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Intralipid Increases Lung Polyunsaturated Fatty Acids and Protects Newborn Rats from Oxygen Toxicity

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ABSTRACT. Intralipid, derived from soybean oil and containing a high percentage of n-6 family polyunsaturated fatty acids (PUFA) and also linolenic acid, an n-3 family PUFA, is commonly the first fat source provided to very low birth weight premature infants. Following up on our previous reports that newborn rats born to dams fed high-PUFA diets demonstrate superior tolerance to hyperoxia, we examined whether the high-PUFA fat source Intralipid might also protect against oxygen toxicity. Adult female rats were fed either regular Rat Chow or fat-free diet containing 20%-Intralipid as the fat source for 3 wk before and then throughout pregnancy and lactation. One- and 5-d-old offspring of Intralipid diet-fed dams demonstrated significant increases in lung lipid n-6 family PUFA plus elevated linolenic acid compared with regular diet-fed offspring. These characteristic fatty acid patterns, apparent in total lung lipids, were even more pronounced in the triglyceride fraction compared with the phospholipid fraction. Associated with these fatty acid changes were significantly improved hyperoxic survival rates (89 out of 95 = 94% survival after 7 d of >95% O_2 exposure) in Intralipid offspring (*versus* 89 out of 106 = 84%, $p < 0.05$ in regular diet offspring) and evidence of superior clinical/pathologic status. No differences in pulmonary antioxidant enzyme or surfactant system development, response of antioxidant enzymes to hyperoxic exposure, or lung prostaglandin E_2, 6-keto PGF_1-α or leukotrienes C_4–F_4 were present. These findings continue to support the hypothesis that increasing lung PUFA content may provide increased O_2 free radical scavenging capacity, thus protecting against hyperoxic lung damage. The results also suggest a role for Intralipid administration in protecting the lungs of high oxygen-exposed very low birth weight premature infants. (*Pediatr Res* 30: 413–417, 1991)

Abbreviations

PUFA, polyunsaturated fatty acids
VLBW, very low birth weight
SOD, superoxide dismutase
CAT, catalase
GP, glutathione peroxidase
DSPC, disaturated phosphatidylcholine
PGE_2, prostaglandin E_2
6-keto-PGF_1-α, 6-keto-prostaglandin F_1-α
LTC_4–F_4, leukotrienes C_4–F_4
BPD, bronchopulmonary dysplasia
P/S, polyunsaturated/saturated fatty acid ratio

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With the progression of modern neonatal intensive care, greater numbers of VLBW oxygen- and ventilator-requiring premature infants are surviving. However, despite favorable outcomes in terms of mortality, these infants are at extremely high risk of developing the chronic lung process known as BPD, considered to be related, at least in part, to the damaging effect of oxygen on the lung (1). Because of the overwhelming clinical problems that these VLBW premature infants experience in the first several days of life, nutritional needs are frequently not the highest priority in their care. In fact, common nursery practices consist of a several-day interval between the birth of a VLBW oxygen-requiring premature infant and the initial administration of lipid nutrition. Because of the vulnerability of the VLBW premature infant to the effects of a delay in lipid nutrition (2) and the possible role that lipids might play in protecting the lung from oxygen toxicity, the early withholding of lipid nutrition from these critically ill neonates might increase their risk of developing the chronic lung disease and damage of BPD.

Evidence is available from the literature to suggest that lung lipid composition is related to hyperoxic tolerance and protection against hyperoxic lung damage in experimental animals. We have performed a series of studies in newborn rats that has demonstrated that lipid nutrition, containing high concentrations of PUFA, confers protection against pulmonary oxygen toxicity. Specifically, newborn rat offspring of dams fed diets high in PUFA demonstrated elevated concentrations of PUFA in their lung lipids and significantly improved survival in hyperoxia compared with offspring of dams fed regular Rat Chow; conversely, newborn offspring of dams fed low PUFA, high saturated fatty acid diets were found to be most susceptible to pulmonary oxygen toxicity (3, 4). The improved hyperoxic tolerance of newborn rats was found to be more or less constant with qualitatively different high PUFA diets (*i.e.* high n-6 family PUFA diet containing safflower oil and high n-3 family PUFA diet containing menhaden fish oil). With these studies as background and in an attempt to use the newborn experimental animal to examine a common nutritional intervention of the human premature infant, we have now tested whether a high-PUFA diet based on Intralipid (the commercially available initial fat preparation administered to premature infants, containing high n-6 family PUFA but also considerable amounts of the n-3 fatty acid, linolenic acid) would produce substantial increases in

lung PUFA content and provide a meaningful degree of protection against O_2 toxicity in newborn rats.

MATERIALS AND METHODS

Animals and diets. Adult Sprague-Dawley albino female rats (~200 g) were fed one of two diets *ad libitum*: *1*) standard Rat Chow (no. 5001; Ralston-Purina, Co., St. Louis, MO) [5% fat as a mixture of animal and vegetable fats, containing 27% linoleic acid (18:2n6), 3% linolenic acid (18:3n3), P/S = 1.0]; or *2*) Intralipid diet ["fat-free" test diet no. 15750; United States Biochemical Corp., Cleveland, OH, plus 10% lipid (by weight), using 20%-Intralipid (KabiVitrum, Inc., Alameda, CA) as the fat source, containing 50% linoleic acid (18:2n6), 9% linolenic acid (18:3n3), P/S = 4.4]. The two diets were essentially comparable in protein and carbohydrate content. Rats were fed the respective diets for at least 3 wk before breeding and then throughout pregnancy and lactation. Breeding was accomplished by placing male and female animals together overnight, checking for sperm-positive vaginal smears the following morning, and considering the midpoint of the cohabitation period as the onset of pregnancy. Newborn rats were nursed exclusively, thereby receiving rat milk from respective diet-fed dams.

Several litters from the two diet groups were killed prematurely at 20 or 21 d of gestation, after term delivery at 22 d of gestation, or at 1 or 5 d of postnatal age. Premature pups were delivered by hysterotomy under pentobarbital anesthesia; newborn rats were obtained after normal parturition (either within 6 h of delivery of the first pup or after 24 h or 5 d of life). Fetal, newborn, 1- or 5-d-old rat pups were killed with an overdose of pentobarbital, and their lungs were perfused immediately *in situ* via the pulmonary artery using cold saline. The perfused fetal and newborn lungs were removed, stripped of nonpulmonary tissue, and homogenized in 20–30 times their weight of cold saline in a Brinkmann polytron (high speed, 90 s) (Brinkmann Instruments, Inc., Westbury, NY). For preterm and term newborns, two to four lungs were pooled and homogenized to obtain adequate tissue for the antioxidant enzyme and phospholipid analyses. One-d-old and 5-d-old rat pup lungs were utilized for fatty acid analyses (as described below).

Antioxidant enzyme, phospholipid, and fatty acid analyses. Aliquots of lung homogenate were analyzed for SOD (5), CAT (6), and GP (7) activities and for DNA (8) and protein (9) content using standard spectrophotometric techniques. Purified standards for SOD, CAT, and DNA assays were obtained from Sigma Chemical (St. Louis, MO) and GP was obtained from Boehringer-Mannheim (Houston, TX). Results of antioxidant enzyme analyses are expressed as activity units/mg DNA (and also calculated per mg protein and per g lung). For analyses of lung tissue DSPC content, separate aliquots of lung homogenates were lipid extracted using the method of Bligh and Dyer (10), reacted with osmium tetroxide according to Mason (11), and assayed for inorganic phosphorus as described by Morrison (12). A known quantity of ^{14}C-dipalmitoyl-phosphatidylcholine (New England Nuclear, Boston, MA) was added before lipid extraction, and aliquots were counted at each step to estimate and correct for sequential losses. DSPC was expressed as mg/g lung weight.

Lungs from 1-d-old and 5-d-old rat offspring from both diet groups were perfused with iced saline as described above, and then frozen in liquid nitrogen in preparation for fatty acid analyses. The lung tissue was subsequently homogenized, aliquoted for protein [assayed according to the method of Lowry (13)], and then subjected to lipid extraction using the procedure of Folch (14). Total fatty acids were converted to their respective methyl esters with methanolic HCl and separated and quantitated by capillary column gas-liquid chromatography (15). C17:0 was used as the internal standard. The fatty acid composition of phospholipid and triglyceride lipid subclasses was determined after separation of phospholipids from the other lipid classes by plate chromatography (16).

Exposure to hyperoxia. Multiple litters (nine to 11 pups/litter) of newborn rats from the two diet groups were placed with their dams into >95% O_2 (or room air) at 6–12 h of life. Details of the continuous 7-d exposure conditions have been previously reported (3). Dams from the same diet group were rotated between room air and >95% O_2-exposed litters every 24 h to prevent poor mothering resulting from O_2-induced maternal illness. Weight gain and hyperoxic survival of newborn rats from each diet group were recorded daily for 7 d. In addition, after an exposure period of 7 d, random surviving offspring of both diet groups were removed from hyperoxia and room air-exposed litters, were killed, and their lungs then perfused, homogenized, and assayed for DSPC and antioxidant enzyme activities as described above. Using separate litters of >95% O_2-exposed newborns from the two diet groups, we evaluated the clinicopathologic status after 7 d of hyperoxic exposure. Pups were scored in a blinded fashion by two investigators, and received a score of 0 (normal), 0.5, 1.0 (moderately abnormal), 1.5, or 2 (markedly abnormal) on each of five parameters of O_2 toxicity: degree of respiratory distress, pleural fluid quantitation, and gross appearance of lung edema, atelectasis, and lung hemorrhage. Potential range of total clinicopathologic score was 0 to 10.

In a separate experiment, offspring from the two diet groups were killed after 5 d of either room air or >95% O_2 exposure, and their lungs were removed and homogenized as described above, with the homogenizing buffer containing acetylsalicylic acid (15 mM). Fresh homogenates were assayed for 6-keto-PGF_1-α, PGE_2, and LTC_4–F_4 using specific RIA test kits (Seragen, Inc., Cambridge, MA).

Statistics. Statistics were performed using χ^2 analysis for survival data and unpaired t test for fatty acid, antioxidant enzyme, disaturated phosphatidylcholine, and clinicopathologic data. When multiple t tests were performed (*i.e.* with fatty acid, antioxidant enzyme, and DSPC data), a Bonferoni-type correction was made, allowing as significant a p value of less than 0.01 (17).

RESULTS

Growth and biochemical development of the offspring of the two diets. The Intralipid-based diet was well accepted by the female rats. Maternal weight, fetal weight and number, and neonatal growth were not different between the Intralipid experimental diet group and the group fed regular Rat Chow.

Antioxidant enzymes, surfactant, and fatty acid analyses. The comparative late gestational development of the pulmonary antioxidant enzyme and surfactant systems in the experimental Intralipid diet and regular diet fetuses and newborns revealed no significant differences between the two diet groups in SOD, CAT, or GP activities or in lung DSPC content during gestational d 20–22, the final 3 d of gestation.

Table 1 shows the major fatty acid components of total lung lipid in 1-d and 5-d-old offspring of the Intralipid diet and regular diet groups. Significant elevations in linoleic acid (18:2n6), linolenic acid (18:3n3), and total n-6 family PUFA were present in the 1-d-old Intralipid group and, by 5 d of age, most of these fatty acid differences were more pronounced and included an unexpected significant decrease in arachidonic acid (20:4n6) and in total n-3 family PUFA in the Intralipid offspring.

Analysis of the fatty acid composition of the triglyceride fraction and the phospholipid fraction of total lung lipid in 1-d and 5-d-old offspring of the two diet groups can be seen in Table 2. In the lungs of the 1-d-old offspring, those of the Intralipid group demonstrated PUFA elevations in the two lipid fractions compared with the regular diet group. However, the fatty acid composition of the lung triglyceride fraction in 1-d-old Intralipid offspring *versus* regular offspring demonstrated more marked elevations in 18:2n6, 20:4n6, total n-6 family PUFA, and total PUFA compared with the fatty acid differences seen in the phospholipid fraction. By 5 d of life, the elevations in 18:2n6,

Table 1. *Major fatty acid components of total lung lipid in 1- and 5-d-old offspring of two diet groups**

	16:0	18:2n6	18:3n3	20:4n6	Total PUFA	Total n − 6	Total n − 3	P/S
1-d								
Intralipid	39	8†	0.25†	11	30	24†	5	0.6
Regular	39	5	0.09	10	27	19	7	0.5
5-d								
Intralipid	35	16†	0.64†	7†	34	29	5†	0.7
Regular	29	7	0.11	13	34	25	8	0.7

* Values are expressed as % of total and are means; n = 4–8 lung samples/group. Fatty acids: 16:0, palmitic acid; 18:2n6, linoleic acid; 18:3n3, linolenic acid; 20:4n6, arachidonic acid.

† $p < 0.01$ or less between diet groups.

Table 2. *Major fatty acid components of triglyceride and phospholipid fractions of lung lipid in 1- and 5-d-old offspring of diet groups**

	Triglyceride		Phospholipid	
	Intralipid	Regular	Intralipid	Regular
1-d-old				
18:2n6	15†	10	8†	6
18:3n3	0.51	0.37	0.34†	0.17
20:4n6	6†	4	14	13
Total PUFA	38†	29	32	28
Total n − 6	30†	20	26	21
Total n − 3	6	8	6	7
P/S	1.0	0.7	0.6	0.5
5-d-old				
18:2n6	27†	12	11†	7
18:3n3	1.3†	0.4	0.4†	0.2
20:4n6	6†	4	16	18
Total PUFA	51†	41	37	38
Total n − 6	41†	26	31	30
Total n − 3	8†	13	5†	8
P/S	1.8†	1.2	0.8	0.9

* Values are % of total and are means; n = 4–8 lung samples/group.

† $p < 0.01$ or less between diet groups.

18:3n3, total n-6 family PUFA, total PUFA, and P/S and the depression in total n-3 family PUFA in the Intralipid *versus* regular groups had become even more pronounced in the triglyceride compared with the phospholipid fraction of lung lipids.

Exposure to hyperoxia. Offspring of Intralipid diet dams demonstrated significantly superior survival (89 out of 95 = 94%) during 7 d of >95% O_2 exposure compared with regular diet offspring (89 out of 106 = 84%), $p < 0.05$. In addition, Intralipid offspring showed evidence of superior clinical/pathologic status after 7 d of hyperoxic exposure (Table 3), with an average clinicopathologic score of 1.56 ± 0.14 compared with an average score of 2.55 ± 0.25, for regular diet offspring exposed to hyperoxia for 7 d ($p < 0.05$). [Note that the high survival rate and relatively low clinical/pathologic score in regular diet newborns is a reflection of the innate relative O_2 tolerance of neonatal *versus* adult animals. For adults in >95% O_2, the comparable survival rate would be ~10–20% and the clinical/pathologic score in the survivors ~7–9 (18)].

The improved 7-d hyperoxic survival in Intralipid offspring was not associated with a significantly improved capacity to mount a protective antioxidant enzyme response to hyperoxia compared with regular diet offspring (Table 4).

Lung tissue levels of PGE_2, 6-keto-PGF_1-α, and LTC_4–F_4 in 5-d-old offspring continuously exposed to either room air or >95% O_2 since birth are shown in Table 5. No significant differences were found in baseline (room air) levels of these eicosanoids, nor in levels after 5 d of hyperoxic exposure in the two diet groups.

DISCUSSION

Intralipid is a commercial fat emulsion designed for i.v. administration. It is derived primarily from soybean oil, with the addition of glycerin and egg yolk phospholipids as emulsifiers. This fat preparation (or its equivalent from other commercial sources), containing a generous amount of the n-6 PUFA linoleic acid (50%) and the n-3 PUFA linolenic acid (9%), represents the initial fat source provided to most VLBW premature infants to ensure adequate caloric intake and prevent essential fatty acid deficiency before the onset of oral feedings. As a logical follow-up to our previous nutritional studies (3, 4), we chose to examine in the newborn rat the effect of this common nutritional intervention in terms of influencing lung fatty acid patterns and tolerance to high oxygen exposure. We utilized Intralipid as the total fat source added to the fat-free experimental diet that was fed to rat dams before and throughout pregnancy and lactation [We had previously determined that rat milk reflects quite closely the fatty acid composition of the diet of the rat dam (3, 4)]. One-d-old offspring of dams fed the Intralipid diet demonstrated increased linoleic and linolenic PUFA in lung lipids; the 5-d-old offspring of Intralipid diet dams demonstrated more marked increases in lung PUFA than 1-d-old offspring, suggesting the importance not only of the intrauterine fatty acid milieu but also a substantial postnatal dietary influence as well. The most striking difference in lung PUFA enrichment in the Intralipid diet rat pups was noted in the triglyceride (nonmembrane) lipid fraction as opposed to the phospholipid (membrane-associated) lipid fraction.

The outcome of the present study using Intralipid is consistent with our previous findings indicating that increasing lung lipid PUFA (especially the triglyceride fraction) of newborn rat offspring is associated with significant protection against hyperoxic lethality and the pathologic effects of high oxygen exposure on the lung (3, 4). Whereas previous investigations examined saf-

Table 3. *Clinicopathological (CP) score of offspring of Intralipid and regular diet rats after 7 d in >95% oxygen**

Diet group	Clinical status	Pleural fluid	Edema	Hemorrhage	Atelectasis	Total CP score†
Intralipid	0.53	0.12	0.54	0.20	0.17	1.56 ± 0.14‡
Regular	0.27	0.35	0.75	0.35	0.82	2.55 ± 0.25

* Values are mean values for regular (n = 20) and intralipid (n = 30). Possible scores for each item: 0 = normal; 0.5, 1 = moderately abnormal; 1.5, 2 = markedly abnormal. Possible total score: 0–10.

† Mean ± SEM.

‡ $p < 0.05$ between diet groups.

Table 4. *Response of antioxidant enzymes and surfactant (DSPC) after exposure to 7 d >95% O_2 in Intralipid and regular diet rat pups**

	Activity units/mg DNA			DSPC (mg/g lung)
	SOD	CAT	GP	
Air-Intralipid diet	13.2 ± 0.8	238 ± 36	0.29 ± 0.07†	3.01 ± 0.17
O_2-Intralipid diet	18.9 ± 1.3	476 ± 81	0.98 ± 0.23	4.65 ± 0.29
	↑43%	↑100%	↑238%	↑55%
Air-regular diet	13.3 ± 1.0	262 ± 54	0.63 ± 0.11	3.74 ± 0.42
O_2-regular diet	17.4 ± 2.3	442 ± 45	1.40 ± 0.14	4.72 ± 1.92
	↑30%	↑69%	↑123%	↑26%

* Mean values ±1 SD and % change for O_2 group *vs* air group values after 7-d exposure to >95% O_2 in Intralipid and regular diet groups; n = 4 lung samples/group; all enzyme values after hyperoxia are significantly higher than air values in both diet groups.

† $p < 0.01$; baseline (air) value for GP is significantly lower in the Intralipid group; it is because of this that the response of GP to hyperoxia in the Intralipid group is significantly greater than hyperoxic response of GP in regular group.

flower oil (high n-6 PUFA oil) and menhaden fish oil (high n-3 PUFA oil), the present study using Intralipid examined the effect of a preparation high in both n-6 family PUFA (linoleic acid) and n-3 PUFA (linolenic acid). Thus, a third high-PUFA fat source has now been shown to confer hyperoxic protection of essentially similar magnitude as the other tested high-PUFA diets (*i.e.* 94% survival after 7 d of >95% O_2 for Intralipid *versus* 95% for safflower oil *versus* 91% for menhaden fish oil) compared with the 7-d hyperoxic survival rate for regular diet rats, which has ranged from 67 to 84%.

The present and previous studies from our laboratory are among several reported *in vivo* investigations linking unsaturation of lung lipids to improved hyperoxic tolerance (18–20). In addition, our present *in vivo* investigation is in agreement with the findings of a recent *in vitro* study using rabbit tracheal epithelial cells. These cells, when cultured in lipid-supplemented media, demonstrated increased PUFA content and increased hyperoxic viability (21).

The mechanism by which PUFA lipid enrichment works to confer hyperoxic protection on the lung is not fully known. Results from this study and previous ones do not indicate that elevated PUFA work through accentuating the development of the pulmonary antioxidant enzymes or surfactant production, nor through enhancing the ability of the newborn animal to mount a protective increase in pulmonary antioxidant enzymes in response to hyperoxic exposure. Other investigators have suggested a role for lung eicosanoids in the development of (or protection from) oxygen toxicity (22–25). In our present study, we were unable to demonstrate differences in PGE_2, 6-keto-PGF_{1}-α (the metabolite of prostacyclin), or in the LTC_4–F4 in lung tissue of either diet group either in air or hyperoxia (Table 5). In addition, in our previous studies, we found a similar degree of protection against pulmonary O_2 toxicity in newborn offspring of rat dams fed either a safflower oil-based or fish oil-based high-PUFA diet, even though the safflower diet pups demonstrated significantly elevated lung eicosanoids whereas the fish oil diet pups had significantly decreased lung eicosanoid levels compared with regular diet pups (3, 4). Our studies thus strongly suggest that eicosanoids do not appear to be playing a major role in the increase in hyperoxic tolerance found in the Intralipid offspring.

Having shed doubts on the role that antioxidant enzymes, surfactant, and lung eicosanoids might be playing in the hyperoxic protection of Intralipid offspring, results of our present (and previous) studies suggest that the increased PUFA content in lung tissue itself may, at least in part, be an explanation for the consistently observed increased oxygen tolerance in our neonatal rats. Dormandy (26) has proposed, in a theory diametrically opposite to traditional biochemical theory, that intracellular PUFA, located in noncritical, nonmembrane sites, could serve to avidly scavenge excess O_2 free radicals and thereby prevent their toxic interaction with critical membrane PUFA, thus protecting cells from hyperoxic or O_2 free radical-mediated damage. Our lung fatty acid data from the Intralipid (*versus* regular diet) offspring are consistent with the hypothesis of Dormandy because in the present study (and our previous ones) hyperoxic protection was clearly associated with increases in total PUFA and P/S of the lung triglyceride (intracellular, nonmembrane) lipid fraction in the high PUFA diet offspring. Other possible mechanisms by which Intralipid nutrition and the resultant increased lung PUFA might influence cellular tolerance to high O_2 exposure, such as by alterations in membrane enzyme systems (27), membrane permeability (28), or membrane fluidity (29) have not been explored in the present investigation.

Our present findings of increased hyperoxic protection of newborn experimental animals associated with Intralipid nutrition raises questions as to whether the early administration of Intralipid to VLBW oxygen-requiring premature infants (during their period of maximum need for supplemental oxygen) might provide protection against the damaging effects of oxygen on their lungs. The likely immaturity of their antioxidant defense systems (*e.g.* antioxidant enzymes, vitamins E and A) would tend to make these VLBW prematures extremely vulnerable to lung damage associated with high O_2 therapy. Any antioxidant advantage that might be derived from early nutritional intervention might make a difference in lessening the susceptibility of these premature newborns to the development of chronic lung disease or BPD.

The results of two clinical studies using Intralipid treatment, each with 20 babies or less per group, have appeared in the recent literature (30, 31). One study demonstrated that early postnatal lipid supplementation was associated with a decreased incidence of severe BPD (30), but the other suggested that lipid therapy during the 1st wk of life might actually increase the risk of developing chronic lung disease (31). However, the results from this latter study have been questioned because of apparent differences in the initial severity of respiratory distress in the control *versus* Intralipid-treated group (32). More extensive and larger scale investigations will be necessary to clarify this disparity in clinical outcome. Nonetheless, our findings of protection by Intralipid against pulmonary O_2 toxicity expand on our previous studies linking high-PUFA dietary treatment with hyperoxic protection, and add further impetus to the continued investiga-

Table 5. *Lung tissue prostaglandin and leukotriene levels in 5-d-old Intralipid and regular diet offspring exposed to >95% O_2 or air**

Group	6-keto PGF_{1}-α	PGE_2	LT C_4–F_4
Room air exposed			
Intralipid	19 160 ± 1640	35 350 ± 2950	18 190 ± 5250
Regular diet	17 140 ± 2160	33 750 ± 2950	14 090 ± 4270
>95% O_2 exposed			
Intralipid	18 240 ± 2500	34 810 ± 4510	7220 ± 1930†
Regular diet	20 700 ± 3380	31 830 ± 6090	6200 ± 2910†

* pg/g lung; n = 8/group; no differences between the two diet groups are statistically significant.

† $p < 0.01$: O_2-exposed groups *vs* respective air groups.

tion of the role that early clinical use of Intralipid might play in protecting the vulnerable oxygen-requiring VLBW infant from oxygen toxicity and the development of the chronic lung process of BPD.

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