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Acute generalized microvascular injury by activated complement and hypoxia: the basis of the adult respiratory distress syndrome and multiple organ failure?

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Summary. It has been suggested that generalized endothelial damage and permeability changes, induced by prolonged activation of the complement system and ensuing release of lysosomal enzymes, prostaglandins and toxic oxygen products, underlie the genesis of the Adult Respiratory Distress Syndrome (ARDS) and Multiple Organ Failure (MOF). The effects in New Zealand white rabbits were investigated of a 4 h infusion of activated complement and its combination with a short hypoxic episode on respiratory function, leukocyte count, platelet count and morphology of the lungs, heart, liver, kidney and spleen. Prolonged activation of the complement system induced hyperventilation with respiratory alkalosis and hypocapnia, depletion of granulocytes (PMN), and a variable accumulation PMN in the capillaries of all organs examined, in combination with interstitial, and, in the liver, cellular oedema. Electron microscopy of the lungs revealed degranulation of PMN, endothelial swelling and widening of the alveolar septa. The combination of hypoxia and systemic complement activation appeared to aggravate this microvascular injury with the occurrence of protein rich alveolar oedema and haemorrhage in the lungs and accumulation of PMN debris containing macrophages in the spleen. The alterations in respiratory function and pulmonary morphology in these rabbits, imitate the clinical and morphological characteristics of the early phase of ARDS. The inflammatory reaction, found in all other organs examined, might represent the early phase of MOF. If so, ARDS and MOF—clinically closely interconnected syndromes—might be interpreted as manifestations of the same syndrome and as the clinical expression of an uncontrolled whole body inflammation.

Keywords; adult respiratory distress syndrome, multiple organ failure, complement activation, polymorphonuclear granulocyte, hypoxia

In vitro and *in vivo* studies have shown that polymorphonuclear granulocytes (PMN), exposed to activated complement, damage the vascular endothelium, thereby increas-

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ing permeability (Goldstein *et al.* 1977; Hammerschmidt *et al.* 1981; Kuehl *et al.* 1979; Sacks *et al.* 1978). This mechanism is recognized as an important pathway in the genesis of ARDS (Hammerschmidt 1983; Hohn *et al.* 1980). However, attempts to imitate this syndrome, by use of prolonged complement activation in animal models, have yielded contradictory results even when the same model was used (Borg *et al.* 1984; Bowers *et al.* 1983; Hohn *et al.* 1980; Opdahl *et al.* 1984; Webster *et al.* 1982). Since an activated complement system (Bjornson *et al.* 1981; Heideman 1978; Heideman 1979; Heideman *et al.* 1982, Lankisch *et al.* 1981, McCabe 1973; Sharma *et al.* 1980; Solomkin *et al.* 1981) is frequently accompanied by hypoxic episodes in clinical situations predisposing to ARDS, hypoxia may enhance the deleterious effects of complement activation.

It has been suggested that ARDS is the first organ failure to occur in MOF (McMenamy *et al.* 1981), and that a common pathophysiologic mechanism could underlie both syndrome (Nuytinck & Goris 1985*b*. Goris *et al.* 1985).

In the current study the early phase of ARDS was mimicked in unanaesthetized rabbits by use of a 4 h period of complement activation in combination with a 20 min episode of hypoxia. In addition, searching for early morphological characteristics of MOF, the effects of the combination of complement activation and hypoxia on the morphology of organs other than the lungs was studied. The complications of general anaesthesia and surgical manipulations were avoided by use of local anaesthesia and a 2 h recovery period, before starting the experiment.

Materials and methods

In New Zealand white rabbits zymosan activated homologous plasma (ZAP), obtained by exsanguination of donor rabbits, was infused at a rate of 1 ml/min over 4 h ($n=5$). The control group received unactivated plasma (UP, $n=5$). Slight changes were seen in leukocyte count and arterial blood gas values of rabbits receiving an infusion with UP, possibly due to complement activation in the donor blood (Nuytinck & Goris 1985*a*), and so another group of control animals was added. These rabbits received the plasma expander haemaccel (HAE), infused at a rate of 1 ml/min ($n=5$). The effects of a hypoxic insult plus complement activation were studied by applying a 20 min period ($t_{10}-t_{30}$) of spontaneous hypoxic breathing, with an inspired oxygen fraction of 0.1, resulting in another two groups: one with zymosan activated plasma and hypoxia (ZAP.H, $n=5$) and one with haemaccel and hypoxia (HAE.H, $n=5$).

Using 1 ml of Xylocaine 1% for local anaesthesia, the femoral artery and vein were cannulated for blood sampling, monitoring of blood pressure and pulse rate, and infusion. Thereafter the animals were restrained in a prone position and allowed to recover for 2 h. The experiment was then performed without anaesthesia.

During the 4 h infusion blood samples for arterial blood gas analysis were withdrawn at 30 min intervals. Platelet count, total and differential white cell count were performed at t_0, t_5, t_{15}, t_{60} and then hourly. Normal values for rabbits in this laboratory are: leukocytes $6.2\pm 1.9\times10^9$/l and platelets $210\pm65\times10^9$/l ($n=9$, mean $\pm$SD).

Animals were killed with 4 ml of intravenous Nembutal. Lungs, trachea and heart were removed *en bloc* after ligating the aorta and the pulmonary artery and veins. The spleen, right kidney and about 1 cm^3 of liver tissue were then removed and fixed in 4% formaldehyde. The lungs were fixed by intratracheal instillation of 4% formaldehyde. Blocks of lung tissue (1 cm^3) were taken from the right middle lobe. Tissue samples were embedded in paraffin after dehydration, and 4 μm sections were prepared and stained with haematoxylin and eosin (H&E). The PMNs in the lungs were counted in five fields at a magnification of $\times400$.

Only lung tissue was examined by electron microscopy. About 10 fragments of fresh lung tissue (1 mm^3) were removed from the

right middle lobe and fixed in 2.5% glutaraldehyde, postfixed in osmium tetroxide, dehydrated through graded ethanols, passed through a propylene oxide/Epon mixture, and embedded in Epon. Thin sections (approximately 60 nm) were cut and stained with lead citrate and uranyl acetate.

To compare PMNs in the microvessels with those in peripheral blood, 8 ml samples of venous blood were taken at the end of each experiment and anticoagulated with sodium citrate. The buffy coat from these samples, obtained by centrifugation (10 min at 1500 ct/min), was fixed for 4 h at 4°C in 2.5% phosphate-buffered glutaraldehyde and embedded in Epon. The ultrathin sections were stained as described above.

All sections were examined blind.

Statistics. For comparisons within and between groups the sign ranked and Kruskal-Wallis tests were used, respectively. P values ≤ 0.05 were considered significant.

Statistical evaluation was performed using medians of the absolute parameter changes with respect to the baseline values at t_0. In the figures, for the sake of clarity, means $\pm$SEM are depicted. The statistically significant differences in the figures refer to evaluation of the medians.

Results

No difference between the five groups was observed in the baseline values ($n=25$) of any of the parameters studied.

Arterial blood gases

Baseline values of P_{O_2} were 11.3 (8.8–15.4) kPa ($n=25$). Significant decreases were found in the HAE.H- and ZAP.H rabbits during the hypoxic episode ($P<0.01$), after which P_{O_2} returned to baseline values. No significant decreases occurred in the remaining groups, nor were significant differences observed between groups.

Baseline values of P_{CO_2} were 4.1 (3.4–5.5) kPa ($n=25$). P_{CO_2} decreased significantly in

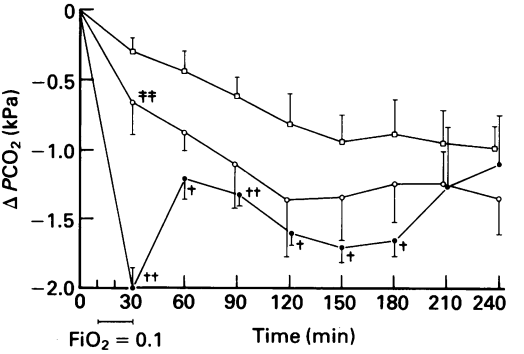

Fig. 1. Changes in P_{CO_2} levels (kPa). Baseline values UP, 4.2 (3.9–5.5); ZAP, 3.5 (3.4–4.8); ZAP.H: 3.7 (3.4–4.5).
*, UP versus ZAP, $P<0.05$; †, ZAP versus ZAP.H, $P<0.05$; ‡, ZAP.H versus UP, $P<0.05$. Double symbols, $P<0.01$ □, plasma; ○, activated plasma; ●, activated plasma + hypoxia.

all groups ($P<0.01$). No significant differences were found between the UP-, HAE- and HAE.H groups, except for the hypoxic episode. Complement activation alone did not decrease the P_{CO_2} below that of the UP rabbits, whereas the addition of hypoxia caused significantly more severe hypocapnia from t_{30} till t_{180} (Fig. 1).

Baseline values of pH were 7.40 (7.37–7.50) ($n=25$). Apart from a temporary peak into the alkaline range in the hypoxic animals, the pH of the HAE- and HAE.H rabbits remained constant at baseline level (data not shown). The pH of the UP-, ZAP- and ZAP.H groups increased significantly ($P<0.05$). At the end of the experiment the pH of the ZAP.H group returned to the baseline. Except at t_{30} and t_{240}, no significant differences were found between the UP-, ZAP- and ZAP.H groups (Fig. 2).

Baseline levels of bicarbonate were 19.6 (14.8–27.1) mmol/l ($n=25$). Again no differences were found between the UP-, HAE- and HAE.H rabbits, except for a significantly greater fall in bicarbonate concentration in the HAE.H animals at t_{30}. In all groups bicarbonate levels decreased steadily during the first 2 h. Complement activation alone did not decrease the bicarbonate concentra-

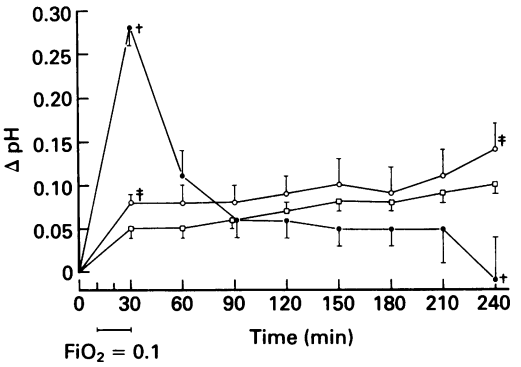

Fig. 2. Changes in pH levels. Baseline values UP, 7.42 (7.40–7.44); ZAP, 7.39 (7.37–7.45); ZAP.H, 7.46 (7.41–7.50).

*, UP versus ZAP, $P<0.05$; †, ZAP versus ZAP.H, $P<0.05$; ‡,ZAP.H versus UP, $P<0.05$. Double symbols, $P<0.01$. □, plasma; ○, activated plasma; ●, activated plasma + hypoxia.

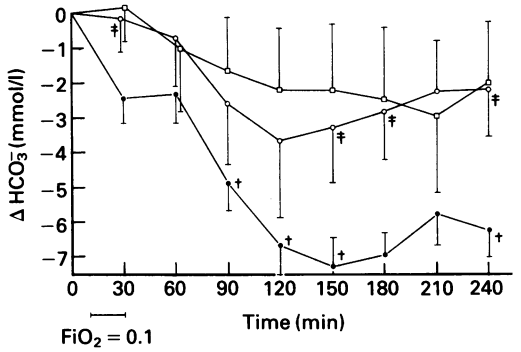

Fig. 3. Changes in bicarbonate concentrations (mmol/l). Baseline values UP, 19.4 (17.5–27.1); ZAP, 16.9 (14.8–21.1); ZAP.H, 19.4 (17.2–21.2).

*, UP versus ZAP, $P<0.05$; †, ZAP versus ZAP.H, $P<0.05$; ‡, ZAP.H versus UP, $P<0.05$; Double symbols, $P<0.01$. □, plasma; ○, activated plasma; ●, plasma + hypoxia.

tions below those of the UP animals, whereas adding hypoxia did cause a further reduction (Fig. 3).

Granulocytes and platelets

Baseline numbers of leukocytes were 5.8 (4.0–8.2) × 10^9/l ($n=25$). An immediate and pronounced drop in leukocyte count, mainly due to PMN depletion, was observed in the ZAP- and ZAP.H rabbits, whereas leukocyte count remained at or above baseline level in controls, apart from a significant decrease in the HAE.H group at t_{180} and t_{240} ($P<0.05$, data not shown).

Baseline numbers of PMNs were 2.3 (1.0–4.3) × 10^9/l ($n=25$). PMN count showed a sustained decrease of approximately 100% in the ZAP- and ZAP.H rabbits (Fig. 4). Significant differences in leukocyte and PMN count between the ZAP/ZAP.H animals and the others were found at all times, except at t_0. The reductions in PMN count of the ZAP- and ZAP.H rabbits did not differ significantly.

Baseline numbers of platelets were 210 (110–326) × 10^9/l ($n=25$). Platelet counts showed a significant decrease in all groups ($P<0.05$), without significant differences between the ZAP- and UP rabbits nor between the ZAP- and the ZAP.H rabbits (Fig. 5). In the first hour only there was a significantly larger fall in platelet count in the ZAP.H group than in the UP group. Those in the HAE- and HAE.H rabbits did not differ significantly from the UP animals.

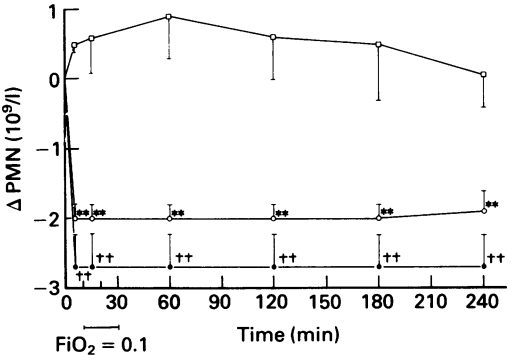

Fig. 4. Changes in PMN count (10^9/l). Baseline values UP, 1.1 (1.0–2.5); ZAP, 2.0 (1.6–2.8); ZAP.H, 2.6 (1.5–4.3).

*, UP versus ZAP, $P<0.05$; †, ZAP versus ZAP.H, $P<0.05$; ‡, ZAP.H versus UP, $P<0.05$; Double symbols, $P<0.01$. □, plasma; ○, activated plasma; ●, activated plasma + hypoxia.

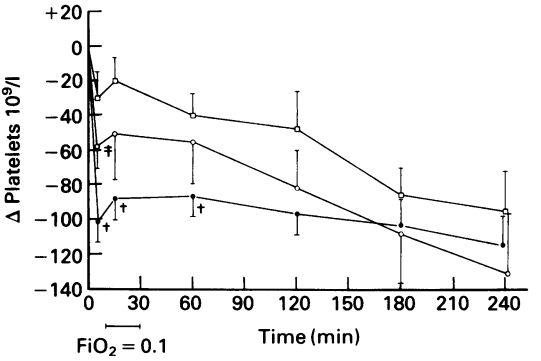

Fig. 5. Changes in platelet count (10^9/min). Baseline values UP, 223 (197–326); ZAP, 192 (110–326); ZAP.H, 206 (121–253).

*, UP versus ZAP, $P<0.05$; †, ZAP versus ZAP.H, $P<0.05$; ‡, ZAP.H versus UP, $P<0.05$; Double symbols, $P<0.01$. □, plasma; ○, activated plasma; ●, activated plasma + hypoxia.

Morphological studies

Pink frothy sputum, draining from the nose and mouth, was seen in ZAP.H rabbits only and the lungs of these animals had extensive haemorrhagic areas. Tiny haemorrhagic spots were found also on the lungs in ZAP rabbits. The ZAP.H rabbits showed more extensive haemorrhagic areas.

The lungs

Morphological changes were focal, so that the sections studied may not be fully representative. The number of PMNs in the lung vasculature was significantly higher ($P<0.05$) in the ZAP rabbits (85 ± 15= mean ± SD) than in other groups (UP, 44 ± 16; HAE, 49 ± 12; ZAP.H; 51 ± 8; ZAP.H, 29 ± 25). Aggregates of PMN were observed only in ZAP and ZAP.H rabbits; in other groups mainly individual cells were found (Fig. 6). All five ZAP.H rabbits had slight septal and extensive perivascular and subpleural interstitial oedema, whereas ZAP.H rabbits also had protein-rich alveolar oedema (Fig. 7).

At electron microscopy, solitary or aggregated PMNs, adherent to the vascular endothelium, were observed in all groups, but were most numerous in ZAP and ZAP.H rabbits. Degranulation and endothelial swelling occurred only in these two groups. The alveolar septa of ZAP- and ZAP.H rabbits were widened (Fig. 8). As PMNs had virtually disappeared from the circulation in the ZAP and ZAP.H rabbits their morphology in peripheral blood could not be studied in these groups. In the remaining groups PMNs in

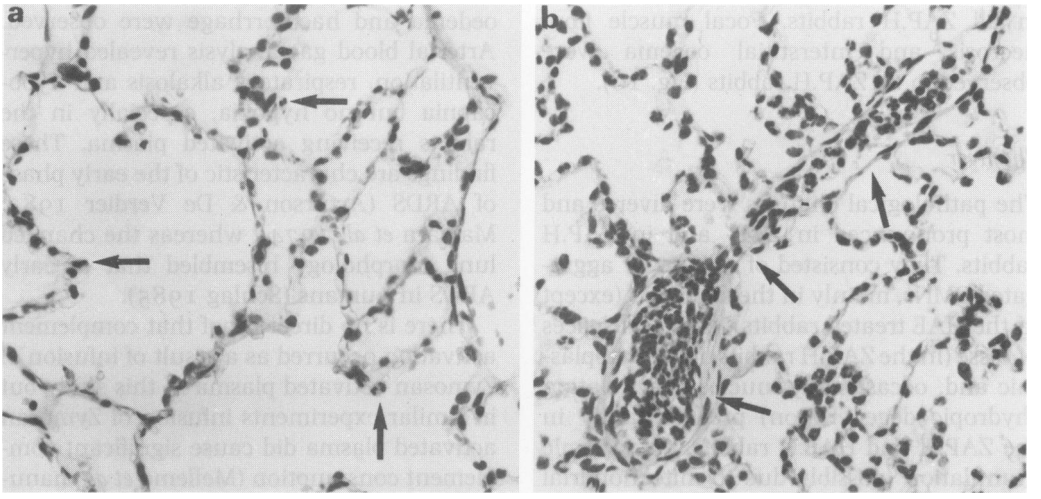

Fig. 6. Lung tissue *a*, UP-treated animal. Arrows, solitary PMN. *b*, ZAP-treated animal. Arrows, intravascular, aggregated PMN. Many PMN in the alveolar septa. × 255.

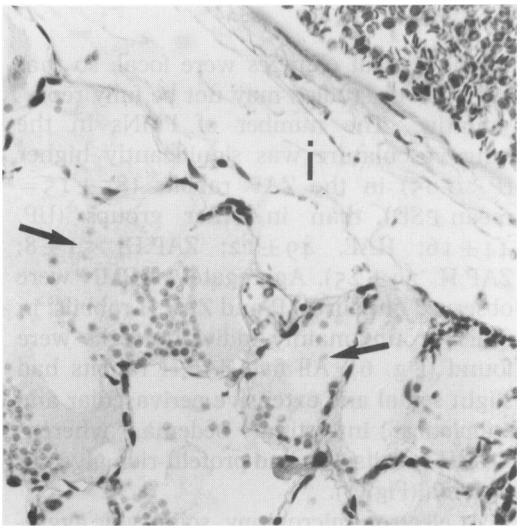

Fig. 7. Lung tissue. ZAP.H-treated animal. Arrows, alveoli, filled with protein rich edema and erythrocytes. i, interstitial oedema. ×270.

peripheral blood showed no degranulation (Fig. 9).

The heart

Accumulations of PMNs were not seen in UP and HAE rabbits, were occasional and focal in the ZAP and HAE.H-, and were moderate in all ZAP.H rabbits. Focal muscle fibre necrosis and interstitial oedema were observed in all ZAP.H rabbits (Fig. 10).

The liver

The pathological changes were diverse and most pronounced in ZAP- and in ZAP.H rabbits. They consisted of solitary or aggregated PMNs, mainly in the sinusoids (except in the HAE treated rabbits), enlarged spaces of Disse (in the ZAP.H rabbits only), cytoplasmic and, occasionally, nuclear vacuolation (hydropic degeneration) predominantly in the ZAP.H and HAE.H rabbits, cytoplasmic granulation (possibly due to mitochondrial swelling), and interstitial oedema in the ZAP.H rabbits only (Fig. 11).

The kidney

Slight accumulation of PMNs in the glomerular capillaries, widening of the tubules and intratubular protein precipitation were found consistently in ZAP- and especially in ZAP.H rabbits but only occasionall in UP-, HAE- and HAE.H rabbits. The tubular cells of ZAP.H animals had an unusually granular cytoplasm. Interstitial oedema was observed only once in the ZAP group (Fig. 12).

The spleen

Spleens of ZAP rabbits and most markedly those of ZAP.H rabbits, had remarkable accumulations of macrophages containing cellular debris, probably remnants of PMNs and platelets. The number of PMNs increased consistently in ZAP rabbits and occasionally in UP-, HAE- and HAE.H animals (Fig. 13).

Discussion

In rabbits, 4 h infusion of Zymosan activated plasma combined with a 20 min period of hypoxia induced a variable degree of PMN accumulation in the capillaries of many organs plus interstitial and, in the liver, cellular oedema; ie., a whole body inflammatory reaction. In the lungs also alveolar oedema and haemorrhage were observed. Arterial blood gas analysis revealed hyperventilation, respiratory alkalosis and hypocapnia but no hypoxia, especially in the rabbits receiving activated plasma. These findings are characteristic of the early phase of ARDS (Arturson & De Verdier 1983; Mazzara *et al.* 1974), whereas the changed lung morphology resembled that of early ARDS in humans (Schlag 1985).

There is no direct proof that complement activation occurred as a result of infusion of Zymosan activated plasma in this study but in similar experiments infusion of Zymosan activated plasma did cause significant complement consumption (Mellema *et al.* manuscript in preparation). In the experiments of Mellema *et al.* rabbit-C_3 was determined by

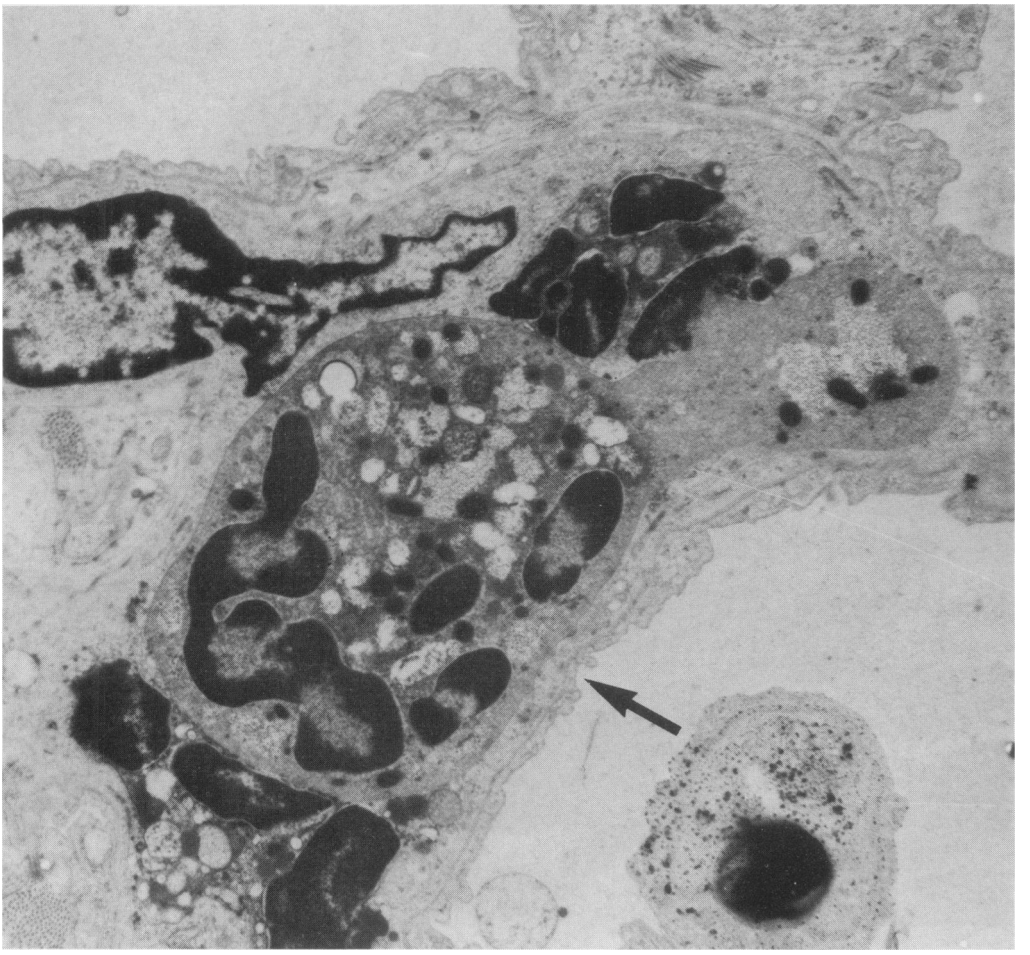

Fig. 8. Aggregate of degranulating PMN (arrow), adherent to the slightly swollen pulmonary vascular endothelium, in a ZAP rabbit. × 8840.

laser nephelometry and goat antilapine C_3, as described earlier (Nuytinck & Goris 1985*a*). Moreover, acute depletion of granulocytes is regarded as a specific sign of complement activation (O'Flaherty *et al.* 1979) and it is established practice to determine the level of C_{5a} formation indirectly *in vitro* by measuring the intensity of PMN aggregation (Craddock *et al.* 1977).

The complement activation product C_{5a} induces PMN depletion by causing PMN aggregation. *In vivo*, C_{5a} causes PMNs to adhere to vascular endothelium. These activated PMNs subsequently release lysosomal enzymes, prostaglandins and toxic oxygen species, which may cause endothelial cell damage and permeability changes (Henson 1982; Jacob 1980; Sacks *et al.* 1978). This would explain the granulocytopenia, PMN accumulation in the microvessels and PMN degranulation in the pulmonary capillaries. The slow decrease in platelet count in the ZAP- and ZAP.H rabbits may be the result of secondary activation of the coagulation system following activation of the complement cascade. Furthermore, the number of plate-

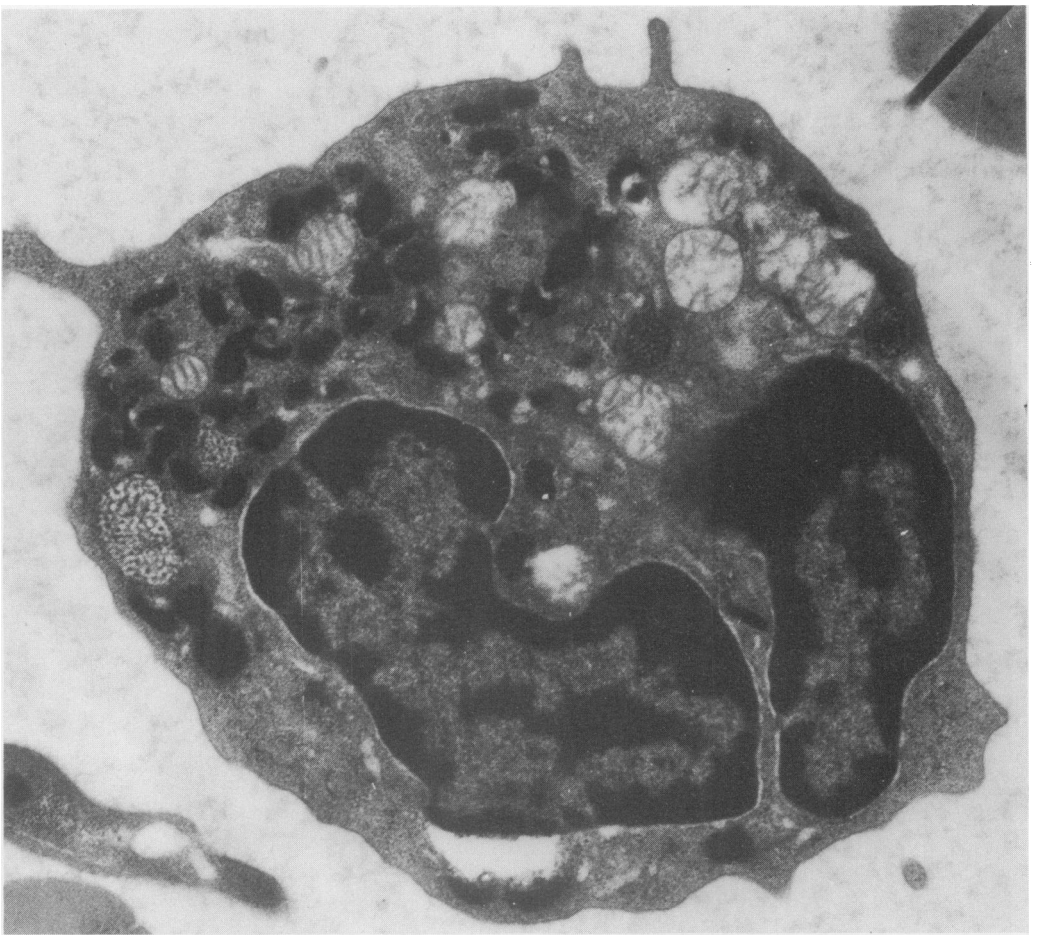

Fig. 9. Normal PMN in peripheral blood of a UP rabbit. No degranulation. × 17680.

lets may fall by adsorption to the vascular endothelium, already damaged by activated PMNs. The slight and sometimes significant changes in the various parameters in the UP group may result from a modest activation of the complement system (Nuytinck & Goris 1985*a*). Hence, the UP animals could receive slightly activated, instead of unactivated plasma. The minor changes in the HAE- and HAE.H rabbits may be the results of overhydration and hypoxia respectively.

Such data support the concept that complement activation plays a central part in the development of ARDS. However, there are a few caveats. Complement activation alone induces only minor changes in this particular model, whereas other investigators (Hohn *et al.* 1980) reported ARDS-like physiological and morphological changes after complement activation alone. Webster *et al.* (1982) showed that systemic complement activation in rabbits, by either cobra venom factor or infusion of Zymosan activated plasma or purified rabbits C_{5a} failed to produce significant pulmonary inflammation as indicated by PMN emigration or increased vascular permeability, unless these agents were combined with anaesthesia, surgical manipulation or intubation. These additional insults were avoided in our model, but

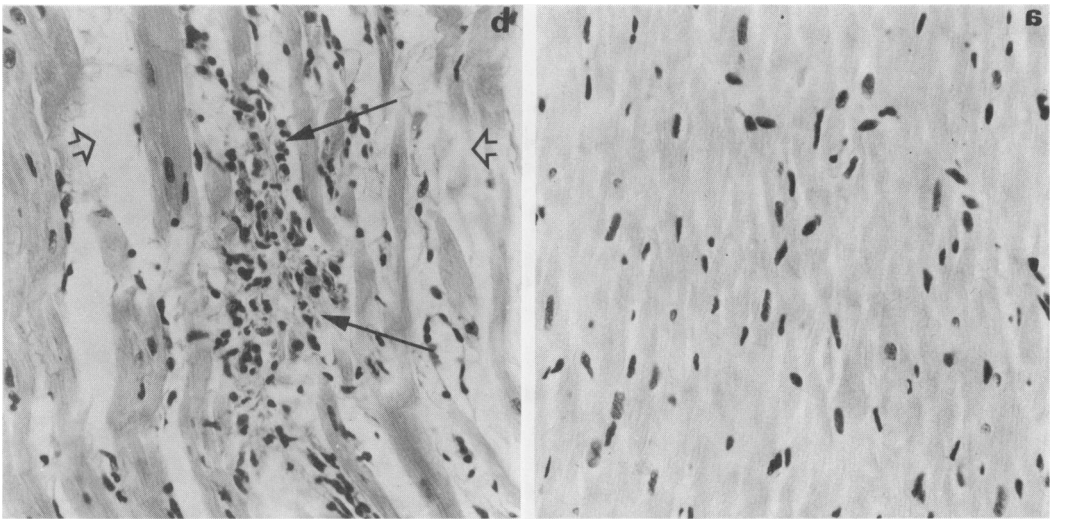

Fig. 10. Heart. *a*, UP-treated animal; *b*, ZAP.H-treated animal. Long arrows, necrotic heart muscle fiber, surrounded by PMN. Open arrows, interstitial oedema. × 270.

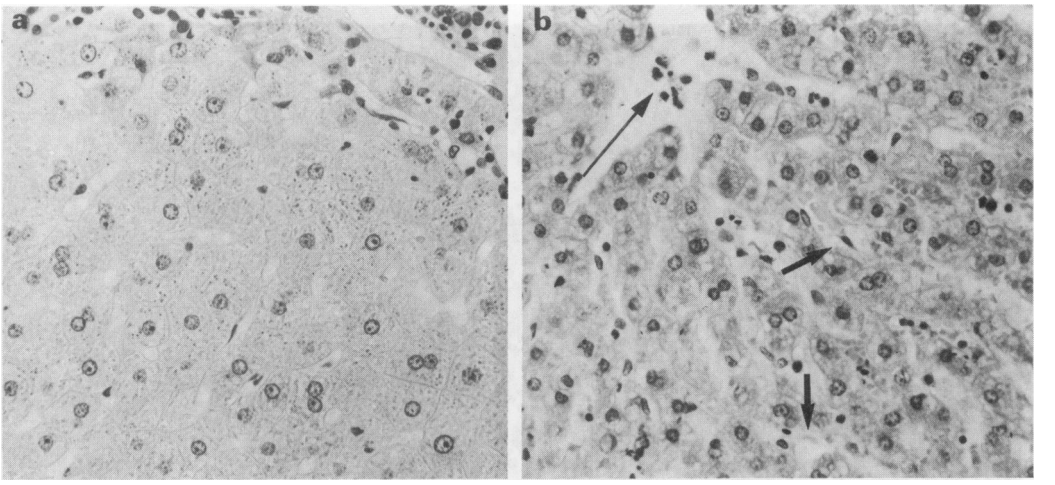

Fig. 11. Liver. *a*, UP-treated animal; *b*, ZAP.H-treated animal. Hydropic degeneration of liver cells. Long arrow: PMN aggregate. Short arrow: increased space of Disse. × 250.

evidently hypoxia alone was sufficient. The pathophysiological background of this synergism needs further study. Increased oxygen radical production during reoxygenation (Parks *et al.* 1983) and/or increased prostaglandin synthesis during hypoxic ventilation (Conzen *et al.* 1984; Said *et al.* 1974) might be contributing factors.

The role of PMNs in complement activation-induced microvascular injury needs further clarification for the measure of PMN accumulation, in the lungs at least, did not parallel the other morphological changes. Other pathophysiological mechanisms may thus contribute to the development of ARDS, eg., activation of the coagulation system,

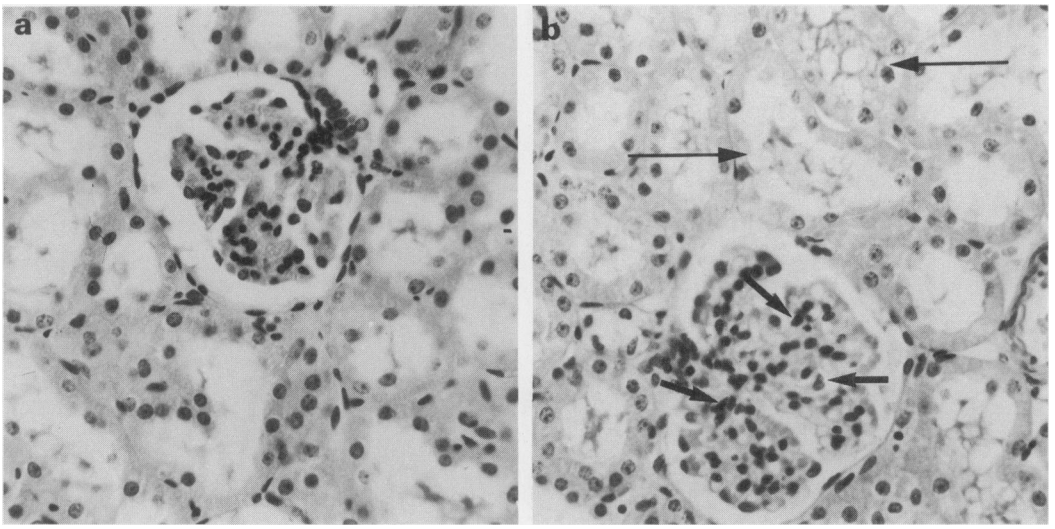

Fig. 12. Kidney. *a*, UP-treated animal; *b*, ZAP.H-treated animal. Long arrows, widened tubules, containing protein precipitate. Accentuated cytoplasmatic granularity. Short arrows, PMN. × 270.

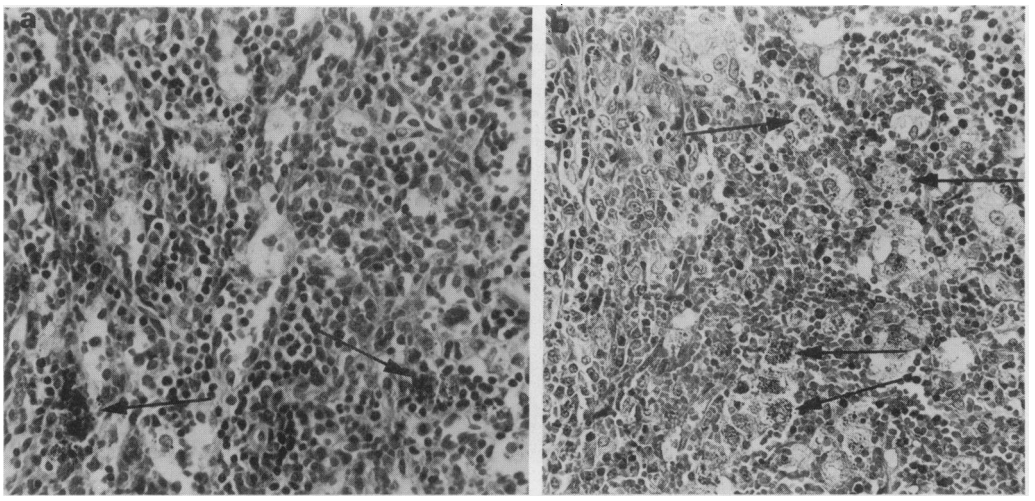

Fig. 13. Spleen. *a*, UP-treated animal. Modest number of makrophages (arrows); *b*, ZAP.H-treated animal. Large number of makrophages (arrows), containing debris of PMN and platelets. × 255.

activation of the arachidonic acid cascade, or production of fibrin degradation products after systemic activation of the complement system. Alternatively, the combination of complement activation and hypoxia could result not only in aggregation (as illustrated by the acute granulocytopenia, to the same extent as in the normoxic rabbits) and degranulation of PMNs but also in their lysis thus causing more extensive microvascular damage, lower numbers of PMNs in the lung and, as demonstrated above, removal of PMN debris by splenic macrophages.

The morphological changes observed in other organs can be described as 'whole body inflammation'. This inflammatory response

could well be the early morphological indication of MOF. If so, clinical MOF should also be accompanied by signs of inflammation; furthermore inflammation would be expected in patients suffering from diseases that predispose in the development of MOF; complement activation should be demonstrable in patients suffering from MOF, and clinically, MOF should be paralleled or preceded by ARDS.

Patients with MOF have been reported to show classical signs of inflammation such as fever, oedema, widespread peripheral vasodilatation and functio-laesa of multiple organ systems (Goris *et al.* 1985). The first organs to fail are usually the lungs (McMenamy *et al.* 1981; Goris *et al.* 1985). Complement activation has been demonstrated in patients with ARDS (Hammerschmidt 1980) and also in patients with MOF (Heideman & Hugli 1985; Nuytinck *et al.* 1986). Recently, total body inflammation was reported in early trauma deaths (Nuytinck 1985). These morphological abnormalities are indicative of early MOF, for severe trauma frequently causes MOF to develop in surviving patients. Rats receiving intraperitoneal Zymosan develop advanced MOF (Goris *et al.* 1986). The morphological abnormalities found in the rat model are strikingly similar to those reported here.

Collectively, such data support the suggestion that the morphological abnormalities reported here in an experimental animal model do indeed represent early MOF. If so, ARDS and MOF should be interpreted as manifestations of one and the same syndrome and as the clinical expression of an uncontrolled whole body inflammatory reaction, mediated, at least in part, by generalized activation of the complement system.

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