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Studies on the Mechanism of Endotoxin-Induced Increase of Alveolocapillary Permeability[1] (38421)

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The respiratory insufficiency which accompanies clinical gram-negative septic shock remains a poorly understood but often lethal complication. The administration of endotoxin to experimental animals results in several types of pulmonary changes. Thus, Kuida *et al.* (1) and Kux *et al.* (2) demonstrated an increase in vascular resistance. The occurrence of structural modifications in the capillaries resulting in pulmonary edema was reported by Snell and Ramsey (3) and a decrease in surfactant was observed by Harrison *et al.* (4). McKay *et al.* (5) noticed the development of clotting as part of a disseminated intravascular coagulation syndrome. Most of these changes do not occur in the isolated perfused lung in the absence of cellular elements of the blood (6). However, in an isolated perfused lung system, it was demonstrated in this laboratory (7, 8) that endotoxin caused an increase in alveolocapillary permeability using plasma as perfusate. Therefore the question was raised as to which plasma components are required for the endotoxin-induced increase in alveolocapillary permeability. Because many of the effects of endotoxin are known to be mediated by the activation of the complement system (9–12), the possibility was entertained that complement might be required for the changes in alveolocapillary permeability induced by endotoxin. When plasma preheated at 56° for 30 min was used to perfuse the lung, endotoxin caused no increment in permeability (8). This was attributed to the decomplementation of the plasma by the heat treatment. Furthermore, the mechanism of steroid protection of endotoxin-induced cell damage was postulated to be due to a steroid-mediated decrease in complement activity or complement fixation, as suggested by the *in vitro* study of Schumer *et al.* (12). However, Kitzmiller *et al.* (13) reported that the integrity of the complement system is not necessary for the development of endotoxin shock in cats.

The present study was planned to investigate further the importance of plasma factors and to ascertain whether complement plays a role in the increment of alveolocapillary permeability produced by endotoxin in the isolated perfused canine lung system.

Materials and Methods. Lung perfusion system. The isolated perfused canine lung was used, as originally described by Taylor *et al.* (14) and modified by Goodale *et al.* (7, 8, 15, 16). Adult mongrel dogs were anesthetized with sodium pentobarbital (30 mg/kg) and injected with 10,000 USP units of heparin. The dogs were ventilated on a respirator with 100% oxygen for 30 min and then the endotracheal tube was clamped and anoxia was induced. Total lung collapse was facilitated by creating a pneumothorax. The atelectatic left lower lobe was removed and connected to an isolated vascular perfusion circuit. Then the lobe was placed in a saline-filled plethysmograph. The vascular system was perfused at 22° with the aid of a pulsatile pump at a flow of 140 ml/min utilizing different types of perfusate, as described later. The collapsed tracheo-alveolar compartment was filled with Tyrode's solution containing 50 μCi of ^{131}I-labeled serum albumin (Abbott Laboratories, North Chicago, IL) previously dialysed to remove free iodine. The liquid perfusate was gassed with room air containing 5% CO_2. The perfusion pressure in the arterial side was monitored throughout with a Statham strain gauge transducer connected to a Sanborn recor-

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der. The venous effluent drained by gravity into a reservoir kept at constant height. Previous studies showed that venous pressure was constant for each experiment (15, 16). Three-milliliter samples of vascular perfusate were taken every 30 min and the uptake of radioactive albumin from the alveolar compartment was determined in a gamma scintillation detector (2804 Scintillation Detector, Picker Nuclear Instrument, White Plains, NY), after which each sample was returned to the perfusate. After an initial perfusion period of 2.5 hr, purified *Escherichia coli* endotoxin (Difco Laboratories, Detroit, MI) was added to the perfusate in a dose of 10 mg/kg of the combined weight of lung tissue and perfusate. The endotoxin was dissolved into 3 ml of 5% dextrose and injected into the arterial side of the preparation.

Composition of perfusion solutions. Before performing an experiment, the vascular compartment was perfused first with 400 ml of Ringer's solution and then with 400 ml of a solution identical to the perfusate used in the actual experiment, in an effort to remove all blood components.

The lung preparation was perfused with dog serum instead of plasma because of the potential anticomplement activity of the agents used to prepare the plasma. To obtain serum, unheparinized blood was collected in 250-ml plastic bottles and left at room temperature to clot for 30 min. Then it was placed at 4° for 30 min and finally centrifuged at 1000*g* for 20 min. The serum was removed and stored at 4°, to be used within 24 hr.

To study the role of complement, one group of experiments was carried out using dog serum that was heated at 56° for 30 min prior to perfusion. Another group was perfused with serum that was treated with potassium thiocyanate to inactivate the complement components C3, C4, and C5 (17). To preculude the occurrence of a large degree of dilution during treatment with KSCN, the original method was modified as follows. One volume of 10 *M* KSCN solution at 22° was added over a period of 5 min to 9 vol of fresh dog serum, with constant mixing. Immediately before this step, the temperature of the serum was raised to 22° to prevent insolubilization of the KSCN. After mixing, the temperature was rapidly brought to 4° and the reaction mixture was kept at this temperature for 16 hr. Then it was dialysed three times, each time against 300 vol of a buffer composed of 145 m*M* NaCl, 10 m*M* tris–HCl, 0.5 m*M* $MgCl_2$, and 0.15 m*M* $CaCl_2$, pH 7.4. The KSCN-treated serum had no hemolytic activity against optimally sersitized sheep erythrocytes (17). As observed previously with human serum (17), restoration of hemolytic activity of KSCN-treated dog serum was obtained by addition of purified human C3, C4, and C5, but not when any of the three components was omitted.

Experiments were also performed using the following solutions for perfusion: human serum albumin at a concentration of 5.8% prepared by dilution of 25% normal human serum albumin (Cutter Laboratories, Berkeley, CA) with Ringer's lactate solution; 5.8% human serum albumin containing hydrocortisone sodium succinate (Solu-Cortef, The Upjohn Co., Kalamazoo, MI), 150 mg/kg of the combined perfusate–lung tissue weight; and finally, 6% dextran in isotonic saline (Gentran 75, Travenol Laboratories, Inc., Morton Grove, IL).

Calculation of alveolocapillary permeability. The calculations and assumptions employed to measure alveolocapillary permeability in this perfusion system have been previously described in detail (7, 8, 14–16). Permeability (P) was calculated as follows: $P = 0.693\, V_a V_b / t_{\frac{1}{2}} A (V_a + V_b)$. P is expressed in cm/sec and is related to the diffusion coefficient (D) derived from Fick's first law. The calculation of P involves computing alveolar volume (V_a) and vascular volume (V_b). The half time ($t_{\frac{1}{2}}$) to reach equilibrium concentration of isotope in the alveolar and vascular compartments is obtained from the rate of appearance of the isotope in the perfusate. The log of the difference between the calculated counts per minute at equilibrium and the observed counts per minute in the perfusate yields a straight slope when plotted over time. Surface area (A) was obtained after the method of Taylor *et al.* (14) by multiplying the lung weight (g) by 661. The assumptions underlining the use of weight-to-surface conversion ratio have been discussed by Taylor *et al.* (14) and Goodale *et al.* (15, 16). Since surface area is constant for each experiment, each dog serves as his own control, and the effect of endotoxin on the appearance rate of the isotope in each perfusate can be used to calculate the change in half times, and thus the corresponding relative permeability changes.

Results. Table I shows the values of alveolocapillary permeability before and after ad-

TABLE I. Endotoxin-Induced Increment of Alveolocapillary Permeability in the Isolated Dog Lung System Perfused with Normal and Decomplemented Dog Serum.

Perfusate	Expt No.	Permeability × 10^{-9} cm/sec			Mean arterial pressure (mm Hg)	
		Control	Endotoxin	% Change	Control	Endotoxin
Dog	1	0.266	0.405		21	20
serum	2	0.131	0.302		13	11
	3	0.160	0.325		10	10
	4	0.126	0.455		15	15
	Mean	0.171	0.372	+118	15	14
Dog	5	0.228	0.503		24.5	25
serum	6	0.331	0.269		24	24
heated at	7	0.306	0.320		9	13
56° for	8	0.722	0.339		11	12
30 min	Mean	0.397	0.358	−10	17	18.5
Dog	9	0.326	0.485		14	14.5
serum	10	0.107	0.330		13	13
treated	11	0.448	0.987		17	22
with	12	0.192	0.552		22	21
KSCN	Mean	0.268	0.589	+120	16.5	17.6

ministration of endotoxin in lungs perfused with fresh dog serum and serum subjected to procedures that inactivate the complement system. An increase in alveolocapillary permeability was produced by endotoxin when fresh serum was used. The permeability increased by 118%, which was similar to the increase obtained previously using plasma (8). Pretreatment of serum at 56° for 30 min abolished the endotoxin-mediated increase of permeability in three of four experiments (Table I). The heat treatment of the serum caused a reduction in the endotoxin-mediated change of permeability that was statistically significant with respect to the change obtained using fresh serum ($P < 0.05$). In contrast, inactivation of complement by pretreatment of serum with potassium thiocyanate did not abolish the endotoxin-mediated increment in alveolocapillary permeability (Table I).

In view of the latter results indicating that the complement system was probably not the mediator responsible for the effect of endotoxin upon alveolocapillary permeability, we investigated whether this effect would also occur in the presence of human serum albumin and dextran 75. The results (Table II) demonstrate that these perfusates lacking complement still allowed the endotoxin-induced permeability increment. However, when hydrocortisone sodium succinate was added to the perfusion system 30 min before endotoxin administration, the increase of permeability was lower than in the experiment using albumin alone ($P < 0.02$). Figure 1 shows the permeability changes in representative experiments with serum, KSCN-treated serum, and human serum albumin. Endotoxin caused increased permeability after a short lag period. With heat-treated serum there was no increase observed.

No significant changes could be detected in the arterial perfusion pressure after administration of endotoxin throughout the various experimental manipulations (Tables I and II).

Discussion. These studies using the isolated perfused canine lung preparation were directed to ascertain the role of plasma factors in the modifications of alveolocapillary permeability after administration of endotoxin. A potential role of the complement system as mediator of permeability changes induced by endotoxin was suggested by the previous finding that no such permeability change occurred when plasma was preheated at 56° for 30 min to inactivate complement (8). Several lines of evidence, however, indicate that complement is not required. Thus, perfusion with human serum albumin, dextran, or dog serum subjected to exhaustive decomplementation by treatment with potassium thiocyanate, all were associated with increased alveolocapillary permeability following endo-

TABLE II. Endotoxin-Induced Increment of Alveolocapillary Permeability in the Isolated Dog Lung System Perfused with Albumin and Dextran.

Perfusate	Expt No.	Permeability × 10^{-9} cm/sec			Mean arterial pressure (mm Hg)	
		Control	Endotoxin	% Change	Control	Endotoxin
Human albumin[a]	13	0.086	0.204		15	15
	14	0.512	1.258		12.5	12.5
	15	0.221	0.621		13	12.5
	16	0.233	0.445		21	19
	Mean	0.263	0.632	+140	15	15
Human albumin plus hydrocortisone[b]	17	0.162	0.256		11.5	11
	18	0.332	0.488		10	11
	Mean	0.247	0.372	+51	11	11
Dextran 75	19	0.247	0.676		—	—
	20	0.379	0.741		14	16
	Mean	0.313	0.709	+127	—	—

[a] Human serum albumin, 5.8% in Ringer's lactate solution.

[b] Hydrocortisone sodium succinate, 150 mg/kg of combined perfusate–lung weight given 30 min before endotoxin.

toxin. These findings support the concept that the effects of endotoxin on permeability are not necessarily mediated by the complement system. Evidence for depletion of complement components in endotoxin states has been found by many investigators (9–12). However, the exact role of complement in endotoxin states still remains a complex problem. Although most investigators feel that many of the *in vivo* effects of large doses of endotoxin are complement dependent, Kitzmiller *et al.* (13) noted little difference in the severity and outcome of the endotoxin shock induced in cats decomplemented by treatment with cobra venom factor when compared to normal controls.

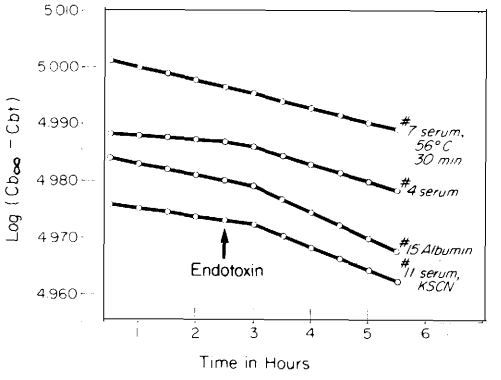

FIG. 1. Effect of perfusate composition on the effectiveness of endotoxin to induce changes in alveolocapillary permeability. The isolated perfused dog lung preparation was used. The slopes were obtained from the log of perfusate radioactivity differences, as a function of time. A steeper slope denotes a shorter half time and therefore an increased permeability. Cb∞ is concentration of isotope in vascular perfusate at equilibrium and Cbt that at a particular time.

Our data show that endotoxin acts directly on the alveolocapillary membrane and is not dependent on the mediation of circulating complement. There is, of course, a possibility that small amounts of complement or other plasma factors may yet remain absorbed on the endothelial cells, despite copious prior lavage. It is also possible that endotoxin enhances permeability through a cell enzyme inhibition mechanism. Thus, it has been reported that endotoxin produces uncoupling of oxydative phosphorylation in liver mitochondria (18) and inhibition of liver glucose-6-phosphatase activity (19). Furthermore, treatment of the isolated lung preparation with iodoacetic acid, which is a potent inhibitor of glyceraldehyde phosphate dehydrogenase, caused a profound enhancement of alveolocapillary permeability (15). The nature of the injurious effect of endotoxin on endothelial cells is still unclear (3).

Our results indicating that the enhancement of permeability caused by endotoxin can be reduced significantly by a large dose of hydrocortisone sodium succinate is in accord with the present concepts of the beneficial effects of large

doses of corticosteroids in the treatment of gram-negative septic shock (8, 20, 21). That this effect was achieved in lung preparations perfused with albumin solution suggests that the beneficial effect of corticosteroids may be due to a direct protection of the capillary or lysosomal membranes (20, 22) or reduction in destruction of mitochondria in alveolar cells (23). This mechanism is as reasonable as that postulated by Schumer *et al.* (12), who felt that inhibition of a complement-mediated process by steroid was involved.

It is of great interest that the endotoxin-mediated increase of alveolocapillary permeability did not occur in the lung preparations perfused with dog serum that was subjected to 56° for 30 min. It is possible that mildly heated serum proteins have the ability to detoxify endotoxin, perhaps simply by binding it and thus preventing its access to the alveolocapillary membrane.

No changes in arterial perfusion pressure occurred after administration of endotoxin throughout the various experiments with lung preparations perfused with dog serum, human serum albumin, or dextran. These findings are in agreement with previous observations (6, 24).

Summary. Using an isolated perfused dog lung system, studies were carried out to ascertain the role of plasma factors in the increase in alveolocapillary permeability induced by the administration of endotoxin. Heat treatment of dog serum (56° for 30 min) abolished the capacity of endotoxin to induce permeability increase. In contrast, serum treated with potassium thiocyanate to inactivate the complement components C3, C4, and C5 did allow the increase in endotoxin-induced permeability, as did a 5.8% solution of human serum albumin and a 6% dextran solution. Pretreatment with a massive dose of hydrocortisone sodium succinate decreased, but did not abolish, the endotoxin-induced permeability increase in albumin-perfused lung. The studies suggest that endotoxin may affect the alveolocapillary membrane directly, by a process which is independent of the complement system. Heat treatment of serum impairs the endotoxin effect due to a mechanism other than complement inactivation.

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