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A Comparative Study of the Morphology of the Systemic Anaphylactic Reaction (SAR) and the Shock Reaction Induced by Antigen-Antibody Complexes in Rabbits

Ján Jakubovský, Miroslav Brozman, Drahomír Chorváth, Mária Ružičková, Eva Surmíková, and Milan Belošovič

Research Laboratory of Histochemistry and Ultrastructural Pathology VÚLB – Institute of Pathology, Comenius University, CS-88424 Bratislava, Czechoslovakia (Head: Prof. MUDr. M. Brozman, DrSc)

Summary. Differences in the morphological picture of the systemic anaphylactic reaction (SAR), and of the shock reaction induced by antigen-antibody (ag-ab) complexes in rabbits, are described. In rabbits SAR is characterized by acute distension of the lungs, with oedematous swelling in the vicinity of bronchi and veins, intravascular stasis of basophils without degranulation or visible fixation of the inducing antigen, small clusters of blood platelets, leukostasis, minimal decomposition of neutrophil granulocytes and absence of circulating or fixed ag-ab complexes. The shock reaction induced by ag-ab complexes is characterized by acute distension of the lungs without bronchospasm, swelling of the vicinity of bronchi and veins, hyaline thrombi particularly in the pulmonary microcirculation, leukostasis, formation of antigen-antibody complexes and thrombocyte clusters in the microcirculation, and by phagocytosis of these complexes and thrombocytes by various cells.

Key words: Systemic anaphylactic reaction – Shock induced by antigen-antibody complexes – Rabbit – Light and electron microscopy

Introduction

Discrepancies between the morphological descriptions of anaphylactic reaction have attracted the attention of several authors. In particular, Letterer (1967) has pointed out in his monograph that anaphylactic shock can occur both in the presence and absence of ag-ab complexes in the circulation of experimental animals. He has come to the conclusion that what is designated as a systemic anaphylactic reaction can also be termed serum sickness, both in experimental and human pathology. Despite new information on the pathogenesis of SAR and of the reaction induced by ag-ab complexes, in descriptions of the morphological manifestations of SAR, one process is frequently mistaken for the other.

Send offprint requests to J. Jakubovský at the above address

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Thus processes indisputable induced by ag-ab complexes are considered to be SAR (Germuth and McKinnon 1957; Sabesin 1964; Goodman et al. 1979a, b). Weigle et al. (1960) described SAR produced in rabbits by soluble antigen-antibody complexes or nonprecipitating antibody in an immunological and histological study. The present paper is concerned with the differences in the morphological picture of the two types of reactions observed in model experiments, defined by immunological methods in rabbits.

Materials and Methods

Antigens. Bovine serum albumin (BSA, ÚSOL Praha) and ferritin from horse spleen without cadmium (SERVA, No. 21319).

Animals. Rabbits from our own breeding stock (Chinchilla).

Laboratory Examinations. Thrombocyte and leukocyte counts immediately before and 1–5 min after inducing the corresponding reaction by a specific antigen, as described in Goodman et al. (1979b).

Detection of Precipitating Antibodies. Immunoelectrophoretically, by double diffusion in agar gel (Ouchterlony) and by inducing the Arthus reaction.

Detection of Reaginic Antibodies. By performing homologous and heterologous passive cutaneous anaphylaxis (PCA) as described in Pinckard et al. (1972).

Inducing SAR. Within 24 h postnatally, 14 rabbits were administered $1 \cdot 10^{-3}$ g of alum bovine serum albumin (ABSA) intraperitonelly (i.p.). On their postnatal days 14, 21, 35, 49 and 63, they received i.p. $5 \cdot 10^{-3}$ g BSA in 0.5 ml phosphate buffered saline (PBS), and on postnatal day 77 they were given $1 \cdot 10^{-2}$ g ABSA in 0.5 ml PBS subcutaneously (s.c.). 10 rabbits received $1 \cdot 10^{-3}$ g of alum ferritin (AF) within 24 h postnatally, and then on days 7, 14, 21, 35, 49, 54 and 61, they were administered i.p. $1 \cdot 10^{-3}$ g of ferritin in 0.5 ml PBS. After SAR had been induced, the animals were sacrificed by i.p. injection of Thiopental (SPOFA) within 2–80 min.

Performing the Shock Reaction Induced by ag-ab Complexes. Three rabbits of 3,000 g average weight were immunized with $5 \cdot 10^{-3}$ g ferritin in complete Freund's adjuvant. One month later, they were reimmunized in the same way s.c. and intramuscularly (i.m.). After a week the antibody titre was determined. The following week, each of them received $17 \cdot 10^{-3}$ g·kg^{-1} body weight ferritin i.v. Thirty minutes later the rabbits were sacrificed by i.p. injection of Thiopental.

Four rabbits with average weight of 3,200 g were immunized with $1.6 \cdot 10^{-3}$ g·kg^{-1} BSA in 1 ml of complete Freund's adjuvant administered s, c, and i.m., and reimmunized after 4 weeks with $1 \cdot 10^{-3}$ g·kg^{-1} BSA in complete Freund's adjuvant administered s.c. and i.m. After determining the antibody titre, the reaction was induced by i.v. injection of $3.3 \cdot 10^{-3}$ g·kg^{-1} BSA in 2 ml PBS and the animals were sacrificed by i.p. injection of Thiopental within 10–30 min.

Control material. Ten rabbits with average body weight of 3,100 g. Two of them received $1.6 \cdot 10^{-3}$ BSA in 2 ml PBS, two were immunized with ferritin in the same way as the animals in the experiment, but in the course of experiment they received only 2 ml PBS each, and two further rabbits were given $17 \cdot 10^{-3}$ g·kg^{-1} ferritin in PBS without previous immunization. In four rabbits, blood platelet and leukocyte counts were made after BSA administration without preceding immunization.

Morphological Examinations. The lungs, liver, spleen, kidney, myocardium, bone marrow, and in some cases the brain and pituitary were examined. Specimens were taken from 3 to 4 lobes of the lungs so as to involve the main bronchi and vessels and peripheral parts of the parenchyma of the upper and lower lobes with macroscopically apparent abnormalities. Fixation was carried out with buffered formalin, paraffin sections were stained with haematoxylin and eosin, and with

Mallory's phosphotungstic haematoxylin. The reaction for bivalent and trivalent iron was performed. Electron microscopic examinations were done after usual double fixation with phosphate buffered 3% glutaraldehyde (SERVA) solution and phosphate buffered 1% OsO_4 (Johnson-Mathey) solution, at pH 7.2–7.4, for 2 and 1 h respectively. The specimens examined were similar to those studied under the light microscope. From the lungs five different tissue blocks were examined from the more peripheral parts of the lobes at sites displaying the most pronounced findings on dissection. After dehydrating the tissue with sequence of acetone, it was embedded in Durcupan ACM (FLUKA). The ultrathin sections were stained by uranyl acetate and lead citrate (SERVA, Venable and Coggeshall 1965).

Results

Regardless of the nature of antigen and mode of sensitization used, the animals became restless within 30–60 s, their breathing was labored and its rate increased. 2–3 min later they developed striking derangements in the coordination of hind limb movements and dropped to the side. Simultaneously some of them urinated and defecated. In approximately 30 min, some of them showed signs of overall improvement, yet the condition of the others continued to deteriorate. SAR differed from the shock reaction induced by ag-ab complexes only by the intensity with which individual animals were affected.

The thrombocyte and leukocyte count in peripheral blood showed considerable variations (Table 1).

Shock Induced by Antigen-Antibody (ag-ab) Complexes

Immunoelectrophoretic examination of sera from rabbits immunized to ferritin in the presence of Freund's adjuvant, displayed precipitation lines in the cathodal area. Comparable examinations of sera from rabbits immunized in the same way with BSA demonstrated the presence of specific anti-BSA precipitating antibodies. Double diffusion in agar gel (Ouchterlony) displayed the equivalence zone of antigen and antibodies in the dilution 1:64 in sera of rabbits with antiferritin as well as with anti-BSA antibodies.

Dissections of the animals showed acute distension of the light-gray lungs that complete filled up the thoracic cavity. In the inferior lobes, minute subpleural haemorrhages were observed and 2–3 mm large brownish-red spots were

Table 1. Number of thrombocytes and leucocytes in samples of peripheral blood (average rates) before and 1–5 min after, injection of antigen

Reaction	Leucocytes		%	Thrombocytes		%
	For	After		For	After	
Shock induced with BSA-anti BSA	7,475	2,075	27.4	135,000	52,750	39.0
Shock induced with F-antiF	4,220	2,200	52.1	206,000	168,000	81.5
SAR to BSA	5,170	4,210	81.4	142,000	170,800	119.8
SAR to ferritin	3,983	2,033	51.0	214,166	184,166	86.0
BSA (unsensitized animals)	9,925	9,150	92.2	115,250	76,000	66.4
Ferritin (unsensitized animals)	7,680	6,720	87.5	290,750	224,750	77.1

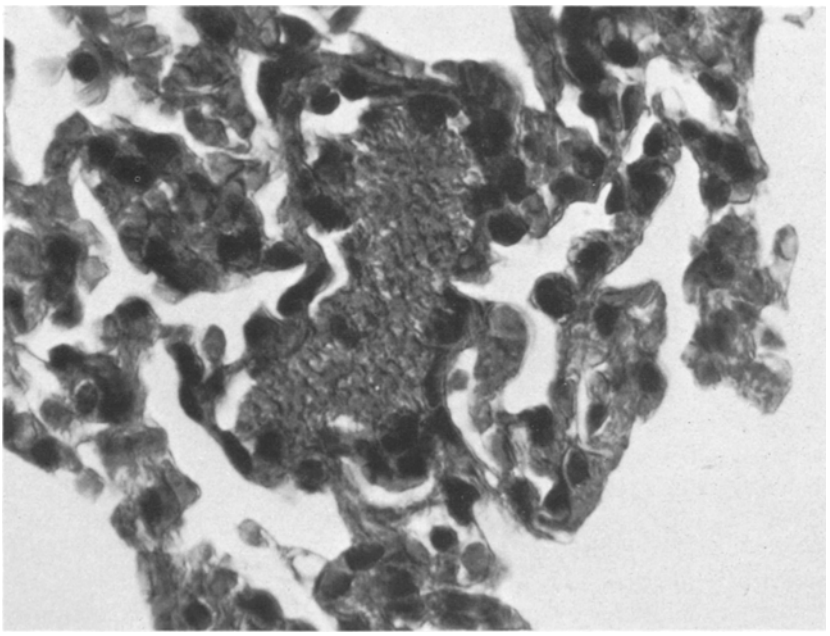

Fig. 1. Pulmonary microcirculation plugged with hyaline thrombus. Shock induced by ferritin-antiferritin complexes. ×450 HE

shining through the pleura and were also seen on the cut surface. The right side of the heart was markedly dilated and sporadic haemorrhages were observed under the epicardium. The other organs were unchanged.

Histological examination of the lungs confirmed the signs of acute distension of pulmonary tissue. No pronounced bronchospasm was found. Focal stasis in the microcirculation and small haemorrhages into the alveolae, together with hyaline thrombi (Fig. 1) with varying amounts of polymorphonuclear neutrophil granulocytes (PMN), were observed. In the other parts of the lungs, sticking of PMN to endothelium and minute haemorrhages in the walls and into the lumen of bronchi were seen. The reaction to iron was strongly positive in hyaline thrombi and PMN in the rabbits with ferritin-antiferritin complexes (F-antiF). Fibrous fibrin was not detected. In the kidneys, an anaemic cortex was seen in some instances, in others there was pronounced stasis. Kidney from rabbits with F-antiF complexes had hyaline thrombi in the microcirculation with a strikingly positive reaction to iron. Otherwise the histological findings did not differ greatly with the antigen applied.

On electron microscopic examination, the lung microcirculation in the reaction induced by F-antiF complexes showed abundant presence of these complexes (Fig. 3). Ag-ab complexes were engulfed particularly by PMN (Fig. 4) or were surrounded by small aggregates of thrombocytes (Fig. 5). Complexes were also abundant in the liver microcirculation and ingested by Kupffer cells. In the spleen, macrophages and PMN were involved in removing the immune complexes from the lumen of the sinuses and in the cords, and there was intensive phagocytosis of blood platelets by macrophages. The sinus were filled

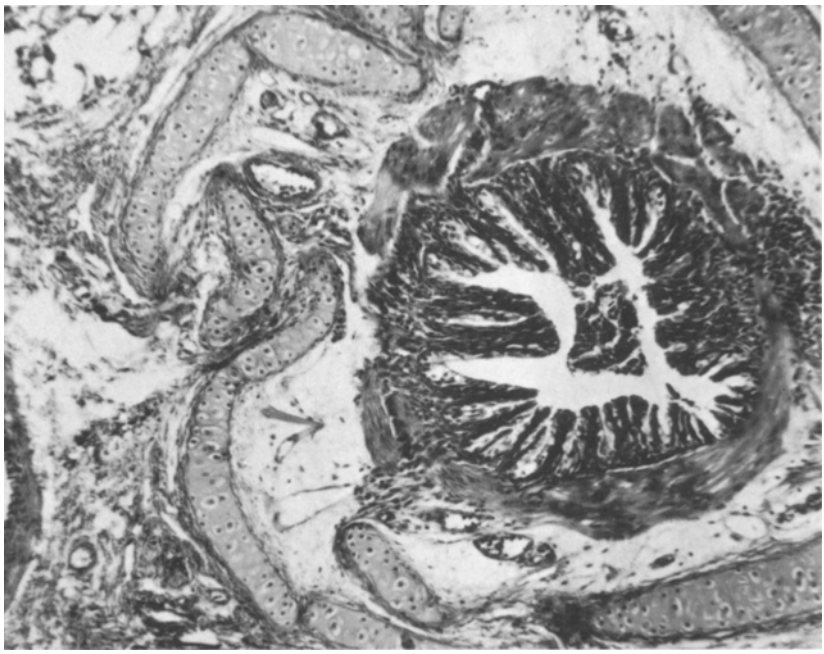

Fig. 2. Bronchospasm. SAR to ferritin. ×50

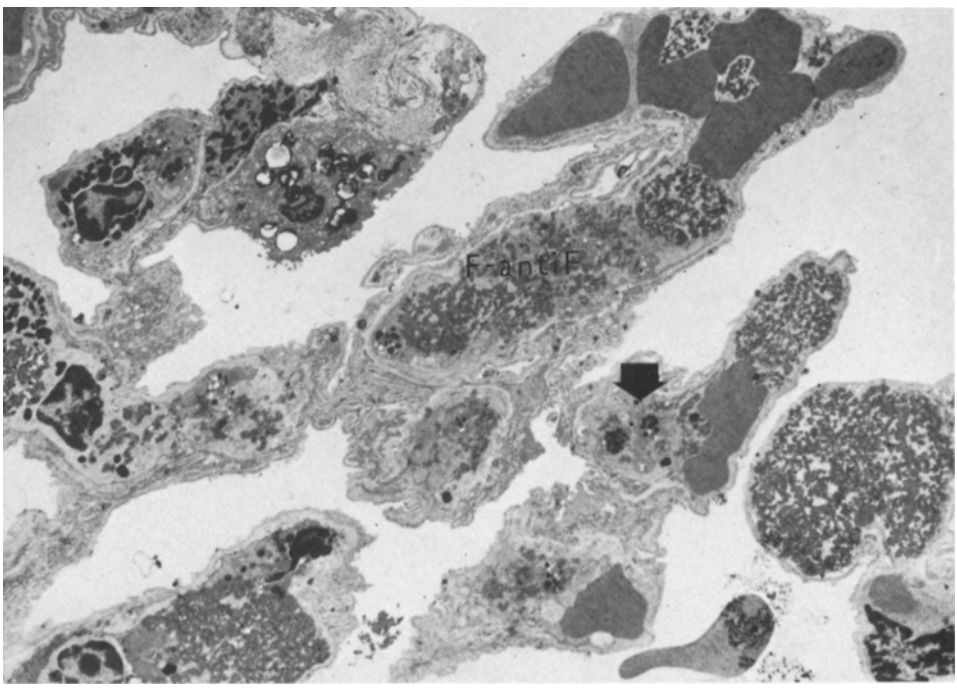

Fig. 3. Abundant presence of ferritin-antiferritin complexes and thrombocyte clusters in the lung microcirculation (arrow). ×3,000

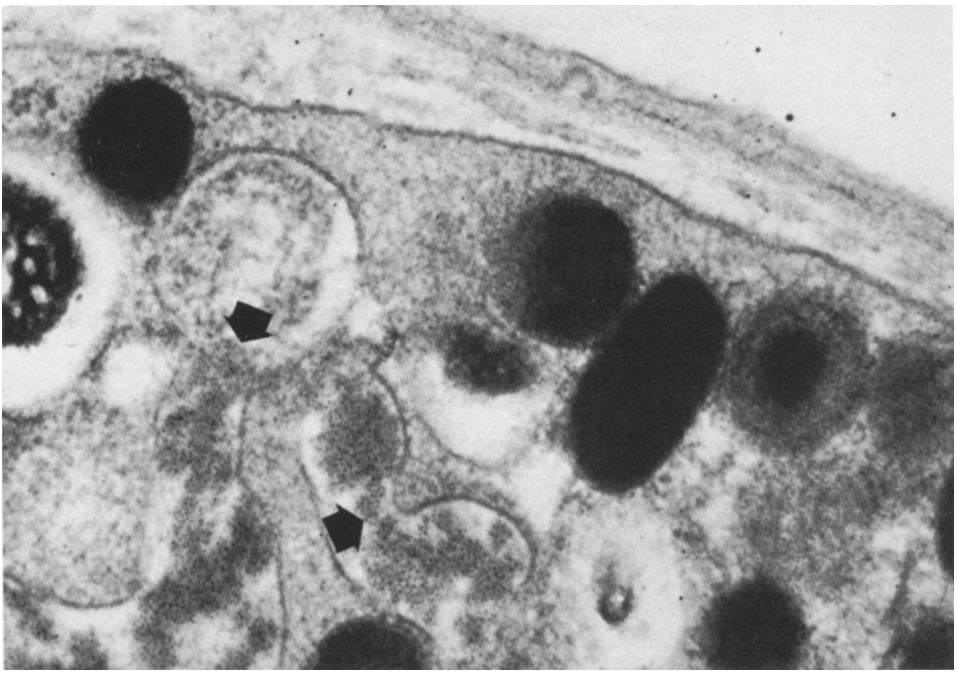

Fig. 4. Ferritin-antiferritin complexes in phagolysosomes of PMN (*arrows*). ×180,000

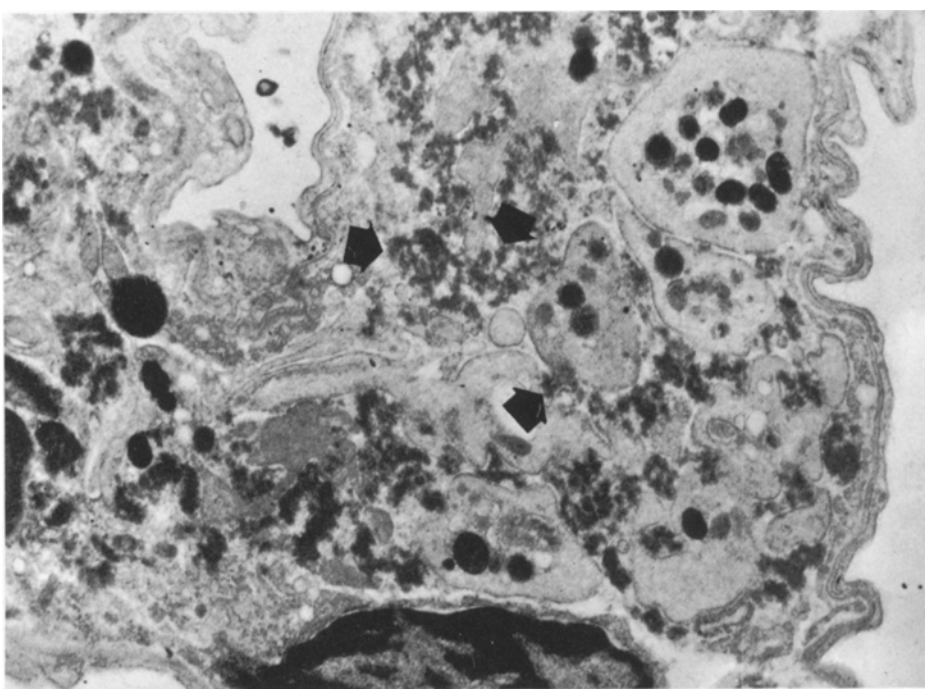

Fig. 5. Ferritin-antiferritin complexes between thrombocytes in a clusters (*arrows*). ×36,000

with numerous erythrocyte ghosts. Numerous thrombocyte clusters and F-antiF complexes were also found in the portal circulation of the pituitary and in the kidneys, especially in the capillary loops of the glomeruli. A remarkable finding was the intravascular thrombocyte wreaths around the PMN phagocytyzing the ag-ab complexes in the microcirculation. The basophils in the lung microcirculation did not engulf the ag-ab complexes, were not degranulated, and no ferritin was found on their surface. The endothelium was mostly undamaged. When the microcirculation was overfilled with clusters of thrombocytes, the endothelium of the capillaries and postcapillary venules showed loss of pseudopodia, diminished pinocytic activity, and swollen mitochondria. The interendothelial junctions were not significantly enlarged. The bone marrow did not show phagocytosis of immune complexes or degranulated basophils. The findings induced by the production of BSA-antiBSA complexes were comparable to those induced by F-antiF complexes.

Administration of antigens to nonimmunized animals failed to induce their aggregation in the microcirculation or the development of ag-ab complexes.

Systemic Anaphylactic Reaction (SAR)

Immunoelectrophoresis and Ouchterlony's test of the sera of rabbits sensitized with ABSA and AF failed to show the presence of specific precipitating antibodies. Heterologous PCA to ferritin and BSA was negative, homologous PCA to the given antigens yielded positive results in all these samples from rabbits sensitized agains ferritin and in 9 of 14 rabbits sensitized against BSA.

In the animals with SAR, the brownish-red spots were not seen in the lungs, yet but findings on dissection were comparable with those induced by ag-ab complexes. During histological examination signs of acute distension of the lungs of varying degrees were recorded. The majority of the affected animals were without extensive bronchospasm, which were seen occasionally (Fig. 2). In the vicinity of some bronchi and smaller veins there was considerable swelling. No marked stasis or hyaline thrombi were found in the pulmonary microcirculation. Focal leukostasis was present in the lungs, liver and spleen. The reaction to iron was also negative in animals sensitized with AF. Fibrous fibrin was not detected.

Regardless of the antigen applied, stagnation of individual platelets and their clusters (Fig. 6) mostly without very dense granules (VDG) were found in the microcirculation of alveolar septa. There was sporadic stasis of PMN, sticking to the endothelium (Fig. 7) and containing granules. There were, however, also individual decomposing PMN. Nondegranulated basophils were seen to accumulate sporadically in scattered groups. Their granules showed partial loss of content density (Fig. 8), and were also found extracellularly in small amounts.

In the capillary network, there was a considerable occurrence of erythrocyte ghosts and of fragments of membranes. The endothelium was not severely damaged, with the exception of the different cell cluster sites. In those places where basophils and thrombocyte aggregates attached to the endothelium, it exhibited very low or no pinocytic activity,was remarkably thined, sporadically fenestrated, or showed largely opened intercellular clefts. The endothelium of

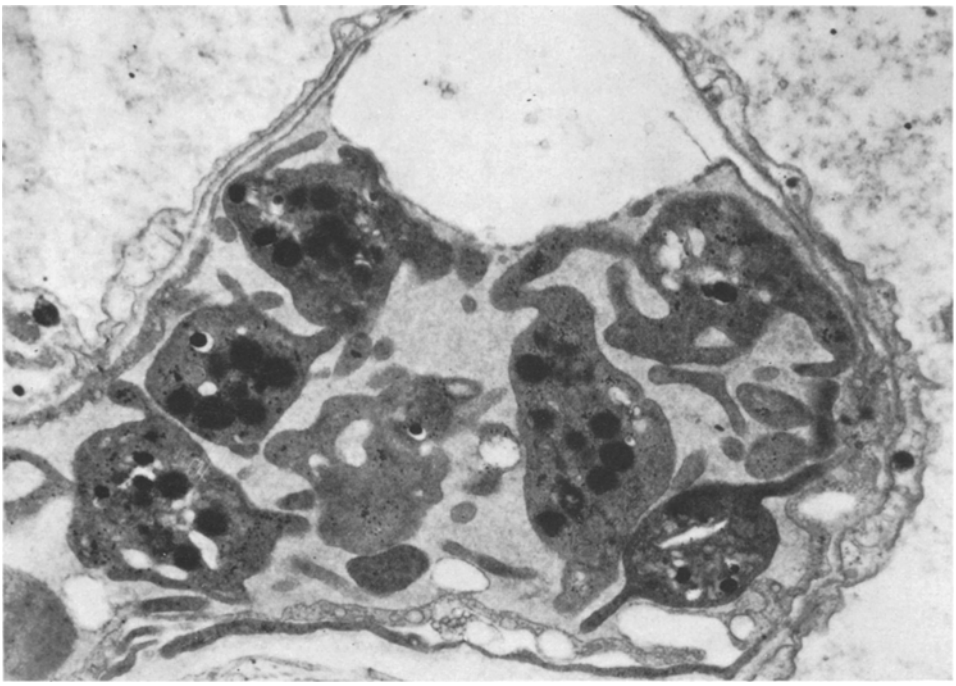

Fig. 6. Stagnation of small thrombocyte cluster in lung microcirculation. SAR to BSA. ×36,000

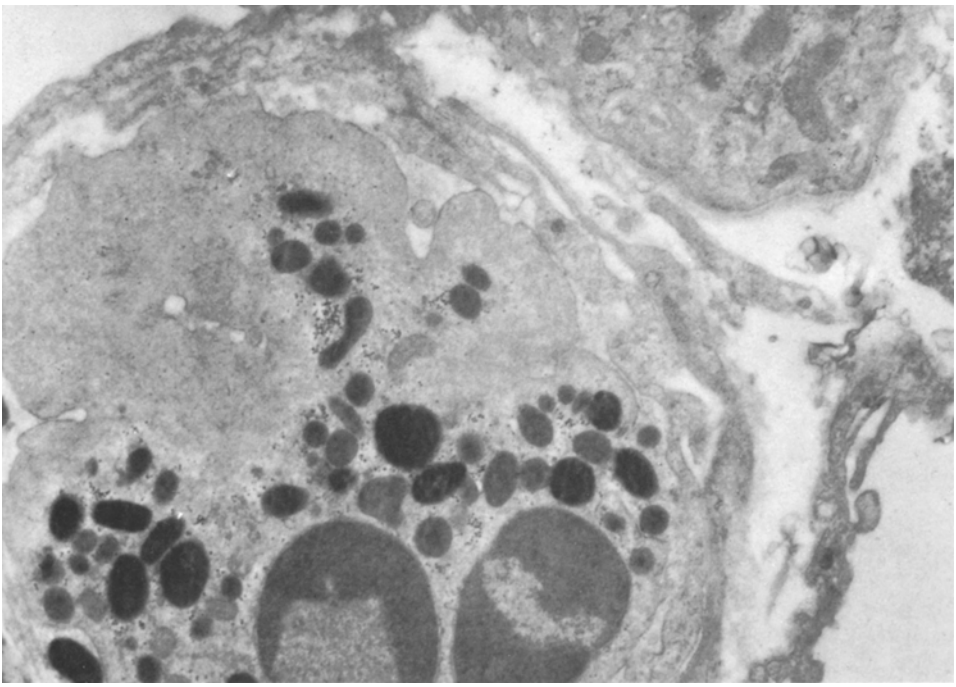

Fig. 7. PMN sticking to endothelium. No signs of ag-ab or antigen phagocytosis by PMN. SAR to BSA. ×16,000

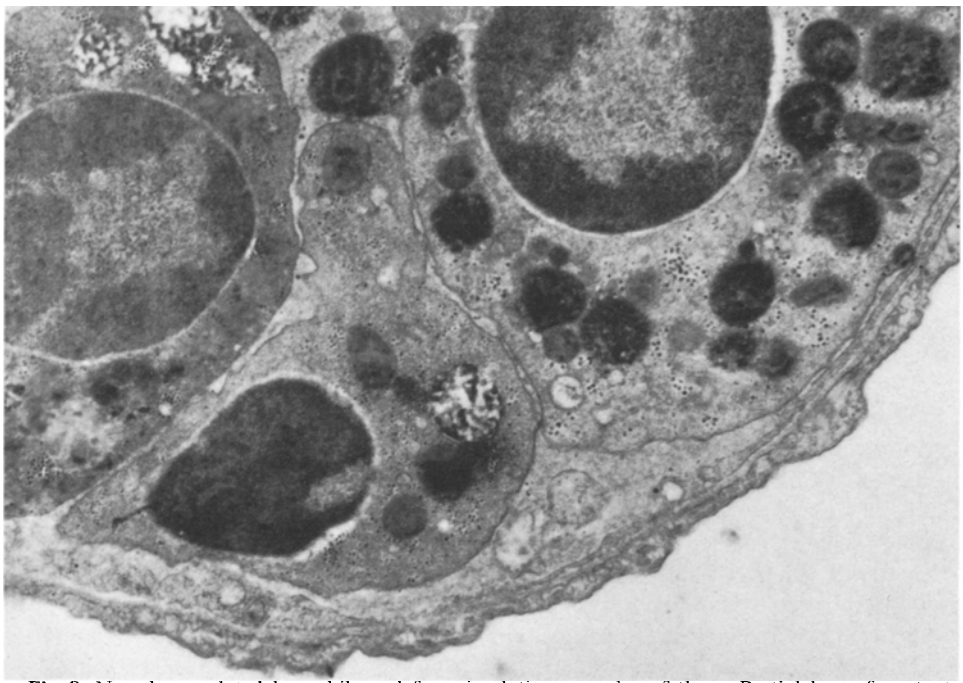

Fig. 8. Non-degranulated basophils and free circulating granules of these. Partial loss of content density of basophil granules. ×28,000

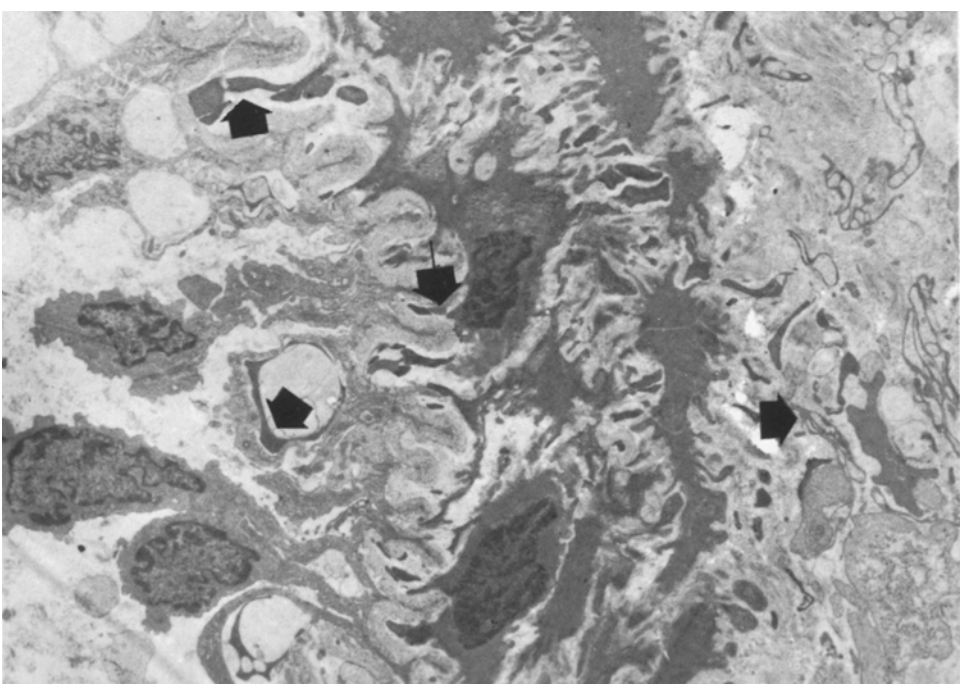

Fig. 9. Penetration of erythrocytes into vascular wall (*arrows*). ×6,000

smaller pulmonary arteries was frequently seen to be disengaged from common bundles. Erythrocytee penetrated the vascular walls (Fig. 9). In the animals sensitized with ferritin, this antigen was either found freely circulating, or it was seen between the thrombocyte clusters. The spleen displayed numerous thrombocyte aggregates, sometimes engulfed by macrophages. Some basophils in the bone marrow exhibited remarkably diminished density of granule content.

Discussion

A picture similar to that we have obtained in the presence of ag-ab complexes was described by several authors as light or electron microscopic findings of the manifestations of SAR (Germuth and McKinnon 1957; Weigle et al. 1960; Sabesin 1964; Goodman et al. 1979a, b). At present, however, the processes induced by ag-ab complexes are generally considered to be manifestations of serum sickness and have been investigated by electron microscopy in vitro (Henson 1971a, b), in vivo by light microscopy (Stadecker and Leskovitz 1973), and in vivo by electron microscopy (Brozman et al. 1976). These studies concerned type 3 hypersensitivity reaction (Gell and Coombs 1968), or type 4 hypersensitivity reaction according to the classification of Sell (1972, 1978).

Anaphylaxis in man and rat is nowadays a precisely defined response of the organism to antigens reacting with antibodies of the IgE class (reagins), bound to the surface of target cells (mast cells). It concerns type 1 hypersensitivity reactions of Coombs and Gell's classification and the type 1 hypersensitivity reaction of Sell's classification (1972, 1978). There is no complete description of the morphological picture of SAR (except for a short account on electron microscopic findings in the lungs of the rabbit (Pinckard et al. 1977)). We have attempted to describe the morphological picture of such a reaction in rabbits under precisely defined conditions.

To compare the morphological picture of SAR in rabbits sensitized according to Pinckard et al. (1972), we examined rabbits in the shock reaction induced by ag-ab complexes containing the same antigens as used in inducing SAR. Comparison of the two types of shock reaction revealed that they do not differ with the nature of the inducing antigen. The Pinckard et al. (1972) method gives a high yield of animals with circulating reagins, without the presence of a detectable amount of precipitins in the microcirculation determined by immunoelectrophoresis, Ouchterlony's test, or electron microscopic examination. Immunization of rabbits with antigens in complete Freund's adjuvant yielded animals with sera containing precipitating antibodies. In conformity with findings of Goodman et al. (1979b), we have also recorded changes in thrombocyte and leukocyte counts in SAR, following development of ag-ab complexes, but also after administration of an antigen to nonsensitized animals. These changes appear to be related to the decrease of blood platelets, as they were observed in amniotic fluid embolism (Brozman 1963) and after colloidal carbon injection (Donald 1972).

The changes in the behavior of rabbits with SAR or shock reaction induced by ag-ab complexes differed only in the intensity of response to antigen. The animals with shock reaction induced by ag-ab complexes were generally more severaly affected. No pronounced differences were observed on dissection. Our

findings were similar to those observed by Weigle et al. (1960) in the classical passive and active anaphylactic reaction of guinea pigs or rabbits.

The histological picture of the individual types of shock reactions did not differ according to the inducing antigen. Differences were however found to exist between SAR and shock reaction induced by ag-ab complexes. Hyaline thrombi occured during the shock reaction induced by ag-ab complexes, and were not to be present in SAR.

The most prominent difference on electron microscopy consisted of the presence of ag-ab complexes in the microcirculation of various organs of the animals affected with shock induced by these complexes. They were absent in animals with SAR. The thrombocyte clusters occuring during the reaction induced by the ag-ab complexes were usually more massive and tended to establish various intercellular relationships between PMN and thrombocytes, between thrombocytes and ag-ab complexes, between thrombocytes and monocytes, and between thrombocytes and endothelial cells. There were characterized by marked changes in the structure of the thrombocytes, which were frequently phagocytized by Kupffer cells and macrophages in the red pulp of the spleen. In an other experiment with peroxidase-antiperoxidase complexes (Jakubovský et al. in press), we also observed, in guinea pigs, ingestion of thrombocytes by PMN. The majority of these findings were not present in SAR. Although thrombocyte clusters did occasionally form in the lung microcirculation during SAR, they were small and accompanied by slight or absence of changes in the blood platelets. Pinckard et al. (1977) described a similar picture in the lungs of rabbits with SAR induced by IgE antiHRP antibodies. The morphological appearance of the platelets suggests that thrombocyte aggregation during SAR is of a reversible nature, in contrast to the irreversible changes induced in platelet by ag-ab complexes. The transient character of blood platelet aggregation is also indicated by the findings of Pickard et al. (1977) who showed that thrombocytopenia appearing 3–5 min after induction of SAR is followed by platelet deaggregation and their return to the peripheral circulation within normal prechallenge levels by 60 min. No relationship of ferritin molecules to any one type of assumed target cells (blood basophils or platelets) was observed. In SAR the relationship of antigen to target cells has been elucidated in man and rat, but is unclear in other experimental animals (Austen 1978). However, after the specific antigen has become bound to IgE fixed to the surface of target cells, an extensive redistribution of the reaction sites can occur and under in vivo conditions, the binding of antigen need not necessarily be permanent. In our experiments, no degranulation of blood basophils was observed, yet there were changes in granule density. Likewise, Henson and Pinckard (1977) had described a disappearance of the metachromatic staining properties of the circulating basophils accompanied by marked but transient thrombocytopenia in rabbits with SAR. Pinckard et al. (1977) concluded that the IgE-induced platelet alterations, probably induced by the intravascular release of basophil – derived platelet-activating factor, play a major role in the pathogenesis of SAR in the rabbit.

Further differences observed concerned neutrophil polymorphonuclear granulocytes. During shock reaction induced by ag-ab complexes the complexes were phagocytized by these cells. This is in contrast to SAR, where apart

from the sticking of PMN to endothelium and decomposition of a small number of these cells accompanied by liberation of their granules into the circulation, no phagocytosis was observed.

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