PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 167, 137–142 (1981)

Platelet Blockade of Particle Absorption from the Peritoneal Surface of the Diaphragm (41138)

ALLAN E. DUMONT, EDITH ROBBINS, AMALIA MARTELLI, AND HENNY ILIESCU

Departments of Surgery and Cell Biology, New York University School of Medicine, 550 First Avenue, New York, New York 10016

Abstract. Particulate material is absorbed from the peritoneal cavity via respiration-induced gaps between mesothelial cells on the peritoneal surface of the diaphragm and is then collected into diaphragmatic and retrosternal lymph vessels. The effect of platelets on this process was examined by (i) comparing the appearance of these lymph vessels after intraperitoneal (ip) injection of Radiopaque or carbon particles in control and experimental rats receiving, respectively, an ip injection of either platelet-rich (PRP) or platelet-poor (PPP) plasma or saline solution 1 hr prior to the injection of particles; and by (ii) inspecting lymph vessels on the pleural side of 20 freshly excised pieces of rabbit or dog diaphragm (secured pleural side down, to the mouth of a suction flask) 5 min after application of India ink with either PRP or PPP (or saline) to the peritoneal surface. In each case injection or application of PRP either blocked or markedly restricted particle absorption. Electron microscopic (EM) observations indicate that platelets block absorption of particles by plugging gaps between adjacent mesothelial cells.

Absorption from the peritoneal cavity into lymphatics occurs predominantly at the peritoneal surface of the diaphragm. Proteins and other large molecules, particles, and formed blood elements leave the peritoneal cavity predominantly via gaps between mesothelial cells on this surface and then enter diaphragmatic and in turn, retrosternal lymph vessels (1–3).

Mesothelial cell junctions on the peritoneal surface of the diaphragm are in part similar structurally to junctions in venular endothelium (4). Unlike endothelium however, the diaphragmatic mesothelium normally contains intercellular gaps.These probably include the "stomata" recognized by Von Recklinghausen (5) and more recently described by Leak and Rahil (6). While particles and formed blood elements do not normally escape across vascular endothelium, they do so extensively in platelet-deficient animals (7, 8). Functionally, therefore, the leaky vascular endothelium of such deficient animals tends to resemble the mesothelium on the peritoneal surface of the diaphragm. Intravenous infusion of fresh platelets rapidly stops the abnormal leak across vascular endothelium (9, 10) but whether platelets applied to the peritoneal side of the diaphragm similarly block the absorption of particles across the mesothelial surface is unknown. In this communication we describe experiments which indicate that platelets either completely block or markedly restrict the absorption of particles from the peritoneal cavity and that such action derives from formation of plugs on the basal lamina exposed at the level of gaps between mesothelial cells.

Methods. Initial experiments were carried out *in vivo* in 20 healthy adult Wistar rats (200–300 g) divided equally into two groups as follows: in Group 1 each animal received a single intraperitoneal (ip) injection of 4 ml of fresh platelet-rich plasma (PRP) obtained from citrated (0.32%) human, dog, or rabbit blood by centrifugation at 150*g* for 10 min at room temperature. Examination under a light microscope disclosed that platelets remained intact after this procedure. Animals in Group 2 received a single ip injection of either isotonic saline solution or platelet-poor plasma (PPP), prepared by further centrifugation at 2000*g* for 20 min. One hour later animals in both groups received an ip injection of 1 ml of either cream (Von Recklinghausen's

0037-9727/81/050137-06$01.00/0

method) (5) or a 0.4% suspension of Radiopaque lipid particles (particle size, 0.5 μm—prepared by a method described earlier) (11). Animals receiving an ip injection of cream were sacrificed 2 hr later. The anterior chest wall was incised laterally, reflected, and diaphragmatic and retrosternal lymph vessels and intercostal lymph nodes were examined grossly and with the aid of a stereomicroscope. After 24 hr animals that received an ip injection of Radiopaque lipid were lightly anesthetized and were examined radiographically in the anterior–posterior and lateral positions. Following radiography these animals were sacrificed and examined as described above.

In subsequent *in vitro* experiments, 20 freshly excised hemidiaphragms were obtained from anesthetized dogs and rabbits. A 6-cm-diameter full-thickness piece of the muscular portion in dogs and the tendinous area in rabbits was secured with the pleural side down to the mouth of a 500-ml suction flask (12). One and a half milliliters of either PRP (10 experiments) or PPP or isotonic saline solution (10 experiments) was placed on the peritoneal surface. Two milliliters of undiluted India ink. (0.7 mg/ml–particle size 50 μm) was then added. In order to simulate the effects of respiration of the diaphragm, the specimen was intermittently stretched and relaxed 10–15 times per minute for 5 min by applying suction sufficient to draw the diaphragm slightly into the mouth of the flask. These experiments were carried out at room temperature and the tissues were not oxygenated. The specimen was then removed, washed in saline for 5 min, blotted, and the pleural surface inspected grossly and with the stereomicroscope. Specimens were then fixed in 10% formalin, sectioned, stained with hematoxylin and eosin, and examined under the light microscope.

To study the phenomenon at the cellular level sections of diaphragm were obtained for electron microscopic (EM) studies as follows: in six male Swiss-Webster mice (25–30 g), 3 ml of undiluted India ink were

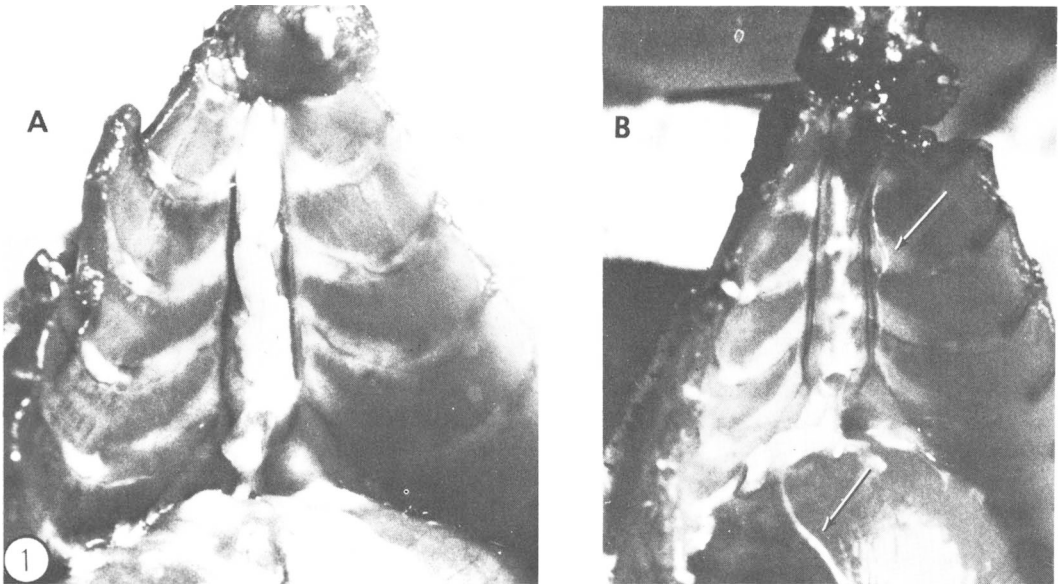

FIG. 1. On the left (A) is the pleural surface of the diaphragm and the retrosternal region in a mouse that received an ip injection of PRP followed in 1 hr by an ip injection of cream. No lymph vessels are opacified. The same area in a PPP-treated mouse on the right (B) shows diaphragmatic and retrosternal lymph vessels (arrows) prominently distended with cream.

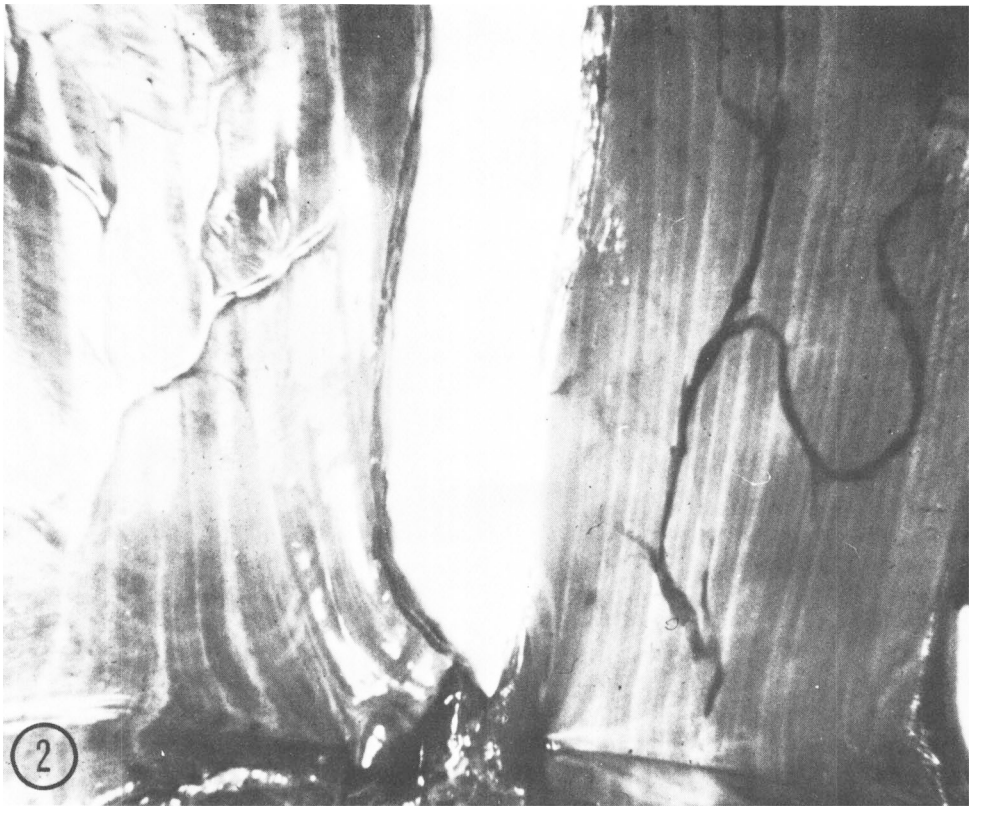

FIG. 2. On the left is the pleural surface of a freshly excised dog hemidiaphragm tested *in vitro* by placing PRP and then India ink on its peritoneal surface. No carbon-containing lymph vessels are seen. On adjacent area from the same hemidiaphragm tested with PPP and India ink (control) on the right shows lymph vessels filled with carbon.

injected ip 30–60 min after ip injection of 3 ml of either PRP (three animals, Group 1) or isotonic saline solution (three animals, Group 2) and the mice were sacrificed 10–60 min later. Diaphragms were excised and fixed with cold 2% glutaraldehyde in 0.1 *M* sodium cacodylate buffer containing 0.1 *M* sucrose. In some instances the fixative was administered ip to anesthetized animals a few minutes prior to sacrifice and fresh fixative was then used to gently rinse the peritoneal surface of the diaphragm just prior to excision. After removal, diaphragms were kept in cold fixative for at least 24 hr. Subsequently they were rinsed in buffer, postosmicated in 1% aqueous OsO_4, dehydrated in graded ethanol series, and embedded in Epon 812. Sections were cut on a Porter–Blum MT2 ultramicrotome, doubly stained with uranyl acetate and lead citrate, and examined in a Phillips 300 electron microscope.

Results. Gross, stereomicroscopic, and radiographic examination of the diaphragmatic and retrosternal lymphatics disclosed striking differences between animals that received PRP (Group 1) and those that did not (Group 2). Two discrete retrosternal channels together with corresponding lymph nodes in the second intercostal space were especially prominent grossly and/or radiographically in every animal in Group 2. In contrast, these structures were either not identifiable or barely visible in animals in Group 1 (Fig. 1).

Similarly, when India ink was added to

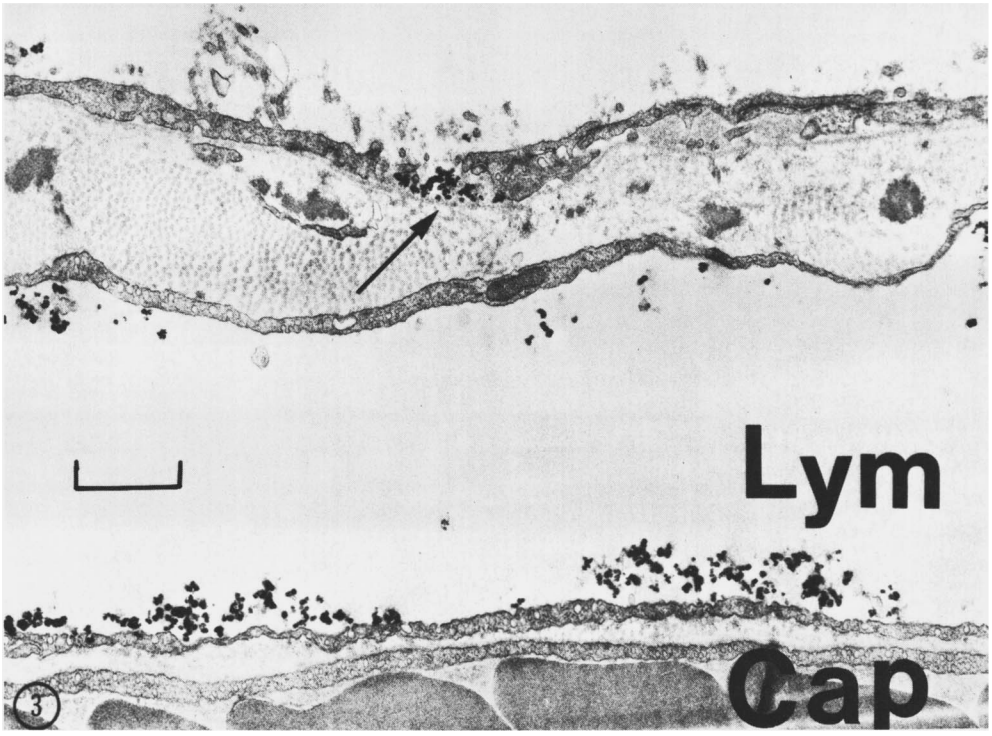

FIG. 3. Peritoneal surface of the diaphragm in a control mouse that received IP saline and India ink. Carbon particles are seen penetrating the diaphragm via a gap between mesothelial cells (arrow) and in the lumen of a subadjacent lymphatic (Lym). Blood capillaries such as the one (Cap) below the lymphatic were never seen to contain carbon. Bar = 1 μm. ×17,750.

PPP or saline on the peritoneal surface *in vitro*, subpleural lymph vessels became distended with carbon particles and were easily identified grossly as distinct black channels. These vessels invariably remained colorless when India ink was added to PRP-treated diaphragms (Fig. 2). Histologic examination of sections of the diaphragm confirmed that significantly more carbon had accumulated in the interstitial spaces and lymph vessels when platelets were absent.

When examined by EM, the peritoneal surface of the diaphragm of Group 2 animals (controls) (Fig. 3) showed dense accumulations of carbon in intercellular gaps, connective tissue, and lymph vessels, regardless of whether the overlying mesothelium was cuboidal or squamous. In contrast, diaphragms from PRP-treated Group 1 animals showed only traces of carbon in connective tissue and lymph spaces and gaps between mesothelial cells were plugged with degranulating platelets, carbon, and some amorphous material (Fig. 4). The basement appeared intact in all specimens.

Discussion. These experiments indicate that when platelets are applied to the underside of the diaphragm, the absorption of lipid and carbon particles from this area is either completely blocked or markedly restricted. Viewed in the light of current information, this action of platelets is understandable. Absorption of particles from the peritoneal surface of the diaphragm predominates at sites which overlie lymph spaces (2). It is clear from earlier studies that mesothelial cells in these areas separate more readily than cells elsewhere on the peritoneal surface (2) and that particles as well as formed blood elements pass into diaphragmatic lymphatics intermittently

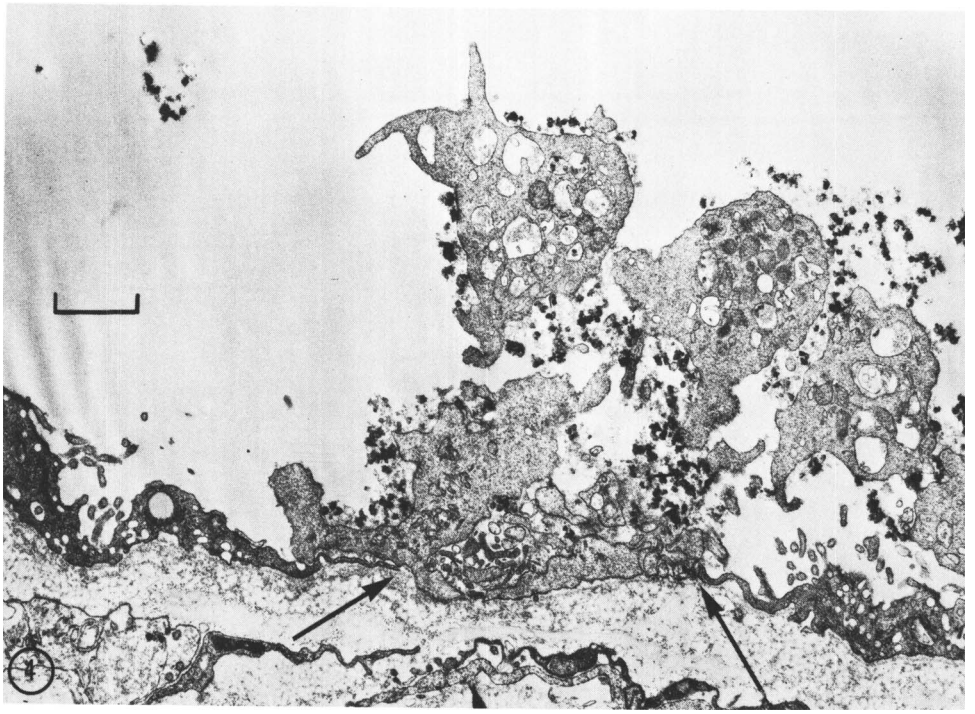

FIG. 4. Peritoneal surface of the diaphragm in a mouse that received PRP and India ink. A plug of partially degranulated platelets, carbon particles, and other material occupies the gap between two mesothelial cells. Arrows indicate the area of contact of this plug with the basal lamina. Lymphatics in such animals (not illustrated) contained little or no carbon. Bar = 1 μm. ×14,250.

through respiration-induced gaps between these cells (6, 12). Our EM observations indicate that platelets plug the gaps between adjacent mesothelial cells and thus block the absorption of particles.

Since the peritoneal surface of the diaphragm has predictable properties with respect to the intercellular passage of particles and formed blood elements, the observation that it can be converted into a barrier simply by the application of platelets is not without interest. The effect of platelets on this surface might be utilized clinically for example, in attempts to prevent the spread of bacteria (14) and tumor cells (15) from the peritoneal cavity. Applied experimentally, it might serve as a model to help clarify the mechanism by which platelets regulate permeability of postcapillary venular endothelium (16, 17). Other similarities between the latter and mesothelium were outlined by Simonescu and Simonescu (4). The attraction of the peritoneal surface of the diaphragm for platelets, attributable in all likelihood to an exposed mesothelial basal lamina, could in addition provide the basis for a new experimental model for studies of platelet adhesion.

1. Courtice, F. C., and Simmonds, W. J., Physiol. Rev. **34**, 419 (1954).
2. French, J. E., Florey, H. W., and Morris, B., B. Quart. J. Exp. Physiol. **45**, 88 (1960).
3. Casley-Smith, J. R., Quart. J. Exp. Physiol. **49**, 365 (1964).
4. Simonescu, M., and Simonescu, N., J. Cell Biol. **74**, 98 (1977).
5. Von Recklinghausen, T., Virchows Arch. Pathol. Anat. **26**, 172 (1863).
6. Leak, L. V., and Rahil, K., Amer. J. Anat. **151**, 557 (1978).
7. Van Horn, D. L., and Johnson, S. A., J. Lab. Clin. Med. **71**, 301 (1968).
8. Kitchens, C. S., J. Clin. Invest. **60**, 1129 (1977).

9. Woods, M. C., Gamble, F. N., Furth, J., and Bigelow, R. R., Blood **8**, 545 (1953).
10. Johnson, D. P., Sorensen, D. K., Cronkite, E. P., Bond, V. P., and Fliedner, T. M., J. Clin. Invest. **38**, 1689 (1959).
11. Dumont, A. E., Jacobs, R., and Gordon, F., J. Surg. Res. **7**, 313 (1967).
12. Durden, C., Laslie, M., and Allen, L., Anat. Rec. **150**, 335 (1964).
13. Bettendorf, U., Lymphology **11**, 111 (1978).
14. Buxton, B. H., and Torrey, J. C., J. Med. Res. **15**, 5 (1906).
15. Saldeen, T., Acta Pathol. Microbiol. Scand. (Suppl.) **161**, 26 (1963).
16. Maca, R. D., Fry, G. L., and Hoak, J. C., Blood **48**, (Abstr.) 977 (1976).
17. Gore, I., Takada, M., and Austin, J., Arch. Pathol. **90**, 197 (1970).

Received June 16, 1980. P.S.E.B.M. 1981, Vol. 167.