Changes in the Fibrinolysin System During Experimental Pulmonary Hyaline Membrane Formation in Guinea Pigs.* (32001)

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Pulmonary hyaline membranes (HM) described in respiratory distress syndrome due to hyaline membrane disease of infants (HMD), were shown to consist primarily of fibrin(1,2). Several investigators reported deficiencies in the fibrinolysin system of infants suffering from this disease(3-7). We have proposed earlier that plasminogen develops rather late in ontogeny thus leaving premature infants without defense against pulmonary fibrin deposition which may occur from pulmonary exudates developing in relation to birth trauma(8,9). A pathologic entity similar to HMD can be induced in guinea pigs by prolonged exposure to high concentrations of oxygen and aerosolized human amniotic fluid(10,11). The purpose of this study was to investigate possible changes which may occur in the fibrinolysin system during the development of experimental HMD.

Materials and methods. English smooth hair male guinea pigs of 250-350 g body weight from our own colony were used. The animals were kept in air conditioned quarters and allowed Purina guinea pig pellets, green salad leaves and water *ad lib.*

Hyaline membranes (HM-s) were produced by the following method. Exposure to 95% oxygen was undertaken in specially constructed plastic cages, through which gas flow was continuously maintained at a rate of 5 lit/min. Oxygen concentration in the chamber was periodically checked using a Beckman Model D2 oxygen analyzer. Human amniotic fluid was nebulized into the chamber from a 24850S N.C.G. Nebulizer at a rate of 10cc/hour using oxygen as propellant. All experiments were performed at atmospheric pressure. Human amniotic fluid was immediately frozen after collection and stored at —70°C. Thromboplastic potency was determined before use and only batches with significant activity employed. Commercially available bovine amniotic fluid showed little thromboplastic activity.

All guinea pigs subjected to such combined treatment died; the mean time of death was 4.6 days. HM-s were found in only 45-50% of the dead animals, a somewhat higher ratio than that obtained by oxygen exposure alone (30%). Therefore the combined method was adopted for all experiments described. Autopsies were performed on guinea pigs as soon after death or sacrifice as possible. Fig. 1 shows pulmonary histology in an infant who succumbed to HMD. Fig. 2 shows a pulmonary histology in an infant who succumbed to HMD. Fig. 2 shows a pulmonary section from a guinea pig which died on the 4th day of oxygen exposure. The two pictures are remarkably similar.

Factors of the blood coagulation and fibrinolysin systems (plasminogen, plasminogen activators, plasmin, antiplasmins) were determined by methods described previously (12). Tissue activator of plasminogen was determined as described before(9) by measuring the area of lysis zone produced by 0.050 ml tissue homogenate of 1 mg dry weight on a 0.3% fibrin plate prepared with human fibrin and human thrombin with known amounts of plasminogen contamination. The lysis zones were read after 24 hours incubation at 37°C. The activator inhibitory activity was measured by the simultaneous determination of the lysis zone produced by (1) the tissue homogenate alone, (2) 10 Ploug(13) U urokinase (UK) alone, and (3) a mixture of the tissue homogenate with 10 Ploug U UK. The lysis zone of the mixture is compared to the sum of the lysis areas produced by the individual components, and, if inhibition is present, it is expressed as % of the lysis zone expected

* This study was aided by grant HE-05672 and FR 00262 from Nat. Inst. Health, USPHS.

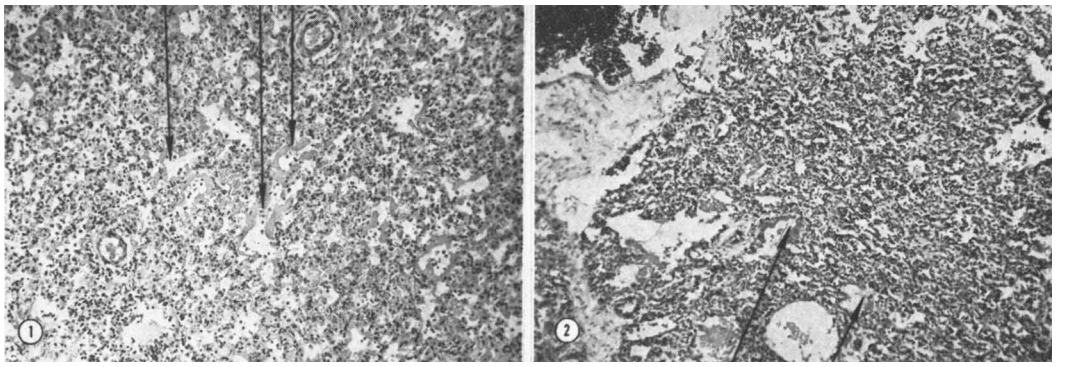

FIG. 1. Section from guinea pig lung. Hyaline membranes are located in terminal bronchioles and alveoli similar to Fig. 2, but the atelectasis is not as severe (70 ×).

FIG. 2. Section from human lung. Note hyaline membranes (see arrows) in terminal bronchioles and alveoli with atelectasis of surrounding lung parenchyma (70 ×).

by the components alone. UK was selected as a standard because of its similarity to tissue activators and its availability in relatively pure form.

Results. Table I shows changes in components of the fibrinolysin system in the circulation of guinea pigs exposed to O_2. As mentioned before the blood samples were taken before the animals were sacrificed. Serum plasminogen, plasmin, plasminogen and plasminogen activator in the euglobulin fraction of plasma show some degree of fluctuation from day to day within a relatively narrow range. The only fibrinolytic factor which showed progressive change correlated with O_2 exposure time was serum antiplasmin. There was a daily increase in the mean antiplasmin values as well as an increase in spread of the individual levels as reflected by the increased standard errors.

Fig. 3 shows changes in pulmonary plasminogen activator (PAA) activity and activator inhibitor (AI) in the course of O_2 exposure. With prolonged exposure PAA decreases, until on day 6 it completely disappears. AI is not present in normal guinea pigs but appears after the first day of exposure. Its increase during the consecutive days is inversely proportional to the decrease in PAA. These changes are more pronounced in lung samples from animals which died than in those which were sacrificed. There seems to be no correlation with the presence or absence of HM-s.

Since the level of circulating antiplasmin increased with the days of O_2 exposure (Table I) the question arose, whether the appearance and steady increase of a pulmonary activator inhibitor could be partly the result of local antiplasmin accumulation due to pulmonary edema and congestion.

Therefore in some of the sacrificed animals one lung was washed with saline, through both the pulmonary artery and the main bronchus, and PAA and AI levels were determined on both the washed and intact lungs. Fig. 4 shows the results of these experiments.

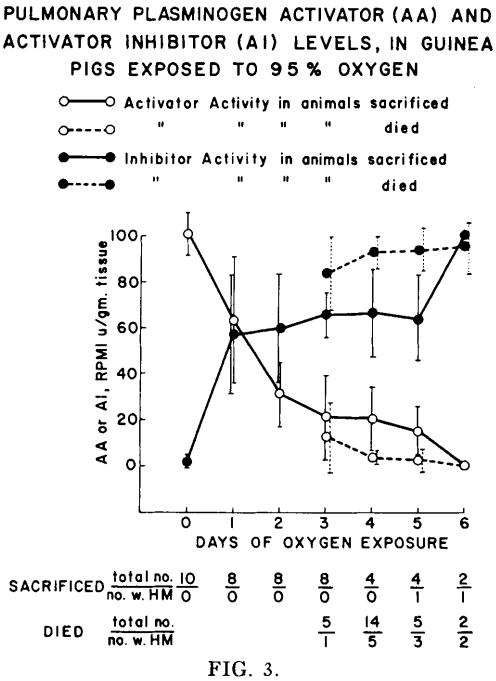

FIG. 3.

It seems that PAA is consistently higher in the washed lung samples, indicating the removal of an inhibitor; nevertheless there is a steady decrease in PAA from day to day. The increasingly severe pulmonary pathology during O_2 exposure of course results not only in greater accumulation of circulating components, but may also render their removal by washing more difficult.

TABLE I. Changes in the Fibrinolysin System in the Blood of Guinea Pigs During Experimental Pulmonary Hyaline Membrane Formation. English smooth hair female guinea pigs 250-350 g. Human amniotic fluid: 0.5 cc into each nostril, then by aerosol for 24 hr. O_2: 95% at 1 atm.

Days of O_2 exposure		0	1	2	3	4	5
No. of samples analyzed		4	6	6	7	3	4
		RPMI, U/ml (mean ± S.E.)					
Plasmin	In plasma	0	0	0	0	0	0
Plasminogen Activator	In serum	5.3 ± .44 0	4.3 ± .29 0	4.6 ± .71 0	4.76 ± .79 0	5.9 ± .88 0	4.2 ± .61 0
Antiplasmin		34 ±1.0	35 ±3.3	46 ±9.6	45 ±9.9	64 ±13.3	65 ±14.0
Plasmin	In euglobulin fraction of plasma	.89 ± .52	.53 ± .19	.12 ± .03	.37 ± .18	.57 ± .28	.87 ± .48
Plasminogen Activator		1.4 ± .30	.64 ± .28	.48 ± .19	.74 ± .12	.73 ± .19	.95 ± .21
		.69 ± .07	.70 ± .06	.65 ± .08	.72 ± .19	.59 ± .18	.62 ± .15

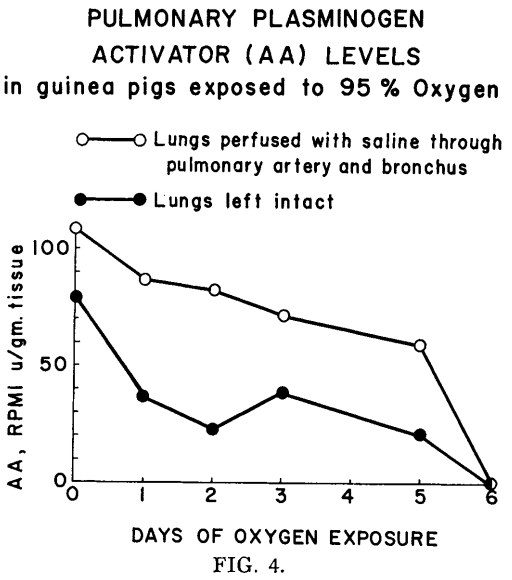

FIG. 4.

This study demonstrates that during exposure to O_2 serum antiplasmin levels progressively increase and pulmonary plasminogen activator levels decrease. Increased appearance of antiplasmin in the pulmonary edema fluid and decreased pulmonary plasminogen activator activity may impair the guinea pig's fibrinolysin system, thus reducing the activity of an important defense against pulmonary fibrin deposition. Lieberman(14) suggested that an activator inhibitor in the lung of infants with HMD may play an etiological role in the development of this disease. Our findings suggest that this pulmonary inhibitor develops in the course of the disease and may derive at least partly, from the circulation, since increased antiplasmin levels were found in the clinical respiratory distress of newborns(9) as well as the experimental study reported here.

The mechanism whereby exposure to O_2 increases antiplasmin levels in the blood is unknown. In a previous study(15) we have demonstrated that circulating antiplasmin is produced by the isolated, perfused liver. No attempt was made in the experiments to measure inhibitors of plasminogen activators or to distinguish them from antiplasmins. Such experiments are presently underway.

Summary. Pulmonary hyaline membranes developed in guinea pigs exposed for several days to 95% oxygen at atmospheric pressures. This process was accompanied by increased antiplasmin activity in plasma and decreased pulmonary plasminogen activator activity. The latter phenomena may promote the formation of alveolar hyaline membranes by interfering with enzymatic removal of fibrin derived from pulmonary exudation.

We wish to acknowledge the devoted assistance of G. Hauser, H. B. Lassman, I. B. Mink, and V. Zaniewski.

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Received August 24, 1966. P.S.E.B.M., 1967, v125.

Ventilation of Newborn Lambs by Means of a Donor Lung.* (32002)

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Isolated lungs of dogs and cats have been shown to retain their diffusion capacity for many hours(1,2), and excised dog lungs have also displayed a fair ability to function as human kidney(3). In view of the durability and adaptability of pulmonary tissue, an investigation was performed in which donor lungs were attached to the circulation of live animals, and the effects of this shunt upon blood oxygenation and upon survival of the recipient animals were studied.

Although time and circumstances did not permit additional experiments for resolution of the problems involved in this procedure, this report is presented in the belief that the findings may be of use to other investigators working in this field.

* This work was supported by Contract NASw-674 and, in part, by Grant NsG-722 from National Aeronautics and Space Administration.

Experimental procedures. Mongrel laboratory dogs were used to supply the donor lungs. The animals were anesthetized (pentobarbital 30 mg/kg i.v.), heparinized (10 mg/100 ml blood), and exsanguinated; the chest was opened through a midsternum incision. The thoracic aorta was ligated in several places in order to block fluid outflow from the bronchial arteries; ligatures were also placed on the superior and inferior venae cavae, and on the azygos vein. The pulmonary artery was cannulated *via* the right ventricle, and a second cannula was placed in the left atrium. Polyethylene tubes were attached to the cannulae, the chest walls were sewn together with tubes emerging, and 4 to 6 liters of a 6% dextran-saline perfusion solution were pumped at low flow rate and pressure (less than 20 mm Hg) *via* the tubes through the lungs for several hours in order