The Role of Hypovolemic Stress in the Production of Fat Embolism in Rabbits*

1. Morphologic Alterations of the Lungs

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In rabbits, an experimental model of fat embolism was produced that simulates the course of events in the clinical situation. Small doses of fat-cell suspension (0.075 ml/kg of body weight), prepared by collagenase treatment of homologous adipose tissue, were injected intravenously. Concomitantly, hypovolemia was produced in two animal groups by either withdrawing 20 percent of the estimated blood volume or by application of a hind-limb ischemic tourniquet for 90 minutes. The presence of pathoanatomic characteristics typical of fat embolism was evaluated by recording lung/body weights, macroscopic appearance, and semiquantitative microscopic estimation in the lungs of edema, hemorrhage, atelectasis, intravascular coagulation, and leukocytic thrombi. Mean indices of lung/body weight were higher in all animals receiving injections of fat-cell suspension, as compared to controls. The score for microscopic generalized pulmonary damage was significantly higher in rabbits exposed to both fat-cell injections and hypovolemia than in controls or after fat-cell injections alone. It is concluded that hypovolemia enhances the development of fat embolism in rabbits subjected to small doses of fat-cell suspension.

The experimental production of fat embolism has been a subject of intensive studies during the last few years. Various laboratory animals and a multitude of experimental procedures have been used. These experimental models failed to reproduce human fat embolism, either because the pathogenetic mechanisms used were dissimilar from those playing a role in the clinical situation (ie, pulmonary injections of free fatty acids1-4) or because the typical pulmonary morphologic and pathophysiologic alterations of fat embolism were not obtained.5-14

In humans, fat embolism, with some degree of pulmonary insufficiency, almost universally follows trauma and fat marrow embolization of the lungs.15,16 It has been emphasized that the incidence of fat embolism after long-bone injury increases with the severity of the associated stress factors, such as hemorrhagic shock15,17-21 or delayed immobilization of the fractured bone.16,17

The purpose of this study was to reproduce in the experimental model the course of events and the morphologic and pathophysiologic changes that occur in human fat embolism. We report in this paper the morphologic alterations in the lungs of rabbits following intravenous injections of homologous intact fat cells in association with two forms of experimental hypovolemic stress.

**MATERIALS AND METHODS**

*Animals*

Randomly bred local rabbits weighing between 1.75 and 3.65 kg were used. The animals were given regular chow and water ad libitum. All rabbits were weighed, and arterial blood samples were obtained from the central ear artery in the awake state. Blood gas levels and pH were measured using conventional electrodes (Radiometer BMS-3). No animal was accepted for the study unless the arterial oxygen pressure while the rabbit was breathing room air was above 75 mm Hg (ambient barometric pressure in our laboratory is around 700 mm Hg). All animals showed some respiratory alkalosis during the initial blood gas determination in the awake state, but this is considered normal in the rabbit.

*Anesthesia*

Anesthesia was induced using intravenous administration of pentobarbital sodium (30 mg/kg of body weight) and was maintained with supplemental doses as required.
Preparation and Injection of Fat-Cell Suspension

Homologous epididymal or perirenal fat tissue was used for the preparation of fat-cell suspension, as described by Rodbell. Small sections of fatty tissue were incubated and gently stirred for 1½ to 2 hours at 37°C in an albumin-bicarbonate buffer containing collagenase. The buffer was freshly prepared on the day of the experiment from bovine albumin poor in free fatty acids (Armour Pharmaceutical Co. or Miles Laboratories) and bicarbonate (pH 7.4). The cells were then separated from the medium by centrifugation and were washed several times with albumin buffer. The fat-cell suspension was then incubated at 37°C until injection (for no longer than 90 minutes). Cell integrity is demonstrated in Figure 1 and could be verified even after five to six hours of in vitro incubation. The free fatty acid content of this suspension, as determined in our laboratory, was about 0.2 μEq/ml, which is negligible when compared to the total circulating pool of free fatty acids. The triglyceride concentration of the suspension (concentrated by centrifugation) ranged from 750 to 850 mg/mL. From the concentrated floating fat cells, 0.05 ml was drawn for each injected dose and was diluted to 0.7 ml with the albumin buffer, also kept at 37°C. Each dose was slowly injected (15 seconds) and flushed with 2 ml of saline solution every five minutes to a total dose of 0.075 ml of concentrated fat cells per kilogram of body weight.

Experimental Groups

Group A. Fat-cell suspension was injected according to the previously explained protocol in 11 rabbits.

Group B. In ten rabbits, hypovolemia was induced by arterial bleeding. The femoral artery was cannulated, and blood was drawn into a syringe containing 5 to 7 ml of anticoagulant citrate dextrose solution and stirred periodically. The amount of blood withdrawn was based on estimating the animal's total blood volume as 7 percent of body weight; 20 percent of this blood volume was drawn at a rate of 1.94 ml/min with an infusion-withdrawal pump (Harvard). Blood pressure was measured before arterial bleeding, during hypovolemia, and at the end of the bleeding, using transducers (Statham) and a polygraph (Grass). The blood was kept for one hour in a water bath at 37°C. At the end of that period, the blood was reinfused at the same rate. The mean systolic blood pressure was 113 ± 18 mm Hg (SD) before blood withdrawal, 81 ± 20 mm Hg during hypovolemia, and 104 ± 13 mm Hg at the end of reinfusion.

Group C. This group consisted of 14 rabbits. An arterial ischemic tourniquet was applied for 18 hours over the proximal thigh, using a band-and-screw device tightened to produce a cold ischemic limb.

Group D. This group included 15 rabbits in which the procedures of groups A and B were combined. During the hypovolemic period, as produced in group B, fat cells were injected as in group A. The mean systolic blood pressure in this group was 107 ± 17 mm Hg before hypovolemia, 78 ± 19 mm Hg during hypovolemia, and 89 ± 21 mm Hg after reinfusion.

Group E. In 20 rabbits, injections of fat-cell suspension were made as in group A, about half an hour before tourniquet release as in group C.

Group F. Eight rabbits, serving as controls, were subjected to anesthesia only.

Animals of all groups were allowed to wake up at the end of the respective experimental procedures and were returned to their cages.

Morphologic Examination

All surviving animals were weighed and then killed by an overdose of pentobarbital sodium, either on the first or the third day following the procedure. The lungs were weighed, and the pulmonary index was calculated as lung weight/body weight × 100. The lungs were then inspected grossly and fixed in a 4-percent solution of formaldehyde. Frozen sections of representative samples were stained by oil red O for neutral fat, and paraffin-embedded sections were stained with hematoxylin-eosin.

RESULTS

Animals in groups D and E showed higher mortality prior to their planned death, the incidence being 66 percent (ten rabbits) in group D and 40 percent (eight) in group E. Groups A, B, C, and F had death rates of 18 percent (two rabbits), 20 percent (two), 7 percent (one), and zero, respectively.

Figure 1. Fat cell in suspension demonstrating cellular integrity (oil immersion, original magnification × 1,000).

Figure 2. Macroscopic appearance of lungs from rabbit in group E.
Macroscopic Evaluation

The mean pulmonary indices (± SD) for all groups of rabbits, either at death or upon sacrifice, were as follows: group A, 0.61 ± 0.12; group B, 0.54 ± 0.06; group C, 0.51 ± 0.13; group D, 0.63 ± 0.26; group E, 0.66 ± 0.42; and group F, 0.49 ± 0.05. Mean pulmonary indices of groups D and E were statistically higher (P < 0.05) than those of groups B, C, and F, but not statistically different than that of group A. In general, the lungs of rabbits in groups D and E presented a rubbery consistency and showed multiple, small, subpleural petechial hemorrhages (Fig 2). In some instances, their cut surface exuded frothy hemorrhagic fluid. The lungs of animals in group A had, on occasion, large areas of hemorrhage, while other areas appeared macroscopically normal.

Microscopic Evaluation

Morphologic alterations were observed in the lungs of rabbits of all experimental groups. These changes consisted of intravascular fat emboli; focal or widespread interstitial and intra-alveolar edema (Fig 3) and hemorrhages (Fig 4); large or small areas of focal (Fig 5) or diffuse atelectasis and compensatory emphysema; thrombotic occlusion of pulmonary vessels of various sizes, often admixed with fat emboli (Fig 6); and leukocytic stasis or "thrombi" (Fig 7). The incidence, as well as the intensity and extent, of the changes varied from one animal to another within the same group, as well as between the various groups. Of the various histologic changes, five features were singled out for analysis, and the severity of involvement of each feature was evaluated semiquantitatively on an arbi-
FIGURE 7. Lung of rabbit in group D. Note leukocytic “thrombus” in medium-sized vessel (hematoxylin-eosin, original magnification × 100).

Intravascular scale ranging from 0 to ++ (ie, 0, no change; +, mild; + +, moderate; and + + +, severe). Intravascular fat emboli were observed in the lungs in an almost constant intensity in all animals of the three groups (A, D, and E) in which fat cells were injected, whereas no such emboli were seen in any animals of groups B, C, and F. Therefore, this morphologic criterion was not computed for the comparative evaluation of the total generalized pulmonary damage.

The results of the histologic examinations are summarized in Table 1. In order to assess the severity of the lesions in each group, the generalized pulmonary damage was calculated as follows: the scores of each histopathologic criterion were summed, the highest possible score for an individual rabbit being 15 (ie, + + + damage in each of the five pathologic features). The arithmetic mean of the generalized pulmonary damage was computed for each group. As can be seen in Table 1, the highest score for generalized pulmonary damage was found in group D (11), followed closely by group E (9). The intravascular injection of fat cells alone (group A) caused pulmonary damage of a moderate degree (generalized pulmonary damage, 6), whereas only mild damage to the lungs was observed in groups B, C, and F (generalized pulmonary damage of 3, 1, and 1, respectively). Statistical evaluation of the significance of the differences between the generalized pulmonary damages of the various groups is presented in Table 2. The generalized pulmonary damage of groups D and E were significantly higher than those of all, and any of groups A, B, C, and F. The generalized pulmonary damage of group D was somewhat higher than that of group E (P < 0.02).

Discussion

Various animal models for the production of fat embolism have been described in the literature. Trauma to bone in the anesthetized animal is followed by ample evidence of pulmonary fat embolization, but with only minimal alterations in pulmonary function characteristic of fat embolism. Similarly, the intravenous injection of either neutral fat or of homogenized homologous or autologous fat did not produce changes that are typical of fat embolism unless very large doses of fat (> 0.2 ml/kg of body weight), much in excess of estimated fat embolization in the clinical situation, were used. Under these circumstances, early hypoxia and death were usually observed, probably related to massive obstruction of the pulmonary vascular tree, and not due to the gradual development of fat embolism. It seems significant, then, that the experimental introduction in anesthetized animals of reasonable amounts of either neutral fat or homogenized fat cells, or even

Table 1—Incidence and Severity of Histopathologic Changes and Mean Generalized Pulmonary Damage (GPD) in Various Experimental Groups

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>No. of Animals</th>
<th>Edema</th>
<th>Hemorrhage</th>
<th>Alveolar Atelectasis</th>
<th>Intravascular Coagulation and Thrombosis</th>
<th>Leukoeytic “Stasis”</th>
<th>Mean GPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Fat cells</td>
<td>11</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td>21</td>
</tr>
<tr>
<td>B. Hypovolemia</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>C. Tourniquet</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>14</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>D. Hypovolemia and cells</td>
<td>15</td>
<td>2</td>
<td>2</td>
<td>7</td>
<td>4</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>E. Tourniquet and cells</td>
<td>20</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>8</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>F. Anesthesia</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>
duced hypovolemia or ischemic tourniquet-induced result in a moderate degree of pulmonary damage, fat-cell injections, together with intravascular leukocytic stasis, while the administration of fat cells alone resulted in a moderate degree of pulmonary damage. In rabbits subjected only to hypovolemia, tourniquet, or merely anesthetia, mild to minimal pathologic changes were observed.

Pulmonary morphologic alterations were observed in all animals subjected to injections of homologous fat-cell suspension. The presence of some changes in animals subjected to fat-cell administration alone without hypovolemia might possibly be attributed to our use of whole fat-cell suspension as the source of fat, in contrast to the triglyceride particles employed by other investigators. It is difficult to assess at this time whether or not embolization by whole fat cells, as used in our study, is significantly different from the use of homogenized fat cells; however, it has been established experimentally and has been suggested in the clinical situation of fat embolism that pulmonary embolized fat cells might liberate thromboplastin, which secondarily induces intravascular coagulation, and this is considered by some authors to be a central event underlying fat embolism. In our experiment, pulmonary leukocytic stasis and intravascular coagulation were prominent features whenever fat-cell embolization was induced. The relationship of intravascular aggregation of leukocytes to the production of intravascular coagulation has already been suggested by other investigators. Interestingly, in our rabbits, hypovolemia alone also caused a mild degree of intravascular coagulation, together with intravascular leukocytic stasis. These findings are in agreement with those of Wilson.

Fat-cell embolization alone also resulted in an increased lung weight. The seemingly paradoxical observation that increased mean lung weight in this group was not accompanied by microscopic evidence of either severe edema or of significant pulmonary hemorrhages is probably explained by the macroscopic finding of an occasional large, red pulmonary infarct contributing to increased lung weight, but not sampled for microscopic examination and not associated with other criteria of generalized pulmonary damage.

The observed findings in group A were not sufficient to produce the fulminating morphologic picture that is customarily associated with fat embolism. Reidbord similarly could not produce significant pulmonary damage in rats exposed to intravenous injection of autologous fat-cell emulsion. We believe that the addition of hypovolemic stress is essential for the production of the full-blown pathologic picture of fat embolism. The role of stress, or hypovolemia, in this context might well be attributed to the enhancement of the toxic effect of free fatty acids liberated upon lipolysis of the fat emboli.

It has been frequently shown that the incidence of clinical fat embolism increases whenever hypovolemic shock complicates long-bone trauma. Our experimental results are consistent with

<table>
<thead>
<tr>
<th>Group</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Group E</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>NS*</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>C</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>D</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>E</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.02</td>
<td>...</td>
</tr>
<tr>
<td>F</td>
<td>P &lt; 0.001</td>
<td>p &lt; 0.01</td>
<td>NS*</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

*NS, Not significant.
this clinical observation. We feel that hypovolemia, in this regard, is but one form of stress, as is the ischemic tourniquet applied to animals in group E. Robinson and Solowy21 combined triolein embolization of the lungs with hypovolemia in rabbits. The pathophysiologic alterations of fat embolism were not obtained in their study. Possibly, neutral fat particles in this form are not as effective as the fat cells used in our experiment, because they lack the coagulant effects of whole fat cells. In another similar study, we combined embolization of fat emulsion (Intralipid) in a similar dose as in groups A, D, and E with 20-percent hypovolemia and did not obtain any evidence of the production of fat embolism (unpublished data).

Recently, several reports have emphasized the occurrence of milder, and often subclinical, forms of fat embolism.16,22-32 This form of human fat embolism might well be analogous to fat-cell embolization without hypovolemia in our study.

In a subsequent report,36 we shall describe the pathophysiologic alterations that occur in the respiratory system in our animal model.

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