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Pulmonary and Systemic Embolism After Deliberate Intravenous Fluorocarbon Administration

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Summary. After intravenous injection of 0.1 ml Fluorocarbon (FC) into the caudal vein of rats clear droplets which are reminiscent of gas emboli appear in the pulmonary and cerebral arteries. These droplets cannot be stained with Azan, haematoxylin-eosin, Nile blue sulfate, Sudan black B, and Sudan III in Paraplast embedded or frozen sections. Gas chromatography of affected lung tissue reveals a high concentration of FC. The clear droplets are the histological correlates of FC emboli which lead to haemorrhagic lung infarction and ischaemic brain infarcts. After intralienal injection FC induces haemorrhagic infarcts of the spleen near the injection site and massive embolization of the intrahepatic portal veins with consequent liver cell necrosis. FC droplets are phagocytosed by hepatic sinusoidal lining cells. Due to the absence of a specific method for identifying FC embolization of renal vessles is difficult to assess.

Key words: Fluorocarbon – Extracorporeal circulation (EEC) – Pulmonary and systemic embolism – Rat.

Introduction

In open-heart surgery oxygenators are used to arterialize the blood in an extracorporeal circulation. The main types of oxygenators used clinically, membrane and bubble-oxygenators, are limited, especially for long-term application (Galletti et al., 1962; Pierce, 1969; Peirce, 1972; Mottaghy et al., 1976). An alternative method is liquid-liquid oxygenation. Here, the blood is oxygenated by an inert liquid which acts as the gas carrier. The advantages are that there is neither direct blood-gas contact nor membrane resistance. The choice of the gas carrier liquid is a major problem. It should be biologically inert, immiscible with blood

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and other biological fluids and should be non-toxic. After early attempts using silicon-oils, fluorocarbons (FCs) have found a wide spectrum of application for biological purposes. They are chemically stable, have a high specific density (usually $>1.5\ g/cm^3$), a high boiling point and a low vapor pressure. FCs are already used experimentally in animals as synthetic blood, in organ perfusion in the form of emulsions, in liquid breathing and liquid-liquid oxygenation (Clark, 1970; Geyer, 1975). FCs in emulsified form were found to be remarkably non-toxic. When they are used as a gas carrier in liquid-liquid oxygenators, however, the main potential danger arises from FC-microemboli obstructing vital arteries. Therefore, we have undertaken this study to show 1) whether FC microemboli can be detected and if 2) typical histological changes result from FC embolism. A similar investigation on the effect of silicon in the lung of rabbits has been already published in this journal by Preiss et al. (1977).

Material and Methods

Ten male Sprague-Dawley rats, 15 months old, with body weights from 500–700 p were used in our experiments. Two animals received an intravenous injection of 0.1 ml FC into the caudal veins under halothane narcosis, over a period of 30 min, using a motor driven microinjection pump. The FC used was FC-47 (Perfluorotributylamine) 3 M-Company. Similarly, 0.1 ml physiologic saline solution was injected into the caudal vein in two control rats. A further animal was laparotomized under halothane narcosis and 0.1 ml FC was injected near the caudal pole of the spleen. A control animal was treated in the same way, but 0.1 ml physiologic saline solution was injected into the spleen. The wounds were closed by a Mersilene suture and all animals were allowed to survive 24 h. The animals were then sacrificed by an overdose of Nembutal intraperitoneally and the lungs, heart, right lobe of the liver, spleen, kidney, and brain were fixed by immersion in Bouin's fluid (Romeis, 1948). The specimens were dehydrated in ethanol, cleared in methyl benozoic acid and embedded in Paraplast. Fifty serial section, 7 μ thick, were made of each organ. The brain sections were alternately stained with gallocyanin-chromalum according to Einarsson (Romeis, 1948) and Azan, the other organs were stained by Azan. The remaining four animals were processed in a different way, Two animals received an intravenous injection of 0.1 ml FC into the caudal vein, the remaining two 0.1 ml physiologic saline solution. The animals were sacrificed by an overdose of Nembutal intraperitoneally. One lung, right or left alternately was immersed in liquid nitrogene immediately after death and prepared for cryostat sections. The remaining lungs were homogenized and prepared for gas chromatography (Mottaghy et al., 1978). Cryostat sections from control animals and FC-injected animals were stained with haematoxylin-eosin, Nile blue sulfate, Sudan black B, and Sudan III.

Results

1. Macroscopic Findings

Three of the four animals receiving intravenous FC injections were somnolent and dyspnoic even 24 h after. Only one animal was running in the cage and tried to remove the Mersilene thread from the tail. The control animals were alert.

Changes in the lungs were a constant feature in all animals which had received intravenous injections of FC into the caudal vein. The lungs of the FC animals did not collapse when the thorax was opened. Several haemorrhagic

infarcts were regularly observed on the surface of the large lungs. The infarcts were preferentially located near the free margins of the pulmonary lobes. The brain, liver, spleen, and kidneys of the FC injected animals did not show striking macroscopical changes; nor did the organs of the control animals.

The spleen of the experimental animal which had received and injection of 0.1 ml FC near its caudal pole was slightly enlarged. At the injection site, the sharp margin of the spleen was rounded and several haemorrhagic infarcts could be recogneized on the surface of the caudal pole. Some fine strands of fibrin covered the capsule. The liver of this animal was pale and finely nodular. The lungs collapsed when the thorax was opened. No infarcts were observed on the pulmonary surface. Brain, heart, and kidneys did not exhibit gross pathological changes.

The surface of the spleen of the control animal which had received 0.1 ml 0.9% NaCl into this organ was also covered by fine fibrin strands. A small subcapsular haematoma had developed at the injection site. Liver, lungs, heart, brain, and kidneys were not changed.

2. Histological Observations

The lobar pulmonary arteries of animals which had received an intravenous injection of 0.1 ml FC into the caudal vein contained transparent droplets which occupied nearly the whole lumen of these vessels. The droplets showed the tendency to coalesce and formed monstrous globules studded with peripheral subglobules. On arrival at the smaller branches of the pulmonary artery the globules appeared to be readily deformable as they extended, wormlike, into the branches. They were not stained by haematoxylin-eosin or Azocarmine G, and aniline blue. We could not recognize a membrane surrounding the droplets, which were highly suggestive of emboli from their manner of deformation. We shall henceforth call the droplets emboli.

Multiple emboli obstructing the lumina of segmental pulmonary arteries were frequently segregated by clumps of granulocytes (Fig. 1). Although emboli were wedged into the lumen of segmental arteries highly deformed nuclei of granulocytes could be discovered between the embolus and vascular endothelium.

At fortuitous sections straight arterial branches were observed which emerged from segmental arteries and ran radially to the subpleural regions. If the segmental artery was occluded by an embolus, the radially running lobular arteries were likewise filled with sausage-like emboli. Starting at the transitional zone between arteriole and capillary the walls of the pulmonary capillaries were enormously dilated by wedged emboli, this zone being an apparently crucial point. Proximal to this transition a zone of haemorrhagic infarction began, surrounding the segmental pulmonary artery as a cone.

The inner walls of the pulmonary arterioles were frequently glued up by squeezed erythrocytes and fibrin (Fig. 2). The capillaries surrounding the embolized arterioles were massively dilated and filled with erythrocytes. It was sometimes difficult to recognize the degenerated smooth muscle cells in the

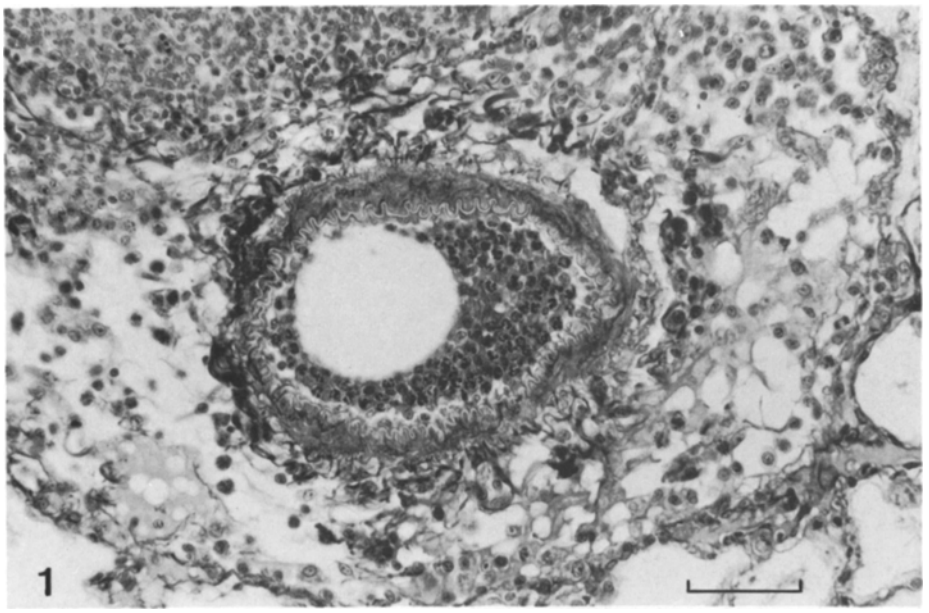


Fig. 1. Segmental pulmonary artery with FC embolus and aggregated granulocytes. Azan. Bar 50 μ

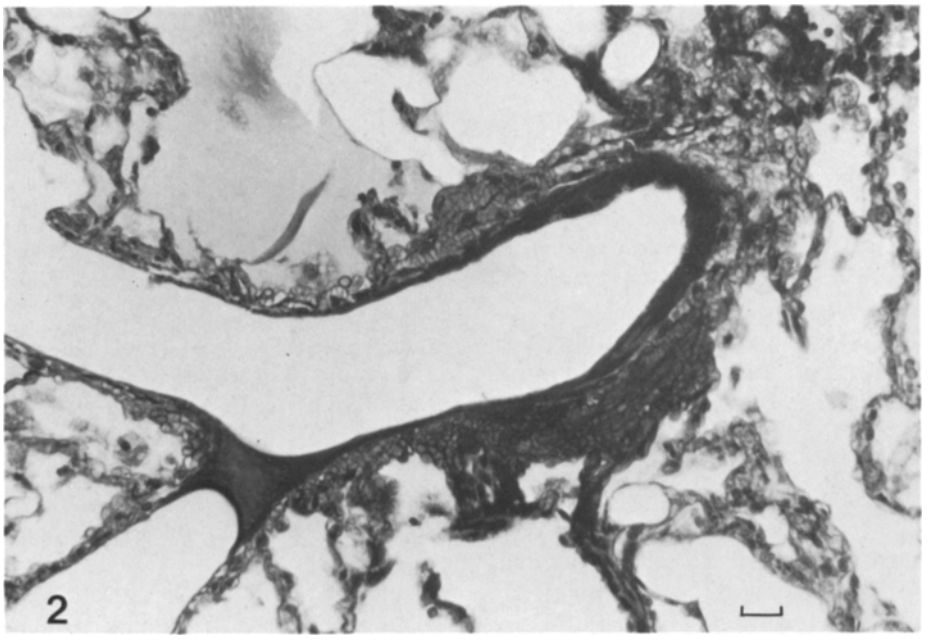
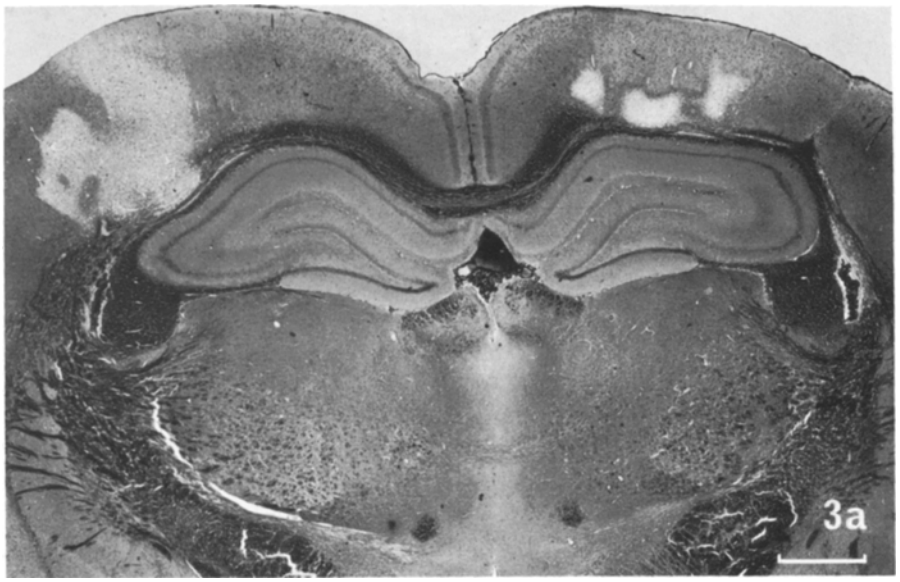


Fig. 2. Transition of pulmonary arteriole into capillary. Vascular wall of arteriole is glued up by erythrocytes and fibrin. Wedged FC embolus dilates vascular walls maximally. Bar 10 μ



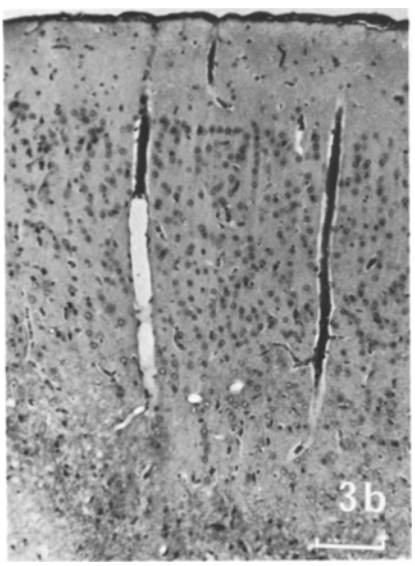


Fig. 3.
a Frontal section through the brain at the level of the rostral end of the infundibulum. Ischaemic infarcts in Krieg's areas 17 and 18. Azan. Bar 1.0 mm.
b Neocortical vessels at higher magnification. Left vessel contains FC embolus reminiscent of gas embolism. Right vessel exhibits normal aspect of unaffected cerebral vascular system. Azan. Bar 100 μ

wall of embolized arterioles. But serial sections clearly revealed that slugded erythrocytes dilated the walls of a periarteriolar capillary plexus.

In the frozen sections of lungs of those animals which were injected intravenously with 0.1 ml FC we distinguished haemorrhagic infarcts and alveolar and vascular oedema. The droplets within the obstructed vessels were not stained by haematoxylin-eosin, Nile blue sulfate, Sudan black B, and Sudan III. Fluorocarbon is not stained by these solutions in in-vitro experiments. Gas chromatography of the contralateral lung revealed high concentrations of FC in the homogenized pulmonary tissue. Control sections and gas chromatography of

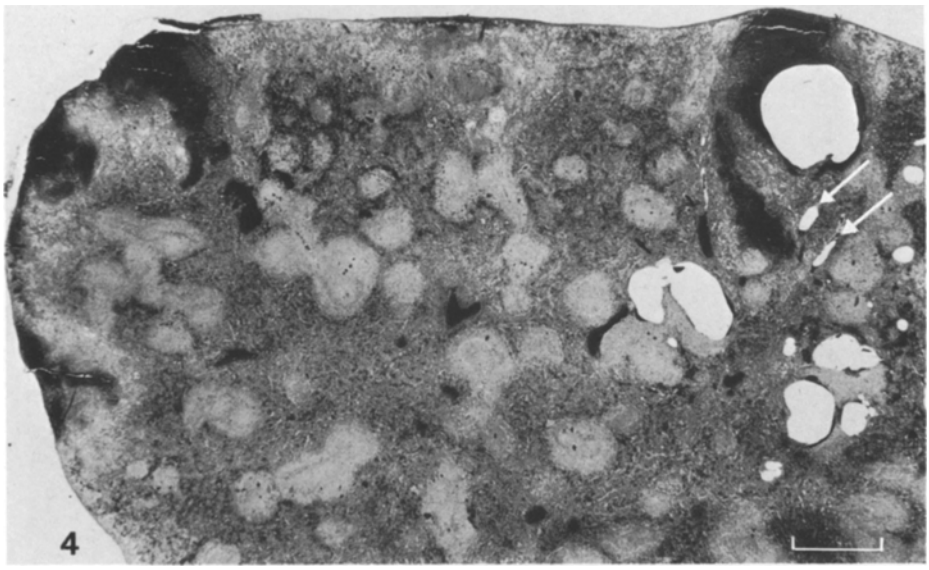


Fig. 4. Spleen. Azan. Injection site at the upper right corner. FC drops within the red and white pulp. Arrows point to FC emboli in two trabecular veins. Multiple haemorrhagic infarcts at the caudal pole (Left part of the picture). Bar 1.0 mm

the control animals did not show infarcts, oedema or traces of FC. Therefore, we conclude that the clear intravascular droplets in Fig. 1 and 2 which are reminiscent of gas emboli and which do not stain with conventional histological stains are the morphological equivalents of FC droplets and emboli.

Ischaemic brain infarcts were found in both animals which had received an i.v. injection of 0.1 ml FC into the caudal vein. In the first animal necrotic tissue was confined to the neocortex of the frontal and anterior parietal lobes of both hemispheres. In the second animal extensive ischaemic infarcts were found in the frontal, parietal, temporal and occipital lobes (Fig. 3a) on each side. Smaller infarcts were located in the right hippocampus and the rhombencephalon. The cortical areas 24, 23, and 29b of Krieg (1946) were regularly spared. Cortical vessels running to necrotic areas were filled by clear, long ellipsoidal emboli which prevented collapse of the vascular walls after fixation and Paraplast embedding (Fig. 3b). Ischaemic cellular changes were most pronounced in the deeper cortical layers and the subcortical white matter. Neurons in different infarcted areas showed varying degrees of necrosis. Large clear vacuoles were most frequently found in the white matter. We could not find emboli in the liver, spleen, and kidneys of animals into which FC was injected via the caudal vein, nor could we see ischaemic or haemorrhagic necroses. We sometimes had the impression that the glomerular capillary loops were distended but these changes could be also due to fixational or preparative artifacts.

A rather conspicuous sphere, about 1 mm in diameter, was observed at the injection site of the spleen of the rat which had received a dose of 0.1 ml FC intralienally. The sphere was bordered by a layer of clumped erythrocytes and fibrin (Fig. 4). Smaller drops surrounded the large sphere in a semi-circle.

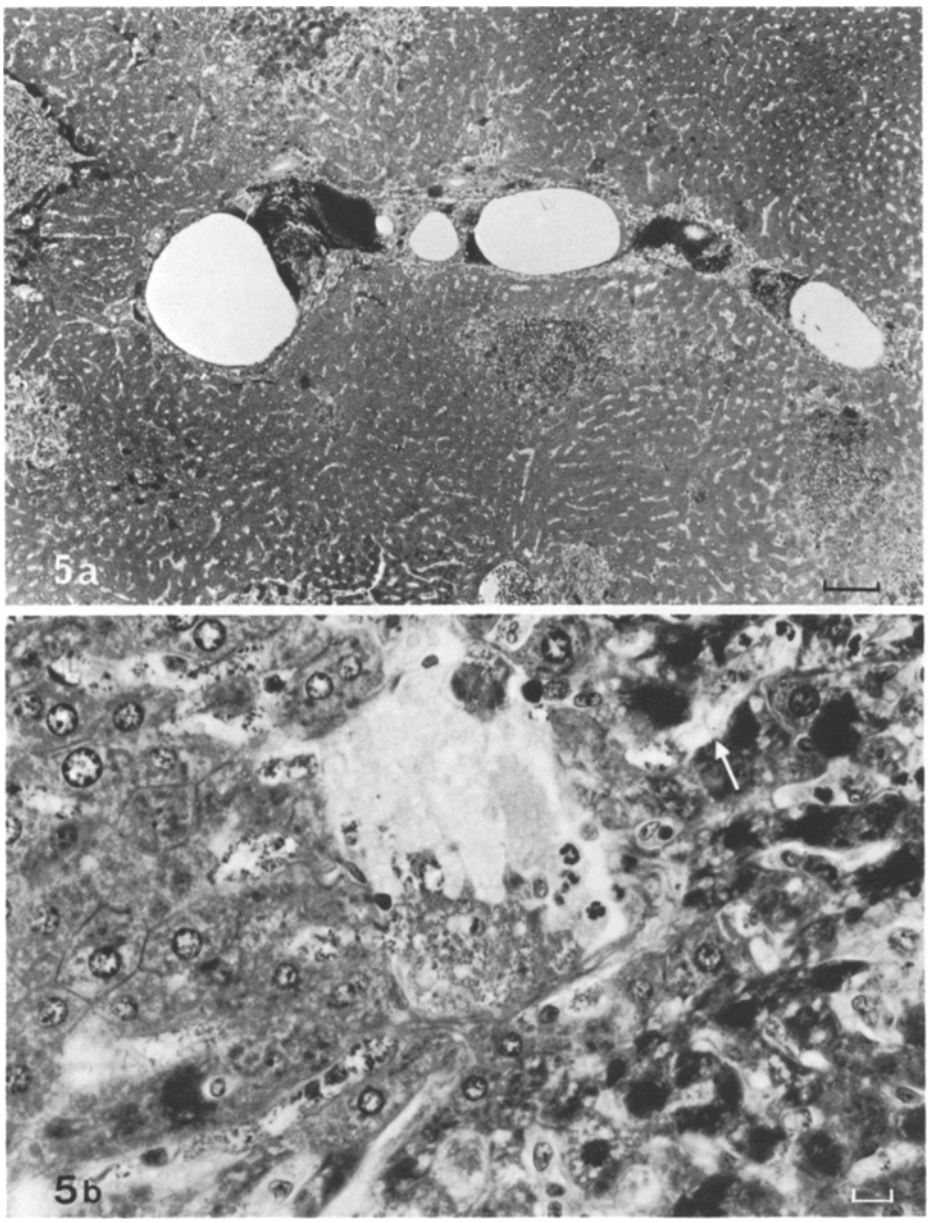


Fig. 5. a Liver. Azan. Longitudinally sectioned branch of a portal vein obstructed by FC emboli, parietal thrombi and aggregated erythrocytes. Necrosis of zone III of Rappaport's lobule. Bar 100 μ. **b** Hepatic vein and surrounding liver tissue. Arrows point to phagocytosed FC droplets in cells lining the sinusoids. Azan. Bar 10 μ

These drops were more frequently found in the red pulp of the spleen and in this case carried caps of compressed erythrocytes. Drops which were located in the white pulp separated the lymphatic tissue. The drops did not bear caps of erythrocytes in this case, nor could we see macrophages or giant cells surrounding the FC drops. Oval emboli could be observed in the trabecular veins (Fig. 4) which were running towards the hilus of the spleen. Several haemorrhagic infarcts pointed with their apices towards the FC drops in the centre of the spleen.

In the control animal, which had received a single injection of 0.1 ml physiologic saline solution, only a small subcapsular haematoma and some fibrin was found on the capsule. The lumina of the intrahepatic portal veins were filled with clear drops and droplets in the animal which had previously received an intralienal injection of 0.1 ml FC (Fig. 5a). Neither drops nor droplets were limited by a membrane. Parietal thrombi and agglutinated erythrocytes separated single drops one from another. About 20% of the hepatic tissue was necrotic, necroses were located in zone III of Rappaport's lobule. Liver cells were small and acidophilic (Fig. 5b) and the sinusoids seemed to be enlarged (Fig. 5b). Small clear droplets were discovered in the sinusoids and their number and diameter seemed to increase with increasing distance from the portal veins. Focussing with oil immersion objective suggested that the small droplets were captured in the delicate cytoplasm of cells lining the wall. An acidophilic, granular and sometimes threadlike material was seen winding in the small space left between the protruding endothelial lining cells. The hepatic veins regularly contained erythrocytes, granulocytes, some necrotic parenchymal cells and granular material. The hepatic arteries were open and filled with erythrocytes.

Two small haemorrhagic infarcts were discovered in the lung of the animal which had received 0.1 ml FC intralienally. Brain and kidneys did not show necrotic areas or gross embolization.

Discussion

The obvious purpose of our research is to develop a liquid-liquid oxygenator that does not embolize FC droplets into the circulation. It is the purpose of this study to investigate whether such embolization would cause characteristic morphological changes subsequent to deliberate FC administration.

Pulmonary arterial embolism, haemorrhagic infarcts, oedema of the alveoli and vascular walls, as seen in our study (Figs. 1, 2) have been shown to be the result of pulmonary fat embolism (For a review see Ceelen, 1931; Sevitt, 1962; Collard, 1973; Brinkmann et al. 1976; Chomette and Oriol, 1977).

The development of haemorrhagic pulmonary infarction is a consequence of elevated pulmonary arterial pressure (Ceelen, 1931; Meesen, 1940) and the double blood supply of lung tissue by pulmonary and bronchial arteries (Lapp, 1951; Weibel, 1959). Furthermore, Ceelen (1931) and Meesen (1940) stated that the failure of the left ventricle is a prerequisite for the pathogenesis of haemorrhagic lung infarctions. Recent publications (Lee et al., 1979) cast doubt upon this view. We could not find emboli in the coronary arteries, nor myocar-

dial infarcts. Apart from these simple mechanical factors, toxic or chemical and shock-inducing influences have been attributed to cause haemorrhagic and alveolar and interstitial oedema. In particular free long-chain fatty acids are said to be toxic for the vascular endothelium and promote extravasation of erythrocytes and plasma (For a review see Wehner, 1968 and Brinkmann et al., 1976). FCs are chemically inert and we may therefore exclude toxic effects. However, we cannot explain the accumulation of granulocytes around the FC emboli (Fig. 1) especially within the segmental pulmonary arteries. Granulocytes and thrombocytes seem to have an affinity for embolic material (Thompson et al., 1969) and leucocytes migrate readily against the blood stream towards inflammatory regions (Cliff, 1966) which may explain the frequent occurrence of granulocytes between vascular endothelium and FC emboli. Lee et al. (1979) suppose that humoral factors are responsible for the development of oedema. Apart from humoral factors, Saldeen (1976) discusses the role of fibrin and fibrin degeneration products in the increase of vascular permeability. In this context we found, especially in the wall of the arterioles at the transitional zone to pulmonary capillaries (Fig. 2) a threadlike material which showed the staining characteristics of fibrin. Capillaries surrounding the arteriole were maximally dilated and filled with erythrocytes, and proximal to these embolized arterioles the region of haemorrhagic infarction began. We suppose that in this transitional zone terminal branches of the bronchial artery communicate by a periarteriolar plexus with the pulmonary artery. Embolic obstruction, together with fibrin hampers the blood flow and rupture of the delicate capillaries causes the outflow of erythrocytes. Nosé et al. (1970) discuss the role of mechanical obstruction of the capillaries, intravascular gas emboli due to FC vapor and intravascular clotting induced by FC vapor in the development of haemorrhagic infarcts. Sloviter et al. (1970) consider multiple platelet microemboli to be responsible for sudden death.

Emboli may leave the pulmonary vascular bed and enter the systemic circulation with serious effects (Sevitt, 1960). In at least two rats which had received 0.1 ml FC intravenously into the caudal vein massive embolization in the cerebral hemispheres ensued (Fig. 3a). It is remarkable that areas 24, 23, and 29 of Krieg (1946) were spared and that cortical areas lateral to the cingular region were preferrentially affected by ischaemic infarcts. We conclude that no emboli had found their way into the anterior cerebral artery and preferential involvement of the medial cerebral artery and its branches had occured. Neurons in infarcts of about the same size but located in different areas exhibited different signs of necrosis. In one infarct large pyramidal cells were already phagocytized by microglia, other neurons were in the stage of simple ischaemic cell change. This may be indicative of repeated cerebral embolism, a process which is not complete 24 h after i.v.-administration of FC.

Naito et al. (1978) and Miller et al. (1974) found FCs phagocytized by spleen reticular cells and hepatic Kupffer cells. We could not demonstrate FC in either organ in those rats which received FC intravenously into the caudal vein. To circumvent the pulmonary capillary filter we injected 0.1 ml FC into the spleen. Multiple haemorrhagic infarcts in the spleen near the injection site resulted (Fig. 4) and FC drops were found in the red and to a lesser degree

in the white pulp. No "foam cells" (Naito et al., 1978) were encountered in the red pulp nor did phagocytes or giant cells surround the FC drops (FCs do not have antigenic properties). These findings are in marked contrast to the findings in gas embolism which presents a comparable histological picture but is accompanied by a foreign body reaction (Huhn et al., 1963; Golenhofen, 1971). Only after intralienal injection of 0.1 ml FC- an injection technique which circumvented the capillary filter of the lungs – could we find FC drops in the portal vein and massive embolization of even the smallest intrahepatic portal veins (Fig. 5a). Embolization of the smallest intrahepatic veins is a prerequesite for liver cell necrosis (Gerlach, 1930) which also demonstrated Fig. 5a and b. Phagocytosis of FCs by Kupffer cells (Fig. 5b) has already been reported by Miller et al. (1974) and by Naito et al. (1978).

We could not discover unequivocal signs of FC embolism in serial sections of the kidney. Collard states that even after massive renal fat embolism ischaemic infarcts will not develop because collaterals supply embolized areas richly. Specific fat stains reveal multiple intravascular fat emboli. There are no specific stains for FCs probably due to their chemical inertia and thus a certain diagnosis of FC-embolization is difficult. The toxic effects of FC after i.v.-administration seem to be dependent of the physical properties of long and short-chained perfluorocarbons (Clark et al., 1974) and the particle size of the FC (Naito et al., 1978). The "toxicity" is inversely proportional to the particle size and proportional to the amount and injection velocity of FC. We think that haemorrhagic and ischaemic infarcts are the outcome of embolism. Further combined autoradiographic and electron microscopic study are needed to elucidate the mechanisms of phagocytosis of FC by sinusoidal lining cells and to study the systemic long-term effects of FC.

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