CARDIAC OUTPUT AND VASCULAR RESPONSE TO TRAUMA

HARVARD MEDICAL SCHOOL, BOSTON, MASSACHUSETTS

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CARDIAC OUTPUT AND VASCULAR RESPONSE TO TRAUMA

Final Comprehensive Report

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) By clinical observation of traumatized, septic, and burned patients, correlated with animal experiments, the nature and clinical course of the adult respiratory distress syndrome and abnormal energy metabolism were described. Initially interstitial pueumonaitis (Phase I) is accompanied by a clear chest x-ray hypoxemia due to pulmonary shunting, and an elevation of pulmonary vascular resistance. Histologically this lesion is characterized by diffuse focal abveolar collapse, and alveo-

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lar septal congestion. Surfactant synthesis is reduced. If unchecked by appropriate respritory therapy, Phase I progresses to bronchopneumonia (Phase II). Among other etiological agents, circulating vasoactive peptides play a major role in establishing the lung injury. Their presence was demonstrated in the plasma of injured and septic patients or animals by bioassays in which dog or rabbit lung was perfused by a non-blood perfusate to which the test plasma was added. The greatest activity resides in the fraction of plasma containing substances of molecular weight between 1,000 and 10,000. Positive plasma assays correlate closely with the development of pulmonary shunting and phenmortis in the patients. A variety of substances have been assayed including E. coli endotoxin kinins, and prostaglandins and found not to be the responsible agent. The active peptide remains unknown. Above others the presence of nonthoracic sepsis appears to be the single greatest cause of this syndrome, and the most effective treatment beside ventilatory support is elimination of the septic focus.

In a second set of observations, insulin resistance typical of sepsis was found to reside principally in skeletal muscle. The energy fuel deficit in muscle results in consumption of branch chain amino acids and leads to failure of protein synthesis. Among other systems affected by this mechanism is a reduction of surfactant synthesis.

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INTRODUCTION

The physiological responses to trauma, shock, or sepsis which lead to recovery have been measured and documented to delineate the situations in which death occurs for lack of adequate responses. Whether due to to abnormalities of energy metabolism or to failure of a vital organ system death supervenes when the dynamic balance between injury and the responses essential to survival is upset in favor of the former. Previous work conducted in Cleveland, Ohio, and in Charleston, South Carolina by the principal investigator under the auspices of the U. S. Army R and D Command demonstrated the requirement of an elevated cardiac output for survival from trauma or sepsis (2) (3) (8). The relationship of increased circulatory demands to respiratory and metabolic abnormalities encountered under these conditions was investigated during this period. As shown by data in Table I derived from a series of 103 very sick patients who were completely studied, circulatory and respiratory complications constitute the greatest contributory causes for death among such patients (8). Similar findings were obtained in a review of more than 8,000 consecutive admissions to two general surgical services. In that group it was observed that the overall mortality was 2.1% but that respiratory complications contributed to more than 65% of all deaths which took place.

The principal research undertaking during tenure of the present contract was study of the clinical manifestations and etiology of the "adult respiratory distress syndrome". Now recognized as a

TABLE I STATISTICS OF SEPTIC, BURN AND POSTSHOCK PATIENTS ANALYZED FOR COMPARISON WITH UNCOMPLICATED RECOVERY IN 30 PATIENTS AFTER MAJOR OPERATIONS

	TOTAL NO.	SEPSIS		POSTSHOCK HYPOVOLEMIA AND TRAUMA		BURNS	
		NO.	MEAN AGE	NO.	MEAN AGE	NO.	MEAN AGE
Total	103	49	52(11-90)	31	40(16-78)	23	38(2-70)
Survivors	57	30		20		7	
Deaths	46	19	57(29-88)	11	44(16-78)	16	42(2-70)
Mortality	44.7%		39%		36%		70%

NUMBER OF FATAL COMPLICATIONS OCCURRING DURING CONVALESCENCE

SYSTEM INVOLVED	SEPSIS	HYPOVOLEMIA AND TRAUMA	2° AND 3° BURNS	TOTAL INCIDENCE IN 103 PATIENTS
Cardiovascular	7	5	3	14.6%
Acute respiratory failure	5	1	4	9.7%
Renal failure	3		1	3.9%
Bleeding diathesis	3	6	1	9.7%

common and often fatal complication in septic or injured patients, this form of pulmonary dysfunction is otherwise known as "shock lung", "stiff lung", "Da Nang Lung", etc. (7) (41) (59). The investigative work was carried out both by clinical observations as well as by controlled animal experiments. The purpose of this report is to review in separate sections the clinical syndrome of altered respiratory and circulatory function, the pulmonary morphological abnormalities, changes in pulmonary metabolism, and the evidence for a circulating peptide as the principal etiological agent. This agent which probably is a small peptide initiates a sequence of pathological events in the lung. An early interstitial pneumonitis (Phase I) is followed, if unchecked, by bronchopneumonia (Phase II). Finally, based on these findings the value of certain therapeutic measures for the prevention or treatment of this dangerous complication are presented.

CLINICAL PATTERN OF RESPONSES TO TRAUMA, SHOCK, AND SEPSIS

The elevated demands for function by multiple systems in the seriously injured patient, especially the hectic febrile course of those who are seriously infected, contrast with the benign convalescence after uncomplicated major surgical operations. The stresses imposed are emphasized by a comparison of the respiratory, hemodynamic, and metabolic data presented in Table II. From these findings it is evident that clean non-traumatic surgery makes very little extra demand on respiratory or circulatory function, since the metabolic rate is scarcely elevated above normal. On other hand, in post shock states, and particularly in sepsis, the circulatory demand is

TABLE II RESPIRATORY, HEMODYNAMIC, AND METABOLIC VALUES IN COMPARING UNEVENTFUL SURGICAL CONVALESCENCE WITH VARIOUS STATES OF SEVERE SEPSIS (AVERAGE VALUES ± STD. DEV. OF MEAN, WITH NUMBER OF OBSERVATIONS)

MEASUREMENTS	UNEVENTFUL CONVALESCENCE (30 Patients)		POST SHOCK	SEPSIS (61 Patients)			
	Maximal Response	Convalescence	(27 Patients)	Onset-Day 1 (15 Patients)	High Cardiac Output (55 Patients)	Low Cardiac Output* (30 Patients-18 Deaths	Convalescence (40 Patients)
Respiratory:							
P_aO_2(F_1O_2=0.2 mm Hg)	76±8(26)	84±6(58)	62±8(12)	81±17(27)	58±13(57)	47±15(30)	73±9(61)
P_aO_2(F_1O_2=1.0 mm Hg)	388±44(6)	420±51(7)	198±50(24)	270±51(14)	125±42(19)	86±16(14)	240±60(29)
P_aCO_2(mm Hg)	36±6(28)	38±4(72)	35±3(27)	31±6(27)	30±5(57)	35±5(22)	34±5(61)
Arterial Blood pH (spontaneous respiration)	7.39±0.03(38)	7.43±0.05(72)	7.43±.03(18)	7.39±0.08(21)	7.49±0.08(21)	7.31±0.12(9)	7.45±0.04(56)
Hemodynamic:							
Cardiac index ($L/M^2/min$)	3.6±0.3(30)	3.2±0.2(48)	5.1±1.3(16)	2.6±1.9(29)	4.7±1.8(56)	1.8±1(20)	3.6±0.08(40)
Central venous pressure (cm H_2O)	5±3(21)	3±2(41)	7±4(18)	3±5(33)	10.1±5(66)	17±6(38)	5±3(59)
Pulmonary arterial systolic pressure(mm Hg)	23±4(6)	-	24±3(4)	-	41±5(15)	40±15(5)	29±5(5)
Pulmonary arterial mean pressure (mm Hg)	19±3(5)	-	22±4(6)	21±4(3)	28±5(12)	30±8(11)	-
Left atrial (wedge) pressure(mm Hg)	8±2(5)	-	12±4(5)	5±4(3)	10±3(9)	16±4(10)	-
Mean arterial blood pressure(mm Hg)	86±10(32)	87±11(64)	78±15(18)	63±18(32)	84±13(58)	61±8(20)	86±10(80)
Metabolic:							
Metabolic rate ($kcal/M^2/day$)	980±210(18)	928±146(28)	1121±200(10)	-	1206±221(15)	1106±178(8)	1141±151(15)
Body temp. (Tr^oF)	100.7±1.4(32)	100.2±0.7(32)	100.8±2.10(27)	101.3±1.6(30)	101.9±1.7(105)	99.1±2.3(30)	100.3±1.1(80)
Excess lactate (mM/L)	1.4±1.2(21)	0.5±0.2(34)	2.1±0.8(12)	1.9±0.7(10)	1.6±1.1(42)	3.0±1.4(21)	0.7±0.3(38)

*23 patients were studied in both high and low output states.

proportionately much greater than the elevation of metabolic rate. Severe pulmonary shunting is also characteristic of these patients.

During the course of these studies it has become evident that the majority of patients pass through a series of events typical of the post traumatic or septic course. These four phases are outstanding: 1) shock and septic invasion, 2) localization of the site of necrosis or infection, 3) resolution, and 4) recovery. It is important to realize that these phases are not distinct entities but that they may overlap. Reversion from one to another may also occur.

Phase I: In the early phase of tissue necrosis or bacterial invasion agents disseminated by the blood stream include: endotoxin, the products of tissue necrosis, and activated vasoactive agents. The circulatory demand is greater than normal. A shock like state may exist in which circulatory demands by all tissues are not satisfied. Commonly circulatory insufficiency is caused by 1) translocation of fluid, 2) an increase of pulmonary vascular resistance, and at times,3) by myocardial failure (27). This may be a form of high output shock. Hypoxemia and reduced oxygen delivery as well as other metabolic abnormalities may contribute to lactacidemia and acidosis. Some degree of compensation is obtained by respiratory alkalosis as long as the respiratory system remains competent. If the responses mediated through the central nervous system and the sympatho-adrenal systems, supplemented by supportive therapy, are sufficient to maintain cellular metabolism in vital tissues, the second phase may be entered.

Phase II: Aided by the inflammatory reaction as well as the reticulo-endothelial and immunological systems, the necrotic or infectious processes are contained and localized. However, the stress remains, for toxic agents continue to diffuse from the traumatic or septic focus to all parts of the body. Characteristically metabolic abnormalities continue. These include lactacidemia (8), muscle insulin resistance (27), and proteolysis (23). The metabolic rate and oxygen requirements as shown in Table II are elevated on the average to 32% above the normal resting value. To complicate this picture, there is an inappropriate vasodilation, characteristic of the post traumatic and septic states. Under these conditions the average cardiac output has been observed in patients who do well to be approximately 68% above the normal resting value (8) (12). If for any reason the cardiac index falls below the normal of 3 liters per meter square per minute, hypotension and vaso constriction with severe metabolic consequences ensue (8) (23) (27). (See Table II) The mortality of patients remaining hypotensive, especially those which are septic, for more than 24 hours has until recent times been more than 70% (23) (62). For this reason the data in Table II concerning the septic patients are divided according to the high cardiac output state or the low output shock state. Renal failure usually takes place in conjuction with the low output state.

Phase III: The usual course of recovery continues by resolution of the septic or traumatic focus. Pus may be drained or reabsorbed, and necrotic tissue is sloughed. Healing by the deposit of collagen completes the cycle. In the late phase of localization or resolution

the commonest cause of death is protein-calorie depletion. This state of affairs is accompanied by inadequate protein synthesis and failure to maintain immunological competence or ability to heal wounds.

Phase IV: With the ultimate return to normal metabolism and function throughout the body, first protein structure and subsequently fat deposits are restored.

PULMONARY FUNCTION: Moderate hypoxemia frequently occurs after serious trauma or fulminating sepsis. Arterial blood oxygen tensions (PaO_2) fall to values below 60 mm Hg. while breathing air. This effect is far less pronounced in our experience after pure hypovolemic shock. Despite hypoxic evidence of shunting (35) at this early pneumonitis (Stage I), examination of the chest seldom discloses more than coarse breath sounds and occasionally diffuse ronchi. The x-ray of the lung fields may appear normal and seldom shows more than a ground glass appearance or evidence of mild edema. From histological studies, to be presented, it appears that the diffuse focal alveolar collapse typical of the Stage I lesion is not sufficiently confluent to show as an x-ray density. Subsequently if it proves impossible to check the progress of the pulmonary process bronchopneumonia, referred to as Stage II, develops. Usually the bronchopneumonia is manifested by coarse ronchi and decreased breath sounds. The x-ray then shows the usual appearance of coarse density characteristic in this lesion.

With progression of the lung lesion the pulmonary vascular resistance increases. This is manifested by elevation of peak inspir-

atory pressure (PIP). (26) (28). Measurement of the mean pulmonary artery pressure with a Swan-Ganz catheter indicates elevation from the normal values of 16-19 mm of mercury up to levels of 30 mm of mercury or more, as shown in Table II. The P. A. pressure and pulmonary vascular resistance under these conditions contrast with the usual behavior of the lung vasculature. In normal people an elevation of cardiac output leads to the recruitment of additional capillaries progressing from the dependent portion of the lung upward. Thus an elevation of pulmonary artery pressure is prevented. In the septic or post traumatic state elevation of pulmonary vascular resistance not infrequently leads to right heart failure. Commonly the central venous pressure starting at 15 may reach values as high as 25 cmH_2O or more, and the mean P.A. pressure may rise to 30 mm Hg.

The typical respiratory alkalosis accompanied by hypoxemia as shown in Table II, is evidence of shunting. Calculation of shunts in the observed patients seldom exceeded 25% in the post shock state, but rises to between 35 and 40% in the severely traumatized or septic patients. Furthermore, it has become evident that the cardiac output is of importance in relation to the arterial oxygen tension and the degree of shunting. When the cardiac index is low, as in the premortem patients shown in Table II, oxygen extraction by the tissues may be greater than usual. In the mixed venous blood pO_2 is low. Because the oxygenating capacity of the lung appears to be limited, the arterial PaO_2 is lower than it would have been at a higher cardiac output Measures such as infusion of dopamine or GKI (glucose, potassium and insulin) often result in a simultaneous rise of cardiac output and an increase of arterial oxygen tension. (26)

The importance of sepsis as a cause for the development of the pulmonary lesions resulting in severe shunts was further supported by the work of Proctor et al (54). They observed in wounded military personnel who became septic that the increase of shunting rose from 10% to 30% or more during the course of five days. At the same time this was accompanied by an elevation of the work of breathing. Powers et al (61) observed a similar relationship with regard to functional residual capacity (FRC). The highest shunts in their series were accompanied by FRC values falling from the normal of two liters to less than 500 ml.

PULMONARY MORPHOLOGY

Pulmonary biopsies obtained from eight patients who underwent thoracotomy after shock and trauma, or during severe sepsis demonstrate a pneumonitis (Stage I) lesion which was characterized by interstitial septal edema, vascular congestion, round cell mononuclear leukocyte infiltration, and diffuse focal alveolar collapse. At autopsy or in the later stages of the disease typical full blown bronchopneumonia (Stage II) with polymorphonuclear infiltration of the alveoli and alveolar septa is found. Considerable fluid exists in those alveoli which remain open. This is the confluent lesion seen subsequently in the x-ray of the chest.

It has proved possible to reproduce the pulmonary lesions found clinically in a variety of animals. Following the induction of peritonitis by cecal ligation or by the injection of bile mixed with coloform organism into the peritoneum, in dogs, rats, and pigs the sequence of physiological events is very similar to that observed

in man (5) (7). In dogs and pigs the oxygen tension declines from the normal fasting state and is accompanied by an increase of pulmonary vascular resistance on the average to 152 ± 34 percent of the basal value. The changes were greatest in those animals which died (21). When hypoxemia and the evidence of shunt first appeared between 12 and 18 hours following the induction of peritonitis, pulmonary biopsies revealed an early pneumonitis in almost every respect similar to that described for man. Diffuse alveolar collapse was evident within a few hours after the appearance of capillary congestion and alveolar septal edema. As time passed, polymorphonuclear leucocytes adhered to the vascular endothelium and migrated into the septa and alveoli. Thereafter, the pulmonary lesion frequently progressed to the full blown picture of bronchopneumonia

Aerobic and anaerobic cultures in more than 60 percent of the biopsies from the lungs of experimental animals, obtained at the early stage of pneumonitis, failed to demonstrate the presence of culturable organism (5). Thus, it appears that factors other than pulmonary bacterial invasion (hematogenous pneumonia) are responsible for the early (Phase I) post traumatic and septic lung lesion (26).

Studies by Rubin et al (15) in our laboratory, using radioactive phosphorous, demonstrated in rats that the rate of its secretion into the airways was reduced in the starved state and even more so in the septic condition. In part this accounts for the focal alveolar collapse as well as for the development of bronchopneumonia. Surfactant not only reduces surface tension, but is bacteriostatic and is required for the normal function of cilia. (See Table IV)

TABLE III PERCENTAGE OF LUNG SECTIONS WITH ABNORMAL FINDINGS

Animals	Focal Alveolar Collapse	Septal Edema	Perivascular and Septal Leukocyte Infiltration	Vascular Congestion
Fed (n=7)	15	30	30	15
Starved (n=8)	25	60	40	35
Septic (n=11)	75	90	75	65

TABLE IV SPECIFIC ACTIVITY OF TOTAL PHOSPHOLIPID (PL) AND PHOSPHATIDYL CHOLINE (PC) IN AIRWAY WASH AND BLOOD-FREE TISSUE

	Hours after injection of ^{32}P									
	Three		Six		Twelve		Eighteen		Twenty-Four	
	PL	PC	PL	PC	PL	PC	PL	PC	PL	PC
Airway wash										
Fed	512	497	1,680	1,723	3,492	3,310	3,634	3,647	2,840	3,310
Starved	-	-	1,478	1,596	1,702	1,564	2,330	2,914	2,300	2,608
Septic	242	196	1,099	1,185	1,852	2,341	2,166	2,964	2,069	2,570
Lung tissue										
Fed	1,181	1,566	1,420	2,185	2,745	3,589	2,085	3,122	1,717	-
Starved	-	-	1,186	1,689	1,175	1,916	1,482	1,980	1,414	1,750
Septic	1,233	1,775	1,041	1,696	1,315	2,099	1,585	2,278	1,233	2,033

ETIOLOGY

Farrington with Cossette et al (11) in our group employed an isolated perfused canine pulmonary lobe in situ to demonstrate that whole blood from shocked or septic dogs produced within half an hour the complete histological pattern of the early pneumonitis. At the same time the perfusion pressure increased on the average 71 ± 12%, indicating a rise in pulmonary vascular resistance. This sequence of events occurred despite the fact that the remainder of the dog's lung through which its own blood was circulating remained normal.

There seems little doubt that peptides contribute to the induction of this effect. Voss (13) using the same dog lung perfusion preparation showed that the administration of a protease inhibitor (Trasylol 50,000 units KIU) to a septic dog two hours before drawing blood almost entirely prevented development of the lung lesion and the elevation of pulmonary vascular resistance in the perfused lobe. This finding strongly suggests that peptidases or proteases are involved in activation of the substances responsible for the Stage I pneumonitis.

In subsequent experiments the plasma from normal or septic patients was separated into fractions by cold vacuum filtrations employing viscose membranes of graded pore size (39). Only the non-protein fraction of plasma from septic patients and animals containing substances of molecular weight from 1,000 to 10,000 when added to normal perfusing blood was capable of inducing the same pulmonary changes (26) (28). Subsequently it was observed that the same frac-

tion was capable of inducing pneumonitis when added to a non blood perfusate. These findings suggest a number of possibilties as being responsible for the induction of the early Stage I lung lesion.

1. The presence of bacterial endotoxin working directly upon the capillary membrane of the lung (60).
2. The presence of activated Factor XII and the secondary induction of diffuse intravascular coagulation (D.I.C.). (58) (36)
3. The presence of fibrino-peptides produced by the activation plasmin, concomitant with D.I.C. (38)
4. Aggregation of platelets and release of serotonin, ADP or postaglandins, and histamine in some species including man. (50) (55)
5. Activation of the kinin system. (37) (43)
6. The possibility that a variety of vasoactive peptides are generated from other proteins by release of proteolytic lysosomal enzymes particularly from damaged macrophages.

To assess the possible role of each of these factors in the light of present day knowledge it is necessary to review briefly the literature and to consider a series of experiments then undertaken by the principal investigator and his colleagues. Cuevas et al (59) found that whereas endotoxin infusion causes rabbits to develop the characteristic lung lesion, the induction of endotoxin resistance prevented the development of pneumonitis when endotoxin was injected.

However, Kuida (29) and Hinshaw (32) demonstrated that endotoxin does not directly affect the lung when it is perfused with a non-blood perfusate. More recently we have shown experimentally that when endotoxin is added to whole blood the rise of perfusion pressure and development of edema are virtually eliminated if excessive quantities of heparin are added to the preparation (21). A similar effect is produced if the blood cells are eliminated from the perfusate leaving only heparinized plasma. Thus, one is led to the conclusion that endotoxin acts via the activation of Factor XII by a combination with an antibody and complement (48), through action of lysozymal enzymes, or platelets release. Plasmin is activated also by Factor XII producing fibrino-peptides. Platelet aggregation and release may well produce the substances enumerated above which also can injure the lung.

D.I.C. occurs in many clinical situations associated with the lung lesion under consideration including endotoxemia (33) (36). The presence of thrombin may be the ultimate pathway (38). Olsson et al (53) have found that infusion of thrombin experimentally produces peripheral vasodialtion and at the same time an increase of pulmonary vascular resistance. Both fibrin formation and platelet aggregation are induced by thrombin infusion. The roles of intravascular obstruction by micro emboli and thrombosis in the elevated vascular resistance is doubtful. The reaction has been found to occur in animals defibrinated with reptilase and can also be rapidly reversed by the administration of heparin. Both findings argue against obstruction of the

pulmonary vessel and favor a reaction of pumonary vascular constriction.

Platelet aggregation is recorded frequently in septic shock and other states involving D.I.C. (42) (50). With radioactive chromium marked platelets Bergentz, Lewis and Ljungqvist (55) demonstrated platelet accumulation in the lung as the blood platelet count falls. ADP and serotonin both pulmonary vascular vasoconstrictors (53) (60) are released from aggregated platelets. Both also produce peripheral dilation. Acetylsalicylic acid (ASA) does inhibit the aggregation of platelets as demonstrated by the presence of thrombocytopenia, but it does not prevent the pulmonary hypertension and airway constriction after the infusion of thrombin into dogs (53). Thus, one is led to the conclusion that most of the release of pulmonary vascular resistance is related to the release of vasoactive substances from platelets. However, inhibition of serotonin by methylsergide also fails to block the reaction. Another possible series of substances from platelets may be the prostaglandins, E_2F_{2a} (40). Their release can be blocked by A.S.A. These agents have been found to be pulmonary vasoconstrictors as well as bronchoconstrictors in the dog.

Although Bayley (38) found that infusion of fibrino-peptides increased pulmonary vascular resistance, Olsson (53) observed that defibrinated dogs responded to thrombin infusion by pulmonary hypertension in the absence of evident clotting. Thus the role of fibrino-peptides remains in doubt.

Activation of the kinin system through Hageman Factor (XII)

(56) may play a role in the pulmonary reaction. It has been clearly demonstrated that the infusion of Bradykinin into the pulmonary artery causes an increase of the pulmonary vascular resistance (43). Our earlier work with the isolated perfused dog lung gave the same response and in addition produced a morphological change in the lung characterized principally by interstitial edema. Evidence from experiments in primates carried out by Mason (52), indicate that endotoxin infusion causes a reduction of pre-kallikrein. Attar et al (37) have shown the Bradykinin, activated in significant quantities in shocked and septic patients, may contribute to plasma loss. Measurements made by ourselves in cooperation with Drs. Richard Talamo and Robert Coleman of the Massachusetts General Hospital in seriously ill septic patients demonstrated a significant reduction of pre-kallikrein which was most marked in those patients who were hypotensive at the time of the observation. In the same group, Bradykinin was elevated above three nanograms per ml. in 60% of the patients who were hypotensive with or without liver disease. This is in contrast to an elevation of 28% and 12% respectively in the patients without hypotension. However, no exact correlation between the plasma Bradykinin concentration and the extent of pulmonary shunting could be made.

The possibility that any of these agents of small molecular weight circulating in the non-protein fraction of the plasma are capable of injuring the lung directly is suggested by recent experiments employing the in vitro rabbit lung perfusion. If the perfusate contains gelatin (5% in Ringer lactate solution) and if the blood has been

carefully washed out of the lung, the addition of endotoxin produces no edema or vasoactivity. This is an effect previously observed by Kuida et al (32) and Hinshaw et al (29), also using a perfusate of gelatin. Following a long series of experiments during the past year a very effective bioassay system has been developed which is sensitive to the circulating vasoactive agents but not to endotoxin per se.

Rabbit Lung Bioassay: Anesthetized rabbits of approximately 1.5 Kg. are employed. Rabbits of 1.5 Kg body weight are anesthetized with pentobarbital, and an endotracheal tube is placed through a tracheostomy. A Harvard respirator is connected, being adjusted to maintain normal aeration. Through a midline sternal splitting incision the heart and great vessels are exposed. Ligatures are loosely placed around the aorta and pulmonary artery. Purse string sutures for subsequent cannulation are placed in the right and left ventricular walls. Great care is exercised to avoid stressing the animal by blood loss or inadequate anesthesia.

When all dissections are completed the animal is given intracardiac heparin 5 mgm/Kg body weight. A cervical spine column section is then carried out in order to avoid sympathetic discharge during the remainder of the procedure and to preserve catecholamine stores in the lung. A cannula of the largest diameter possible is quickly introduced into the main pulmonary artery through the right ventricular purse string suture and ligated in place in the pulmonary artery. A large bore cannula (5/16" I.D.) is placed in the left ventricle through the purse string suture which is then tied. The aorta just distal to

the valve is ligated. Perfusion fluid (5% gelatin in Ringer lactate solution buffered to pH 7.4) is then pumped into the pulmonary artery. More recently 6% dextran 75 in 0.9% NaCl has been diluted 50% with Ringer lactate solution. This mixture has an oncotic pressure equal to rabbit plasma, and is very satisfactory for perfusion. Fluid is allowed to escape from the left heart cannula until the effluent is clear. The outflow is then connected to the reservoir to permit recirculation.

The circuit consists of a large bore polyvinal tube leading from the left heart to the reservoir which is a plastic transfusion bag or polyethylene cylinder. From the reservoir perfusate is pumped by a rotary pump through a silicone rubber tube to the pulmonary artery cannula. The volume of perfusate in the circuit is approximately 100 ml. The reservoir is immersed in a water bath to maintain perfusate temperature at 35 to 37°C. Carbondioxide is bubbled through the reservoir perfusate at a rate sufficient to maintain the pH between 7.3 to 7.4. The reservoir is placed at 15 cm. below the left heart to be certain that positive pressure does not exist in the left atrium. Pressure in the pulmonary artery is recorded continuously on a polygraph through a transducer attached to a small tube which passes through the cannula. The tip lies beyond the cannula in the lumen of the pulmonary artery. Ventilation of the lungs with air is continued at a volume adequate to fill but not distend them. Insufflation pressure is continuously recorded through a side arm in the endotracheal tube.

Perfusion rate (usually 100 to 150 ml/kg rabbit weight) is

established to produce a pulmonary artery mean pressure between 18 and 20 cm. H_2O. The perfusion is then allowed to continue for 45 minutes to be certain that no artifactual reaction of edema or pulmonary vascular constriction occurs. The sample of plasma (3 ml.) or plasma fraction (0.5 ml.) to be tested is then injected into the reservoir. A positive reaction is clearly demonstrated within 15 to 25 minutes by a rise of pulmonary artery pressure 50% or more above the baseline. Edema formation in the lungs becomes evident by a rise of insufflation pressure, as compliance is reduced. This effect is apparent before florid edema takes place in the lung which subsequently becomes manifest by failure of the lungs to collapse and by the presence of bubbles in the tracheal tube. Any test, in which no reaction takes place after 45 minutes, is checked by the administration of histamine (0.3 mgm) into the reservoir to be certain that the preparation is reactive. It is rare not to get a reaction with histamine, but in such a case the test is disregarded and is repeated with a new preparation.

With this preparation the perfusion with gelatin solution can be continued for three hours or more without evidence of edema or change in PA pressure. When a reaction does occur, vasoconstriction is recognized by a rise of PA pressure. When a test substance such as histamine or serotinin is introduced into the perfusate, within 20 minutes a rise of PA pressure takes place followed by an increase of insufflation pressure. The test is objective, and no doubt exists when vasoactive substances are present. Multiple tests with the intro-

duction of endotoxin into the system have failed to give a positive reaction.

The initial studies with this assay were carried out using 3 ml. whole plasma from starved normal or septic pigs with induced peritonitis. The results of this experiment are presented in Table V

It is apparent that a close correlation of positive results in the test rabbit lung perfusion exists with the presence of a pulmonary shunt in the septic pigs with peritonitis induced by cecal ligation. The presence of an active factor in the plasma is demonstrated thereby. No positive reactions occurred with the plasma from any normal pig nor from any septic pig who failed to develop hypoxemia, evidence of a pulmonary shunt. All negative assays were tested by the addition to the perfusate of histamine to be certain that the rabbit lung preparations was capable of a response.

A second aspect of this experiment which is of considerable interest, is that only the fraction of active plasma containing molecular weights from 1,000 to 10,000 was capable of eliciting a response. Neither the fraction containing molecular weights below 1,000 nor the protein containing fraction of molecular weights greater than 10,000 produced any positive reactions.

When a Ringer lactate solution containing 5% human albumin was substituted for the gelatin solution, no positive reactions were produced in the rabbit lung either by whole septic plasma or by the "active fraction" (molecular weight 1,000 to 10,000). Thus, it appears that whatever the circulating agent may be, it is bound to

TABLE V

ASSAY OF PIG PLASMA BEFORE AND AFTER THE INDUCTION OF PERITONITIS

	RABBIT LUNG ASSAY			
	CONTROL NORMAL PLASMA		SEPTIC PLASMA	
	REACTIVE	NON-REACTIVE	REACTIVE	NON-REACTIVE
PIG BLOOD GASES (at time plasma obtained for test)	n=0	(n=10)	(n=4)	(n=6)
Art. pO_2 (mm Hg)	-	66.2 ±3.8	47.5 ±7.7	72.7 ±4.9
Art. pCO_2 (mm Hg)	-	36.7 ±4.0	40.1 ±1.62	39.2 ±2.5

the albumin and is no longer active. This finding may have significant therapeutic implications.

Thus, the exact nature of the substance or substances present in the blood after injury, shock or sepsis which cause interstitial edema and pulmonary vasoconstriction remains unknown. Endotoxemia in the presence of whole blood causes these same pulmonary changes. Whether endotoxin activates the complement to produce the injury to the lung through the mediation of leucocytes, or whether lysozymal enzymes are released, it is almost certain that other substances including vasoactive peptides are present which directly cause the early pulmonary abnormalities.

Experiments performed by Dr. Calvin Saravis using sephadex column chromatography of the plasma fraction containing molecular weights from 1,000 to 10,000 exhibited a peak of activity at molecular weights between 4,000 and 6,000 not present in normal blood. The fact that experimentally trasylol is capable of blocking the development and transfer of the active agent to the perfused lung preparation from a septic animal argues strongly that peptidases are involved in its production. The conclusion then must be reached that somewhere in the chain of inducing this active factor in the plasma whether derived from platelets, clotting activity, or other sources, peptidases and peptides in abnormal concentrations must be involved.

CLINICAL OBSERVATIONS WITH BIOASSAY:

Previous studies with perfusion of the isolated canine pulmonary lobe (10) and the in vitro perfusion of the rabbit lung, (21) in which

human plasma or "active fraction" (molecular weights 1,000 to 10,000) were introduced into the perfusate, gave evidence of the presence of vasoactive substances which produced vasoconstriction and edema in the test lung. Since development of the present sensitive rabbit lung bioassay, 20 patients have been studied in some detail. The data from these patients are summarized in Table VI . Although insufficient in numbers for definite conclusions it becomes evident that some correlation of plasma activity exists with the development of a pulmonary shunt. Furthermore, the presence or absence of endotoxin in the blood as determined by the Limulus Lysate Technique (58) apparently bears little relationship to the activity of the patients' plasma in the bioassay system.

METABOLISM

From the description of the clinical responses in postshock, post-traumatic, and septic states, comparing the pattern of recovery to that which leads to death, it is evident that disturbances of metabolism in tissues must play a major role in the elevated circulatory demand. As shown in Table II, lactacidemia, so characteristic of these patients, reflects not only insufficient perfusion of tissues or hypoxemia, but also in all probability alterations of glucose utilization in the periphery or failure of the liver to clear glucose via the Cori cycle.

Confirming the findings of Dr. John Howard in the Korean war, glucose tolerance tests carried out in ten patients who were severely injured and septic demonstrated flat glucose tolerance curves which

TABLE VI

RELATIONSHIP BETWEEN PLASMA BIO-ASSAY AND CARDIO-PULMONARY FUNCTION IN SEPTIC AND INJURED PATIENTS

	PLASMA BIO-ASSAY	
	Active	Non-active
% Shunt (Estimated)*	27 ± 1.5 (n = 33)	18.0 ± 1.5 (n = 25)
Cardiac Index; l/M /min**	2.6 ± 0.4 (n = 18)	4.0 ± 0.5 (n = 14)
Mean Pulmonary Artery Pressure, mm Hg**	32 ± 1.8 (n = 18)	25 ± 2.5 (n = 12)
Peak Inspiratory Pressure, cm H_2O	40 ± 1.8 (n = 25)	31 ± 3.3 (n = 12)

*$p < 0.01$
**$p < 0.05$
(n = Number of observations)

persisted as long as they were septic. Without exception the diabetic like glucose tolerance curves disappeared when recovery took place. This response occurred despite the fact that the blood insulin values rose in normal fashion to levels of 50 μU/ml or more within five minutes of the glucose infusion. It is evident that insulin resistance exists under these conditions.

Further studies, presented in Table VII, in seriously septic patients demonstrate that the metabolic pattern of energy production differs not only from that of normal starvation but that significant differences exist between that associated with the usual high cardiac output response and the situation when hypotension and low output are present. In the high output state blood insulin is three times that found in starvation. Blood sugar is normal or high, and lipolysis is suppressed. Experimental measurements of limb substrate utilization shown in Table VIII indicated that oxygen and glucose uptake are the same as in fasting while net fat utilization is almost zero. It is suggested by negative nitrogen balance in patients that the apparent peripheral fuel deficit is satisfied by proteolysis and oxidation of amino acids. When the cardiac output is below the normal basal value the blood insulin is as low or lower than in starvation, probably related to intense catecholamine activity. Peripheral oxygen uptake is not significantly reduced and net fat utilization is normal. Although peripheral glucose uptake is normal, the major portion of it appears to be converted to lactate. The fuel deficit again appears to be satisfied by amino acid oxidation which is reflected by the

TABLE VII METABOLIC SUBSTRATES AND URINARY NITROGEN EXCRETION

IN FASTING AND SEPTIC MAN
(Values ± SEM)

		Septic Semi-Starvation (Glucose 100g/24°)	
	Normal 12 Hour Fasting 8 Patients	High Flow n = 20 10 Patients	Low Flow n = 12 8 Patients
Cardiac Index ($L/min/m^2$)	2.8 ± 0.4	4.6 ± 0.8	2.4 ± 0.3
Mean Arterial Pressure (mm Hg)	93 ± 14	110 ± 12	70.2 ± 4
Rectal Temperature (°F)	99.6 ± 0.6	102^4 ± 0.8	100^6 ± 0.6
Blood Insulin (uU/ml)	18 ± 2	42 ± 6.4	12 ± 4
Blood Glucose (uMol/ml)	3.6 ± 0.8	7.2 ± 0.5	7.68 ± 2.3
Blood FFA (uMol/ml)	1.3 ± .15	0.41 ± .06	1.176 ± 0.9
Blood Lactate (uMol/ml)	0.6 ± 0.1	1.15 ± .11	2.79 ± 0.3
Arterial O_2 Tension Breathing Air (mm Hg)	90 ± 3	65 ± 6	55 ± 8
Urinary N (gms/24 hrs)	-	7.4 ± 0.9	16 ± 2

n = number of observations

TABLE VIII PERIPHERAL METABOLISM DURING PERITONITIS IN PIGS
(Values ± SEM)

	Basal n = 10	High Flow n = 9	Low Flow n = 7
Temperature (^{o}F)	101^{4} ± 0.2	102^{8} ± 0.5	100^{8} ± 1.2
Cardiac Output (L/M)	3.9 ± 0.3	5.0 ± 0.6	2.0 ± 0.3
Hind Limb Flow (ml/kg body wt/min)	2.4 ± 0.2	3.1 ± 0.1	1.9 ± 0.2
Mean Arterial Pressure (mm Hg)	115 ± 3.7	103 ± 4.1	103 ± 10.8
Hind Limb Resistance (pres/flow)	49 ± 6	33 ± 2	53 ± 4
Arterial Insulin (uU/ml)	10 ± 4	41 ± 9	14 ± 5
Arterial Glucose (uM/ml)	5.6 ± 0.5	4.0 ± 0.2	4.6 ± 0.6
Glucose Uptake * (uM/kg/min)	1.6 ± 0.3	1.3 ± 0.3	1.5 ± 0.4
Arterial Lactate (uM/ml)	1.0 ± 0.1	0.9 ± 0.1	1.7 ± 0.3
Lactate Production * (uM/kg/min)	+1.2 ± 0.2	+1.2 ± 0.3	+2.5 ± 0.6
FFA Arterial (uEq/ml)	.715 ± 0.8	.398 ± .07	.462 ± .03
FFA Uptake or Release * (uMol/kg body wt/min)	-.101 ± .03	+.067 ± .04	+.126 ± .04
Glycerol Release * (uMol/kg body wt/min)	+.042 ± .011	+.009 ± .001	+.116 ± .01
O_2 Uptake * (ml/kg body wt/min)	.15 ± .03	.14 ± .03	.12 ± .01
Arterial pO_2 (mm Hg)	72.36 ± 2.6	69.5 ± 7.18	75.2 ± 7.2

* Net uptake (-) or release (+) by hind limb.

TABLE IX MAXIMAL AND MINIMAL RESPONSE WITH (TIME): COMPARING PERITONITIS WITH ENDOTOXEMIA INDUCED BY A SINGLE DOSE OR INFUSION.

Measured parameter	Group I Peritonitis		Group II Endotoxin bolus		Group III Endotoxin infusion		Group IV (Control) Saline infusion	
	Survivors	Deaths	Survivors	Deaths	Survivors	Deaths	Survivors	Deaths
Hemodynamic								
Cardiac output	.17 (5d)	*.10 (1d)	.32 (24h)	.22 (6h)	.24 (24h)	*.16 (12h)	.13 (24h)	
l/min/Kg	.11 (1d)	.09 (2d)	.21 (1h)	*.08 (2h)	.15 (6h)	*.07 (6h)	.12 (6h)	
Art. blood pres.	110 (1d)	102 (1d)	97 (4h)	*80 (4h)	119 (8h)	*138 (12h)	116 (12h)	
mmHg. Mean	100 (2d)	98 (2d)	65 (2h)	63 (1h)	95 (2h)	*70 (1h)	108 (6h)	
Metabolic								
Body temp.	103.2 (5d)	106.4 (2d)	104.8 (4h)	102.3 (6h)	105.2 (8h)	105.8 (5h)	102.2 (12h)	
°F. Rectal	102.8 (0d)	103.2 (0d)	102.2 (2h)	*98.3 (2h)	102.6 (24h)	101.0 (12h)	101.7 (6h)	
Blood lactate	0.9 (1d)	* 1.7 (2d)	3.9 (2h)	3.7 (2h)	2.2 (3h)	*5.4 (8h)	0.6 (12h)	
µmol/ml	0.6 (5d)	1.4 (1d)	1.6 (8h)	*2.8 (8h)	0.8 (12h)	*3.5 (6h)	0.3 (24h)	
Blood glucose	140 (5d)	95 (1d)	93 (.5h)	86 (.5h)	66 (24h)	63 (12h)	84 (24h)	
mg. %	76 (2d)	68 (2d)	49 (2h)	*26 (2h)	40 (10h)	*28 (6h)	63 (10h)	
Blood gas								
PaO_2	89 (1d)	86 (1d)	72 (6h)	66 (6h)	72 (24h)	72 (6h)	75 (4h)	
mmHg.	72 (2d)	74 (2d)	56 (1h)	51 (1h)	57 (6h)	54 (12h)	63 (12h)	
$PaCO_2$	36 (5d)	32 (1d)	34 (6h)	33 (1h)	38 (12h)	34 (4h)	36 (12h)	
mmHg.	32 (1d)	30 (2d)	30 (1h)	29 (8h)	30 (6h)	28 (10h)	35 (6h)	
Hematology								
Prekallikrein	146 (0d)	157 (0d)	180 (1h)	210 (1h)	172 (1h)	150 (1h)	180 (2h)	
µmol hydrol/h	116 (2d)	113 (2d)	130 (6h)	170 (6h)	152 (10h)	130 (12h)	170 (12h)	
Bradykinin	2.6 (3d)	2.7 (2d)	3.6 (4h)	3.7 (4h)	1.8 (12h)	3.6 (12h)	2.1 (12h)	
ng/ml	1.3 (5d)	1.9 (1d)	.7 (24h)	-	.9 (1h)	.8 (1h)	1.7 (1h)	
Fibrinogen deg.	-	-	36 (4h)	40 (4h)	29 (12h)	17 (10h)	5 (24h)	
prod. µmol/ml	-	-	6 (24h)	10 (.5h)	11 (1h)	6 (1h)	3 (3h)	

* Indicates significant difference of means of survivors and deaths.

severe negative nitrogen balance in hypotensive septic patients.

Again it should be pointed out that failure of surfactant synthesis under conditions of starvation, trauma, and sepsis, demonstrated by Rubin et al (15) in rats is a failure of metabolism and synthesis in the pneumatocytes (type II). This is one of the many aspects of disturbed protein-calorie nutrition which can be remedied by the correct attention to alimentation in injured and septic patients.

THERAPEUTIC CONCLUSIONS

Preventive measure and treatment of established pneumonitis fall into a number of categories based upon the progressive nature of the lung lesions (Stage I and Stage II). These are outlined in Table X .

TABLE X Respiratory Insufficiency in Trauma and Sepsis
(Alveolar Ventilation-Perfusion Ratio Inequality Reduction)

I. Causes

A. Primary:

1. Pre-existing diseas (emphysema, etc.)
2. Congestive diffuse alveolar collapse (shock lung, wet lung, congestive atelectasis)
   a. Intersitial edema
   b. Capillary congestion
   c. Focal alveolar collapse
3. Fat embolization
4. Aspiration pneumonia
5. Hypostatic edema
6. Pulmonary emboli (micro-,macro-, fat)
7. Inadequate ventilatory exchange

B. Secondary:

1. Bronchopneumonia
2. Hematogenous pneumonia
3. Atelectasis

II. Treatment: Preventive and Therapeutic

1. Cough, tracheal suctions, etc.
2. Ventilator if arterial Po_2 is below 65 mm Hg
3. Avoid 100 per cent oxygen except for "shunt" test
4. PEEP 10 to 15 cm H_2O if no response
5. Maintain cardiac output
6. Avoid reduction of plasma oncotic proessure (use colloid plus saline)
7. Gastric suction to prevent aspiration
8. Filter transfused blood, use new blood
9. Eliminate gangrenous or septic focus
10. Reduce interstitial edema
    a. 25 per cent albumen
    b. Diuretic
11. Hydrocortisone
12. Maintenance of adequate nutrition to avoid loss of immunocompetence and protein synthesis

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