Pulmonary Microembolization in Severe Thermal Injury[1] (36287)

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It is generally agreed that anoxia secondary to pulmonary damage is an important contributing factor in the mortality of severe thermal injury. This complication has been related to the inhalation of incomplete products of combustion in a number of instances (1); such a clearcut etiology has not been evident, however, in a significant number of other cases. Recent investigations of reticuloendothelial system function in burned animals (2) have provided an unexpected opportunity to further examine this question. In the course of these studies, it was observed that the intravenous injection of colloidal carbon particles into rats within one hour after thermal injury resulted in a rapid embolization of the carbon particles to the pulmonary vascular bed, with mechanical vascular obstruction, interference with gas exchange and respiratory death. The implications of this observation are described in the present report.

Materials and Methods. One hundred and fifty adult male open-stock Wistar rats weighing 250 to 350 g were used. The technique of burning consisted of applying through a measured asbestos frame, under ether anesthesia, a thermally regulated metal surface maintained at 250° to a precalculated body surface area of the rat (30%). Control of the duration of exposure and of the pressure applied produced a standard full-thickness burn of the exposed area (3). Three milliliters of normal saline solution per 100 g of body weight were given intraperitoneally to each animal immediately after injury. The burned animals received colloidal carbon particles intravenously at various intervals after injury, and the results were compared with the effects of similar doses of carbon in 88 unburned rats.

The colloidal carbon was prepared by the method of Halpern, Benacerraf, and Biozzi (4). For this purpose, a carbon suspension containing 90 mg of carbon per ml, 4.3% fish glue and 0.9% phenol (Pelikan Werke, Hannover, Germany, Lot C11/1431A) was centrifuged at 1500*g* for 20 min to remove large particles and aggregates. The supernatant suspension, containing particles of 200 to 500 Å in diameter, was adjusted to a final carbon concentration of 30 mg/ml with a 2% gelatin solution neutralized to pH 7.0 with 0.01 *N* NaOH. The suspension was stored at 4°, and was heated to 37° before injection. All rats received a standard dose of 30 mg of carbon per 100 g body wt.

The rate of carbon cleared from the blood of the recipients was studied at 2, 10, 20, 30, 45, 60, 90, and 120 min after injection. For this purpose, 0.025 ml of blood was drawn, and the carbon concentration of each sample was determined by optical density measurements at 750 mμ with a Beckman DU Spectrophotometer. The amount of carbon present was calculated on the basis of a standard curve prepared by optical density readings of known carbon concentrations in heparinized blood. The rate of carbon clearance was expressed as the percentage of the initial postinjection carbon concentration (2-min reading) which was cleared from the blood at subsequent time intervals. The actual amount of carbon deposited per g of liver, spleen and lungs was determined in 36 animals. The recipients were sacrificed 4 hr after carbon injection; the organs were removed and weighed, and were then hydrolyzed under a layer of 95% ethanol, in boiling 30%

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TABLE I. Mortality of Thermal Injury in Rats Treated with Carbon at Different Times after Burning.

Experimental group	Number of animals	Number of burned animals surviving at various intervals after injection of carbon: 15 min	4 hr	24 hr	72 hr	7 days
Rats injected with carbon within one hour after thermal injury	29	15	13	4	4	3
Rats injected with carbon within 4 hr after thermal injury	11	4	4	4	4	3
Rats injected with carbon within 8 hr after thermal injury	8	8	8	8	8	8
Rats injected with carbon within 24 hr after thermal injury	23	23	23	23	23	23
Rats injected with carbon within 48 hr to 21 days after thermal injury	79	79	79	79	79	79

NaOH solution. After complete digestion of the tissues, the carbon remnant was washed in 95% ethanol and in distilled water. The final carbon preparation was resuspended in 95% ethanol and was transferred into standard vials of known weights. The ethanol was evaporated by boiling, and the dried carbon was weighed (4). Histological studies were performed on the liver, spleen, lungs, kidneys, brain and heart of the injected animals.

Results. The mortality observed in rats treated with carbon at various times after thermal injury is outlined in Table I. Fifteen of 29 rats given carbon 1 hr after burning survived beyond 15 min after injection. The early deaths were associated with acute signs of respiratory distress, followed by tachycardia and cardiac arrest. By 24 hr, 25 of the recipients (86%) had succumbed. Similar results occurred in 11 rats given carbon 4 hr after thermal injury. In contrast, 110 rats injected with carbon at 8 hr, 24 hr, and at 48 hr to 21 days after injury showed no untoward effects.

Table II compares the carbon clearance in rats burned one hour before injection with the results observed in normal rats. As reported previously by Halpern, Benacerraf and Biozzi (4), the carbon particles were gradually cleared from the blood of the normal animals, so that 76% of the 2-min carbon concentration remained in the blood at 10-min after injection, 70% at 20, 49% at 45, 26% at 90, and 23% at 120 min. Histological

TABLE II. Comparative Rate of Clearance of Carbon Particles from the Blood of Normal and Burned Rats.

Experimental group	Number of animals	Mean percentage of the initial carbon concentration present in the blood at various intervals after carbon injection (2-min carbon concentration = 100%): 10 min	20 min	45 min	90 min	120 min
Normal rats injected with carbon without thermal injury	88	76%	70%	49%	26%	23%
Rats injected with carbon 1 hr after thermal injury	29 total					
(a) Group that died within 15 min	14	6%	—	—	—	—
(b) Group that died within 24 hr	11	25%	21%	15%	7%	5%
(c) Group that survived past 48 hr	4	71%	60%	42%	20%	13%

TABLE III. Tissue Distribution of Carbon Particles in Normal and Burned Rats.

Experimental group	Number of animals	Mean carbon concentration (mg/g of organ) present in: Liver	Spleen	Lungs	% of total carbon recovered from the lungs
Normal rats injected with carbon	12	8.15	7.05	1.35	8.2
Rats injected with carbon 1 hr after thermal injury	12	4.35	3.00	10.05	57.5
Rats injected with carbon 24 hr after thermal injury	12	6.40	16.30	1.25	5.2

study indicated that most of the injected carbon had become localized in the fixed macrophages of the liver and spleen. In contrast, at 10 min after injection, only 6% of the 2-min carbon concentration remained in the blood of the 14 burned rats in which carbon administration at 1 hr after injury had induced a respiratory death. There was a similar but somewhat less rapid disappearance of carbon from the blood of 11 rats in the same group, which died within 24 hr after carbon injection. In contrast, the carbon clearance in four survivors in this experimental group approximated the results observed in normal animals. Histological study of the organs of the rats dying after the injection of carbon showed massive carbon deposits in the pulmonary vascular bed and severe hepatic congestion.

Table III contrasts the quantitative tissue distribution of carbon particles in the liver, spleen, and lungs of normal and burned rats. Normal rats injected with carbon had a mean carbon concentration of 8.15 mg/g of liver, 7.05 mg/g of spleen, and 1.35 mg/g of lung. Only 8.2% of the total carbon recovered per g of these organs was present in the lungs. Rats injected with carbon at 24 hr after thermal injury had a mean carbon concentration of 4.35 mg/g of liver, 3.0 mg/g of spleen and 1.25 mg/g of lung; 5.2% of the total carbon recovered per g was present in the lungs. In contrast, rats given carbon at 1 hr after injury showed a mean carbon concentration of 4.35 mg/g of liver, 3.0 mg/g of spleen and 10.5 mg/g of lung. In this group, 57.5% of the total recovered carbon per g was deposited in the lungs.

Discussion. The effects of intravenous injections of carbon particles into rats within 1 to 4 hr after thermal injury bear a close resemblance to the results reported by Halpern, Benacerraf and Biozzi (4) in rats given thromboplastin, or carbon particles suspended in commercial shellac. These recipients also suffered acute respiratory deaths, with massive deposition of carbon in the lungs. The authors ascribed this sequence of events to the effects of thromboplastin or the stimulation by shellac of the release of tissue thromboplastin *in vivo*. In both instances, the resulting intravascular microcoagulates caused flocculation of the injected carbon particles, and their embolization to the pulmonary vascular tree. The present study suggests that a similar mechanism may have been triggered by severe thermal injury. In this regard, the results are of pertinence to recent reports that soft tissue injury is associated with the intravascular release of showers of microaggregates into the host's circulation, with subsequent embolization to the lungs (5–9). The observations are also in agreement with Eeles and Sevitt's (10) detection of platelet–fibrin microthrombi in the pulmonary vascular bed of severely burned human subjects, and with the reports of Innes and Sevitt (11) and of Eurenius *et al.* (12) of a decrease in the number of circulating blood platelets in the early period following thermal injury. Reports that severe thermal injury may cause the release of a variety of intracellular and/or toxic tissue components into the host (13, 14) support the possibility that such agents may be implicated in the lethality of colloidal carbon suspensions during the early postburn period.

A number of hitherto-unexplained anoxic

burn deaths may therefore have been a consequence of the aggregation of intravascular microemboli in the host's pulmonary vascular bed. The results suggest that caution may be indicated in the use of blood transfusions and/or high molecular weight colloid solutions during the period immediately following thermal injury. The results also raise the possibility that the lethal affects of pulmonary microemboli in thermal injury may be prevented through the use of anticoagulant agents.

Summary. Intravenous injection of colloidal carbon particles within one to four hours after thermal injury is followed by the precipitous clearance of carbon from the blood of the recipients and respiratory death, secondary to massive embolization of the injected carbon to the pulmonary vascular bed. The results suggest that thermal injury may be associated with the early release of showers of microaggregates into the circulation, with subsequent embolization to the lungs.

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