Effects of Indomethacin *in Utero* on the Pulmonary Vasculature of the Newborn Guinea Pig

DAPHNE E. DEMELLO, JOHN D. MURPHY,[1] MARK J. ARONOVITZ,[2] PAUL DAVIES, AND LYNNE M. REID

Department of Pathology, The Children's Hospital and Harvard Medical School, Boston, Massachusetts

ABSTRACT. The clinical syndrome of persistent pulmonary hypertension of the newborn results from failure of the normal perinatal vascular adaptation, and functionally is characterized by persistent right to left shunting of blood through the foramen ovale and ductus arteriosus. Exposure of the fetus to drugs that inhibit prostaglandin synthesis and cause closure of the ductus arteriosus has been suggested as one cause of persistent pulmonary hypertension of the newborn. We attempted to produce a functional and structural model of persistent pulmonary hypertension of the newborn by administration of indomethacin, a cyclooxygenase inhibitor, to pregnant guinea pigs. Five pregnant guinea pigs received 3.5 mg/kg indomethacin intravenously twice each day for the 12 to 19 days before delivery and seven controls received saline. Hemodynamic studies were performed in eight "treated" newborns and in 12 controls. After sacrifice, the ductus was ligated and, for morphometric studies, the pulmonary arteries were distended with barium/gelatin. The treated animals did not show the intraacinar structural or hemodynamic changes of persistent pulmonary hypertension of the newborn. It seems that the indomethacin did cross the placenta because lung structure was modified. The radial alveolar count and alveolar/artery ratio were increased and the preacinar arteries dilated, with more increase in muscle mass. This could be explained by increased pulmonary blood flow because of ductal constriction but direct effect of indomethacin cannot be excluded. (*Pediatr Res* 22: 693–697, 1987)

Abbreviations

PPHN, persistent pulmonary hypertension of the newborn
Rx, treated
C, control
RAC, radial alveolar count
IV, intravenous
Ppa, pulmonary artery pressure
Psa, systemic artery pressure
ED, external diameter

At birth as the lungs expand with air and mediators that include bradykinin are released, pulmonary vascular resistance

falls because of vasodilatation and increased compliance of small resistance arteries (1–4). The increased compliance is indicated by an increase in the diameter and a drop in wall thickness of arteries at a given distending pressure.

Idiopathic PPHN results when this perinatal adaptation fails and right to left shunting occurs through the foramen ovale and/or ductus arteriosus. Sometimes it is fatal (5–7), and in these cases, in addition to any functional failure, the arterial bed is usually structurally abnormal with a well-developed medial muscle coat present in intraacinar arteries that at birth are normally not muscular. The pre- and intraacinar arteries are abnormally small and arteries of all size have a dense collagen sheath (8, 9), indicating that resistance arteries fail to increase compliance.

Indomethacin and other cyclooxygenase inhibitors have been shown to cause constriction of the ductus arteriosus of experimental animals and a rise in pulmonary artery pressure *in utero* (10, 11). This led these authors and others (12) to suggest that premature closure or constriction of the ductus arteriosus might be a cause of PPHN in humans, although they had not identified in animals the human structural changes described above. In some cases of PPHN the mother had ingested cyclooxygenase inhibitors (13–17). Other authors (18, 19) have not found this association. Most reports describe the functional effects of indomethacin exposure and not the structural changes in the pulmonary arteries. *In vitro*, the guinea pig ductus seems not to be affected by indomethacin (20), but this effect has not been studied *in vivo*. We have administered indomethacin to guinea pigs late in pregnancy and have evaluated the newborns for possible structural and hemodynamic features of PPHN.

METHODS

We used 12 pregnant guinea pigs. About 3 wk before delivery a pregnant guinea pigs was allocated to an Rx or C group. Seven mothers received 2 ml of saline, IV twice a day, until delivery. The 12 guinea pigs born to these mothers who had received IV saline for periods ranging from 6–23 days constituted the Control group.

This study is based on four pregnant guinea pigs treated with IV indomethacin, 3.5 mg/kg twice a day, until delivery and a fifth animal that received indomethacin both orally and IV (2 mg/kg IV, b.i.d. for 9 days and 3 mg/kg b.i.d. orally for 7 days). The dosage was based on a series of preliminary studies in a separate group of pregnant guinea pigs. Starting dose was 6.5 mg/kg once or twice a day. This was reduced by 0.5 mg increments until all offspring were liveborn, at 3.5 mg/kg. The highest dose proved fatal to the mother and the intermediate doses were fatal to the fetuses. In aborted fetuses from at least three litters, the ductus was closed.

The treated group yielded eight newborns that had been exposed *in utero* to indomethacin for 12 to 19 days. All the newborns were delivered spontaneously. Within 5 h of birth the

newborn guinea pigs were weighed and studied hemody- namically. They were maintained under a radiant warmer throughout the study.

The newborn guinea pigs were anesthetized with sodium pen- tobarbital, 10 mg/kg intraperitoneally and lidocaine 0.5% locally. Silastic catheters (external diameter 0.56 mm) were placed in the left carotid artery and the main pulmonary artery using tech- niques similar to those described previously (21). Pressures were recorded from the pulmonary artery, right ventricle, right atrium, and systemic arteries. Blood samples were drawn from the sys- temic arteries, pulmonary artery, and right atrium for blood gas determinations, oxygen saturation, and hemoglobin concentra- tion.

Following the hemodynamic studies, the animals were sacri- ficed with an overdose of sodium pentobarbital (300 mg/kg) at ages ranging from >1 to 5 h. When respiration ceased the chest was opened and the heart and lungs removed *en bloc*, the ductus was ligated and the pulmonary arteries were injected with a barium gelatin suspension at a pressure of 73.5 mm Hg. The lung was fixed fully distended by intratracheal instillation of 10% buffered formalin at 25 cm water pressure (22). Pulmonary arteriograms were obtained. The diameter of the axial pulmonary artery at the first branch and at 50 and 75% of the distance to the periphery was measured on pulmonary arteriograms. After fixation, lung volumes were measured by water displacement and four blocks were taken, one each from the left cranial and caudal lobes and two from the right lower lobe. Four μm sections were cut and stained with hematoxylin and eosin and Miller's elastic-van Gieson stain. Morphometric analysis of the pulmo- nary vasculature using light microscopy was performed according to the methods developed by Hislop and Reid (23-25). Briefly, in each of four sections the barium-filled arteries under 300 μm in diameter were examined (about 70/animal), structure was recorded, medial thickness measured, and percentage medial thickness was calculated using the formula 2 $\times$ medial thickness $\times$ 100/external diameter. The airway was identified and the accompanying arteries grouped by airway level as preacinar (terminal bronchiolus, respiratory bronchiolus), alveolar duct, or alveolar wall. Radial alveolar counts were performed according to the method of Emery and Mithal (26). The structure of the pulmonary veins was also examined.

Each heart was dissected and weights of the right ventricular free wall, and left ventricle + septum, were recorded.

The data in the two groups were compared using the Wilcoxon Rank sum test (27) and Student's t distribution, including a Bonferroni correction for multiple comparisons (28). For the latter, SD was used as the index of dispersion, the tests were unpaired and a 5% significance level was used.

RESULTS

Body weights of the Rx animals were similar to the C group (Rx: 88.5 $\pm$ 8.7 and C: 87.8 $\pm$ 14.1, respectively), as were cardiac ventricular weights. The mean hematocrit for the treated animals was somewhat higher than that of the controls but not signifi- cantly so (Rx: 46 $\pm$ 5.19%, C: 42.6 $\pm$ 2.69%). Arterial pH was similar in animals of both groups (Rx: 7.4 $\pm$ 0.02, C: 7.38 $\pm$ 0.06), as were the arterial pO$_2$, values (Rx: 71.25 $\pm$ 12.9 mm Hg, C: 74.2 $\pm$ 22.2 mm Hg).

Ppa and Psa were similar in the Rx and C groups (Ppa Rx:

26.12 $\pm$ 4.73 mm Hg, C: 26.83 $\pm$ 4.0 mm Hg, Psa Rx: 48.7 $\pm$ 10.3 mm Hg, C: 46.5 $\pm$ 6.34 mm Hg) (Table 1).

Morphometric findings. Lung volumes were similar in the Rx and C groups (Rx: 4.0 $\pm$ 0.6 ml, C: 3.88 $\pm$ 0.031 ml) (Fig. 1).

The ductus was open in all animals and was ligated before the preliminary arterial injection was made. Measurement of axial artery luminal diameter on the arteriograms showed greater values in the Rx group that in the C in five of the six levels. In the left artery at the most proximal level, the difference was very close to significant using the Bonferroni correction (Table 2).

The percentage of muscular arteries at each airway level was similar in both groups indicating that intrauterine exposure to indomethacin had not caused abnormal muscularization. In both groups the intraacinar vessels were similar in external diameter and percentage medial thickness (Table 3).

Although the percent medial thickness of preacinar arteries in both the treated and control animals was similar, the ED of the preacinar arteries in the Rx group was larger indicating some increase in smooth muscle mass in preacinar arteries (Table 3). At this level the medial area was larger in the Rx than in the C group (Rx: 9305.2 $\pm$ 6746.2, C: 5215.1 $\pm$ 5357.9, $p < 0.001$).

In seven of the eight animals in the Rx group alveolar multi- plication had exceeded that of the C animals. The mean RAC for the Rx group was 19, significantly above the control value of 15.68 ($p < 0.05$). The alveoli were smaller and alveolar number per unit area was higher, as was obvious from inspecting fields of similar size (Fig. 2).

The alveolar to arterial ratio was higher in the Rx group than the C guinea pigs (Rx: 124 $\pm$ 58.1, C: 76 $\pm$ 17.1, $p < 0.05$). Since the arterial density per unit area of lung did not differ in the two groups the increased ratio reflects the increased alveolar density. The veins and perivascular collagen were similar in the Rx and C groups.

In summary, the Rx animals had an increase in diameter of the left pulmonary artery and preacinar arteries of both lungs with increased muscle mass in preacinar arteries and an increase in alveolar multiplication to give abnormally high radial alveolar counts. They did not show muscularization of intraacinar arteries or increase in perivascular collagenous sheaths.

DISCUSSION

The administration of indomethacin to pregnant animals causes ductal constriction and an elevation of fetal pulmonary artery pressure (11, 29-33). Indomethacin may also lead to intrauterine ductal constriction in humans (13, 14, 34). Since postnatal Ppa after intrauterine exposure to indomethacin has not previously been reported, the effect of intrauterine indo- methacin on the perinatal pulmonary vascular adaptation is not known.

Pulmonary arterial wall thickness has been reported as in- creased after exposure to indomethacin *in utero* in sheep (11), rats (12), and human infants (14). Since these vessels had not been distended before fixation and since the external diameter of the pulmonary vessels was decreased (11), this effect could be due to vasoconstriction alone. From the data given (11), the calculated mean cross-sectional area of the muscular media in controls is 665.92 μm^2 and in experimental animals, 732 μm^2, a difference that is not statistically significant ($p > 0.05$). In another study in fetal rats, vessels of similar external diameter

Table 1. *Hemodynamic data*

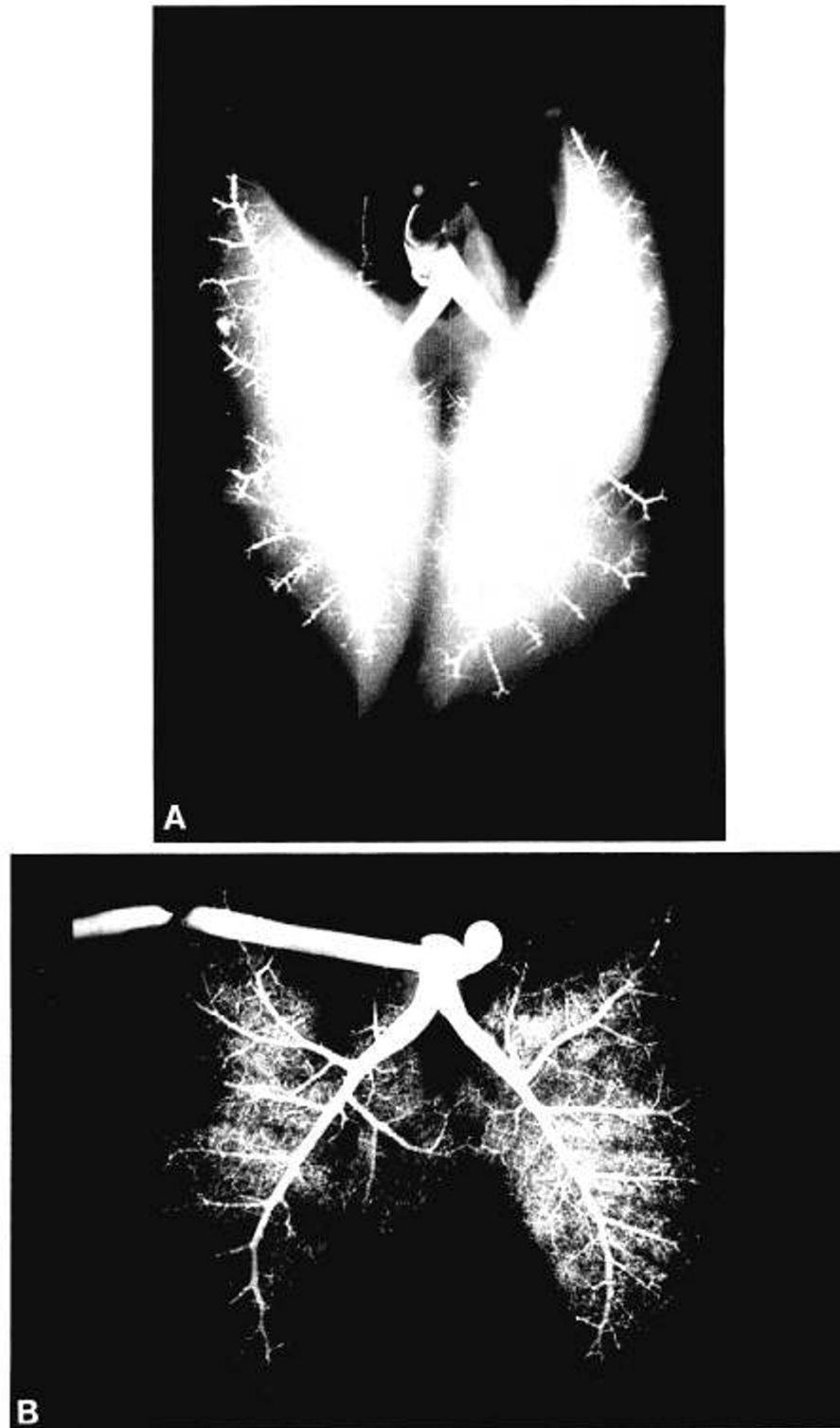|	Mean hematocrit (%)	Mean arterial pH	Mean arterial pO$_2$ (mm Hg)	Mean Ppa (mm Hg)	Mean Psa (mm Hg)
Indomethacin Rx	46.0 $\pm$ 5.1	7.40 $\pm$ 0.02	71.2 $\pm$ 12.9	26.1 $\pm$ 4.7	48.7 $\pm$ 10.3
C	42.6 $\pm$ 2.6	7.38 $\pm$ 0.06	74.2 $\pm$ 22.2	26.8 $\pm$ 4.0	46.5 $\pm$ 6.3

Fig. 1. Pulmonary arteriograms of (A) indomethacin-treated guinea pig showing lumen diameter of left pulmonary artery 799 μm and (B) control, lumen diameter 733 μm (×4).

Table 2. Axial artery—luminal diameter (μm)

	Left			Right		
	At 1st branch	50%	75%	At 1st branch	50%	75%
Indomethacin Rx	924 ± 130.5	649 ± 99.1	408 ± 109.4	774 ± 123.0	633 ± 71.2	391 ± 97.1
C	714 ± 160.7	545 ± 114.7	369 ± 150.0	696 ± 127.6	557 ± 100.0	400 ± 163.1

were compared in experimental animals and controls. Although the calculated percent wall thickness was increased in the experimental group, since the vessels were not landmarked by the accompanying airway, it is not certain that vessels of the same level were being compared. Thus it is again possible that the observed difference is due to vasoconstriction rather than a true

increase in vascular smooth muscle or extension of muscle into normally nonmuscular arteries. We report for the first time an increase in medial muscle mass of normally muscular arteries after indomethacin administration, but it is associated with diffuse enlargement of the lumen in large arteries and not contracture of the arterial bed.

Contrary to our expectations, our study did not produce postnatal pulmonary hypertension or the structural changes, particularly the excessive muscle of intraacinar arteries, that are typical of human PPHN (8, 9, 35). It is unlikely that indomethacin failed to cross the placenta. Our preliminary experiments with higher doses (8 mg/kg/day) resulted in an aborted fetus with closed ductus. The modification to pulmonary structure by indomethacin treatment indicates that indomethacin crossed the placenta and was effective. Several studies have shown that indomethacin crosses the placenta and produces ductal constriction thus increasing pulmonary blood flow (11, 30–33, 36).

Table 3. *Microscopic artery measurements*

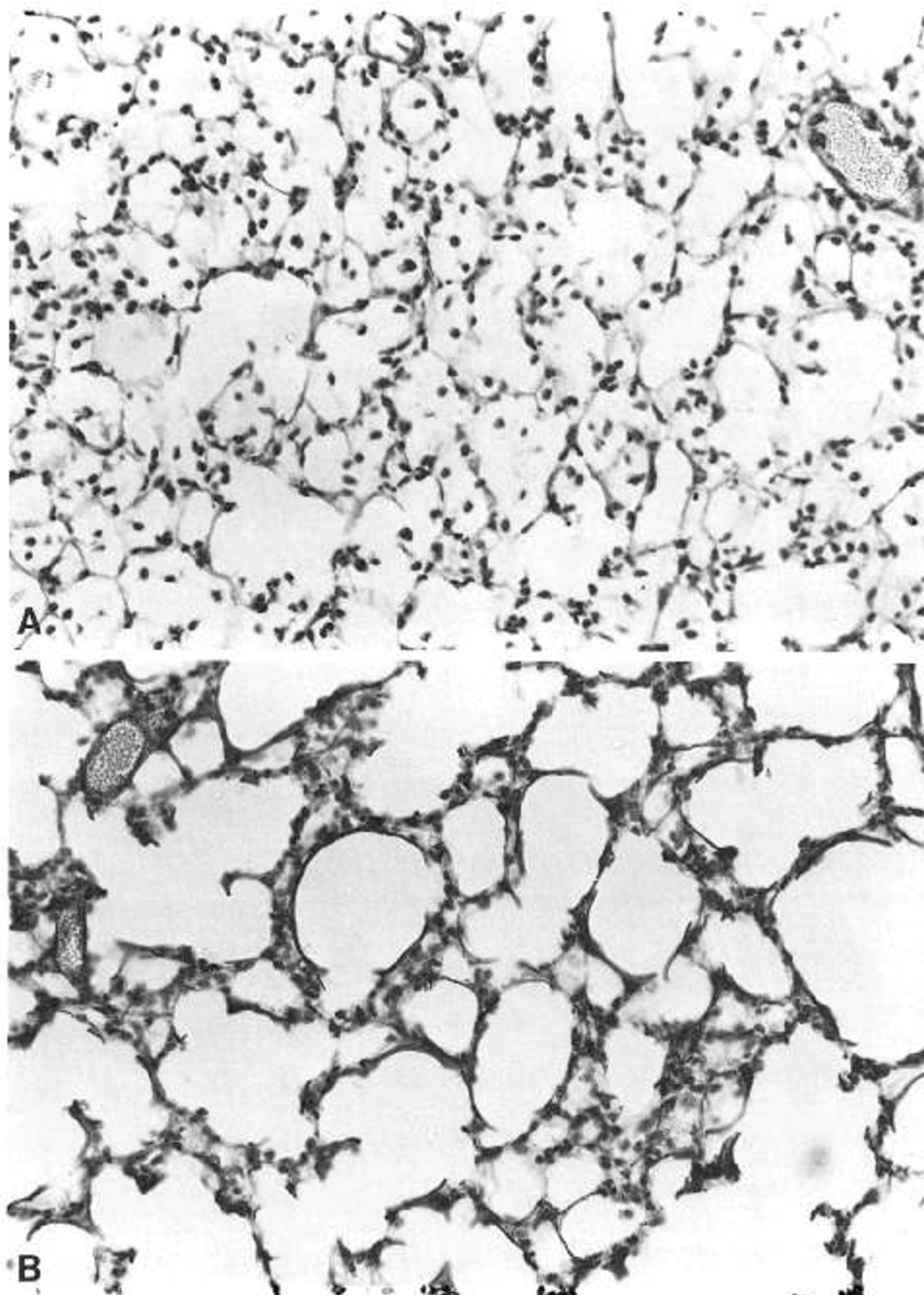| | Preacinar | | Intraacinar | |
	ED (μm)	MT* (%)	ED (μm)	MT (%)
Indomethacin Rx	191.4 ± 52.4*	9.9 ± 3.1	35.3 ± 21.8	20.1 ± 6.4
C	117.5 ± 55.8	10.8 ± 2.6	38.7 ± 11.2	16.4 ± 5.8

* Medial thickness. $p < 0.02$, t test.

Fig. 2. Photomicrograph of lung: (*A*) indomethacin-treated guinea pig showing increased alveolar density per unit area of lung compared with (*B*) control (×250, Miller's elastic-van Gieson stain).

Postnatally the Ppa in both Rx and C animals was similar, indicating that regardless of intrauterine flow postnatal adaptation occurred to give normal Ppa. The increase in RAC in the Rx animals indicates greater than normal alveolar multiplication, a phenomenon not previously described after fetal indomethacin exposure. Whether this is a direct effect of indomethacin or evidence of an increased dose of growth hormones because of increased flow is not known. Rudolph (37) has suggested that there are differences in the biologic response to indomethacin by small pulmonary vessels due to the duration of ingestion, gestational age of the fetus, and time interval between the last dose of the drug and birth; each can affect the nature of the response of the pulmonary circulation.

There is dissociation between multiplication of alveoli and appearance of small alveolar wall vessels. A similar dissociation has been reported in other conditions; an increase in alveolar to arterial ratio was found in polyalveolar lobe where alveolar multiplication is excessive (23), with a raised alveolar to arterial ratio. It is tempting to speculate that the increased pulmonary flow *in utero* consequent to ductal constriction had a similar effect. It could of course be a direct effect on alveolar growth control by indomethacin. A decreased number of pulmonary vessels/cm^2 lung tissue has been described in a human newborn after chronic intrauterine exposure to a cyclooxygenase inhibitor (14). Alveolar number was not estimated.

While the increased diameter of preacinar arteries in our Rx animals could be a rapid change, the increase in muscle mass is likely to have taken place over several days to weeks. Since the animals were killed a few hours after birth, it is likely that these changes occurred *in utero*.

In summary, chronic *in utero* exposure of guinea pigs to indomethacin produced neither the hemodynamic nor structural changes of PPHN. It seems that indomethacin acted long enough *in utero* to modify the pulmonary arterial bed and cause increased alveolar multiplication. These findings suggest that the changes produced by increased blood flow are not the ones that cause PPHN; the structural changes in the latter of muscularized but small peripheral intraacinar arteries and dense collagen sheaths are so different from those seen after intrauterine indomethacin treatment that they do not even suggest early changes of PPHN.

REFERENCES

1. Campbell AGM, Dawes GS, Fishman AP, Hyman AI, Perks AM 1968 The release of a bradykinin-like pulmonary vasodilator substance in fetal and newborn lambs. J Physiol 195:83–96
2. Heymann MA, Rudolph AM, Nies AS, Melmon KL 1969 Bradykinin production associated with oxygenation of the fetal lamb. Circ Res 25:521–534
3. Cassin S, Dawes GS, Mott J, Ross BB, Strang LB 1964 The vascular resistance of the fetal and newly ventilated lung of the lamb. J Physiol 171:61–79
4. Kulik TJ, Lock JE 1984 Pulmonary vasodilator therapy in persistent pulmonary hypertension of the newborn. In: Philips JB (ed) Clinics in Perinatology, Vol 11/3 Symposium on Neonatal Pulmonary Hypertension. WB Saunders, Philadelphia, pp 693–701
5. Gersony WM, Duc GV, Sinclair JC 1969 "P.F.C." syndrome (persistence of the fetal circulation). Circulation 40:87(abstr)
6. Siassi B, Goldberg SJ, Emmanouilides GC, Higashino SM, Lewis E 1971 Persistent pulmonary vascular obstruction in newborn infants. J Pediatr 78:610–615
7. Levin DL, Heymann MA, Kitterman JA, Gregory GA, Phibbs RH, Rudolph AM 1976 Persistent pulmonary hypertension of the newborn infant. J Pediatr 89:626–630
8. Haworth SG, Reid L 1976 Persistent fetal circulation: newly recognized structural features. J Pediatr 88:614–620
9. Murphy JD, Rabinovitch M, Goldstein JD, Reid L 1981 The structural basis of persistent pulmonary hypertension of the newborn infant. J Pediatr 98:962–967
10. Heymann MA, Rudolph AM 1976 Effects of acetylsalicylic acid on the ductus arteriosus and circulation of fetal lambs in utero. Circ Res 38:418–422
11. Levin DL, Mills LJ, Weinberg AG 1979 Hemodynamic pulmonary vascular and myocardial abnormalities secondary to pharmacologic constriction of the fetal ductus arteriosus. Circulation 60:360–364
12. Harker LC, Kirkpatrick SE, Friedman WF, Bloor CM 1981 Effects of indomethacin on fetal rat lungs: a possible cause of persistent fetal circulation (PFC). Pediatr Res 15:147–151
13. Manchester D, Margolis HS, Sheldon RE 1976 Possible association between maternal indomethacin therapy and primary pulmonary hypertension of the newborn. Am J Obstet Gynecol 226:467–469
14. Levin DL, Fixler DE, Moriss FC, Tyson J 1978 Morphologic analysis of the pulmonary vascular bed in infants exposed in utero to prostaglandin synthetase inhibitors. J Pediatr 92:478–483
15. Csaba IF, Sulyok E, Ertl T 1978 Relationship of maternal treatment with indomethacin to persistence of fetal circulation syndrome. J Pediatr 92:484
16. Perkin RM, Levin DL, Clark R 1980 Serum salicylate levels and right to left ductus shunts in newborn infants with persistent pulmonary hypertension. J Pediatr 96:721–726
17. Rubaltelli FF, Chiozza ML, Zanardo V, Cantarutti F 1979 Effect on neonate of maternal treatment with indomethacin. J Pediatr 94:161
18. Wiqvist N, Lundstrom V, Green K 1975 Premature labor and indomethacin. Prostaglandin 10:515–526
19. Kumor KM, White RD, Blake DA 1979 Indomethacin as a treatment for premature labor. Neonatal outcome. Pediatr Res 13:370(abstr)
20. Coceani F, Olley PM, Bishai I, Bodach E, Heaton J, Nashat M, White E 1977 Prostaglandins and the control of muscle tone in the ductus arteriosus. Adv Exp Med Biol 78:135–142
21. Murphy JD, Aronovitz MJ, Reid L 1986 Effects of chronic in utero hypoxia on the pulmonary vasculature of the newborn guinea pig. Pediatr Res 20:292–295
22. Davies G, Reid L 1970 Growth of the alveoli and pulmonary arteries in childhood. Thorax 25:669–681
23. Hislop A, Reid L 1970 New pathological findings in emphysema of childhood. I. Polyalveolar lobe with emphysema. Thorax 25:682–690
24. Hislop A, Reid L 1974 Arterial changes in Crotalaria spectabilis induced pulmonary hypertension in rats. Br J Exp Pathol 55:153–163
25. Hislop A, Reid L 1976 New findings in pulmonary arteries of rats with hypoxia induced pulmonary hypertension. Br J Exp Pathol 57:542–554
26. Emery JL, Mithal A 1960 The number of alveoli in the terminal respiratory unit of man during late intrauterine life and childhood. Arch Dis Child 35:544–547
27. Wilcoxon F 1945 Individual comparisons by ranking methods. Biometr Bull 1:80
28. Wallenstein S, Zucker C, Fleiss JL 1980 Some statistical methods useful in circulation research. Circ Res 47:1–9
29. Friedman WF, Printz MP, Kirkpatrick SE, Hoskins EJ 1983 The vasoactivity of the fetal lamb ductus arteriosus studied in utero. Pediatr Res 17:331–337
30. Levin DL, Mills LJ, Parkey M, Garriott J, Campbell W 1979 Constriction of the fetal ductus arteriosus after administration of indomethacin to the pregnant ewe. J Pediatr 94:647–650
31. Kirkpatrick SE, Printz MP, Friedman WF 1977 Prostaglandins (Pgs) and the fetal ductus arteriosus (PDA). Pediatr Res 11:394(abstr)
32. Sharpe GL, Thalme B, Larsson KS 1974 Studies on closure of the ductus arteriosus: XI. Ductal closure in utero by a prostaglandin synthetase inhibitor. Prostaglandins 8:363–368
33. Sharpe GL, Larson KS, Thalme B 1975 Studies on closure of the ductus arteriosus XII. In utero effect of indomethacin and sodium salicylate in rats and rabbits. Prostaglandins 9:585–596
34. Arcilla RA, Thilenius OG, Ranniger K 1969 Congestive heart failure from suspected ductal closure in utero. J Pediatr 75:74–78
35. Geggel RL, Reid LM 1984 The structural basis of PPHN. In: Philips JB (ed) Clinics in Perinatology, vol 11/3 Symposium on Neonatal Pulmonary Hypertension. WB Saunders, Philadelphia, pp 525–549
36. Parks BR, Jordan RL, Rawson JE, Douglas BH 1977 Indomethacin: Studies of absorption and placental transfer. Am J Obstet Gynecol 129:464–465
37. Rudolph AM 1981 Effects of aspirin and acetaminophen in pregnancy and in the newborn. Arch Intern Med 141:358–363