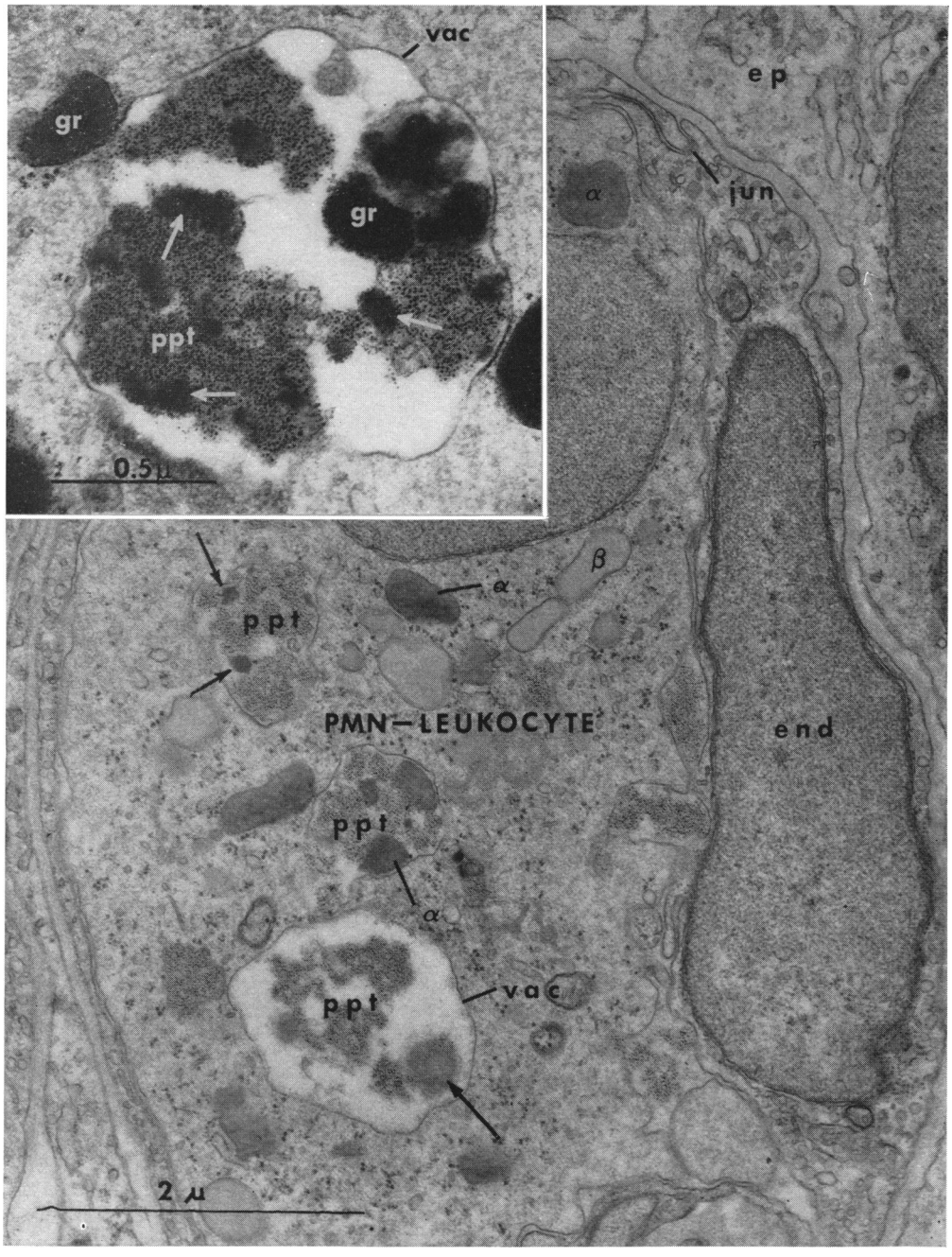

FIG. 5. Portion of pulmonary capillary of the rabbit with a PMN-leucocyte which contains digestive vacuoles (vac), consisting of immune precipitates (ppt) and fragments of lysosomes or granules (arrows). Intact α and β granules are also seen in the cytoplasm. Insert shows high magnification of a digestive vacuole (vac) in PMN-leucocyte of the rabbit.

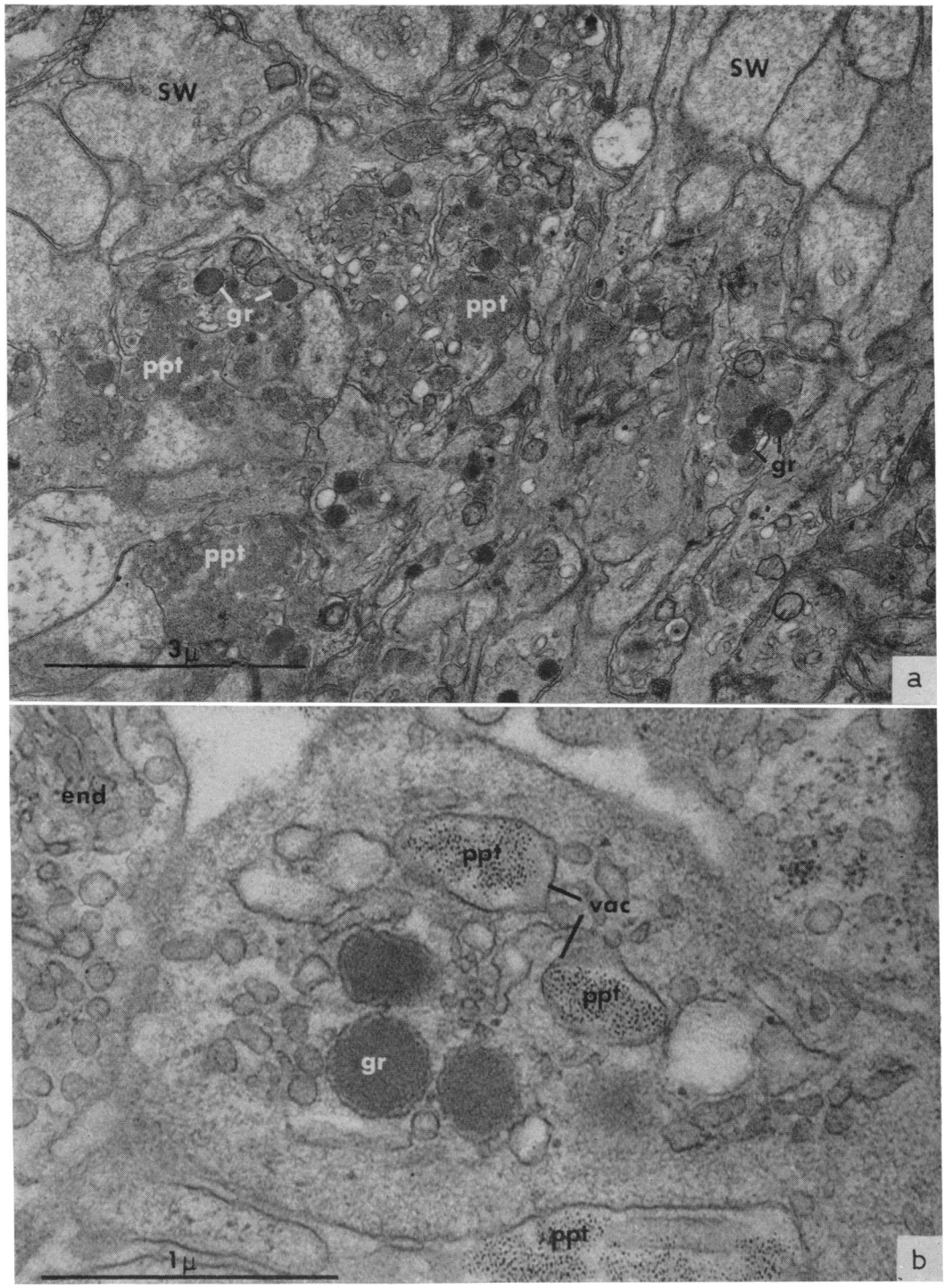

FIG. 6. Low and high magnification of platelets in a pulmonary vessel during anaphylaxis of the rabbit. Note swollen (sw) platelets in (a) and immune precipitates (ppt) in phagocytic vacuole in (b).

TABLE 1

BLOOD PRESSURE IN NORMAL AND LEUKOPAENIC RABBITS DURING SYSTEMIC ANAPHYLAXIS

Group	Blood pressure (mmHg)	
	Pre-anaphylaxis	Minimum pressure
Normal	120	60
	110	0
	110	52
	104	0
	110	75
Mean	110·8	35·4
Leukopaenic	107	80
	105	85
	125	90
	122	67
	110	55
Mean	115·8	75·4

Blood pressure was measured over a 1-hour period and leukopaenia was induced with Nitrogen Mustard (see 'Material and methods').

GROSS PATHOLOGY

Normal rabbits dying within 2–3 minutes after challenge had no detectable changes at autopsy. However, those dying 5 minutes after challenge or later usually showed diffuse and focal haemorrhagic pulmonary lesions. In contrast to normals, leukopaenic rabbits displayed few or no haemorrhagic lesions in their lungs. Haemorrhagic lesions were observed also in swine and to a lesser degree in rats.

FLUORESCENT AND LIGHT MICROSCOPY

By the fluorescent tracer technique it was possible to show that the pulmonary vessels of all animals in anaphylaxis contained variable quantities of fluorescent precipitates. By light microscopy these fluorescent precipitates corresponded to hyaline masses which often completely occluded the pulmonary capillaries, venules and arterioles (Fig. 3). The fluorescent material was seen free and within PMN-leucocytes.

ELECTRON MICROSCOPIC STUDIES

The changes seen by light and fluorescent microscopy could be confirmed at the ultrastructural level. Precipitates of ferritin–anti-ferritin could be detected within the lumina of the pulmonary vessels (Fig. 4). In addition numerous PMN-leucocytes and platelets were found within these vessels. These elements accumulated in such dense aggregates that they often plugged the lumina of rather large vessels. This would explain the fall in the counts of these blood elements during anaphylaxis.

The PMN-leucocytes showed phagocytosis of the immune aggregates. This activity was associated with the formation of phagocytic vacuoles (Fig. 5). These vacuoles contained ingested antigen–antibody precipitates and, in addition, often contained remnants of cytoplasmic granules or lysosomes. During this process the PMN-leucocytes gradually

became degranulated. In some vessels the aggregates consisted mainly of platelets (Fig. 6a). These blood elements also phagocytosed immune aggregates (Fig. 6b).

Antigen–antibody precipitates were also found to be occluding the pulmonary vessels of leukopaenic rabbits. However, phagocytic activity in PMN-leucocytes or platelets as described in normals was rarely detected.

Similar pulmonary changes were encountered in rats (Fig. 4) and in swine. There were only very occasional Ag–Ab aggregates in other organs.

SERUM PROTEASE ACTIVITY

Proteolytic activity against denatured haemoglobin was detected in the serum of normal animals prior to the induction of anaphylaxis. Following challenge an increase in the activity of the serum to denatured haemoglobin was demonstrated (Fig. 7). This activity

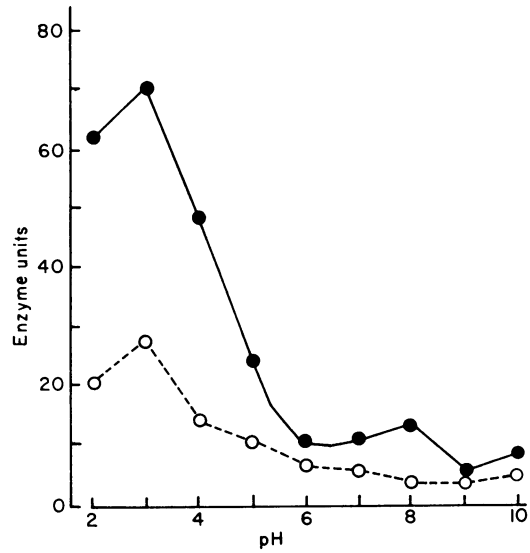

FIG. 7. Acid protease activity of rabbit serum before and during anaphylaxis. Peak activity is seen at pH 3. For description of method and of enzyme units see text and Table 2. ○, Pre-anaphylaxis; ●, 60 minutes post-anaphylaxis.

was seen mainly when the pH of the reaction mixture was low, thereby resembling the peaks of proteolytic activity obtained with isolated lysed PMN-leucocyte or platelet lysosomes as a source of protease (Wasi, Murray, MacMorine and Movat, 1966a). Leukopaenic rabbits, on the other hand, showed only a very slight rise in serum proteolytic activity during anaphylaxis (Table 2).

Increase of hydrolytic enzyme activity of the serum was not confined to the proteases; β-glucuronidase and to a slight degree acid phosphatase were elevated during anaphylaxis (Table 3 and Fig. 8).

PULMONARY CHANGES INDUCED BY INJECTION OF LYSOSOMAL MATERIAL

Material derived from leucocytes (when these phagocytosed Ag–Ab precipitates *in vitro*) was injected intravenously into leukopaenic rabbits challenged with preformed Ag–Ab

TABLE 2

ACID PROTEASE ACTIVITY DURING ANAPHYLAXIS IN THE RABBIT

Rabbits	Activity in enzyme units*			
	Pre-anaphylaxis		60 minutes anaphylaxis	
	Mean	SE†	Mean	SE†
Normal $n = 13$	13·90	1·20	35·10	1·97
Leukopaenic $n = 7$	11·40	2·34	16·20	3·46

Equal volumes of serum, 2 per cent denatured haemoglobin and buffer (citric acid–Na–citrate, pH 4·0) were incubated at 37° for 24 hours, the reaction stopped with 10 per cent TCA and the filtrate read at 280 mμ.

* One enzyme unit gives an optical density (at 280 mμ) of 1×10^{-3}/hr/ml of serum at 37°.

† Standard error.

TABLE 3

LYSOSOMAL ENZYME ACTIVITY IN THE SERUM OF NORMAL RABBITS DURING ANAPHYLACTIC SHOCK

Rabbit	Acid protease (1×10^{-3}/ml/hr)		β-Glucuronidase (1×10^{-3}/ml/hr)		Acid phosphatase (1×10^{-2}/ml/hr)	
	Pre	Post*	Pre	Post*	Pre	Post*
SAR-61	16·0	51·6	0·2	4·8	91·2	155·5
SAR-62	19·6	60·0	1·3	5·8	42·2	67·9
SAR-63	15·9	64·5	0·5	4·0	130·4	142·5
SAR-67	26·0	70·5	5·8	26·8	108·6	151·1
SAR-68	28·5	78·4	4·5	16·9	138·8	200·5
SAR-69	29·5	45·0	3·4	5·0	95·6	108·3
SAR-70	28·3	76·6	3·4	7·2	72·4	130·2
SAR-71	25·2	30·5	1·9	3·6	130·8	158·3

* Serum samples were obtained from animals by cardiac puncture 60 minutes after challenge with antigen save in rabbits SAR-70 and SAR-71. SAR-70 died at 15 minutes and SAR-71 died at 5 minutes after challenge.

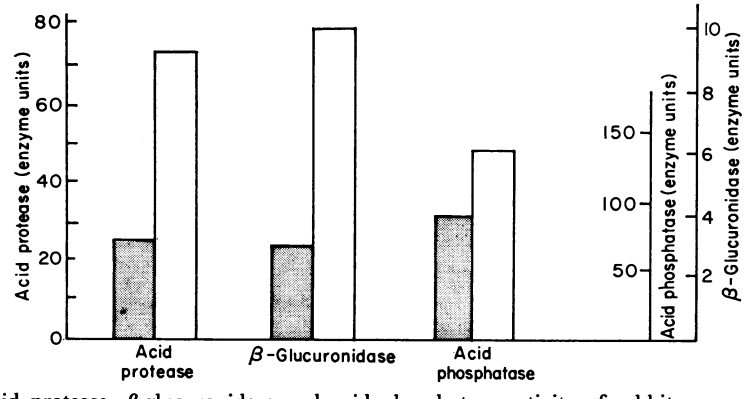

FIG. 8. Acid protease, β-glucuronidase and acid phosphatase activity of rabbit serum before and during anaphylaxis. Methods are described in the text and Table 2. Unlike the data presented in Table 2, acid protease activity was measured at pH 3. Stippled columns, pre-anaphylaxis; open columns, 60 minutes post-anaphylaxis.

precipitates. There was no distinct difference in the clinical picture between these animals and the controls receiving only immune precipitates. However, the animals receiving the material derived from leucocytes had multiple haemorrhagic foci in their lungs. Similar observations were made in the rabbit injected with lysed lysosomal material. The rabbits receiving only leucocyte material and no immune precipitates had no haemorrhagic pulmonary lesions nor definite signs of anaphylaxis.

DISCUSSION

Intravascular aggregation of large quantities of circulating immune aggregates leads to extensive plugging of the pulmonary circulation in the rabbit, swine and rat. This is an important pathogenetic event in this type of anaphylactic shock and can lead to sudden death. However, if the animal is able to survive this initial event, various aggravating factors may potentiate the syndrome. Such factors in the rabbit include release of histamine and serotonin from formed elements of the blood (see 'Introduction') and activation of plasma kinins (Lambert, Otto-Servais, Salmon and Lecomte, 1964; Cîrstea, Suchaiu and Butculescu, 1965). The following data support the participation of PMN-leucocytes in systemic aggregate anaphylaxis:

(1) There is a fall in the number of circulating platelets and PMN-leucocytes during anaphylaxis as shown repeatedly by several investigators (see 'Introduction').

(2) Large numbers of PMN-leucocytes and platelets are sequestered in the pulmonary vessels which are filled with antigen–antibody precipitates. Phagocytosis of these precipitates by platelets and PMN-leucocytes is associated with degranulation. Degranulation is presumably associated with release of lysosomal hydrolases and other biologically active substances from these elements. Studies in this laboratory have shown that lysosomal contents are probably liberated *in vitro* when antigen–antibody precipitates are ingested by PMN-leucocytes or platelets (Movat *et al.*, 1964, 1965; Burke *et al.*, 1964). The acid proteases of PMN-leucocyte lysosomes are capable of degrading various proteins, including antigen–antibody complexes (Wasi *et al.*, 1966a).

(3) A rise in the cathepsin or acid protease activity of serum in normal rabbits can be demonstrated during anaphylaxis. This activity resembles that of isolated leucocytes and platelets (Wasi *et al.*, 1966a; Wasi, Mustard and Movat, 1966 unpublished observations). Leukopaenic rabbits when challenged show much less alterations in serum proteolytic activity.

(4) The hypotension observed during anaphylaxis is more marked in normal than in leukopaenic animals. Whether the release of lysosomal material plays any role in this process is uncertain. It is more likely that the mechanical obstruction caused by the immune precipitates is aggravated by the aggregates of leucocytes and platelets.

(5) The lungs of normal rabbits are haemorrhagic, whereas the lungs of leukopaenic animals show little or no haemorrhage during anaphylaxis.

(6) A haemorrhagic pulmonary lesion can be produced in leukopaenic rabbits injected first with immune precipitates and followed by either lysed PMN-leucocyte lysosomes or by material released from leucocytes during *in vitro* phagocytosis.

(7) Recent experiments in our laboratory indicate that PMN-leucocytes may be the direct or indirect source for SRS. SRS can be released *in vivo* in the rabbit by the same procedure as used in the rat (Stechschulte, Austen and Bloch, 1967) and release of SRS

is markedly reduced in leukopaenic rabbits. Furthermore, an SRS is released also *in vitro* from washed rabbit, rat and guinea-pig PMN-leucocytes during phagocytosis of Ag–Ab aggregates (Macmorine, Movat, Neta and Takeuchi, 1968).

Systemic aggregate anaphylaxis in the rabbit, swine and rat seems to be akin to the cutaneous Arthus reaction. Both the Arthus reaction (Stetson, 1951; Humphrey, 1955; Cochrane *et al.*, 1959; Uriuhara and Movat, 1966) and aggregate anaphylaxis involve the precipitation of immune complexes in the lumina and walls of blood vessels and the participation of PMN-leucocytes and platelets. These structures by degranulating, release their lysosomal contents, which could act directly on tissue components (Cochrane and Aikin, 1966) or as enzymes (proteases, phospholipases) could act on appropriate substrates to release biologically active substances (polypeptides, unsaturated fatty acids). Thus it would appear that systemic anaphylaxis due to intravascular aggregation of immune precipitates should be distinguished from systemic anaphylaxis produced with 'mast cell sensitizing' or 'anaphylactic' antibody, which seems to be mediated primarily by the release of vasoactive amines from disrupted mast cells.

ACKNOWLEDGMENTS

This investigation was supported by the Medical Research Council of Canada (Grant MA-1251) and the Ontario Heart Foundation. The authors are grateful to Miss Safia Wasi for some of the acid protease determinations and to Mrs Otti Freitag and Miss Sue-Ming Weng for technical assistance.

H.Z.M. is a research associate of the M.R.C. of Canada. N.S.T. was a research fellow of the J. P. Bickell Foundation. T.U. was a recipient of a research fellowship from the M.R.C. of Canada.

REFERENCES

ABELL, R. G. and SCHENK, H. P. (1938). 'Microscopic observations on the behaviour of living blood vessels of the rabbit during the reaction of anaphylaxis.' *J. Immunol.*, **34**, 195.

AUSTEN, K. F. and HUMPHREY, J. H. (1963). 'In vitro studies of the mechanism of anaphylaxis.' *Advanc. Immunol.*, **3**, 1.

BECKER, E. L. and AUSTEN, K. F. (1967). 'Anaphylaxis.' In: *Immunopathology* (Ed. by H. J. Müller-Eberhard and P. A. Miescher). Grune & Stratton, New York.

BESSEY, O. A., LOWRY, O. H. and BROCK, M. J. (1946). 'Method for rapid determination of alkaline phosphatase with 5 cubic millimetres of serum.' *J. biol. Chem.*, **164**, 321.

BURKE, J. S., URIUHARA, T., MACMORINE, D. R. L. and MOVAT, H. Z. (1964). 'A permeability factor released from phagocytosing PMN-leukocytes and its inhibition by protease inhibitors.' *Life Sci.*, **3**, 1505.

CAMPBELL, D. H., GARVEY, J. S., CREMER, N. E. and SUSSDORF, D. H. (1964). *Methods in Immunology*. Benjamin, New York.

CÎRSTEA, M., SUHACIU, G. and BUTCULESCU, I. (1965). 'Bradykinin and anaphylactic shock in dogs, guinea pigs and rabbits.' *Arch. int. Physiol. Biochim.*, **73**, 231.

COCHRANE, C. G. and AIKIN, B. S. (1966). 'Polymorphonuclear leukocytes in immunologic reactions. The destruction of vascular basement membrane *in vivo* and *in vitro*.' *J. exp. Med.*, **124**, 733.

COCHRANE, C. G., WEIGLE, W. O. and DIXON, J. F. (1959). 'The role of polymorphonuclear leukocytes in the initiation and cessation of Arthus vasculitis.' *J. exp. Med.*, **110**, 481.

COHN, Z. A. and HIRSCH, J. G. (1960). 'The isolation and properties of the specific cytoplasmic granules of rabbit polymorphonuclear leukocytes.' *J. exp. Med.*, **112**, 983.

DRAGSTEDT, C. A., ARELLANO, M. R., LAWTON, A. H. and YOUMANS, G. P. (1940). 'Passive sensitization of rabbit's blood.' *J. Immunol.*, **39**, 537.

DIXON, F. J. (1953). 'The use of I^{131} in immunologic investigation.' *J. Allergy*, **24**, 547.

EBERT, R. H. and WISSLER, R. W. (1951). '*In vivo* observations of the vascular reaction to large doses of horse serum using the rabbit ear chamber technique.' *J. Lab. clin. Med.*, **38**, 511.

FISCHER, P. and LECOMTE, J. (1956). 'Choc anaphylactique chez le lapin traité par réserpine.' *C. r. Soc. Biol. (Paris)*, **150**, 1026.

FISHMAN, W. H. (1950). *The Enzymes, Chemistry and Mechanism of Action*, pp. 635–652. Academic Press, New York.

GREGORY, J. E. and RICH, A. R. (1946). 'The experimental production of anaphylactic pulmonary lesions with the basic characteristics of rheumatic pneumonitis.' *Bull. Johns Hopk. Hosp.*, **78**, 1.

HUMPHREY, J. H. (1955). 'The mechanism of Arthus reactions. I. The role of polymorphonuclear leukocytes and other factors in reversed passive Arthus reactions in rabbits.' *Brit. J. exp. Path.*, **36**, 268.

HUMPHREY, J. H. and JACQUES, R. (1955). 'The release

of histamine and 5-hydroxytryptamine (serotonin) from platelets by antigen-antibody reactions (*in vitro*).' *J. Physiol. (Lond.)*, **128**, 9.

KATZ, G. (1940). 'Histamine release from blood cells in anaphylaxis *in vitro*.' *Science*, **91**, 221.

KOPELOFF, N. and KOPELOFF, L. M. (1941). 'Blood platelets in anaphylaxis.' *J. Immunol.*, **40**, 471.

LAMBERT, P. H., OTTO-SERVAIS (MME.), SALMON, J. and LECOMTE, J. (1964). 'Sur le role du complément dans le choc anaphylactic du lapin.' *Int. Arch. Allergy*, **24**, 27.

MACMORINE, D. R. L., MOVAT, H. Z., NETA, R. and TAKEUCHI, Y. (1968). '*In vitro* release of permeability factors and a slow reacting substance from phagocytosing PMN-leucocytes.' *Fed. Proc.* (In press).

MCKINNON, G. E., ANDREWS, E. C., HEPTINSTALL, R. H. and GERMUTH, F. G. (1957). 'An immunologic study on the occurrence of intravascular antigen–antibody precipitation and its role in anaphylaxis in the rabbit.' *Bull. Johns Hopk. Hosp.*, **101**, 258.

MOVAT, H. Z., FERNANDO, N. V. P., URIUHARA, T. and WEISER, W. J. (1963). 'Allergic inflammation. III. The fine structure of collagen fibrils at sites of antigen–antibody interaction in Arthus-type lesions.' *J. exp. Med.*, **118**, 557.

MOVAT, H. Z., MUSTARD, J. F., TAICHMAN, N. S. and URIUHARA, T. (1965). 'Platelet aggregation and release of ADP, serotonin and histamine associated with phagocytosis of antigen-antibody complexes.' *Proc. Soc. exp. Biol. (N.Y.)*, **120**, 232.

MOVAT, H. Z., URIUHARA, T., MACMORINE, D. R. L. and BURKE, J. S. (1964). 'A permeability factor released from leukocytes after phagocytosis of immune complexes and its possible role in the Arthus reaction.' *Life Sci.*, **3**, 1025.

ROSE, B. (1941). 'Studies of the histamine-content of the blood and tissues of the rabbit during anaphylactic shock.' *J. Immunol.*, **41**, 161.

ROSE, B. and BROWNE, J. S. L. (1941). 'Studies on the release of histamine from the blood cells of the rabbit by the addition of horse serum or egg albumin *in vitro*.' *J. Immunol.*, **41**, 403.

ROSE, B. and WEIL, P. (1939). 'Blood histamine in the rabbit during anaphylactic shock.' *Proc. Soc. exp. Biol. (N.Y.)*, **42**, 494.

ROCHA E SILVA, M. (1955). *Histamine, its Role in Anaphylaxis and Allergy*. Thomas, Springfield, Illinois.

REUSE, J. J. (1956). 'Antihistamine drugs and histamine release, especially in anaphylaxis.' In: *Ciba Foundation Symposium: Histamine* (Ed. by G. E. W. Wolstenholme and C. M. O'Connor). Churchill, London.

SATO, Y. (1934). 'Ueber die spezifischen Organveränderungen der Kaninchen bei wiederholter parenteraler Eiweisszufuhr.' *Trans. Soc. Path. Jap.*, **24**, 203.

STECHSCHULTE, D. J., AUSTEN, K. F. and BLOCH, K. J. (1967). 'Antibodies involved in antigen-induced release of slow reacting substance of anaphylaxis (SRS-A) in the guinea pig and rat.' *J. exp. Med.*, **127**, 127.

STETSON, C. A., JR (1951). 'Similarities in the mechanisms determining the Arthus and Shwartzman phenomenon.' *J. exp. Med.*, **94**, 347.

URIUHARA, T. and MOVAT, H. Z. (1966). 'The role of PMN-leukocyte lysosomes in tissue injury, inflammation and hypersensitivity. I. The vascular changes and the role of PMN-leukocytes in the reversed passive Arthus reaction.' *Exp. molec. Path.*, **5**, 439.

VAUBEL, E. (1932). 'Die Eiweissüberempfindlichkeit des Bindegewebes.' *Beitr. path. Anat.*, **89**, 374.

WAALKES, T. P. and COBURN, H. (1959). 'The role of platelets and the release of serotonin and histamine during anaphylaxis in the rabbit.' *J. Allergy*, **30**, 394.

WAALKES, T. P., WEISSBACH, H., BOZICEVICH, J. and UDENFRIEND, S. (1957). 'Serotonin and histamine release during anaphylaxis in the rabbit.' *J. clin. Invest.*, **36**, 1115.

WASI, S., MURRAY, R. K., MACMORINE, D. R. L. and MOVAT, H. Z. (1966a). 'The role of PMN-leukocyte lysosomes in tissue injury, inflammation and hypersensitivity. II. Studies on the proteolytic activity of PMN-leucocyte lysosomes of the rabbit.' *Brit. J. exp. Path.*, **47**, 411.

WASI, S., URIUHARA, T., TAICHMAN, N. S., MURRAY, R. K. and MOVAT, H. Z. (1966b). 'Proteolytic activity in the serum of rabbits during anaphylaxis.' *Experientia*, **22**, 196.

WEIGLE, W. O., COCHRANE, C. G. and DIXON, F. G. (1960). 'Anaphylactogenic properties of soluble antigen–antibody complexes in the guinea pig and rabbit.' *J. Immunol.*, **85**, 469.