STUDIORUM PROGRESSUS

Proteolytic Activity in the Serum of Rabbits During Anaphylaxis

Introduction. Soon after the discovery of anaphylaxis by PORTIER and RICHET[1] it was suggested that anaphylaxis in the rabbit was due to acute dilatation and failure of the right heart[2]. This was attributed first to increase in pulmonary artery pressure due to constriction of the pulmonary artery[3]. Later circumstantial evidence pointed to obstruction of pulmonary capillaries by leucocyte-platelet microthrombi and release of histamine[4] and serotonin[5] from the aggregated cells. Since neither antihistamines[6] nor serotonin depletion[7] ameliorate systemic anaphylaxis in the rabbit, attention was subsequently focused on vascular obstruction. It has been known for some time that a high level of circulating antibody is essential to elicit anaphylaxis in the rabbit[8]. It has also been shown that pulmonary vessels become obstructed during anaphylaxis by eosinophilic masses[9]. It remained to be demonstrated that these masses contain I^{131} when labelled antigen was used[10], and that they are fluorescent when fluorescein tagged antigen was used for challenge[11]. Futhermore, anaphylaxis could be produced by injecting preformed antigen-antibody precipitates[11].

Thus obstruction of the pulmonary vessels by antigen-antibody precipitates is presently considered to be the main pathogenetic mechanism responsible for the development of anaphylaxis in the rabbit[12]. However, in other lesions inducted by immune precipitates, such as the Arthus reaction, the precipitates per se, although often plugging vessels[13], cause little tissue injury in the absence of polymorphonuclear(PMN)-leucocytes[14]. Recently evidence was presented that PMN-leucocytes may induce tissue injury when they phagocytose antigen-antibody precipitates[15] and thereby become degranulated, releasing their lysosomal enzymes[16]. These findings prompted us to investigate the possible role of PMN-leucocytic enzymes in anaphylaxis of the rabbit. It was found that anaphylaxis was induced by obstruction of the pulmonary vessels by the immune precipitates. However, PMN-leucocytes which phagocytose the immune precipitates become degranulated and in this process presumably release their lysosomal enzymes. A rise in plasma cathepsin level was detected during anaphylaxis, but was not detected in leucopenic rabbits. These enzymes may be responsible for vascular injury in the lung, particularly hemorrhage, and probably contribute to the development of protracted shock. Preliminary findings were reported recently[17].

Materials and Methods. Albino rabbits (2.5–3 kg) were immunized with antigen given subcutaneously into multiple sites including foot pads: 25 mg of bovine serum albumin (BSA) or horse ferritin (Pentex, Kankakee, Ill.) in complete Freund's adjuvant. Six weeks later the circulating antibody N was determined. Antibody N values varied between 0.2 and 1.2 mg per ml of serum. The antigen dose for intravenous challenge was based on the antigen required for equivalence in the quantitative precipitin test and the estimated plasma volume in each animal. Rabbits immunized with BSA were challenged with fluorescein isothiocyanate (Nutritional Biochemicals, Cleveland, Ohio) conjugated BSA. In order to compare anaphylaxis between leucopenic and normal immunized rabbits some rabbits were paired according to their circulating antibody N level. One rabbit of each pair with similar circulating antibody N level was made leucopenic with nitrogen mustard administered in two intravenous injections (1.75 mg and 1.0 mg/kg) 48 h apart. Hematologic findings on the day of challenge with antigen: total WBC 500–1500, differential count 2–5% neutrophils. At the time each animal was given nitrogen mustard they were also given an intramuscular injection of streptomycin-penicillin. Three days after the first nitrogen mustard injection rabbits were challenged with antigen in pairs (one normal and one leucopenic). All rabbits were observed clinically and signs of anaphylaxis (tachypnea, weakness, nystagmus, weaving of the head, defecation, urination and prostration) were recorded. Serum was collected before challenge and 1 h after challenge, except in those rabbits that died before the hour. Autopsies were performed on all animals.

Serum cathepsin activity was assayed[18] by incubating 1 ml of serum, 1 ml of 2% denatured hemoglobin and 1 ml of buffer, at 37 °C for 24 h. The reaction was stopped by adding 2 ml of 10% TCA. After 5 min the mixture was filtered through Whatman No. 40 filter paper. The optical density of the TCA supernatant was read at 280 nm. Blanks were prepared by adding TCA before the addition of the serum.

Results. Both normal and leucopenic rabbits with high levels (over 0.8 mg Ab N per ml of serum) of antibody died with severe signs of anaphylaxis within a few minutes. The main clinical difference between the normal and leucopenic rabbits that survived was that protracted shock with prostration persisted for hours in normal rabbits but not in those which had been made leucopenic.

At autopsy the lungs of non-leucopenic animals showed gross evidence of severe focal and diffuse hemorrhage, whereas the lungs of leucopenic animals were grossly normal, except for a few areas of atelectasis and a few small focal hemorrhagic areas.

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In animals challenged with fluorescein-labelled BSA, immune precipitates could be detected in the lumina of pulmonary vessels. In hematoxylin-eosin stained sections the pulmonary vessels of all rabbits contained eosinophilic masses. The pulmonary vessels in animals with a normal leucocyte count contained platelets and numerous PMN-leucocytes, both of which had phagocytosed the antigen-antibody precipitates. Many of the phagocytosed precipitates had fused with lysosomes (granules) to form digestive vacuoles (Figure 1). During this process many leucocytes and platelets had become degranulated (Figure 2).

Increase in proteolytic activity of serum during anaphylaxis could be detected only in rabbits with normal leucocyte counts (Table). The protease activity demonstrable in the serum had two peaks of activity: a large peak at pH 3 and a small peak at pH 7 (Figure 3).

Discussion. The demonstration of increased proteolytic activity in the serum of rabbits during anaphylaxis, together with ultrastructural changes indicative of the liberation of lysosomal enzymes is suggestive that the protease or proteases released during anaphylaxis are derived from the PMN-leucocyte lysosomes. Lysed PMN-leucocyte lysosomes hydrolyse various proteins, including antigen-antibody complexes and synthetic substrates. Their activity is inhibited partly by SH-enzyme inhibitors[19,20]. The proteases in leucocytic lysosomes are cathepsins[16]. The proteolytic activity demonstrable in the serum during anaphylaxis resembles that obtained with lysed PMN-leucocyte lysosomes[19,20]. Since there are no acid proteases in the serum[21] the two most likely sources are PMN-leucocytes and platelets[22]. The acid cathepsins released in the serum from PMN-leucocytes are probably the cathepsins D and E described by LAPRESLE and WEBB[23]. It could be argued that proteases with a peak activity of such low pH could not act in vivo. However, low pH values are reported to occur in digestive vacuoles

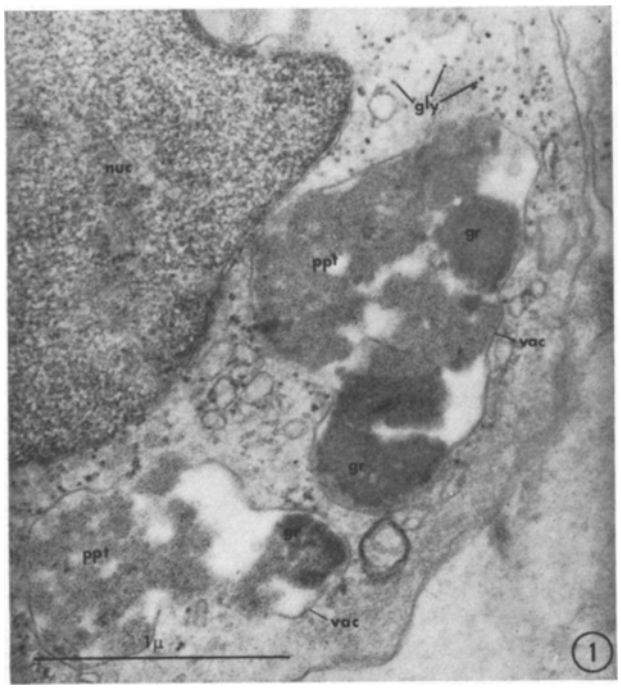

Fig. 1. PMN-leucocyte in capillary of lung. It has a digestive vacuole (vac), which contains ferritin-antiferritin precipitates (ppt), together with fragments of granules (gr). gly = glycogen. × 58,000.

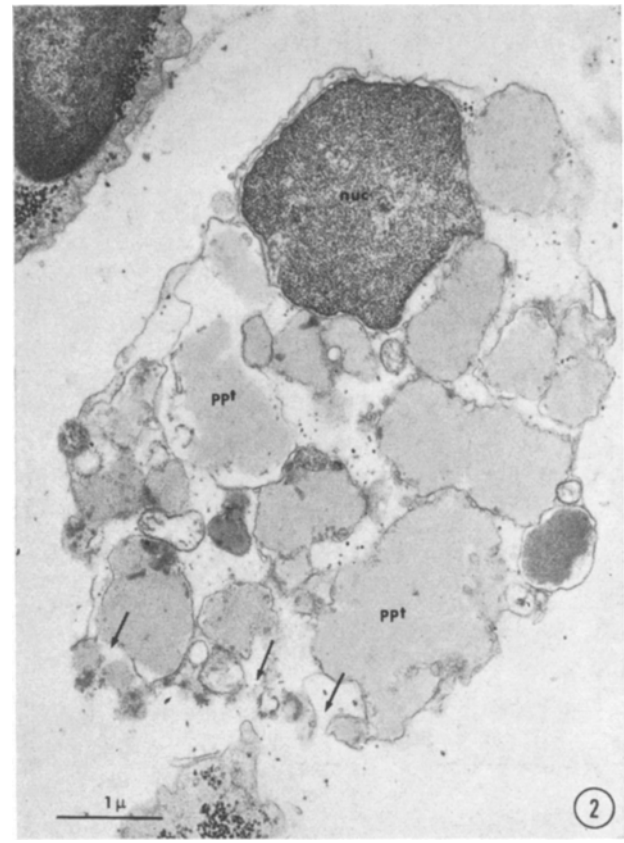

Fig. 2. Completely degranulated PMN-leucocyte showing signs of degranulation. The nucleus (nuc) is piknotic and the cytoplasm, which is almost free of glycogen, has innumerable vacuoles which contain precipitates (ppt) of BSA-anti BSA. The arrows point to disruptions in the cellular membrane. This is not a fixation artifact; note portion of a well preserved cell in the left upper corner. × 24,000.

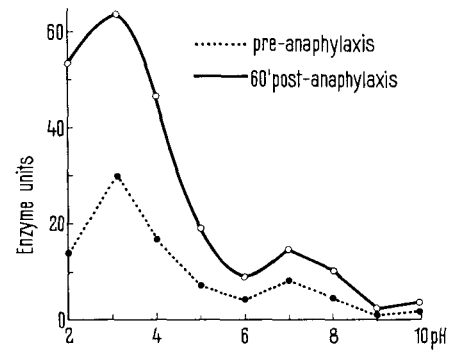

Fig. 3. Serum cathepsin activity during anaphylaxis. Effect on 2% denatured hemoglobin at various pH values (see Table).

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of cells, where substrate and enzyme interact[24] and the intracellular pH of phagocytic cells was said to be 3 or less[25]. If released, acid cathepsin could not act at physiological pH levels. However, when pulmonary vessels become impacted by antigen-antibody precipitates and occluded by aggregated leucocytes and platelets, locally released acid cathepsins could be protected from the buffering capacity of the serum and could cause local alterations in the walls of vessels. A marked drop in pH in inflammatory exudates has been documented[26].

Mechanical plugging of the pulmonary vessels appears to be the major pathogenetic event in systemic anaphylaxis in the rabbit. Obstruction of these channels accounts for the symptoms observed immediately after the challenging dose of antigen and massive obstruction usually ends fatally. However, if the animal survives the first critical phase, other factors besides plugging begin to assume importance. Pharmacologically active substances, such as histamine, serotonin and slow reacting substance seem to play but a minor, if any, role[12]. There is a slight delay in the onset of hypotension in the absence of plasma kinins[27]. However, proteases presumably derived from PMN-leucocytes and platelets may play a significant role in protracted anaphylaxis. Leucopenic rabbits which do not die within a few minutes, due to the mechanical obstruction caused by the immune complexes, rapidly recover. Rabbits with a normal leucocyte count remain in a state of prostration. Furthermore, hemorrhage in the lungs, as in the hemorrhagic skin lesion of the Arthus reaction[13,14] can develop apparently only when PMN-leucocytes are present and show evidence of degranulation.

Finally, it should be noted that the systemic anaphylaxis, as described in this paper, probably occurs to some degree in all species when intravenously injected antigen interacts with circulating antibody and should perhaps be referred to as a systemic[6] 'Arthus reaction'. An 'anaphylactic antibody' has been described in many species and more recently passive cutaneous anaphylaxis, due to such 'anaphylactic antibody' has been described also in the rabbit[28]. It remains to be investigated whether systemic anaphylaxis can be induced in the rabbit with a tissue binding 'anaphylactic antibody'[29].

Zusammenfassung. Während der Anaphylaxie des Kaninchens entstehen intravaskuläre Antigen-Antikörperkomplexe, die von den Leukozyten phagozytiert werden. Die in den Lysosomen (Granula) vorhandenen proteolytischen Enzyme werden dabei freigesetzt, und im Serum nachgewiesen.

Serum cathepsin levels during anaphylaxis in rabbits

No. of rabbits	Enzyme units[a]			
	Pre-anaphylaxis		60 min post-anaphylaxis	
	Mean	S.E.[b]	Mean	S.E.[b]
Normal 10	13.9	1.6	36.5	2.12
Leucopenic 5	9.7	2.67	12.1	3.18

[a] 1 enzyme unit produces an optical density (at 280 nm) of $1 \cdot 10^{-3}$ per h/ml of serum at 37 °C. Equal volumes of serum, 2% denatured hemoglobin and buffer (citric acid-Na-citrate pH 4.0) were incubated at 37 °C for 24 h, the reaction stopped with 10% TCA and the filtrate read at 280 nm. [b] Standard error.

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COGITATIONES

Estimation de la redondance de figures de contour[1]

Nous nous sommes demandé si la structure des formes visuelles pouvait recevoir une expression mathématique. C'est dans la théorie de l'information, qui s'est révélée utile dans l'étude quantitative du langage, que nous avons cherché la solution de notre problème. Dans la transmission du message verbal, ce qui se présente comme de l'information superflue, à savoir l'organisation des signes sous l'action des règles sémantiques, syntaxiques, pragmatiques et autres, constitue la structure à laquelle la théorie de l'information donne le nom de redondance. Elle s'exprime par la formule:

$$R = \frac{H(\max) - H}{H(\max)} \cdot 100 \qquad (1)$$

dans laquelle H(max) représente la somme de l'information contenue dans chacun des signes, et H l'information réelle émise par le message.

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