MIGRATION AND EMBOLIZATION OF MACROPHAGES TO THE LUNG—A POSSIBLE MECHANISM FOR COLLOID UPTAKE IN THE LUNG DURING LIVER SCANNING

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Estrogenic stimulation of the RES in animals will result in the mobilization of large numbers of phagocytic cells from their natural sites of storage, chiefly the liver, spleen, and bone marrow, into the intravascular space. These cells are trapped in the pulmonary capillary bed and retain their ability to phagocytize colloidal particles. This is suggested to be the mechanism whereby increased lung uptake of colloidal radiopharmaceuticals is observed in some patients with liver diseases or other conditions stimulating the RES. It is further postulated that the known increase of estrogenic hormones in some patients with liver disease may be the in vivo stimulation resulting in increased mobilization of RES cells to the lung and subsequent isotope entrapment. It is possible that prognostic information can be obtained from this finding; however, its exact significance cannot be determined from this present study.

An occasional clinical observation is the demonstration of lung isotopic activity during the performance of a liver scan with labeled colloid materials. Lung uptake during liver scanning with technetium sulfur colloid can be due to faulty preparation of the radiopharmaceutical (*1*) resulting in aggregation of the colloid material in a particulate size large enough to be filtered by the pulmonary circulation. Careful radiopharmaceutical preparation and batch control methods will exclude this possibility and it has been estimated that lung uptake is only 1–2% of the injected dose with well-prepared colloid radiopharmaceuticals (*2*). Clinical studies using carefully prepared radiopharmaceuticals have demonstrated lung localization by colloid during liver imaging in patients undergoing liver, spleen, and bone marrow transplants (*3*) and in patients with a variety of primary liver diseases and metastatic carcinoma (*2*). It has been suggested that variations in lung uptake of colloid are due to physiologic and/or chemical variations within the patient resulting in secondary pulmonary microembolization of intravascular aggregated colloids.

Another possible mechanism for increased lung uptake is alteration of the pulmonary reticuloendothelial system (RES) activity. Colloidal radiopharmaceuticals are phagocytized by cells in the

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RES—indeed this is the basis for liver, spleen, and bone marrow scanning (*4*). There are relatively few fixed interstitial macrophages in the lungs of normal humans capable of phagocytosing circulating particles (*5*); however, an increase in their number would allow the colloid to be localized in the lungs (*2,6*). It has been shown that increased accumulation of colloid after RES stimulation can be relatively organ-specific and dependent on the stimulant (*7*). Quinones has demonstrated in rats that stimulation of the RES with endotoxin results in a twenty-fold increase in the deposition of technetium sulfur colloid in the lungs relative to the liver. Previous laboratory investigations by one of the authors (MAM) have demonstrated that stimulation of the RES system produces free intravascular macrophages which can be transported to the lungs (*8–10*). The purpose of this study was to determine if stimulation of the RES could result in the presence of free intravascular macrophages and the trapping of these active macrophages in the lung, therefore suggesting an alternative mechanism to the lung uptake of radiocolloid materials in certain patients.

MATERIALS AND METHODS

Animals. Fifty-two guinea pigs and 64 toads (Buffo regularis) were selected for the experiment. The mean weight of the guinea pigs was 275 gm and the mean weight of the toads was 45 gm.

Intravitum staining material. (A) A 2% solution of trypan blue (BDH) in distilled water was freshly prepared and sterilized for daily use. The daily dose was 2 cc/100 gm body weight for guinea pigs and toads. (B) India ink (commercial, Winsor and Newton Laboratory, London, England) was diluted with saline (1–10 for guinea pigs and 1–4 for toads) and injected in a dose of 0.5 cc/100 gm body weight in guinea pigs and 1.5 cc/100 gm body weight for toads. Trypan blue was selected for histologic studies and India ink for direct microscopic visualization. Sterilization consisted of boiling for 1 min in a water bath, filtering, boiling for another minute, and then allowing solution to cool to body temperature.

Injections of intravitum staining materials were made subcutaneously into the anterior wall of the abdomen in guinea pigs and into the dorsal lymphatic sac of toads.

Estrogenic hormone. Ethinyl estradiol was used as a stimulant to the RES (*11*). The ethinyl estradiol (estrogen) was given daily by introduction of tablets by a long slender forceps to the back of the oral cavity of each experimental animal, making sure the dose was completely swallowed. The daily dose for guinea pigs was 10 μg and for toads 2.5 μg.

Experimental protocol. The guinea pigs and toads were divided into a control group (no estrogen treatment) and a RES-stimulated experimental group (estrogen-treated). Guinea pigs from each group were sacrificed at 24 hr, 48 hr, 3 days, 7 days, 2 weeks, 3 weeks, and 4 weeks. Two days prior to sacrifice, intravitum staining materials were injected to identify RES cells. Histologic sections of the liver, spleen, bone marrow, and lungs were examined and stained RES cells were quantitated by comparing their number counted in 20 adjacent high-power microscopic fields in ten serial sections of the same area, cut at 10-microns thickness, in the different organs examined.

Tissues from both groups of toads were examined at 6 hr, 12 hr, 24 hr, 48 hr, 3 days, 7 days, and 2 weeks. Two days prior to the experimental time, the toads were injected with intravitum staining materials for localization of RES cells. The toads were pithed, a portion of the lung was exposed out of the thoracic cavity through a bloodless wound, and the living pulmonary circulation examined microscopically under strong illumination and recorded microcinematographically using a 16-mm cine camera. The pulmonary capillary network circulation was observed through the camera window and photographed at the rate of 16 exposures per second. Histologic specimens of the liver, spleen, bone marrow, and lungs were made for quantitation of RES cells as previously mentioned.

RESULTS

Results in toads. Dye-bearing macrophages could not be observed in the lung parenchyma of pulmonary circulation at 6–12 hr in either group. An occasional macrophage could be seen in the lung parenchyma at 24 hr; however, no macrophages were noted in the pulmonary capillary circulation. These observations persisted in the control group throughout the period of study.

At 48 hr, intravitally stained macrophages could be observed in small numbers in the lung parenchyma and pulmonary circulation of the estrogen-treated toads. After 72 hr of hormone treatment, increased numbers of macrophages were found in the lung parenchyma and pulmonary circulation, occasionally blocking small arterioles and the capillary circulation supplied by these arterioles (Fig. 1).

After 1 and 2 weeks of hormone stimulation, the main pulmonary artery was found to carry massive numbers of various-sized macrophages. Macrophages larger than 20 microns were trapped in small pulmonary arterioles and accounted for large areas of completely blocked circulation (Fig. 2). Macrophages smaller than 10 microns were observed to circulate across the pulmonary capillary bed and

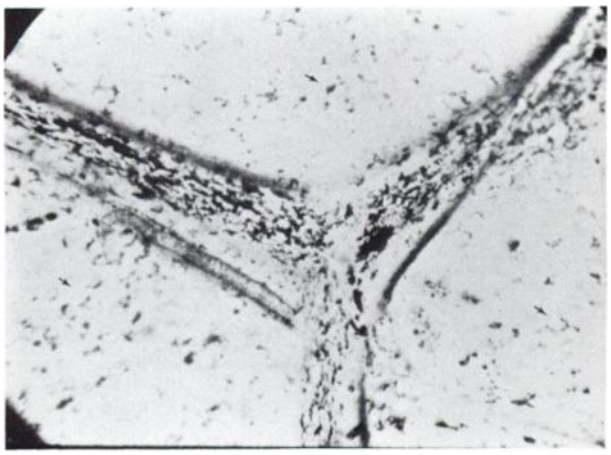

FIG. 1. Pulmonary arteriole containing carbon-bearing macrophages of different sizes. Surrounding pulmonary tissue contains several capillary areas obstructed with same type of cells (arrows). Reproduction from frame of microcinematograph exposed at 16 frames/sec of pulmonary circulation of toad treated with estrogen for 72 hr (X100).

enter the venous circulation. Macrophages between 10 and 20 microns circulated to the venous system or were trapped in the pulmonary capillary system depending on the condition of the capillary bed at their entry point. It was noted that macrophage embolization was a progressive function—a large macrophage will block a portion of the capillary bed, causing irregular circulation in nearby areas and appearing to make it more likely that medium-sized macrophages would be subsequently trapped.

Results in guinea pigs. The histologic observations in the tissues of both groups of guinea pigs were similar at 24 and 48 hr. Liver, spleen, and bone marrow (the primary sites of RES cells) contained macrophages of varying sizes and rare interstitial macrophages were observed in the lungs. These findings did not change in the tissues of control guinea pigs throughout the study.

In the estrogen-treated animals, RES cells in the liver, spleen, and bone marrow were increased in number and size after 2 days of treatment and showed marked degrees of hypertrophy and hyperplasia at 3 days. No significant change was noted in the number of vitally stained cells in the lungs.

After 1 week of estrogen treatment, RES cells in the liver and spleen were markedly hypertrophied, increased in number, and were observed in the venous channels of these organs. Bone marrow RES cells were hypertrophied and hyperplastic but to a lesser extent than the liver and spleen. Small to moderate increases in the number of lung macrophages were noted at 1 week (Fig. 3).

After hormonal treatment of 2, 3, and 4 weeks the histologic findings of the liver, spleen, and bone marrow demonstrated further increase in the number of macrophages in the venous channels. Large numbers of various-sized phagocytic cells, with large nuclei and carrying the injected dye, were seen in the interstitial tissues of the lungs (Fig. 4). The number of macrophages in the lung progressively increased with estrogen treatment and almost all the cells contained the injected dye. In the peripheral areas of the lungs, varying numbers of macrophages were observed in the lumen of bronchioles. The branches of the pulmonary artery contained large numbers of macrophages and the arterial endothelial cells were hypertrophied and contained occasional particles of dye. Smaller pulmonary arterioles were occluded by the hypertrophied endothelium and phagocytic macrophages.

The results of the histologic observations in guinea pigs are summarized in Fig. 5.

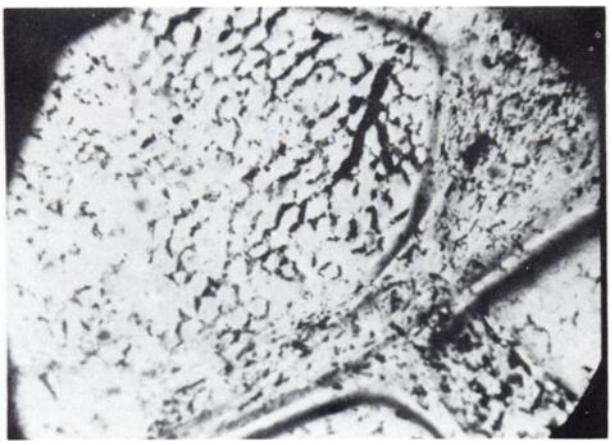

FIG. 2. Pulmonary arteriole containing massive numbers of carbon-bearing macrophages. Surrounding pulmonary capillary area is thronged with intravitally stained cells. Reproduction from frame of microcinematograph exposed at 16 frames/sec of pulmonary circulation of toad treated with estrogen for 2 weeks (X100).

DISCUSSION

The number of free circulating macrophages is normally very small, accounting for approximately 0.2% of the total number of leukocytes in mammals (*9*). Most of the reticuloendothelial cells are found lining the sinuses of the liver, spleen, bone marrow, and lymph nodes and as fixed histiocytes in the interstitial tissues of other organs. The lungs have very few interstitial histiocytes (*5*).

The number of RES cells circulating in the intravascular space and fixed in all organs of the body can be increased by a large number of "stimulants". RES-stimulating factors include vitamin B_{12} (*10*), thyroid (*12*), bacterial endotoxins (*13*), attenuated bacteria (*14*), triglyceride (*15*), dextrose (*16*), yeast extracts (*10*), a variety of foreign proteins (*17*), and

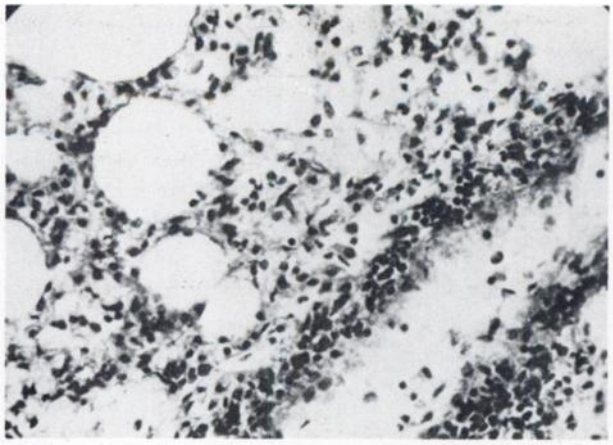

FIG. 3. Section of lung of guinea pig treated with estrogen for 1 week shows infiltration of intra-alveolar spaces with small to moderate number of macrophages. Blood vessel contains same type of cells. (Verhoeff's Van-Gieson stain, X450.)

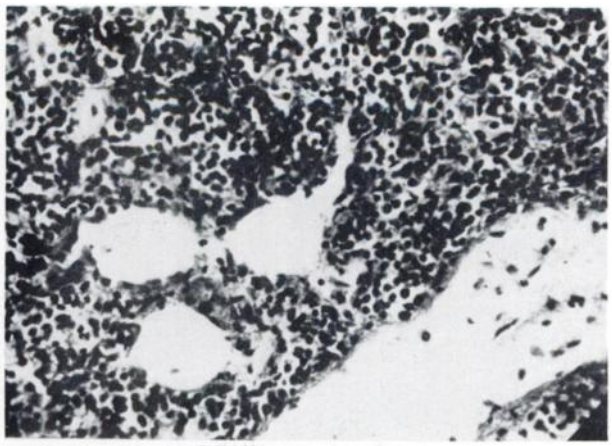

FIG. 4. Section of lung of guinea pig treated with estrogen for 4 weeks. Large numbers of macrophages are observed in interstitial tissue and block some of pulmonary capillaries. Blood vessel contains same type of cells. (Verhoeff's Van-Gieson, X450.)

heparin (*2,4*). Steroid hormones (such as estrogen used in this study) are a potent RES stimulator (*11*) and have been used for this purpose in previous investigations. Estrogen has previously been shown to increase the number of freely circulating macrophages from baseline levels of 0.2–8% of total leukocytes in the right ventricle and 5% in the left ventricle (*9*). The observed difference has been attributed to the trapping of large macrophages in the pulmonary capillaries.

The current study has shown that the lungs of guinea pigs and toads have very small numbers of fixed RES cells capable of phagocytosing circulating particles prior to RES stimulation. Previous investigators (*5,18*) have demonstrated that there are few fixed RES cells in the lungs of mammals confined to the pulmonary interstitial tissues. The alveolar macrophages, on the other hand, are many and are known to be in contact with the bronchial airways rather than the intravascular space as these cells will phagocytize particles injected into the trachea and will not phagocytize particles circulating in the blood (*5,18*).

Our study demonstrates that estrogen treatment results in hypertrophy and hyperplasia of RES macrophages in the liver, spleen, and to a lesser extent, the bone marrow and also results in mobilization of these cells into the intravascular space. A previous study of guinea pigs (including histologic examination of the splenic, portal, and hepatic veins and distal inferior vena cava for the relative numbers of intravascular RES cells together with a quantitative study of the number of macrophages in samples of blood from the previously mentioned veins) treated with estrogens for various periods showed that most of the migrating macrophages reaching the lungs came mainly from the liver and spleen (*9,10*). Nicol and Helmy have also shown that estrogens produce hyperplasia and stimulation of the macrophages in the liver and spleen more than on the bone marrow (*11*). Microphotocinematography demonstrated that large numbers of macrophages reached the pulmonary circulation where they were selectively trapped in the pulmonary capillary bed, related to their size. Macrophages greater than 20–25 microns proceed slowly along the walls of the capillaries and obstruct the small capillary channels; some of these macrophages will penetrate the walls of the capillaries and enter the interstitial tissue of the lungs. Macrophages that are less than 10 microns in size travel freely to the pul-

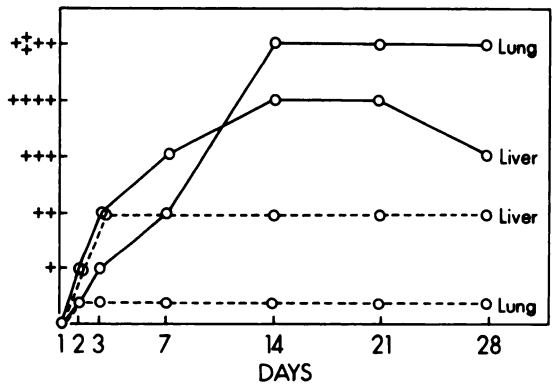

FIG. 5. Histologic observations of trypan-blue-stained macrophages in control (dotted line) and estrogen-treated (solid line) guinea pigs. 0, No macrophages; †, macrophages rarely seen; ††, small number of macrophages (see Fig. 3); †††, moderate number of macrophages; ††††, large number of macrophages (see Fig. 4); and ‡†††, large number of macrophages with areas of massive accumulation corresponding to capillaries blocked by RES cells (see Fig. 4).

monary venous circulation and to the systemic circulation. Macrophages between 10 and 25 microns proceed slowly to the capillary channels and either serve as embolic particles or escape to the venous side depending on the particular condition of the local capillary bed. We further observed that continued estrogenic stimulation leads to a gradual and progressive increase in the percentage of large (greater than 20–25 microns) macrophages with increased chances of their trapping and blocking of the pulmonary bed.

There are three possible mechanisms for increased RES activity in the lungs: (A) The stimulating effect of estrogens could produce hypertrophy, multiplication, and adoption of phagocytic properties in the endothelial cells lining the pulmonary blood vessels. A few isolated reports have demonstrated that the common endothelial cells of the blood vessels are capable of accumulating colloidal materials (*19*). (B) Pre-existing pulmonary RES cells can respond to stimulating agents by hypertrophy and hyperplasia, increasing the phagocytic ability of the lungs. (C) RES stimulation results in large numbers of free intravascular macrophages (mainly from liver, spleen, and to lesser extent bone marrow) and many of these cells are trapped in the pulmonary capillary system.

We believe the third possibility is the most important mechanism for increasing RES activity in the lungs after estrogen stimulation. After estrogen stimulation, RES cells in the liver, spleen, and to a lesser extent bone marrow undergo rapid proliferation as previously mentioned (*11*). Subsequently, large numbers of free intravascular macrophages are present and we have demonstrated their entrapment in the pulmonary capillary system. Studies carried out with injections of chromosome-labeled bone marrow macrophages have shown that approximately two-thirds of lung macrophages, after RES stimulation by foreign proteins, arose in the hematopoietic system (*20*).

This study further demonstrates that transported RES cells to the lungs retain their ability to phagocytize injected colloid particles. In our studies, animals treated with estrogens for 2–4 weeks and injected with colloidal stains prior to sacrifice showed progressively increasing numbers of vitally stained macrophages in the lungs. Nearly all interstitial and intravascular lung RES cells were observed to contain the intravital dye. This observation supports the postulate that migrating RES cells maintain phagocytic ability since the intravital dye was only injected in the final 2 days before sacrifice and it is highly unlikely that a stained migrated cell population completely replaced previously present lung RES cells that had lost phagocytic properties.

The ability of RES cells to maintain phagocytic ability has been shown in another series of experiments using two groups of guinea pigs treated with estrogens for various periods of time varying between 2 and 120 days. One group of animals was treated with trypan blue (vital staining of RES cells) and organ tissues were evaluated by histologic staining at the end of the experiment in the other group. In both groups the number of RES cells in the lungs, vitally and histologically stained, was almost the same after comparable periods of hormonal treatment indicating that the migrated RES cells retained their phagocytic activity (*8,10*). Other authors have postulated that migration of phagocytes from pulmonary capillaries or from transplanted liver, bone marrow, or spleen to the lung does not affect their phagocytic activity (*3,21*).

These experimental findings suggest a mechanism for the clinical demonstration of radiopharmaceutical uptake in the lung during a liver-spleen scan in cases of liver disease or any other condition stimulating the function of RES.

In this study, estrogen was used as an RES stimulant because of its known stimulating properties (*11*). In various forms of liver failure, increased levels of estrogen are found as the result of various mechanisms. It is possible that the increased lung uptake of radiopharmaceuticals during liver scanning is due to the imbalance in estrogen-antiestrogen status of these patients in favor of the estrogens (*22*).

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