Protein Deprivation from the Neonatal Period Impairs Lung Development in the Rat

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ABSTRACT. The effects of early protein-deficiency on lung development were studied in the rat. Newborn male rats were nursed in groups of eight. Control dams and pups received a 15% protein diet during the whole experiment. Test mothers only received 12 and 8% proteins from the 7th and the 14th day of lactation. Test rats fed a diet of 8% protein were weaned at 21 days and maintained on the low protein diet for 1 month. This caused a marked decrease in body weight gain, but lung/body weight ratio was not affected. Lung DNA and protein content, lung protein concentration, but not lung DNA concentration were decreased, suggesting the presence of fewer and smaller cells than in control lungs. Volume-pressure curves were performed on excised lungs. With air-filling, the curve obtained in rats fed a diet with 8% proteins was shifted downward and to the right, even when expressed as percent of maximal volume. Its exponential analysis assessed a decrease in lung compliance. In contrast, with saline-filling, the recoil pressure was decreased in rats fed a diet with 8% proteins. Both with air and saline-filling, the pressure at lung rupture was increased in malnourished rats. It is concluded that protein-deficiency from the neonatal period increases surface forces, decreases tissue elastic forces, and augments resistance of the lung to rupture. (*Pediatr Res* 21: 45–49, 1987)

Abbreviations

V-P, volume-pressure
(P 15) rats, rats fed a diet with 15% proteins
(P 8) rats, rats fed a diet with 8% proteins
V_{RP}, volume at the point of lung rupture
P_{RP}, pressure at the point of lung rupture
V_{30}, air volume at a pressure of 30 cm H_2O
V_{10}, saline volume at a pressure of 10 cm H_2O

Respiratory infections are frequent and often lethal in malnourished infants. However, little is known about the repercussions of protein-calorie malnutrition on the lung function in comparison with its effects on other organs (1, 2).

Several morphological and biochemical stages of lung development have been well characterized during the postnatal period in the rat (3–7). The first stage (between birth and 4 days) corresponds to lung expansion. The second stage (between 4 and 12–13 days) is associated with formation of new alveoli. The

third stage (between 13 and 20 days) corresponds to elastin accumulation. The fourth stage (between 20 and 40 days) is a period of equilibrated growth in which alveolar proliferation slows and surface area increases with lung volume. The functional changes occurring during this lung maturation have been characterized recently (6).

The purpose of this work was to study the influence of early protein deficiency on the development of lung mechanical properties. To this end, dams were fed a diet containing decreasing amounts of proteins during the 3 wk of lactation, and the offspring was thereafter maintained on the low protein-diet for another 4 wk. V-P curves and resistance to rupture were established with air or saline on excised lungs. Protein and DNA content of the tissue were also measured.

MATERIALS AND METHODS

The control diet contained 15% proteins (15% casein, 41.2% starch, 13.8% glucose, 17% cellulose, 5% corn oil, and 8% vitamins and minerals). Test diets contained 12 or 8% proteins, but their carbohydrate content was increased to keep them isocaloric.

Pathogen-free male Wistar rats were obtained on the first postnatal day and nursed in groups of eight. Control mothers were fed the 15% protein diet for the 3 wk of lactation, and the control offspring (P 15) were thereafter maintained on that same control diet. Test mothers received a diet with 15, 12, and 8% proteins during the 1st, 2nd, and 3rd wk of lactation, respectively. Test rats (P 8) were then maintained on that low protein diet (8%) until the age of 49 days. After weaning all rats were housed in groups of four per cage, except between 35–42 days of age, when they were placed in individual metabolic cages permitting daily measurement of food intake. Four weeks later (at the age of 49 days) the animals were anaesthetized with sodium pentobarbital (4 mg/100 g body weight) and exsanguinated by section of the trunk vessels. The trachea was exposed and cannulated just below the larynx with a plastic catheter. The lungs were then excised as described previously (8) and degassed for 2.5 min in a vacuum dessicator.

The tracheal cannula was connected via a T tube to a peristaltic pump (LKB 1200, Bromma, Sweden) and an electromanometer (Philips XM 5321/00, containing a Statham pressure transducer P23 Db). Pressure was recorded on a strip-chart recorder (IFE-LEC 4000, Paris, France). The lungs were then continuously inflated and deflated (three complete cycles) either with air or with saline. With air the flow rate of the pump was 7 ml/min and the pressure varied between 0 and 30 cm H_2O. With saline the flow rate was 4 ml/min and the pressure varied between 0 and 10 cm H_2O. On a fourth inflation lungs were filled to the point of rupture. V_{RP} and P_{RP} were determined on the recording when the pressure curve showed a sudden change in its slope. When saline was used, the lungs were not immersed in fluid, but filled in horizontal position.

V-P curves were constructed from time pressure recordings by

selecting 11 and 9 pressure levels for air and saline fillings, respectively. All measurements were made on the third deflation limb. Maximal lung volume was taken as the volume required to inflate the degassed lungs to a pressure of 30 cm H_2O with air or 10 cm H_2O with saline. V-P curves were plotted with volume expressed in absolute terms (in ml), and as percent of V_{30} or V_{10}. These V-P curves were analyzed by an exponential function to calculate the lung compliance according to the method of Salazar and Knowles (9) as modified by Gibson et al. (10). The equation used was

$$V = Vmax - (Vmax - V_0)e^{-kP}$$

were V is the lung volume at a transpulmonary pressure P, Vmax is the maximal lung volume as defined above, and V_0 is the volume at P = 0. The lung compliance is assessed by the shape constant k of the exponential curve. From V and P values the best-fit, according to χ^2 criterion, was obtained using an iterative exponential regression routine.

After completion of the mechanical tests with air, the lungs were carefully dissected from extrapulmonary airways and vessels, blotted dry, and weighed. The ratio of dry to wet weights was determined by weighing the left lobe before and after drying in an oven at 60° C. The right lobe was homogenized in ice-cold 0.3 M sucrose (10% w/v) buffered at pH 7.4 with 3 mM imidazole-HCl. The livers were homogenized by the same method. The protein content of these homogenates and of the serum was measured (11) with bovine serum albumin as standard. DNA content of the homogenates was determined by fluorometry (12), using calf thymus DNA as standard.

Results are presented as means ± SEM. Statistical significance of differences between control and test groups was assessed by the two-3tailed Student's t test for unpaired values.

RESULTS

Nutritional studies. On the day of weaning the average body weight of the rats fed by a mother receiving a protein-restricted diet was significantly lower (39 ± 1 g, $p < 0.001$, $n = 24$) than that of the rats fed by a mother on a control diet (48 ± 1 g). As shown by Figure 1, the body weight gain during the following 4 wk was markedly smaller in these protein-deficient rats than in controls. At the age of 49 days, the average body weight was only 99 ± 2 g in (P 8) rats as compared to 174 ± 3 g (P 15) rats. Rats fed a low protein diet consumed about 30% less calories than controls, so that their actual protein intake was reduced by 65% (Table 1). Their growth retardation was associated with a better protein-efficiency ratio, but with a clear decrease in the effective-
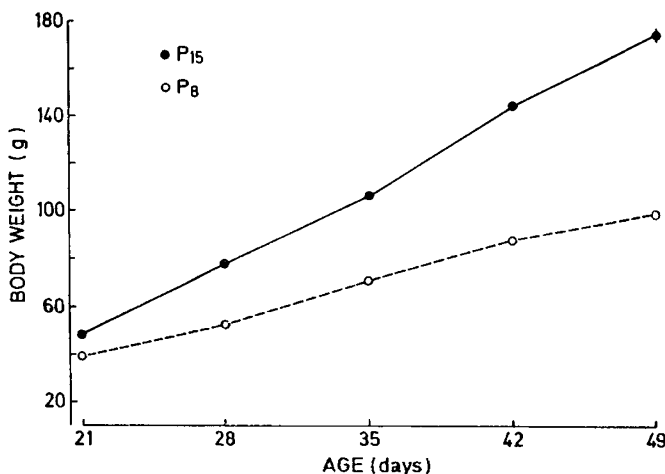

Fig. 1. Body weight of the rats fed a control diet (P 15) or a low protein diet (P 8). Values are means ± SEM (usually within the size of the *symbols*) for 24 rats in each group.

Table 1. *Food consumption and body wt gain by the rats fed a control (P 15) or a low protein diet (P 8)**

	(P 15) rats	(P 8) rats
Daily caloric intake (kcal)	69.2 ± 0.6	47.2 ± 0.9†
Daily protein intake (g)	2.64 ± 0.02	0.97 ± 0.02†
Wt gain		
g/day	5.36 ± 0.10	2.39 ± 0.07†
g/100 kcal	7.76 ± 0.15	5.04 ± 0.16†
g/g proteins	2.01 ± 0.02	2.40 ± 0.16‡

* Rats were housed in individual metabolic cages for 7 days (35–42 days of age). Measurements were made daily and are given as the average for the last 6 days. The first days thus served as adaptation to the new housing conditions. Values are means ± SEM for 24 rats in each group.
† $p < 0.001$ vs rats receiving the control diet (P 15).
‡ $p < 0.005$ vs rats receiving the control diet (P 15).

ness of the ingested calories (Table 1). Total serum proteins were lower ($p < 0.001$) in (P 8) rats (44.1 ± 3.3 g/l) than in (P 15) rats (54.9 ± 3.9 g/l).

Biochemical studies. Table 2 summarizes the biochemical characteristics of the lungs. Protein-deficiency resulted in lung growth retardation as evidenced by a low lung weight (61.6% of controls). However, lung weight was normal relative to body weight, and the dry/wet weight ratio was not altered. Total lung DNA was decreased, but DNA concentration was unchanged. Total lung proteins were decreased by about 50% and their concentration per g of lung or per mg DNA remained lower than in control rats. The liver of these animals was also analyzed for comparison (Table 2). Absolute, but not relative, weight of the liver was decreased in protein-deficient rats. DNA and proteins were decreased to the same extent as in the lungs.

V-P studies. A complete sequence of three air V-P curves could be performed in 8/15 (P 15) rats and 10/14 (P 8) rats. V_{30} was decreased in protein-deficient rats (Fig 2, *left*). However, V_{30}/g of lung was similar in the two groups: 9.5 ± 0.3 and 9.2 ± 0.3 ml/g in (P 15) and (P 8) rats, respectively. Lungs of (P 8) rats contained significantly smaller volumes of air at all transpulmonary pressure levels (Fig. 2, *left*). When the volume of air was expressed as percentage of V_{30}, the V-P curve of (P 8) rats was significantly shifted downward and to the right (Fig. 2, *right*). This indicates a decrease in lung compliance that could be quantitated by the shape constant k (cm H_2O^{-1}) of the exponential function fitting these curves: 0.378 ± 0.018 in (P 15) rats and 0.251 ± 0.013 in (P 8) rats (p < 0.001). Relative volume of trapped air at 0 cm H_2O was lower in (P 8) rats (2.3 ± 0.2 ml/g tissue) than in (P 15) rats (3.1 ± 0.1 ml/g; $p < 0.05$).

Lung volumes and pressures at the point of rupture, recorded during a 4th air-inflation, are presented in Table 3. Absolute V_{RP}, but not relative V_{RP} (per g lung), was decreased in protein-deficient rats whereas P_{RP} was increased.

A complete sequence of three saline V-P curves could be performed in 8/9 (P 15) rats and in 9/10 (P 8) rats. V_{10} was decreased in protein-deficient rats (Fig. 3, *left*). However, V_{10}/g of lung was similar in the two groups: 10.0 ± 0.4 ml/g and 9.2 ± 0.3 ml/g in (P 15) and (P 8) rats, respectively. Lungs of (P 8) rats contained significantly smaller volumes of saline at almost all transpulmonary pressure levels (Fig. 3, *left*). When the volume of saline was expressed as percentage of V_{10}, the V-P curve of (P 8) rats was significantly shifted upward and to the left over a wide range of pressures (Fig. 3, *right*). These curves obtained after saline filling could not be fitted by an exponential function, but tended to be sigmoidal. Relative volume of trapped saline at 0 cm H_2O was higher in (P 8) rats (3.2 ± 0.2 ml/g tissue) than in (P 15) rats (2.4 ± 0.2 ml/g; $p < 0.025$).

During a 4th cycle of saline inflation, absolute V_{RP}, but not relative V_{RP} (per g lung) was decreased in protein-deficient rats, whereas P_{RP} was significantly higher than in controls (Table 3).

Table 2. *Biochemical characteristics of the lungs and liver of rats fed control (P 15) or low protein diet (P 8)**

	(P 15) rats	(P 8) rats
Lung wt		
Wet (g)	0.86 ± 0.02	0.53 ± 0.01†
Wet (% body wt)	0.50 ± 0.01	0.52 ± 0.01
Dry/wet (%)	21.4 ± 0.1	21.0 ± 0.2
Lung DNA content		
Mg/lung	5.20 ± 0.14	3.14 ± 0.10†
Mg/g lung	6.10 ± 0.15	5.98 ± 0.16
Lung protein content		
Mg/lung	91.8 ± 2.8	42.9 ± 1.2†
Mg/g lung	107 ± 2.8	81.6 ± 1.4†
Mg/mg DNA	17.1 ± 0.5	13.7 ± 0.3†
Liver wt		
Wet (g)	7.33 ± 0.15	4.31 ± 0.14†
Wet (% body wt)	4.18 ± 0.06	4.26 ± 0.09
Liver DNA content		
Mg/liver	29.5 ± 0.7	16.1 ± 0.4†
Mg/g liver	4.02 ± 0.10	3.81 ± 0.11
Liver protein content		
Mg/liver	1262 ± 20	632 ± 21†
Mg/g liver	173 ± 2.7	147 ± 1.5†
Mg/mg DNA	43.4 ± 0.9	39.3 ± 1.1‡

* Values are means ± SEM for 15 (P 15) and eight (P 8) rats for lung measurements and for 24 rats for liver measurements.
† $p < 0.001$ vs control rats.
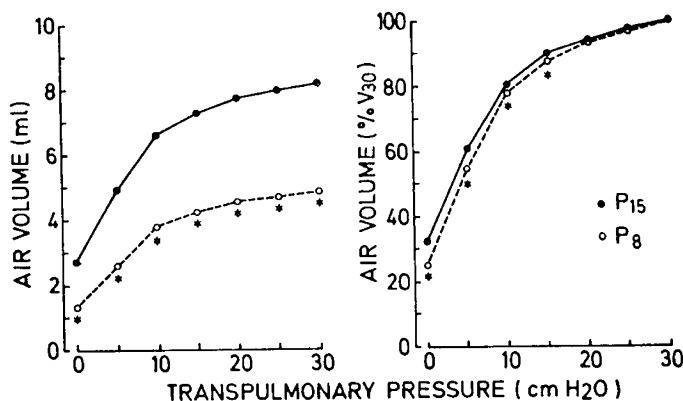‡ $p < 0.005$ vs control rats.

Fig. 2. Deflation V-P curves determined in air-filled lungs of rats fed a control diet (P 15) or a low protein diet (P 8). Volumes are expressed in absolute terms (*left*) or as percentage of V_{30}. Values are means for eight (P 15) or 10 (P 8) rats (SEM were within the size of the *symbols*). * $p < 0.05$ or less from controls.

Table 3. V_{RP} *and* P_{RP} *of lung rupture during air or saline inflation in rats fed control (P 15) or low protein diet (P 8)**

	(P 15) rats	(P 8) rats
Air inflation (n)	(8)	(10)
V_{RP} (ml)	10.9 ± 0.5	6.6 ± 0.2†
V_{RP} (mg/g lung)	12.6 ± 0.5	12.6 ± 0.4
P_{RP} (cm H_2O)	50.8 ± 3.3	60.2 ± 2.7‡
Saline inflation (n)	(8)	(9)
V_{RP} (ml)	11.3 ± 0.4	6.6 ± 0.3†
V_{RP} (ml/g lung)	13.6 ± 0.4	12.4 ± 0.5
P_{RP} (cm H_2O)	18.5 ± 1.7	23.6 ± 1.3‡

* Since the wet weight of the lungs could not be accurately determined after saline inflation, the average wet weight obtained after air inflation was used to calculate V_{RP} in ml/g wet weight. Values are means ± SEM for (n) rats.
† $p < 0.001$ vs rats receiving the control diet (P 15).
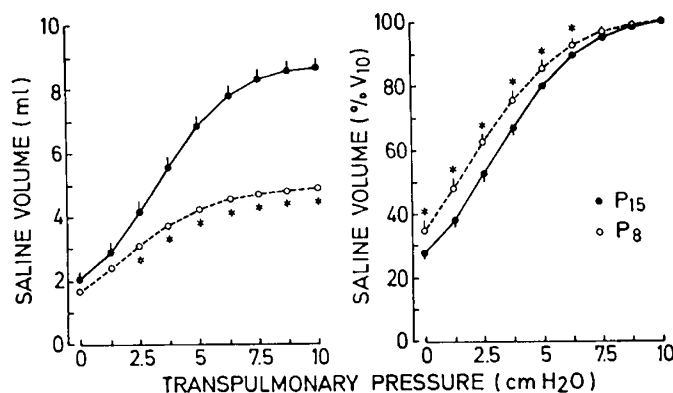‡ $p < 0.05$ vs rats receiving the control diet (P 15).

Fig. 3. Deflation V-P curves determined in saline-filled lungs of rats fed a control diet (P 15) or a low protein diet (P 8). Volumes are expressed in absolute terms (*left*) or as percentage of V_{10}. Values are means ± SEM for eight (P 15) or nine (P 8) rats. * $p < 0.05$ or less from controls.

DISCUSSION

Postnatal malnutrition in rats is usually induced by increasing the number of pups suckling a single mother (13). In the present study we attempted to induce a predominant protein deprivation by a progressive decrease in the protein content of the diet given to the mother and subsequent administration of the same low protein diet to the weaned rats. This caused growth retardation present at weaning and marked decrease in body weight gain thereafter. Daily measurements of food consumption by these animals revealed that the model leads to a mixed protein-calorie deprivation with predominant protein deficiency. As previously observed (14) such a situation was accompanied by a decrease in calorie efficiency and an increase in protein efficiency.

In these rats whose malnutrition started before weaning, lung growth was decreased in proportion to ponderal retardation. Taking DNA content of the tissue as an index of cell number

and the protein/DNA ratio as an index of cell size (15), it appears that these lungs contained fewer cells than control lungs and that these cells were of smaller size. The estimated decrease in cell number was more pronounced than in the lungs of rats submitted to calorie or protein deprivation from the age of 21-28 days only (8, 16). This may reflect impairment of lung cell division that is most important before the age of 21 days (3, 15). Our interpretation is supported by the observation that protein-calorie deprivation, whether started before (this study) or after weaning (8), caused a similar decrease in DNA content of the liver, an organ where cell division continues at about the same rate until the age of 45 days (15).

To ensure optimal uniformity and reproducibility of volume and pressure changes in the lungs, a technique of continuous inflation-deflation was used. To evaluate the relative contributions of surface and tissue elastic forces, V-P curves were performed both with air and saline filling (17, 18). Protein restriction differently altered V-P curves with air or saline filling when these were expressed as percent of maximal lung volume to account for differences in lung size. After air filling, the curve was shifted downward and to the right, and a decrease in lung compliance, assessed by the shape constant k of the exponential function fitted to the P-V curve, was calculated. In contrast, after saline-filling the curve was shifted upward and to the left, the recoil pressure being decreased at almost all volumes.

To our knowledge only one study, published in abstract form (19), has evaluated the repercussions of early malnutrition on

the lung function in the rat. Malnutrition was induced by allowing 18 pups to suckle a single mother. At 21 days, a left shift of the V-P curve obtained with saline filling was observed, as in our study, but no alteration was seen in the V-P curve obtained with air filling. The reason of this discrepancy is difficult to appreciate from an abstract.

The alterations in lung mechanics described herein somewhat differ from those induced by a malnutrition starting at 21–28 days (8, 20, 21). Opposite shifts of the air and saline V-P curves, as produced herein by malnutrition starting before weaning, were not induced by food deprivation in weaned rats. In two studies, a decrease in lung compliance was measured in air- or saline-filled lungs (8, 20) whereas a trend to lower recoil pressures at low volumes was reported in the third study (21).

As proposed to explain the complex alterations in lung mechanics occurring in starved adult rats (22), we suggest that malnutrition starting during the suckling period affects both surface and tissue elastic forces of the lungs. On the one hand, an increase in surface forces, due to impaired production of surfactant or alteration of surfactant quality, could explain the decrease in compliance evidenced after air inflation. Thus, low concentrations of surfactant have been measured in the lungs of malnourished suckling (23), weanling (16), young (24), and adult (25, 26) rats. Similar observations were also made in rats (27) or guinea pigs (28) just born (< 10 h) to mothers that were malnourished during gestation. This fall in surfactant concentration was accompanied by an increase in minimal surface tension of lung extracts from newborn rats (27), but did not alter V-P characteristics of air-filled lungs in guinea pigs (28). Although species differences can contribute to the discrepancy between this latter study (28) and the present one, the period of malnutrition (prenatal and postnatal) and its duration are certainly of importance. In this respect, it should be noted that the concentration of total surfactant phospholipids rapidly falls during starvation in young and adult rats (16, 24–26), but that an increase in surface forces can be evidenced only when food deprivation is maintained for relatively long periods (8, 20, 21). Changes in surfactant quality, and not only quantity, may be involved.

On the other hand, a decrease in tissue elastic forces could explain the decrease in recoil pressure after saline infusion, an observation also made in guinea pigs submitted to prenatal starvation (28). Since lung elasticity is mainly due to elastin (29), and since elastin deposition essentially takes place before the age of 1 month (6), our observations could be accounted for by an alteration of this deposition during early malnutrition. This interpretation is directly supported by the histological demonstration of a decrease in lung elastin in two models of rats submitted to starvation during the suckling period (30, 31). It is also consistent with our previous observation (8) that no such decrease in recoil pressure after saline inflation was recorded in young rats submitted to protein-calorie restriction from the age of 28 days only.

The pressure at lung rupture was higher in protein-deficient rats than in controls. As this difference persisted during saline filling, it is unlikely to be secondary to changes in surface forces only. In normal rats, resistance of the lungs to rupture by saline inflation increases with collagen concentration of the tissue (6). Total collagen content decreases, but collagen concentration slightly increases in the lungs of malnourished rats when food restriction is started after weaning (21) or in adulthood (32, 33). It is possible that early protein deficiency causes desequilibrated growth of the lungs with a similar increase in collagen proportion or alters collagen properties in the lungs as in other tissues (34).

In conclusion, protein deprivation from the neonatal period impaired lung growth and altered lung mechanical properties in the rat. Opposite shifts of the V-P curves obtained after air or saline filling suggest an increase in surface forces and a decrease in tissue elastic recoil, possibly due to deficits in surfactant and elastin, respectively. An increase in lung resistance to rupture

after saline filling also suggests alterations in collagen amount or properties. The mixed protein-calorie deprivation with predominant protein deficiency imposed on our rats was not extreme and is frequently encountered in developing countries. Our results therefore suggest that alterations of lung development may have to be added to the list of the functional consequences of protein-calorie malnutrition in children.

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