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Uptake and Storage of Lung Histamine in Burn Shock* (33561)

V. V. GLAVIANO, A. SUZUKI, AND R. COVA
Loyola University, Stritch School of Medicine, Hines, Illinois 60141

Apart from direct injury of the respiratory tract, the untreated patient with extensive burns is likely to develop pulmonary complications characterized by tachypnea, atelectasis, pulmonary edema, and pneumonia (1–3). Alterations in pulmonary compliance due to degeneration of lung surfactant has been among the causes proposed for pulmonary complications appearing in burn shock (4, 5). On the other hand, thermal injury of tissue is known to be accompanied by the endogenous release of histamine, serotonin, and bradykinin (6, 7), substances proposed as being responsible for vascular permeability responses to injury (8).

The rise in the blood level of histamine in burn shock (9, 10) may play a role in altering pulmonary function, since histamine in the experimental animal has often been cited as the humoral agent responsible for the production of pulmonary edema by epinephrine (11). The apparent lack of information on this aspect of burn shock led us to study the rate of rise in blood and lung histamine content in anesthetized dogs subjected to thermal injury.

Materials and Methods. The hair was shaved from the xiphoid to the base of the tail in 18 mongrel dogs anesthetized with 30 mg/kg of sodium pentobarbital. Burn shock was induced by exposing the lower half of the dog's ventral surface to heat from infrared lamps. A full thickness burn of the skin, verified by histological studies, was produced by exposure to the infrared heat for 10 sec/kg of dog weight. Systolic and diastolic blood pressures were measured with a pressure transducer connected to the left branchial artery. Changes in hematocrit during the course of burn shock were measured from arterial blood samples every 30 min. Blood samples for measuring arteriovenous blood differences of histamine (H) across the lungs were drawn simultaneously from catheters inserted into the right and left ventricles, via the right external jugular vein and left common carotid artery respectively. H levels in blood entering and leaving the lungs were determined during a control period and 15, 60, 180, and 240 min in the postburn period. After 4 hr of burn shock, approximately 2 g of lung tissue from the peripheral border of the left cardiac lobe was excised, frozen in liquid N_2 and analyzed in duplicate for H. Analysis of H in both blood and tissue samples was performed in accordance with the method of Shore *et al.* (12). The H concentration in blood-free lung tissue was calculated by subtracting the H content of blood trapped in the tissue sample from the total tissue concentration of H. Blood was determined by its conversion of hemoglobin to

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TABLE I. Concentration of Blood Histamine in the Right (RV) and Left (LV) Ventricles of the Heart, before and during Burn Shock.

Time (min)	RV (μg/liter)	p	LV (μg/liter)	p	LV—RV (μg/liter)	p
Control (13)[a]	51.2 ± 1.7[b]		54.5 ± 1.9		+3.3 ± 2.2	
15 (13)	80.2 ± 3.5	<.001	72.5 ± 3.5	<.001	−7.7 ± 2.4	<.02
60 (13)	86.3 ± 2.8	>.05	85.6 ± 3.5	>.05	−0.7 ± 2.4	<.02
240 (10)	81.5 ± 3.3	>.05	81.8 ± 3.1	>.05	+0.3 ± 1.7	>.05

[a] Number of dogs given in parentheses.
[b] Mean ± SE.

hematin according to the method of Cohen and Smith (13). In 5 of 18 dogs, the rate of H release from the lungs in μg/min was calculated from the product of the cardiac output (liter/min) and pulmonary arterial–venous blood difference (μg/liter). Cardiac output was measured with the indicator dye dilution technique of Stewart and Hamilton, utilizing a Gilford densitometer and dye computer. In this group, H analysis of lung tissue was performed in the 15-min postburn period. Control lung tissue samples with the omission of the burn injury were obtained from 6 dogs anesthetized for 4 hr with catheterization of the right and left ventricles.

Results. Table I summarizes the levels of H in blood samples taken from the right and left ventricles, before and 15, 60, and 240 min after the burn injury. In the control state, H was generally observed to be higher in blood coming from the lungs than in blood entering the pulmonary artery. As early as 15 min after the dogs were subjected to burn trauma, H levels in both pulmonary arterial and venous blood showed a marked elevation from control ($p<.001$), while the arteriovenous difference across the lungs averaged −7.7 μg/liter ($p<.02$). Within 60 min the concentration of H going in and out of the pulmonary circulation was almost equal. In 10 of the 13 dogs in burn shock for 4 hr, the average pulmonary arteriovenous difference was not significantly different from that measured during the 60-min period, although H levels in both arterial and venous blood remained elevated.

In 6 control dogs H content of lung tissue averaged 15.5 ± 2.8 μg/g, while in 10 dogs in burn shock for 4 hr, H averaged 25.6 ± 4.2 μg/g ($p<.01$). Table II indicates that saturation of the H storage capacity of the lung occurs early in the postburn period. Despite the significant decrease in pulmonary blood flow, the marked rise in rate of H uptake by the lungs occurred entirely within 15 min following burn trauma.

Measurements made on arterial blood pressure, heart rate, hematocrit, and cardiac out-

TABLE II. Rate of Uptake and Storage of Lung Histamine in Five Dogs after Burn Injury (15 min).

	Histamine difference[a] (μg/liter)		Cardiac output (liter/min)		Histamine uptake (μg/min)		Lung histamine (μg/g)
Dog no.	C[b]	15 min	C	15 min	C	15 min	15 min
14	0.3	−8.0	2.4	1.5	0.7	19.2	23.1
15	0.2	−9.1	2.2	1.2	0.4	10.8	29.9
16	2.1	−5.3	1.5	0.9	3.0	4.5	21.4
17	5.3	−12.4	3.1	1.8	6.0	21.6	22.5
18	1.1	−7.2	2.9	1.6	3.1	11.5	30.1
Mean	1.8	−8.4	2.4	1.4	2.6	13.5	25.4

[a] Blood histamine difference across the lungs.
[b] Control.

put showed the observed changes to agree with those made by other laboratories for dogs in burn shock (14, 15). Both mean blood pressure and heart rate rose 30% immediately after the burn injury and remained elevated for approximately 1 hr, after which they returned to near control, and remained at this level until the experiment was terminated. Cardiac output in 5 dogs underwent an average decrease of 42% in the 15-min period following burn injury (Table II), and subsequently, slowly declined in the remaining 3–4 hr to 50% of control. Hematocrits taken during the 4-hr postburn period gradually rose to 40% above control.

Discussion. In the control state, the lungs released into the systemic circulation small quantities of H, an observation previously reported (16). As verified by analysis of blood entering and leaving the lungs, and from the direct analysis of pulmonary tissue, the lungs were found to extract and store H in the dog subjected to thermal trauma. Saturation of the lung H stores in burn shock occurred when the tissue had increased its H content by 68%. In the 1 hr postburn period, blood entering the lungs contained the highest concentration of H, a finding that agrees with the work of Rose and Browne (10). They reported that the highest level of H occurs in the burned patient within 1 hr following a burn injury. In the dog, the increase in blood H stems in large part from the release of bound and unbound forms of H from the mast cells of the burned skin. Experiments that are near completion in this laboratory show that 15 min after thermal injury, skin from a burned area will release 60% of its H stores while a decrease of 20% occurred in skin analyzed from an unburned area.

In the experimental animal the administration of H causes a rise in pulmonary artery pressure that generally has been attributed to constriction of the pulmonary postcapillary vessels (17–19). Although high doses of H are required to induce pulmonary congestion in the normal animal (20), the concurrent presence in burn shock of an elevation in blood catecholamines, bradykinin, as well as H (11, 21) may be sufficient to alter the relationships between pulmonary ventilation and perfusion. On this basis the observed uptake and storage properties of lung tissues for H in the burned shock animal may be related to the pulmonary pathology observed in patients with severe burns.

Summary. In anesthetized dogs in burn shock for 4 hr, the rate of rise in arteriovenous difference of histamine across the lungs was measured from blood entering and leaving the lungs via the right and left ventricles of the heart, respectively. A maximal uptake of blood histamine by the lungs was attained within 15 min following burn injury. In subsequent periods following the burn injury, the pulmonary arteriovenous difference declined while arterial and venous histamine levels remained elevated until the experiment was terminated. Of the hemodynamic alterations recorded, the most significant change was the immediate decrease in cardiac output following burn trauma. The uptake and storage of histamine by the lungs may be related to pulmonary complications observed in patients in burn shock.

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Passive Transfer of Hypersensitivity to Lepromin (33562)

MAURICIO GOIHMAN-YAHR, RODOLFO W. FERRARESI, AND SIDNEY RAFFEL

Departments of Medical Microbiology and Dermatology, Stanford University, Stanford, California 94305

Reactions to lepromin are generally conceded to have importance as an aid to clinical judgment, but there is question about which main component of this complex "antigen," bacilli or dermal tissue, is responsible for the granulomatous inflammatory response (1–3). It is also uncertain whether this phenomenon is immunological in nature, and if so, to which of the recognized types of hypersensitivity it belongs.

In respect to the first question, we had earlier found (4) the bacillary component of a purified lepromin (see below) to be mainly responsible for the tissue response observed following its injection into the foot pads of normal guinea pigs. This was marked by a continuing hypertrophy of the draining lymph node, and increasing numbers of contained pyroninophilic cells. In regard to the second question, it was observed that in guinea pigs sensitized to any of a variety of mycobacteria, the intradermal injection of test lepromin produced granulomas which were grossly and histologically markedly different from reactions observed in control animals, or in the sensitized ones injected with dermis suspension (5, 6). These responses suggested that immunologically specific sensitivity had been induced to lepromin by the mycobacteria. We wished to confirm this point further and to determine the nature of this sensitivity. The work reported here describes efforts to find whether specific hypersensitivity is induced by purified lepromin, and if so, whether this is transferable to normal animals by serum or lymphoid cells.

Methods. For work with lepromin in animals it was requisite to eliminate, as far as possible, the antigenic and phlogistic properties of heated human tissue which is a constituent of this test substance. A purified lepromin (i.e., a suspension of *M. leprae*) was prepared by sequentially digesting heated, ground, epidermis-free lepromata with DNAase, RNAase, and Pronase. This was done in barbitone–barbitone sodium buffer, pH 7.4, with adjustment to the optimal pH of the enzyme being used with NaOH or HCl, and the addition of $CaCl_2$, and $MgCl_2$ as required. Residual particulate matter was washed with phosphate buffered saline, pH 7.4, with 0.05% Tween-80 (PBS-T) and recovered by filtration through Millipore membranes. This consisted essentially of bacillary bodies, as shown by the Ziehl–Neelsen, Wright–Giemsa and acid orcein stains. The final suspension was prepared in phosphate buffered saline, pH 7.4, with 0.5% phenol and 0.05% Tween-80 added.

Two concentrations were prepared: "strong lepromin" and "test lepromin," containing approximately 430×10^6 and 140×10^6