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Long Term Experiment of Perfluorochemicals Using Rabbits

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Summary. Morphological changes six months to two years after the administration of Perfluorochemicals (PFC) in rabbits injected with a large amount of PFC (67 mg/kg) have already been reported by Ohnishi and Kitazawa (1979, 1980, and 1981). In this paper, the authors report on the persistance of large numbers of foamy cells in the liver, spleen and lymph nodes and many in the kidneys, lung and bone marrow, up to two years after administration. Quantitative analysis of PFC in various organs showed that it was retained. The liver revealed progressive fibrosis surrounding the foamy cells in or around the Glisson's sheath.

Key words: Fluorocarbon – Perfluorochemicals – Blood substitute – Foamy cell – Reticuloendothelial system

We have already reported the first autopsy case of a patient who had received a large amount of Perfluorochemicals (PFC) as artificial blood, following the development of haemolytic anemia, occurring after an operation for dissecting aneurysm (Ohnishi and Kitazawa 1979 and 1980). Some experimental studies in rabbits kept for 120 days after the administration of PFC were also described (Kitazawa and Ohnishi 1981).

Perfluorochemicals were developed with high expectations as an artificial blood (so called "white-blood") because of their excellent ability to transport oxygen without any injury to the organs (Ohyanagi et al. 1974). Fluosol-DA (FDA) is the newest of the PFC's, measuring 0.1–0.2 micron in particle diameter. There have been some reports (Ohyanagi and Mitsuno 1975; Yokoyama et al. 1977; Ohyanagi et al. 1980) concerning the pharmacological or biophysical effects of these compounds and their excretion using monkeys, dogs or rats and demonstrating the effective transportation of oxygen, but histopathological descriptions are very few (Kamae 1979).

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Heinsen et al. 1980 reported pulmonary/systemic embolism of fluorocarbon and Schnoy and Pfannkuch 1980 reported the secretion of fluorocarbon from the lung by an electron microscopic study, both groups using rats. Makowski et al. (1978) briefly reported experimental application of PFC in decerebrate patients after severe head injury, with the appearance of a few foamy cells in the liver, spleen and lymph node. The authors have observed morphological changes following prolonged usage of PFC in rabbits.

Materials and Methods

Twenty-six normal healthy, young male rabbits, weighing 2–2.5 kg were used. A PFC Fluosol-DA (FDA) which contained 17.5 volume percent of perfluorodecalin, 7.5 volume percent of perfluorotripropylamine, 3.4 volume percent of pluronic F-68, 0.5 volume percent of yolk phospholipids, and 1.0 volume percent of glycerol was uses as artificial blood. Each rabbit was injected with 67 mg/kg of FDA in two doses two days apart, which corresponded to the dosage of our reported human autopsied case. These rabbits were slowly injected with FDA emulsion into the auricular vein, without blood depletion. Thereafter, they were kept on normal feed and breathing a normal atmosphere. After the administration of PFC, 2 or 3 rabbits were killed with Lavonal® injection; respective two rabbits after 1, 2, 3, 15, 37, 60, 120, 180 days and 1, 2 years, 3 rabbits after 7, 22 days. For the light microscopic observation, tissues from all organs were fixed in 10% buffered formalin. For the electron microscopic observations, materials were immediately fixed in 2.5% glutaraldehyde, in 0.1 M phosphate buffer at pH 7.4, and refixed in 1% buffered osmium tetraoxide, and then dehydrated in graded concentration of ethanol. After embedding in Epon-812, ultrathin sections were cut in series and double stained with both uranium acetate and lead citrate for observation with Hitachi HS-9 type electron microscope. For the quantitative analysis of PFC retention, small pieces of each organ were frozen to $-70°$ C and retention estimated by the courtesy of the Green Cross corporation.

The authors regard foamy cells as the histopathological indicator of PFC content in every organ, based on our previous data.

Results

After 2 years, the liver contained a large number of foamy cells, mainly around or in the Glisson's sheath, compared to 2 week material (Fig. 1a–c). A few such cells were scattered in the sinusoids, but no mobilization of Kupffer cells was found. Dense collagenous fibers, including argyrophilic fibers, surrounded each foamy cells and they extended into lobules from Glisson's sheath. This fibrotic change appeared at first 6 months after the injection of PFC and gradually progressed after one year, becoming more prominent after two years. Complete fibrous bridging or pseudolobular formation was not detectable.

The spleen contained a large number of foamy cells in both red and white pulp shortly after treatment. These were at a maximum at the second week (Fig. 2a) but two years later a large number were still perceptible, mainly in the Billroth cords. Sinuses were almost closed (Fig. 2b). The size and number of the lymph follicles slightly increased from the second week findings.

In the lymph node, there were some foamy cells forming small groups, mainly in the medulla (Fig. 3a, b).

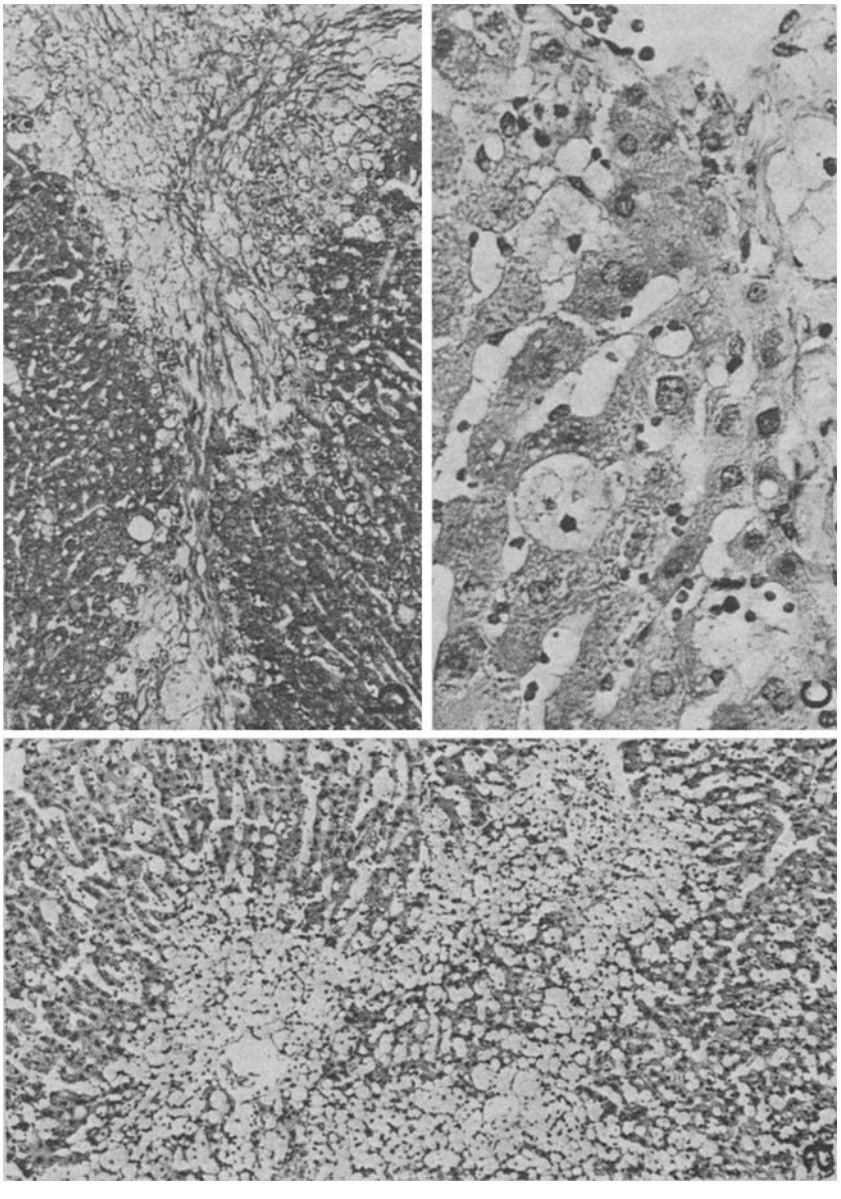


Fig. 1. a Liver: two weeks after PFC administration. A large number of foamy cells were found around the central vein, especially in sinusoids. × 20, H.E. **b** Liver: two years after injection. Foamy cells gathered around the Glisson's sheath. Pericellular collagenosis and elongation of fibers from Glisson's sheath among the foamy cells. There is demarcation, as a nodular mass, from the hepatic acini. × 50, Azan-Mallory. **c** Same material as **b** at high power. In the sinusoid, some of Kupffer's stellate cells and monocytes show a foamy appearance. × 50, H.E

A few foamy cells remained in the bone marrow. The population of haematopoietic series was almost recovered compared to second week material (Fig. 4a, b).

In the kidney, some foamy cells still remained here and there as emboli in the capillary lumina of glomeruli, forming multinucleated giant cells (Fig. 5a, b). There were neither obvious basement membrane thickening,

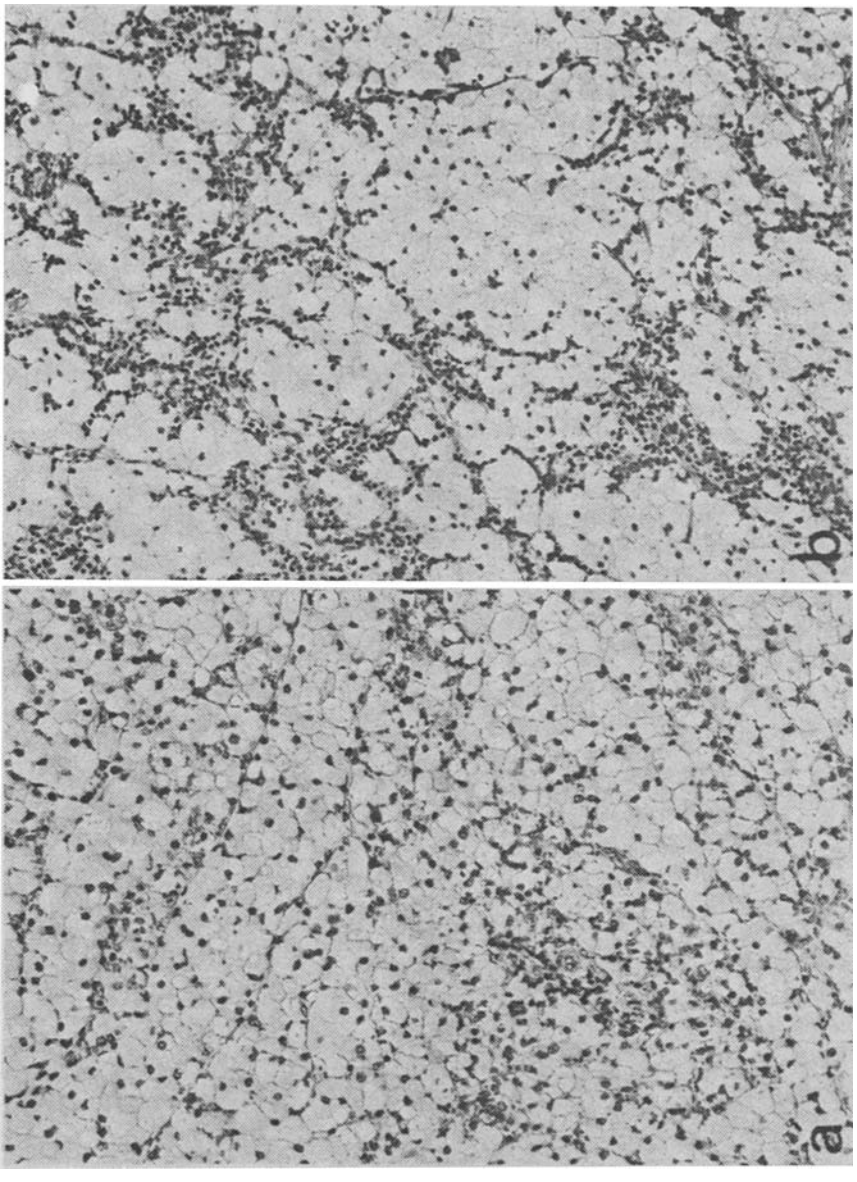


Fig. 2. a Spleen: two weeks after injection. Massive diffuse foamy cells. Foamy cells appeared mainly in the Billroth cords and the sinuses are almost obliterated. Marked depletion of lymphocytes of the lymphatic nodules. × 50, H.E. **b** Spleen: two years after injection. a large number of foamy cells are retained in red pulp, compressing the sinuses. The population of lymphocytes and the size of lymphatic nodule has recovered slightly. × 50, H.E

nor mesangial proliferation. Tubules showed no degeneration or destruction at intervals during the 2 years.

The lungs had a few foamy cells in peribronchial regions, around small pulmonary veins and in the lymphatic apparatus. Also a few foamy cells were found in the capillary lumina of the alveolar wall (Fig. 6a, b). No obvious excretion of PFC particles into the lungs was detected by either light or electron microscopic observation during 2 years.

The relative number of foamy cells assessed histologically with hematox-

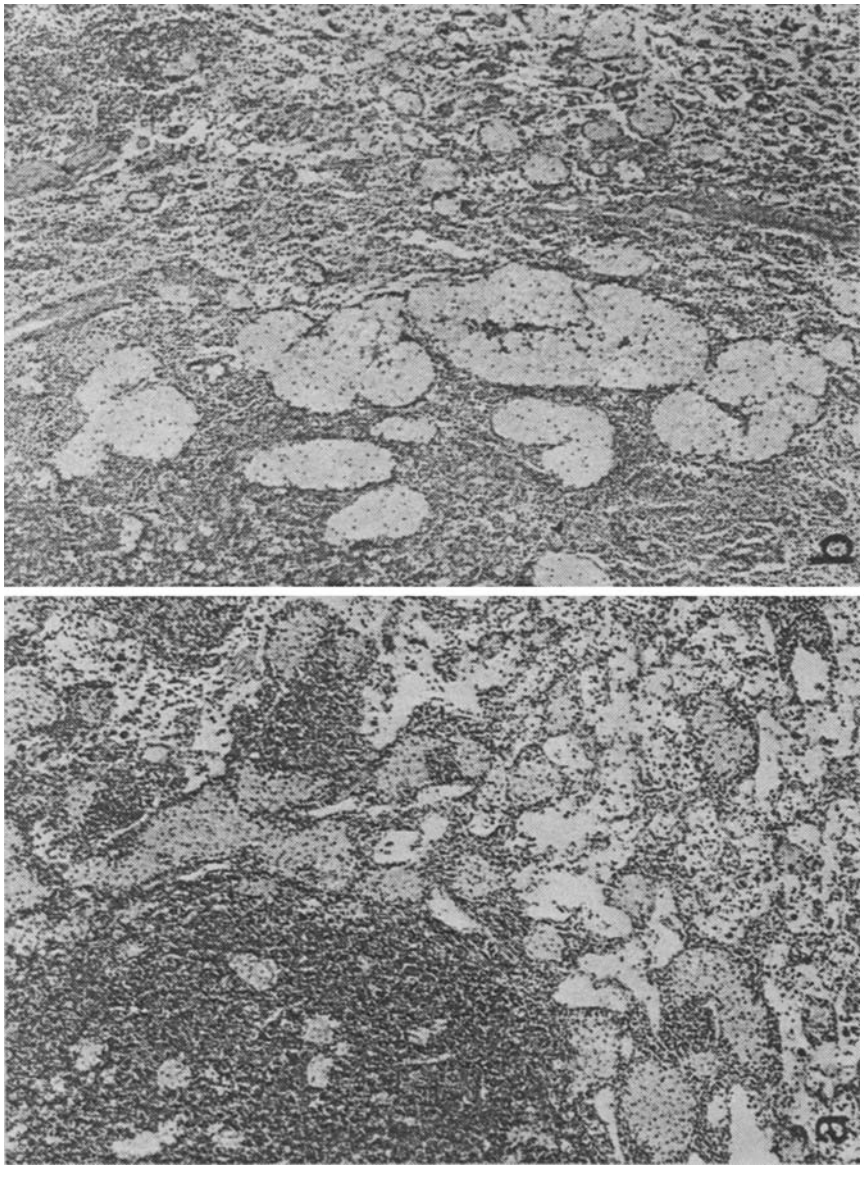


Fig. 3. a Lymph node: one month after injection. A large number of foamy cells mainly in the medulla and perivascular areas. ×20, H.E. **b** Lymph node: two years after injection. Almost the same numbers and distribution of foamy cells. ×20, H.E

ylin eosin staining in the organs of rabbits are shown in Table 1. The spleen, liver and lymph node showed almost the same pattern. At first, the total number of foamy cells increased rapidly, especially in the spleen and remained at a maximum between one week and two months and then gradually decreased. Even two years later, foamy cells were still to be found, showing accumulation or becoming multinucleated giant cells in all organs examined almost in the same numbers as on the first day after the administration of PFC.

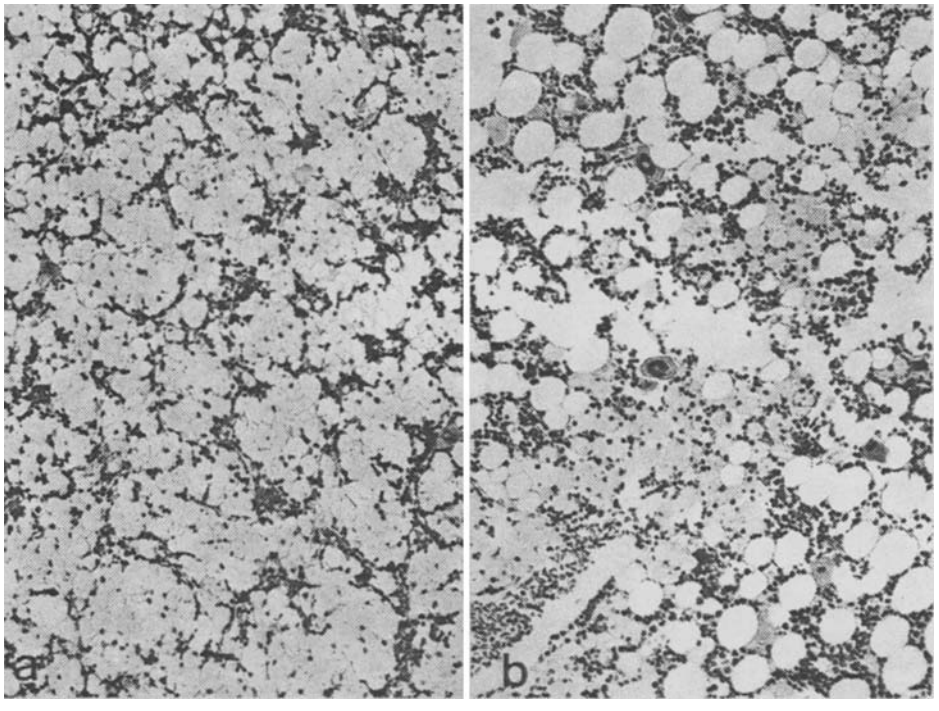


Fig. 4a Bone marrow: two weeks after injection. Massive foamy cells in very hypoplastic haematopoietic tissue. × 50, H.E. **b** Bone marrow: two years after injection. Some foamy cells in moderate hematopoietic hypoplasia. × 50, H.E

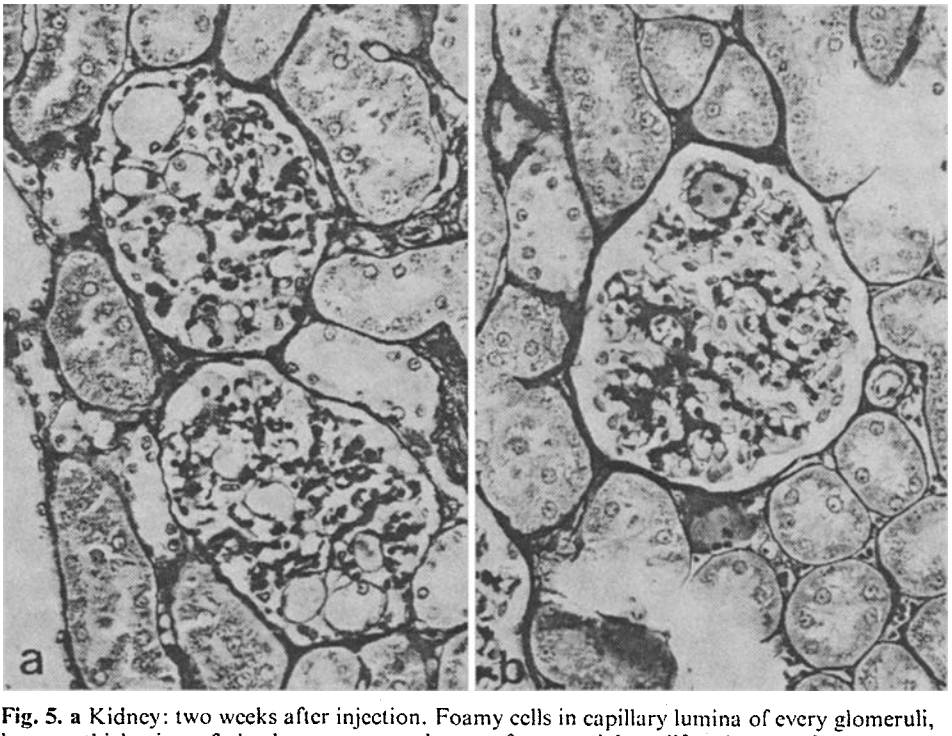


Fig. 5. a Kidney: two weeks after injection. Foamy cells in capillary lumina of every glomeruli, but no thickening of the basement membrane of mesangial proliferation. × 100, PAM. **b** Kidney: two years after injection. A few foamy cells in glomerular capillary showing multinucleated giant cells. × 100, PAM

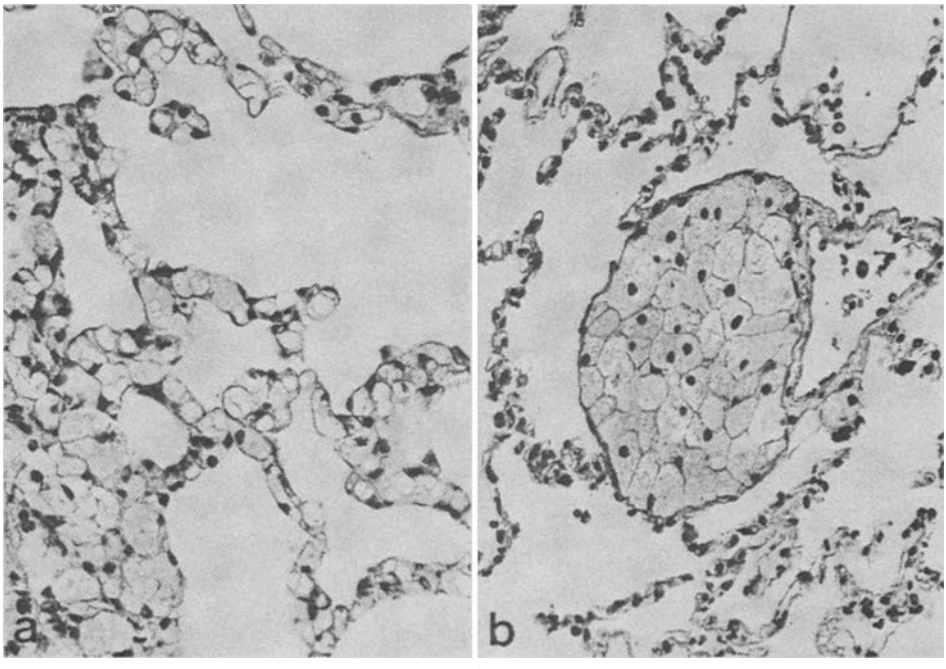


Fig. 6. **a** Lung: two weeks after injection. A large number of foamy cells in the capillary lumina and the alveolar septa. ×100, H.E. **b** Lung: two years after injection. In some places, foamy cells remain around the small vessels but are rarely seen in the capillary lumina. ×100, H.E

Table 1. The distribution of foamy cells in every organ shows peaks and plateau between 2 weeks and 2 months after the injection of PFC

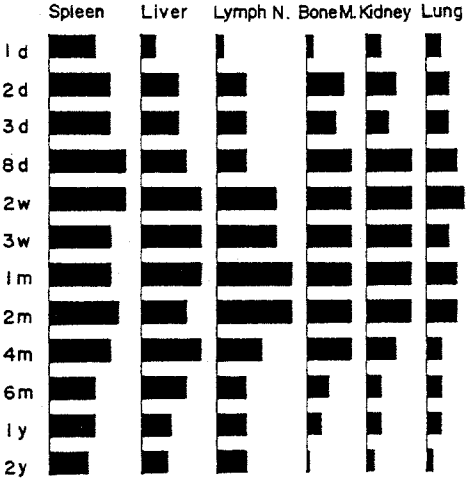


Table 2. The concentration of PFC in organs of experimental rabbits shows 2 maximal peaks, the first at 3 days to 1 week and the second at 1–2 months

	Number	Spleen	Liver	Kidney	Lung
1 day	2	96.7	17.5	6.8	7.6
2 days	2	46.8	23.2	3.4	10.3
3 days	2	164.1	27.7	3.7	11.5
1 week	3	74.3	11.7	1.6	10.0
2 weeks	2	42.0	28.4	0.8	12.5
3 weeks	3	25.7	7.3	1.1	0.8
1 month	2	98.8	14.4	1.1	8.6
2 months	2	84.4	13.4	0.7	25.5
4 months	2	21.7	2.5	0.2	0.6
6 months	2	12.0	2.0	2.1	0.4
1 year	2	3.6	1.3	0.1	0.1
2 years	2	3.2	0.2	0.01	0.03

mg/g wet tissue

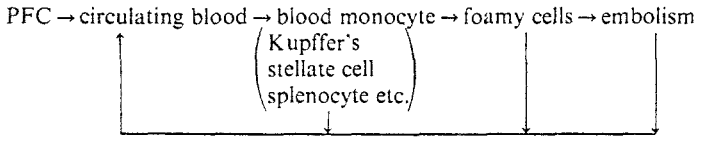
Quantitative analysis of PFC in the spleen, liver, kidneys and lungs of the experimental rabbits (Table 2) showed that even two years after administration still there remained an 0.27% concentration of PFC compared with the maximum contents of the organs examined. To be exact, the PFC concentration in the spleen reached a maximum three days after administration and then gradually decreased, but one or two months later it increased again. The liver showed the same pattern as the spleen. A low titer of PFC remained in both kidneys and lungs.

Discussion

The excellent oxygen transportation and the security of PFC as an artificial blood in animals other than rabbits has been reported (Kamae 1979). Yokoyama et al. 1978 reported that PFC particle is excreted mainly through the lungs as vapor.

In a previous paper, the authors (Ohnishi and Kitazawa 1980) pointed out that a great amount of PFC was retained in human autopsy material. Moreover, the authors suggested long term observations should be made because there were so many foamy cells and marked retention of PFC concentration 4 months after PFC administration into rabbits. From our previous data, the foamy cells appear to be markers of PFC retention, because most of the PFC particles were phagocytized by macrophages in the reticuloendothelial system (RES). Further, PFC particles were retained in circulating blood monocytes, appearing histologically as emboli. As mentioned above, even two years after PFC administration, the foamy cells were still found to contain some PFC. If severe blood depletion had occurred

before PFC was given there might be fewer embolic foamy cells in both the kidneys and lungs, but more prominent changes should be expected in the spleen, liver, bone marrow and the lymph nodes. Both the analytically determined PFC and the foamy cells in all organs increased initially, and one or two months later increased again. This time may represent the life span of monocytes and/or Kupffer's stellate cells. So the long term circulatory cycle of PFC in living body may be as shown in the diagram.



There were some reports of generalized lipid embolization or accumulation after the administration with a large amount of Intralipid (Ohsaki et al. 1981). PFC compounds and "Intralipid" were phagocytized in both blood monocytes and macrophages in RES organs. But the former persisted in the circulating blood as above mentioned, however the latter was shortly metabolized.

Hepatic fibrosis was first found at six months after injection of PFC. This change appeared along with the tendency of foamy cells to gather around or in the Glisson's sheath. The collagenous fibers became dense, surrounding individual or collections of foamy cells as the months increased.

Even after 2 years, there were massive numbers of foamy cells in RES organs and a few in the lungs. Moreover, hepatic fibrosis showed up in long term experimental rabbits. The authors suggest that the use of a large amount of PFC as an artificial blood should be avoided, in view RES accumulation (Ohnishi and Kitazawa 1980).

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