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Role of Fibrinolysis in Development of Hyaline Membrane Disease in Newborn Rabbits

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Summary. The role of fibrinolysis in the pathogenesis of hyaline membrane disease was studied in newborn rabbits at 28- or 29-day gestation. Hyaline membrane was recognized in the lungs of rabbits that were exposed to intrauterine hypoxia and received trans-4-aminomethylcyclohexane carboxylic acid (t-AMCHA), a plasmin inhibitor found to cause a marked decrease in the fibrinolytic activity of lung extracts. In the newborn rabbits subjected to intrauterine hypoxia alone, surface activity of lung extracts was reduced, but hyaline membrane was not seen.

By immunofluorescent examination the hyaline membrane was found to be rich in fibrinogen and its derivatives. Electron microscopic examination revealed that they contained various amounts of cellular debris, bundles of fibrils and electron-dense, finely granular deposits and polymerized fibrin with 230 Å periodicity. Disintegration of pulmonary epithelial cells was also seen.

These findings indicate that diminished fibrinolytic activity of lung tissue contributes to intra-alveolar accumulation of fibrinogen, fibrin and their degradation products, thereby bringing about morphological and physiological disintegration of the terminal airways.

Key words: Hyaline membrane disease – Fibrinolysis – Newborn rabbits – Antiplasmin – Hypoxia.

Introduction

Pulmonary hyaline membrane and atelectasis are characteristic findings in the lungs of newborns dying of hyaline membrane disease (HMD) (Askin, 1975). Many extensive studies on pathophysiology of idiopathic respiratory distress syndrome (IRDS) or HMD support that pulmonary surfactant deficiency pro-

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vides an explanation for pulmonary atelectasis (Avery and Mead, 1959; Reynolds et al., 1965; Farrell and Avery, 1975). Other concepts which proposed to explain the pathogenesis of HMD include pulmonary hypoperfusion (Chu et al., 1965; Stahlman et al., 1964 and 1972), increased permeability of alveolar walls (Stahlman et al., 1972; Lauweryns, 1970), inadequate fibrinolysis (Lieberman, 1967; Ambrus et al., 1971), plasmatic hypercoagulability (Bleyl et al., 1969) and aspiration of gastic juice (McAdams et al., 1973). To understand the precise mechanism of hyaline membrane formation further investigations are required. Hyaline membrane has been reported to develop under certain experimental conditions in the neonates of lambs (Stahlman et al., 1964; Reynolds et al., 1965) and monkeys (McAdams et al., 1973), but not in small animals such as rabbits.

The purpose of this study is to discuss the role of fibrinolysis in the pathogenesis of experimental HMD in newborn rabbits. This has been studied light and electron microscopically, by immunohistochemical methods and by measurements of surface activity and fibrinolytic activity of lung extracts.

Materials and Methods

Animals and Experimental Groups. This study was performed using newborn rabbits at 28- or 29-day gestational age, delivered by cesarean section. Thirty one pregnant rabbits were anesthetized with intravenous injection of pentobarbital, 15–25 mg per kg of body weight. A lower midline abdominal incision was made and the gravid uterus was incised in situ. The chorionic sac was opened to deliver the fetus, and the entire operative procedure was completed within 30 min.

Two hundred and eight newborns were divided into four groups: group 1 received no treatment (control group), group 2 was given trans-4-aminomethylcyclohexane carboxylic acid (t-AMCHA), group 3 was exposed to intrauterine hypoxia and group 4 was exposed to intrauterine hypoxia and t-AMCHA.

Intrauterine hypoxia was produced by giving the pregnant rabbits low concentrations of oxygen (10–12%), regularly checked by a Beckman O_2 analyzer, Model D 2. Twenty eight day fetuses were exposed to hypoxia for 12 h before delivery, those delivered at 29 days were exposed to hypoxia twice at 12 h intervals. T-AMCHA was administered intravenously to group 2 and 4 as 0.1 ml of 10% t-AMCHA solution (Daiichi Seiyaku Co., Ltd., Tokyo, Japan) per 10 g body weight, immediately after delivery.

The rabbits were observed until 8 h after delivery in an incubator, conditioned at 35° C and 90% humidity.

Light Microscopic Study. Formalin-fixed lung tissues were prepared as usual and paraffin sections were stained with hematoxylin and eosin (H.E.), periodic acid-Schiff and phosphotungstic acid hematoxylin.

Electron Microscopic Study. Small lung blocks from newborns were immersed in 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, at 4° C for 4 h. After washing in cacodylate buffer, they were postfixed in 1% chilled OsO_4 buffered with 0.1 M cacodylate solution, pH 7.4, dehydrated in graded ethanol, and then embedded in Epon 812. Ultrathin sections were stained with both uranium acetate and lead citrate, and were examined with an electron microscope, JEM 100 C.

The number of osmiophilic inclusion bodies were counted in a random sample of type II pneumocytes and the average number of inclusion bodies per type II pneumocyte was determined in each group.

Immunohistochemical Study. Anti-rabbit fibrinogen antiserum was prepared by immunizing a goat with purified rabbit fibrinogen (kindly provided by Mochida Pharmaceutical Co., Ltd., Tokyo,

Japan). Immunological specificity of the antiserum was confirmed by immunoelectrophoretic analysis. Goat IgG was separated by column chromatography of DEAE-cellulose equilibrated with 5 mM sodium phosphate buffer, pH 8.0, after preparation with 33% saturated ammonium sulfate and conjugated with FITC (BBL Inc., Maryland, U.S.A.) (Clark and Shepard, 1963). Non-specific IgG was obtained from pooled goat serum and conjugated with FITC by the same procedure as above.

Tissue from the lung was rapidly frozen in dry ice-acetone. Cryostat sections (5 μm) were fixed in cold ethanol and were subsequently washed in 0.1 M phosphate buffered saline, pH 7.2, at 4° C. Tissue sections were incubated with specific conjugate for 12 h at 4° C and then washed three times in the phosphate buffered saline for 45 min. The sections were examined with a Leitz Ortholux microscope.

The following controls were carried out. The sections were incubated with: 1) nonspecific conjugate instead of specific conjugate, and 2) nonconjugated specific IgG prior to the application of the specific conjugate.

Measurements of Surface Tension. The surface tension of lung extracts was measured with a modified Wilhelmy's balance (Brown et al., 1959). Lung tissues were minced as finely as possible with scissors and mixed with 50 ml of normal saline per 3 g of lung tissue. After stirring for 30 min, the suspension was filtered through gauze into a teflon trough (Acoma Med. Ind. Co., Ltd., Tokyo, Japan) and aged for another 30 min. The surface area was varied from 9 to 45 cm^2 with a cycle per 2.5 min. Hysteresis loop was recorded on an X–Y recorder. The highest and lowest surface tension was read and the extract activity index (E.A.I.) (Gruenwald et al., 1962) was estimated.

Measurements of Tissue Fibrinolytic Activity. Fibrinolytic activity of lung extracts was measured by means of plasminogen-rich fibrin plate prepared from 0.4% bovine fibrinogen (Seikagaku Kogyo Co., Ltd., Tokyo, Japan) (Astrup and Albrechtsen, 1957; Kohga, 1974). Lung tissues from newborns, at survival periods from immediately after birth to 8 h after delivery, were used in each group. Fresh lung tissue (100 mg) was homogenized in solution of 2 M KSCN (3.0 ml) followed by mechanical shaking for 2 h. After centrifugation the supernatant was diluted with distilled water (10 ml) and 1 N HCl was added so as to adjust the pH to 1.0. The precipitate was dissolved in solution of 2 M KSCN (1 ml) and neutralized with $NaHCO_3$. The fibrinolytic activity of 0.03 ml of lung tissue extracts and trypsin solution (100 BAEE u/ml), which were separately dropped on the plasminogen-rich fibrin plate, was expressed as the product of diameters of lysis area after incubation for 18 h, at 37° C and then was converted to trypsin units (TU) (Sandberg et al., 1963; Kohga, 1974).

Results

A few newborns in non-asphyxiated groups (group 1 and 2) had mild respiratory distress. By contrast, most of the newborns in asphyxiated groups (group 3 and 4) had severe respiratory distress, associated with persistent cyanosis, marked respiratory efforts and intercostal retraction.

Light Microscopic Study

Histologic findings were summarized in Table 1. Most of the lungs in non-asphyxiated groups looked well inflated, but patchy atelectasis was noted in less than one-third. In the lungs of asphyxiated groups, especially in group 4, atelectasis and oedema were frequently observed. Venous congestion was usually noted, and hemorrhage was noticed in some alveoli. Inspissated oedema-fluid was often present in less well aerated alveolar ducts and alveoli. Hyaline membrane was recognized in 6 animals in group 4 and not in other groups. Survival

Table 1. Histological findings of the lungs in newborn rabbits with intrauterine hypoxia and/or t-AMCHA therapy

Experimental group	No. of animals	Atelectasis			Oedema			Hemorrhage			Microthrombi	Hyaline membrane
		*	**	***	*	**	***	*	**	***		
Non-Asphyxiated												
Group 1	26	5	2	1	3	2	0	1	0	0	0	0
Group 2[a]	25	3	4	0	4	1	0	1	1	0	0	0
Asphyxiated												
Group 3	20	11	6	2	9	5	1	7	2	0	0	0
Group 4[a]	20	3	7	9	10	5	4	9	2	0	3	6

Histological findings of newborn surviving more than 2 h are graded as slight (*), moderate (**) and severe (***)
Number of animals with histological changes of varying severity is shown in Table 1

[a] Treated with t-AMCHA

periods of these 6 varied from 2.5 to 6.5 h. Three cases had been delivered at 28-day gestation and 3 at 29 days. Hyaline membrane covered the dilated alveolar ducts, alveoli and occasionally the respiratory bronchioles (Fig. 1), often associated with gradual transition to intra-alveolar protein-rich oedema-fluid (Fig. 2). Microthrombi, consisting mainly of aggregated platelets, were recognized in the small arteries and arterioles of 3 animals in group 4 and not in other groups. In only 1 animal were microthrombi associated with the formation of hyaline membrane.

Electron Microscopic Study

Non-Asphyxiated Groups (Group 1 and 2). There were no significant differences between lungs exposed to t-AMCHA and those not treated in this way. Development was well advanced at 28 and 29 days. In type II pneumocytes a Golgi complex and multivesicular bodies, some of which occasionally fused small lamellar bodies, were frequently noted, and many mature lamellar bodies were observed. Tight junctions (zonulae occuludentes) were recognized at the luminal side of intercellular junction, between the alveolar epithelial cells (Fig. 3). Alveoli of newborn rabbits 2 h after birth frequently contained myelinlike figures.

Asphyxiated Groups (Group 3 and 4). Immediately after birth a decrease in number and in size of lamellar bodies was notable in type II pneumocytes with occasional dilatation of endoplasmic reticulum. The interstitium was mildly edematous. No significant morphological alteration was observed in type I pneumocytes or the endothelial cells of capillaries.

There was widespread atelectasis. Alveolar septae were thick, and type I pneumocytes and endothelial cells of capillaries were less distended.

The hyaline membrane in group 4 consisted of granular matrix, apparently arising from inspissated oedema-fluid, and various amounts of cellular debris (Figs. 4, 5, 6 and 7). Fibrils with 230 Å periodicity, most of which corresponded

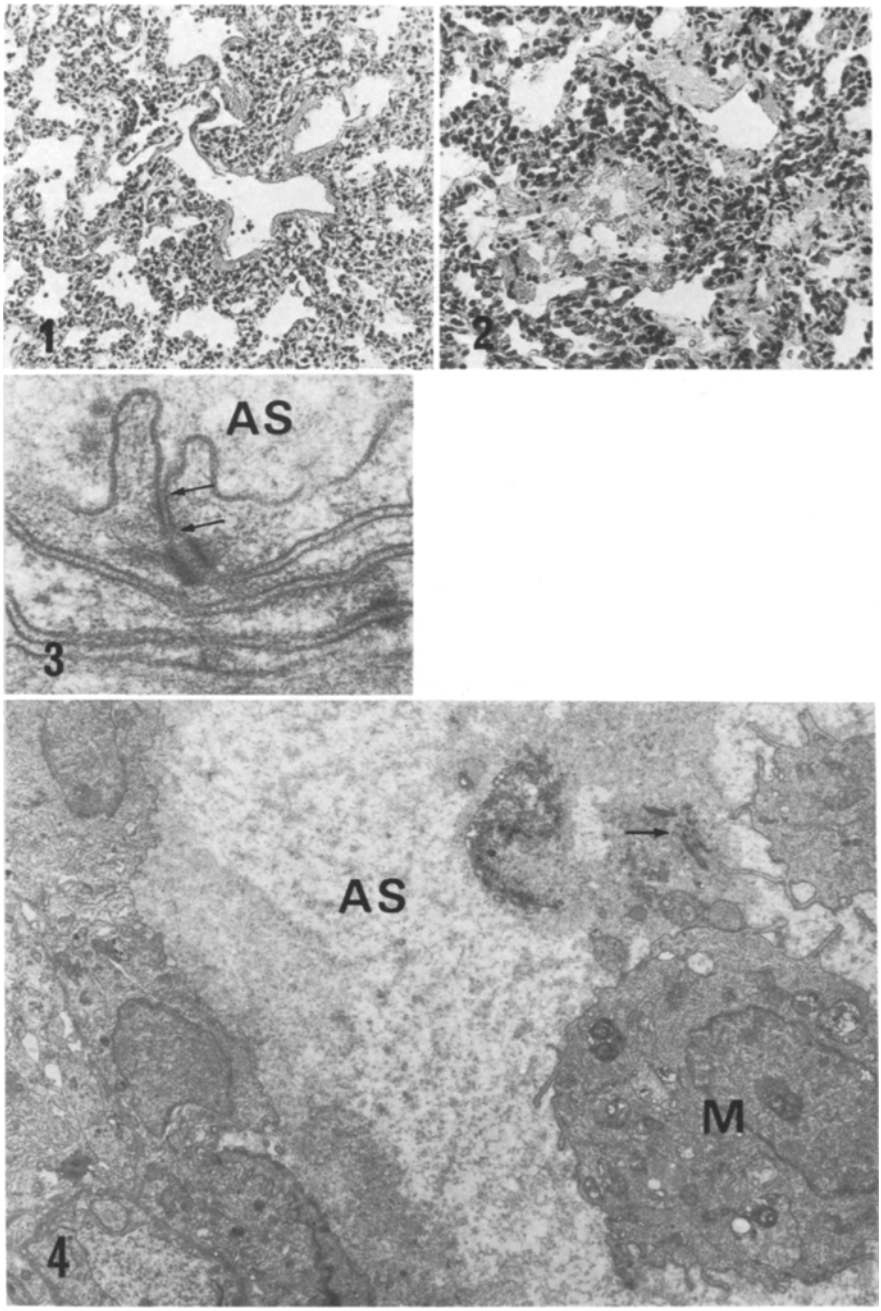

Fig. 1. Lung from a newborn rabbit (29-day gestation) exposed to intrauterine hypoxia and t-AM-CHA, dying in severe respiratory distress 2,5 h after birth. Dilated alveolar ducts and alveoli are covered with well developed hyaline membrane containing nuclear debris. H.E. × 140

Fig. 2. Lung from a newborn rabbit (28-day gestation) exposed to intrauterine hypoxia and t-AM-CHA, sacrificed 3,5 h after birth. Hyaline membrane shows gradual transition to proteinaceous oedema-fluid. H.E. × 260

Fig. 3. Lung from a newborn rabbit (29-day gestation) exposed to intrauterine hypoxia. An intercellular junction between type I pneumocytes is sealed by a tight junction (between arrows) at the luminal side. A desmosome is also seen. *AS:* alveolar space. × 41,600

Fig. 4. Lung from a newborn rabbit (29-day gestation) exposed to intrauterine hypoxia and t-AM-CHA, sacrificed 6 h after birth. Hyaline membrane consists of granular matrix, which seems to arise from inspissated oedema-fluid, containing cellular debris and fibrin (*arrow*). Infiltration of macrophage (*M*) is also seen. AS: alveolar space. × 7560

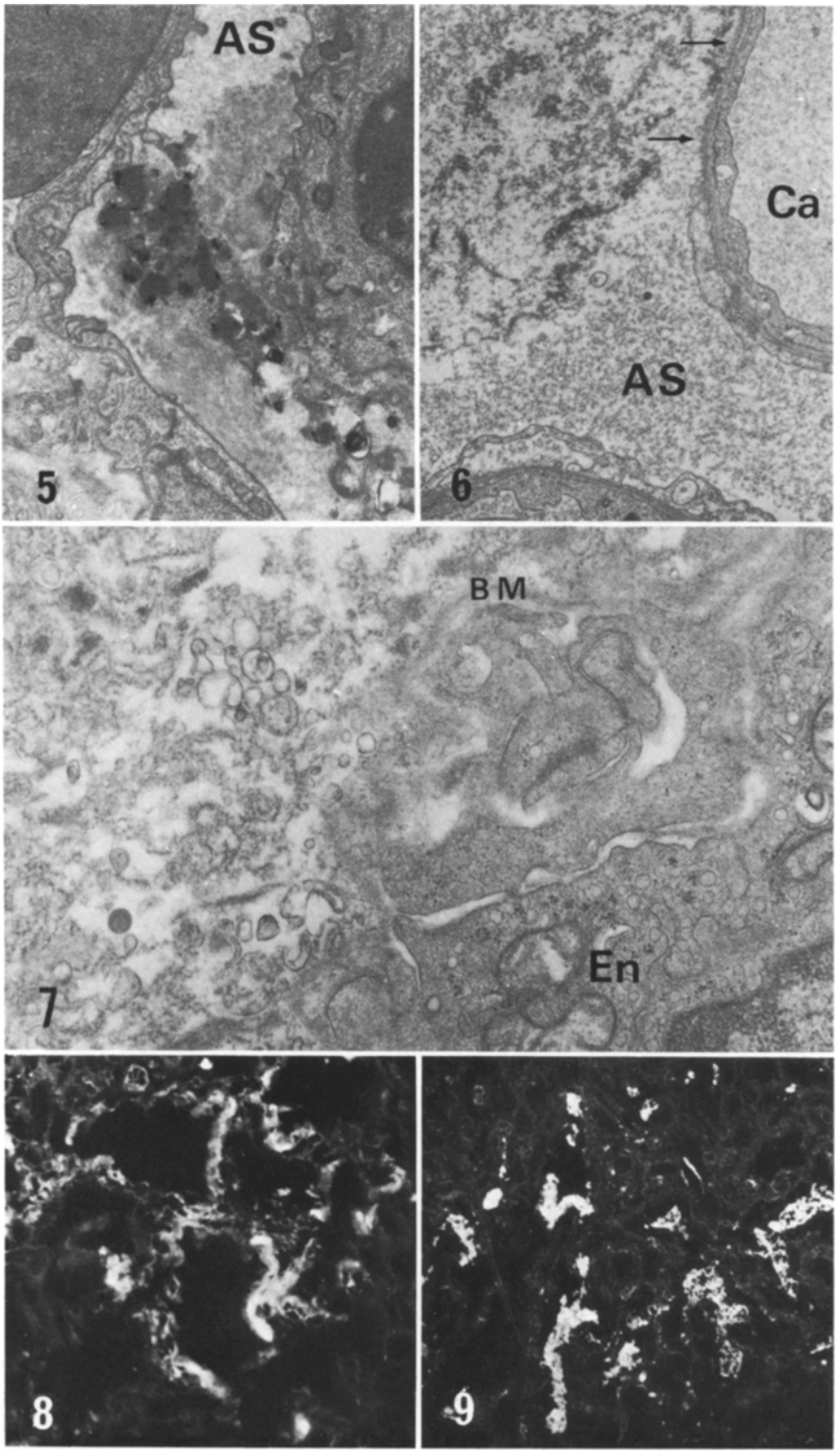
AS
5
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Table 2. Surface tension of lung extracts and average number of osmiophilic inclusion bodies per type II pneumocyte in newborn rabbits

	No. of samples	Surface tension		E.A.I.	Number of inclusions per type II pneumocyte
		γ_{min}	γ_{max}		
Group 1	3	7.2±2.1	47.1±2.8	1.47±0.11	5.8±0.78 $n=300$
Group 3	6	18.1±4.5	41.0±5.1	0.78±0.26	2.3±0.38 $n=300$
Group 4					
with widespread atelectasis	4	22.0±1.6	44.5±5.5	0.68±0.16	3.1±1.10 $n=200$
with hyaline membrane	1	25.4	45.6	0.57	2.8±0.96 $n=200$
					Mean±S.D.

γ_{min} = lowest surface tension (dynes/cm)
γ_{max} = highest surface tension (dynes/cm)
E.A.I. $= \frac{2(\gamma_{max}-\gamma_{min})}{\gamma_{max}+\gamma_{min}}$ (Gruenwald et al., 1962)
n = number of type II pneumocytes observed under electron microscope
Newborns in group 1 and 3 were sacrificed immediately after cesarean section
Each sample of the lung extracts is obtained from lungs of 2 or 3 newborns in each group

to polymerized fibrin, formed a small component of the membrane. Bundles of fibrils and electron-dense, finely granular deposits were often seen in hyaline membrane. Alveolar epithelial cells, especially type I pneumocytes, had apparently disintegrated under the protein-rich oedema-fluid or hyaline membrane. In these areas, the denuded subepithelial basement membrane was directly covered with oedema-fluid or hyaline membrane (Figs. 6 and 7).

The average number of inclusion bodies per type II pneumocyte was 5.8 in the lungs of group I. Further reduction to 3.1 in the lungs with widespread atelectasis, to 2.8 in those lungs with hyaline membrane in group 4, and to 2.3 in the lungs in group 3 was found (Table 2).

Fig. 5. Lung from the same rabbit as in Figure 4. In the alveolar space (*AS*), bundles of fibrils and finely granular, electron-dense deposits are seen. ×13,250

Fig. 6. Lung from the same rabbit as in Figure 2. In the alveolar space (*AS*), accumulation of oedema-fluid containing bundles of fibrils is seen, associated with lifting of type I pneumocyte from the subepithelial basement membrane and denudation of the subepithelial basement membrane with disappearance of a type I pneumocyte (*arrows*). *Ca:* capillary lumen. ×11,000

Fig. 7. Lung from the same rabbit as in Figures 2 and 6. The denuded subepithelial basement membrane (*BM*) is covered directly by hyaline membrane consisting of granular and fibrillar matrix and cellular debris. *En:* endothelial cell. ×26,000

Fig. 8. Immunohistochemical examination using FITC conjugated antirabbit fibrinogen goat IgG. A specific fluorescence is seen in association with a hyaline membrane. ×470

Fig. 9. Immunohistochemical examination using FITC conjugated fibrinogen goat IgG. A specific fluorescence is noted in intra-alveolar oedema-fluid, showing a net-like appearance, and revealing gradual transition to the appearances of hyaline membrane. ×280

Table 3. Fibrinolytic activity of lung extracts from untreated or treated newborn rabbits from immediately after birth to 8 h after birth

Survival hours after birth	0[a]	0–2[a]	2–4[a]	4–8[a]
Experimental group				
Group 1	342±50	377±25	430±25	477±34
Group 2	98±11***	104±10***	95±11***	83±13***
Group 3	465±60	428±43	439±41	475±40
Group 4	138±16**	157±31***	187±40***	276±53*
			Mean±S.E.	

The difference between value of group 1 and those of other groups is significant from student's *t*-test, * $P<0.02$, ** $P<0.01$ and *** $P<0.001$. Fibrinolytic activity is expressed as trypsin units

[a] Number of samples in each group is ten

Immunohistochemical Study

Using FITC-antirabbit fibrinogen goat IgG, bright fluorescence was observed in association with the membrane, in a granular or fibrillar pattern (Fig. 8). Fluorescence was occasionally seen in oedema-fluid (Fig. 9). Control sections showed no significant positivity, confirming immunologic specificity.

Measurement of Surface Tension

As shown in Table 2, the lowest surface tension of newborn lung extracts in asphyxiated groups (group 3 and 4) was abnormal (less than 10 dynes per cm). Values of E.A.I. in these groups were less than 0.8. From these findings it is apparent that there is a decrease in surface activity in the lungs of asphyxiated groups, especially in the lungs with hyaline membrane.

Measurement of Tissue Fibrinolytic Activity

The results of an assay of fibrinolytic activity of 40 newborn lung extracts in each group is recorded in Table 3. Treatment with t-AMCHA (group 2 and 4) apparently reduced fibrinolytic activity of lung extracts. Lungs of asphyxiated groups (group 3 and 4) showed a tendency to higher fibrinolytic activity than those of non-asphyxiated groups. The six lungs with hyaline membrane showed considerable variation in values from 52 to 634 TU. The mean value ± standard error was 200±71.

Discussion

Hyaline membrane has been produced experimentally in prematurely delivered lambs (Reynolds et al., 1965; Stahlman et al., 1964) and monkeys (McAdams

et al., 1973) but not in rabbits. In this study, formation of hyaline membrane was found in mature newborn rabbits (28- or 28-day gestation) that were exposed to intrauterine hypoxia and injected with t-AMCHA.

Several investigations have suggested that impairment of the fibrinolysis system is a contibutory factor in the pathogenesis of HMD. Lieberman (1969) reported a deficiency of pulmonary plasminogen activator in HMD. Ambrus et al. (1971) suggested that as premature infants lacked serum plasminogen, they were unable to develop effective fibrinolysis of intra-alveolar fibrin deposition in HMD. Ambrus et al. have tried to treat newborns with urokinase-activated human plasmin (1971) or human plasminogen (1974) as preventive therapy for HMD. Evans et al. (1972) recognized a possible relationship between decreased levels of α_1-antitrypsin and IRDS, and Mandle et al. (1973) showed a immunohistochemical correlation between hyaline membrane formation and accumulation of α_1-antitrypsin in lungs.

In this study, all newborn rabbits with hyaline membrane were treated with t-AMCHA, an antiplasmin. Treatment with t-AMCHA brought about marked reduction of fibrinolytic activity of lung extracts. Diminished fibrinolytic activity enhanced intra-alveolar accumulation of both fibrinogen and fibrin, followed by necrosis of alveolar epithelial cells. As the major permeability barrier of alveolar walls seems to lie in the alveolar epithelium (Schneeberger and Karnovsky, 1971), its disintegration indicates a marked increase in permeability through the alveolar wall. These events established a vicious cycle, often culminating in death. Thus this study indicates that a diminished fibrinolytic activity of lung tissue might play an important role in the development and progression of HMD by inhibiting fibrinolysis in the alveolar exudate.

In human HMD, plasmatic hypercoagulability has been found (Bleyl et al., 1969; Mahasandra and Hathaway, 1973; Remberger, 1973), possibly associated with increased fibrinolytic activity (Karitzky et al., 1970). Plasma hypercoagulability might cause intravascular polymerization of fibrin monomers and extravascular polymerization of these monomers to hyaline membrane (Bleyl et al., 1969). In this study, microthrombi were recognized in 1 animal with HMD and in 2 without HMD, in group 4. Plasma hypercoagulability, in addition to diminished fibrinolytic activity due to intravenous injection of t-AMCHA, might contribute to the development of HMD in this study. Further investigations should be carried out in order to clarify the role of coagulation and fibrinolysis in HMD.

Asphyxiated rabbits showed a significant decrease in surface activity, which was well correlated with diminution in the number of osmiophilic inclusion bodies in each type II pneumocyte. Since Avery and Mead (1959) demonstrated pulmonary surfactant deficiency in the newborn in HMD, many investigations have supported the hypothesis that pulmonary surfactant deficiency is a pathognomonic factor in the development of IRDS (Reynolds et al., 1965; Gluck et al., 1972; Farrell and Avery, 1975) and this has resulted in distinct approaches to both diagnosis and preventive therapy. Surface active lecithin synthesis in the rabbit fetus is dependent upon a CDP-choline pathway which becomes active at the 27–28 day of the 30-day gestation (Gluck et al., 1972). Asphyxiated rabbits in this study showed pulmonary surfactant deficiency. Intrauterine hyp-

oxia might depress synthesis of surface active lecithin via the CDP-choline pathway (Gluck et al., 1972), and for a critical evaluation of hypoxic and/or an acidotic effect on the synthesis of surfactant, a further investigation must be performed. In addition, the secreted surfactant in alveoli might be inactivated by fibrinogen (Taylor and Abrams, 1966) or degradation products of fibrinogen and fibrin (Miyahara, 1969) present in the alveolar exudate.

The immunohistochemical and electron microscopical examination of hyaline membrane revealed similar findings to those of the previous reports on HMD in human beings (Gitlin and Craig, 1956; Lauweryns, 1970; Askin, 1975) and experimental animals (Stahlman et al., 1964; Kikkawa et al., 1965). Immunohistochemical examination showed that hyaline membrane was rich in fibrinogen and its derivatives but that strands with periodicity of 230 Å, mainly corresponding to polymerized fibrin, were a small component of hyaline membrane. In view of the fact that fibrillar bundles and finely granular, electron-dense deposits were frequently observed, it is suggested that hyaline membrane does not contain much polymerized fibrin, but fibrinogen, non-polymerized fibrin or fibrinogen-fibrin complexes, and their degradation products.

References

Ambrus, C.M., Ambrus, J.L., Weintraub, D.H., Foote, R.J., Courey, N.G., Niswander, K.R.: Thrombolytic therapy in hyaline membrane disease. Thromb. Haemorrh. **47**, 269–282 (1971)

Ambrus, C.M., Weintraub, D.H., Choi, T.S., Eisenberg, B., Staub, H.P., Courey, N.G., Foote, R.J., Goplerud, D., Moesch, R.V., Ray, M., Bross, I.D.J., Jung, O.S., Mink, I.B., Ambrus, J.L.: Plasminogen in prevention of hyaline membrane disease. Am. J. Dis. Child. **127**, 189–194 (1974)

Askin, F.B.: Neonatal respiratory distress syndrome (hyaline membrane disease). In: Pathology of infancy and childhood, ed. by Kissane, J.M., 2nd ed. pp. 486–495. St. Louis: Mosby 1974

Astrup, T., Albrechtsen, O.K.: Estimation of the plasminogen activation and the trypsin inhibitor in animal and human tissues. Scand. J. Clin. Lab. Invest. **9**, 233–243 (1957)

Avery, M.E., Mead, J.: Surface properties in relation to atelectasis and hyaline membrane disease. Am. J. Dis. Child. **97**, 517–523 (1959)

Bleyl, U., Büsing, C.M., Krempien, B.: Pulmonale hyaline Membranen und perinataler Kreislaufschock. Virchows Arch. Abt. A Path. Anat. **348**, 187–204 (1969)

Brown, E.S., Johnson, R.P., Clements, J.A.: Pulmonary Surface tension. J. Appl. Physiol. **14**, 717–720 (1959)

Chu, J., Clements, J.A., Cotton, E., Klaus, M.H., Sweet, A.Y., Thomas, M.A., Tooley, W.H.: The pulmonary hypoperfusion syndrome. Pediat. **35**, 733–742 (1965)

Clark, H.F., Shepard, C.C.: A dialysis technique for preparing fluorescent antibody. Virology **20**, 642–644 (1963)

Evans, H.E., Keller, S., Mandle, I.: Serum trypsin inhibitory capacity and the idiopathic respiratory distress syndrome. J. Pediat. **81**, 588–592 (1972)

Farrell, P.M., Avery, M.E.: Hyaline membrane disease. Am. Rev. Resp. Dis. **111**, 657–688 (1975)

Gitlin, D., Craig, J.M.: Nature of the hyaline membrane in asphyxia of the newborn. Pediat. **17**, 64–71 (1956)

Gluck, L., Kulovich, M.V., Eidelman, A.I., Cordero, L., Khazin, A.F.: Biochemical development of surface activity in mammalian lung. IV. Pulmonary lecithin synthesis in the human fetus and newborn and etiology of the respiratory distress syndrome. Pediat. Res. **6**, 81–99 (1972)

Gruenwald, P., Johnson, R.P., Hustead, R.F., Clements, J.A.: Correlation of mechanical properties of infant lungs with surface activity of extracts. Proc. Soc. Exp. Biol. Med. **109**, 369–371 (1962)

Karitzky, D., Pringsheim, W., Künzer, W.: Fibrinogen and fibrinolysis in the respiratory distress syndrome: Observation during the first day of life. Acta Paediat. scand. **59**, 281–287 (1970)

Kikkawa, Y., Motoyama, E.K., Cook, C.D.: The ultrastructure of the lungs of lambs. The relation of osmiophilic inclusions and alveolar lining layer to fetal maturation and experimentally produced respiratory dustress syndrome. Am. J. Path. **47**, 877–903 (1965)

Kohga, S.: Plasminogen tissue activator and its clinicopathological significance. Jap. J. Clin. Path. **22**, 552–558 (1974)

Lauweryns, J.M.: "Hyaline membrane disease" in newborn infants. Macroscopic, radiographic, and light and electron microscopic studies. Human Path. **1**, 175–204 (1970)

Lieberman, J.: Pulmonary plasminogen activator activity in hyaline membrane disease. Pediat. Res. **3**, 11–18 (1967)

Mahasandra, C., Hathaway, W.E.: Circulating anticoagulants in the newborn: Relation to hypercoagulability and the idiopathic respiratory distress syndrome. Ped. Res. **7**, 670–673 (1973)

Mandle, I., Koller, S., Fierer, J., Evans, H.: The role of proteolytic enzyme inhibitors and connective tissue proteins in the maturation of the lung. In: Respiratory Distress Syndrome, ed. by Villee, C.A., Villee, D.B., Zuckerman, J., pp. 99–115. New York and London: Academic Press 1973

McAdams, A.J., Coen, R., Kleinman, L.I., Tsang, R., Sutherland, J.: The experimental production of hyaline membranes in premature rhesus monkeys. Am. J. Path. **70**, 277–290 (1973)

Miyahara, T.: A study of the pathogenesis of hyaline membrane disease in the newborn infants. Fukuoka Acta Medica **60**, 95–125 (1969)

Remberger, K.: Immunofluorescence studies on intravascular coagulation and microthrombosis in the perinatal period and their relation to hyaline membrane disease. Beitr. Path. **150**, 70–75 (1973)

Reynolds, E.O.R., Jacobson, H.N., Motoyama, E.K., Kikkawa, Y., Craig, J.M., Orzalesi, M.M., Cook, C.D.: The effect of immaturity and prenatal asphyxia on the lungs and pulmonary function of newborn lambs: The experimental production of respiratory distress. Pediat. **35**, 382–392 (1965)

Sandberg, H., Rezai, M., Bangayan, T.T., Bellet, S., Feinberg, L.J., Hunter, S.: Organ distribution of fibrinolytic activity in man. J. Lab. Clin. Med. **61**, 592–603 (1963)

Schneeberger, E.E., Karnovsky, M.J.: The influence of intravascular fluid volume on the permeability of newborn and adult mouse lungs to ultrastructural protein tracers. J. Cell Biol. **49**, 319–334 (1971)

Stahlman, M., LeQuire, V.S., Young, W.C., Merrill, R.E., Birmingham, R.T., Payne, G.A., Gray, J.: Pathophysiology of respiratory distress in newborn lambs. Am. J. Dis. Child. **108**, 375–393 (1964)

Stahlman, M., Blankenship, W.J., Shepard, F.M., Gray, J., Young, W.C., Malan, A.F.: Circulatory studies in clinical hyaline membrane disease. Biol. Neonate **20**, 300–320 (1972)

Taylor, F.B., Abrams, M.E.: Effect of surface active lipoprotein on clotting and fibrinolysis, and of fibrinogen on surface tension of surface active lipoprotein. With a hypothesis on the pathogenesis of pulmonary atelectasis and hyaline membrane in respiratory distress syndrome of the newborn. Am. J. Med. **40**, 346–350 (1966)

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