STATE OF THE MONONUCLEAR PHAGOCYTE SYSTEM AFTER INFUSION OF PERFLUOROCARBON EMULSIONS

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Morphological investigations of the action of perfluorocarbon compounds (PFC) on the body have constantly revealed accumulation of PFC in cells of the mononuclear phagocyte system (MPS), mainly the liver, spleen, lymph nodes, and lungs [1, 2, 4, 6, 12]. Despite the many investigations directly or indirectly devoted to the study of the state of the MPS during the use of various PFC and different methods of their introduction into the body, many aspects of this problem remain unsolved. For instance, the effect of PFC on peritoneal macrophages, on which most experimental studies of MPS have been carried out, remains unexplained [5]. There are considerable contradictions in the assessment of the functional state both of those macrophages which accumulate PFC and of the system of mononuclear phagocytes as a whole. During the first few hours slowing of PFC clearance is observed, and this phenomenon diminishes appreciably in its intensity after 24 h [8, 9]. Similar results were obtained [10, 11] in a study of the phagocytic activity of the macrophages in vitro after administration of PFC. Meanwhile, electron-microscopic investigations have not detected any significant effect of PFC incorporation on macrophage ultrastructure [7]. The possibility cannot be ruled out that disturbances of morphology and function of MPS, recorded during administration of PFC, especially as a blood substitute, are largely linked both with the actual blood loss itself and with the introduction of large doses of the blood substitute into the body. Similar changes have been described [3] during parenteral administration of large doses of the Soviet plasma expanders Gemodez and Polyglucin.

The aim of this investigation was a morphological and histochemical study of different classes of MPS during accumulation of PFC in them after parenteral administration of PFC emulsions to experimental animals.

EXPERIMENTAL METHOD

PFC emulsions were synthesized in the Institute of Biological Physics, Academy of Sciences of the USSR. Experiments were carried out on 350 male Wistar rats, which were subjected to a single replacement of 50-60% of the circulating blood mass, plethoric intravenous injection of PFC emulsions in a dose of 3 mg/kg, and intraperitoneal injection of PFC emulsions in a dose of 4 ml per animal. Rats of the control group (60 animals) received protein-salt solution intravenously in the same doses, and 10% peptone solution (4 ml per animal) intraperitoneally 3 days before the end of the experiment. The animals were killed by decapitation under ether anesthesia from 12 h to 24 months after the beginning of the experiment. Sections were stained with hematoxylin and eosin and the PAS reaction; total protein was determined with Fast green, and RNA, DNA, and lipids also were estimated. Activity of succinate dehydrogenase (SDH), lactate dehydrogenase (LDH), NADH and NADPH diaphorases, and acid and alkaline (AlP) phosphatases was determined in unfixed frozen sections. Blood and bone marrow films were stained by the Romanovsky–Giemsa method, and concentrations of glycogen (PAS reaction), nonspecific esterase (NE), peroxidase (PO), and AlP were determined in blood monocytes and bone marrow macrophages. To obtain peritoneal macrophages the peritoneal cavity of the killed

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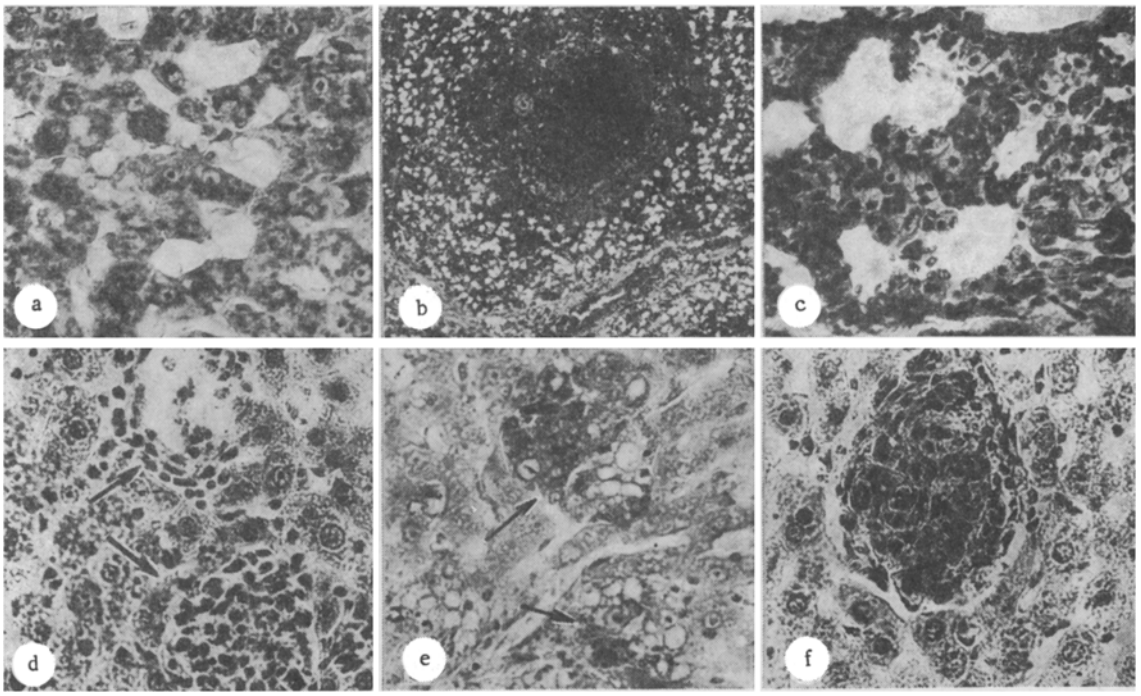

Fig. 1. Morphological changes in organs at various times after blood replacement with PFC emulsions. a) Absence of nucleoproteins in PCM. Stained by Brachet's method. Liver after 24 h. 200× (Here and in Fig. 1d, e); b) "starry sky" arrangement of PCM. Hematoxylin and eosin (here and in Fig. 1c, d, f). Spleen after 24 h. 40×; c) PCM in interalveolar septa. Lung after 3 days. 100×; d) Accumulation of lymphocytes at periphery of aggregate of PCM and macrophagal granuloma. Liver after 2 months; e) neutral mucopolysaccharides in PCM. PAS reaction. Liver after 4 months; f) macrophagal granuloma formed with remnants of PCM. Liver after 6 months. Hematoxylin–eosin.

rats was washed out with Hanks' solution, the exudate thus obtained was centrifuged, and films were prepared from the resiude. The films were stained by the Romanovsky–Giemsa method and cytochemical reactions carried out for SDH, LDH, and glucose-6-phosphate dehydrogenase (G6PDH), and of substrate-free reduction of nitroblue tetrazolium (the nitro-BT test). The intensity of the histochemical reactions was estimated by a semiquantitative method.

EXPERIMENTAL RESULTS

During exchange transfusion, during the first few hours after injection of PFC large macrophages with colorless, vacuolated cytoplasm and an eccentrically placed nucleus were found in the liver and spleen. The dimensions of the macrophages reached a maximum after 12 h of the experiment. After 2-3 h their cytoplasm appeared to be filled with small vacuoles, after 6-8 h, it was filled with larger vacuoles, and after 12-24 h it contained one or two large vacuoles. Histochemical reactions for polysaccharides, nucleoproteins, total protein, and lipids in the vacuolated cytoplasm were negative (Fig. 1a). After 24 h these perfluorocarbon-containing macrophages (PCM) had completely colonized the red pulp so that it resembled a "starry sky," and a few of them also were located in the follicles (Fig. 1b). After 3 days the number of PCM in the liver and spleen showed a small decrease, and at the same time they began to be found in lymph nodes, the adrenal cortex, bone marrow, lungs and thymus (Fig. 1c). By the 5th-7th days the appearance of lymphoid-macrophagal cells was observed around the PCM, and by the 14th day aggregates of groups of PCM, lymphoid cells, and macrophages with the usual structure were identified in the organs (Fig. 1d). In the late stages the PCM gradually disappeared and lympho-macrophagal granulomas were formed (Fig. 1f), and these later were absorbed without any trace of scar formation. During exchange transfusion absorption of the granuloma took place most rapidly with a mixture of perfluorodecaline and perfluoroparamethylcyclohexylpiperidine, and slowest of all with perfluorotributylamine.

After plethoric injection of small doses of the emulsions the degree of accumulation of PFC in the organs was much less and they were eliminated more rapidly. The histochemical investigation revealed solitary granules of dye in a narrow rim of cytoplasm around the vacuoles

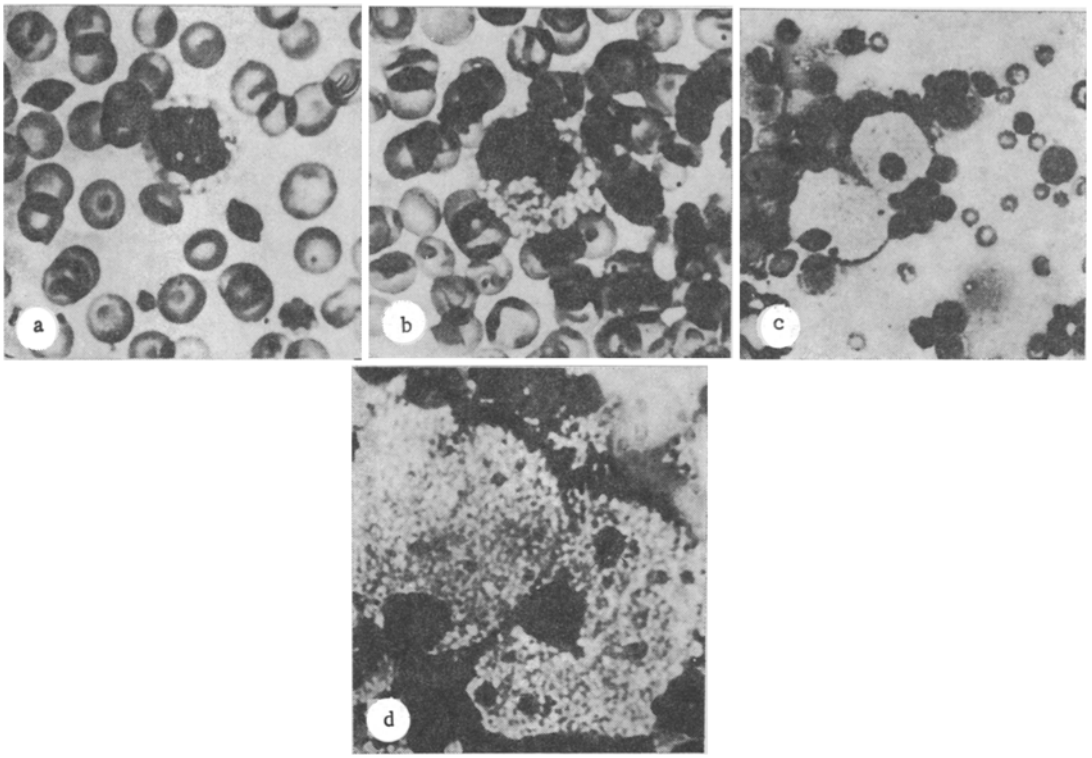

Fig. 2. Blood and bone marrow at different times after blood replacement by PFC emulsions. Stained by Romanovsky—Giemsa method. a) Peripheral blood after 24 h. Particles of PFC emulsion in cytoplasm of a monocyte. 1000× (Here and in Fig. 2b); b) peripheral blood after 5 days. Monocytes with finely vacuolated cytoplasm, containing PFC particles; c) bone marrow after 7 days. PCM with coarsely vacuolated cytoplasm. 400× (Here and in Fig. 2d); d) bone marrow after 8 months. Aggregates of finely vacuolated PCM.

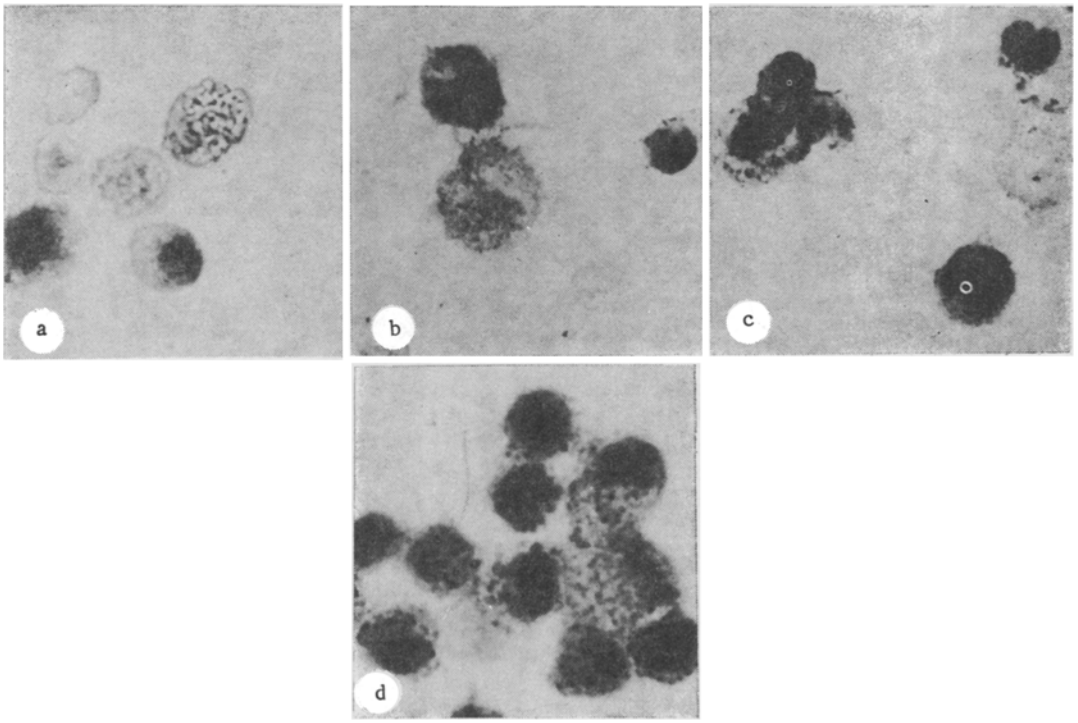

Fig. 3. Peritoneal macrophages after intraperitoneal injection of PFC emulsions. a) PCM with different degrees of vacuolation of cytoplasm on 4th day. Romanovsky—Giemsa. 400× (Here and in Fig. 3b-d); b) different degrees of G6PDH activity in PCM on 4th day; c) nitro-BT test in PCM on 10th day; d) nitro-BT test in peritoneal macrophages not containing PFC, on 10th day.

containing PFC, evidence of minimal activity of oxidative and hydrolytic enzymes. During the formation and regression of the granulomas the vacuoles with PCM became smaller and fragmented, and this was accompanied by activation of the enzyme systems.

Starting from the first few hours after exchange blood transfusion and plethoric injection, monocytes and macrophages with vacuolated cytoplasm respectively were found constantly in films of peripheral blood and bone marrow. Theae cells were distinguished by their large size and by the eccentric position of their nucleus (Fig. 2a, b). Similar cells were identified in the blood during 5-7 days of the experiment. Macrophages containing vacuoles with PFC in the bone marrow were much more numerous and were recorded for a long time (from a few months to 1.5 years). In the later stages of the experiment aggregates of PCM began to be observed, their cytoplasm finely vacuolated. Cytochemical investigation of PFC-containing blood monocytes and bone marrow PCM revealed (compared with the monocytes and macrophages of animals of the control group) a considerable (by 1.5-1.7 times) increase in NE activity and a sharp decrease, in some cases to a completely negative reaction, of PO and AlP activity.

The glycogen content in the cytoplasm was the same as that in control animals.

After intraperitoneal injection of PFC emulsions PCM were constantly found in the peritoneal exudate (Fig. 3a), and their number of the 4th day was 26%, on the 7th day 34%, and on the 10th day 40-50%. Enzyme-chemical analysis revealed biochemical heterogeneity of the cells: PCM with both high and low activity of individual dehydrogenases were observed (Fig. 3b). The results of the nitro-BT tests were demonstrative. The principle of this test is that the macrophage ingests nitroblue tetrazolium and reduces it into diformazan, which is deposited in the form of granules on the surface of the cells. In control experiments with intraperitoneal injection of a 10% solution of peptone the nitro-BT test in the peritoneal macrophages was negative. Marked activity of the nitro-BT test was observed in the peritoneal PCM at all times of observation (Fig. 3d). The nitro-BT test was negative on the 4th and 7th days in peritoneal exudate macrophages not containing PFC. On the 10th day after intraperitoneal injection of PFC emulsions deposition of formazan granules was observed in some cells not containing the fluorocarbon (Fig. 3e), and this can evidently be regarded as a state of "readiness" for phagocytosis. The results of these investigations show that endocytosis of PFC emulsions by blood monocytes and bone marrow and peritoneal macrophages is accompanied by activation of the enzyme systems of the cell. The relative enzyme-histochemical inertia of the PCM in aggregates of the liver, spleen, and lungs after massive blood replacement by PFC emulsions must be regarded as blocking of elements of the PMS responsible for clearance of PFC from the blood. The presence of a positive nitro-BT test in peritoneal macrophages containing PFC is evidence of the stimulating effect of small doses of perfluorocarbon compounds on the mononuclear phagocyte system.

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