

Preliminary communication

Influence of the central nervous system on fetal lung development

Experimental study

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SUMMARY Upper cervical cord injury was produced in fetal rabbits at 22-26 days' gestation. In 11 fetuses with severe cord injury delivered at 28-29 days' gestation there was a median reduction in lung weight (expressed as a proportion of body weight) of 43% and a median reduction in estimated total lung DNA of 16% in comparison with paired operated littermates with intact cords. The hypoplastic lungs showed collapse on histology; if cord damage had been inflicted before 24 days' gestation there was retarded maturation. We conclude that the central nervous system plays a vital role in fetal lung growth and maturation, probably by maintenance of fetal respiratory movements.

The liquid secreted into the airways of the mammalian fetal lung is believed to aid growth and development of the lung lobule in the latter part of gestation (Alcorn *et al.*, 1977). The recognition that the human fetus undertakes respiratory movements from early in the second trimester (Boddy and Dawes, 1975) and the observation that human fetal lung growth is retarded in conditions where respiratory activity is known to be depressed have prompted the senior author to postulate that respiratory movements and lung liquid secretion act in conjunction to promote normal fetal lung growth (Wigglesworth, 1976). The experiment reported here was designed to test this hypothesis by interrupting central nervous pathways to the fetal lungs without causing mechanical obstruction to lung growth and without interruption of the pituitary-adrenal axis.

Materials and methods

New Zealand white rabbits were subjected to laparotomy under fentanyl and fluanisone veterinary anaesthetic (Hypnorm: Crown Chemical Co., Lamberhurst, Kent) at 22-26 days' gestation. The fetus within its segment of uterus was delivered from the

abdomen, and a 1 cm incision made in the uterine wall over the fetal neck with care to avoid rupturing the amniotic sac. A 19-gauge needle or plastic cannula size 3 FG was inserted through the sac, advanced into the neck to the level of the upper cervical cord (C1-C3) and a small quantity of tissue aspirated. The uterine wound was repaired using a fine purse string suture. Control fetuses were treated similarly but the needle was inserted into the subcutaneous tissue without aspiration. Loss of liquor was minimal and the sac remained turgid at completion of the procedure. Up to 6 operations were performed on any one litter with an equal number of control and experimental fetuses.

The animals were killed at 28 or 29 days' gestation (term=30-31 days) and the uterus removed intact. The fetuses were retained within the amniotic sacs until attempts at respiration had ceased (usually 30 minutes). After weighing each fetus the lungs were dissected out and weighed to 0.01 g and the spinal canal and cranial cavity opened and inspected for macroscopical damage. Comparison was made between experimental fetuses with severe upper cervical cord injury (total or hemitranssection, or a grossly atrophic cord segment) and experimental or control operated fetuses with macroscopically normal cords and brain stems. Each cord-injured fetus

was compared with the operated fetus with an intact central nervous system nearest in weight to it in the same litter. Fetuses with minor cervical cord damage are not considered further here.

The left lung was fixed intact for routine light microscopy while the right lung was reweighed and frozen for nucleic acid measurements after removal of a small portion for histological assessment of lung maturation by the p-phenylene diamine method (Gandy *et al.*, 1970). DNA was measured in triplicate by the method of Le-Pecq and Paoletti (1966), to allow assessment of variations in lung cell population according to the principle of Enesco and Leblond (1962).

Results

Surviving fetuses with severe cord injury made no visible attempt to breathe at any time after excision of the uterus, whereas all other fetuses whether they had been operated on or not were seen to make periodic gasps involving synchronous mouth opening and thoracic movements. Control and experimental operated fetuses showed no apparent differences in appearance from each other and from non-operated littermates. There was no evidence of fetal compression, malformation, or growth retardation in the cord-injured fetuses. The thoracic cages appeared normal.

Fetal weights did not differ between cord injury and control groups (Table). 10 of the 11 cord-injured fetuses had very small lungs (expressed as lung weight/10 g body weight) with a median reduction of 43% when compared with their controls (Table). Estimated total lung DNA/g body weight was also significantly decreased (median reduction 16%, Table), indicating a genuine reduction in lung cell population.

Histology showed collapse of the small lungs in the cord-injured group; if operation had been performed before 24 days' gestation the lungs appeared less mature than those of the controls. Preliminary examination of semi-thin lung sections showed fewer osmiophilic inclusions in the type II alveolar cells of cord-injured fetuses operated on before 24 days than in their controls (confirming delayed maturation) but no obvious differences in those operated on at 25 or 26 days.

The findings in the cord-injured fetus with lungs of normal weight (pair no. 8 in Table) deserve special mention. The damage to the central nervous system included complete cord transection and extensive destruction of the left half of the medulla, associated with maldevelopment of the tongue and pharynx and obstruction of the larynx by a mucus plug. Although the lung DNA content was normal, histology showed fluid distension of the air spaces with hypercellular septa and retarded maturation of alveolar epithelium.

Discussion

Upper cervical cord destruction should stop fetal respiratory movements without impairing musculo-skeletal nutrition, since the spinal reflex arc is preserved. Vagal parasympathetic lung innervation also remains intact if the medulla is unharmed. However, the sympathetic supply to the lungs which leaves the spinal cord via segments T1-T5 (Widdicombe and Sterling, 1970) will be affected in this experiment, so we are unable at present to exclude the possibility that interruption of sympathetic pathways has played a role. Nevertheless, we believe that cessation of fetal respiratory activity is the most logical explanation for the collapse and growth retardation of the fetal lungs which follows cervical cord transection.

Table *Body weight, lung weight, and lung DNA content in cord-injured fetuses as compared with operated littermate controls*

Pair no.	Fetal weight (g)		Lung weight (mg/10g body weight)		Lung DNA (μ g/g body weight)	
	Control	Cord injury	Control	Cord injury	Control	Cord injury
1	16.87	27.84	249	194	277	198
2	32.80	35.68	314	188	228	210
3	36.73	34.16	316	179	264	243
4	31.73	33.21	312	166	198	233
5	29.13	31.55	309	158	219	174
6	33.81	33.98	396	215	304	251
7	53.25	40.65	366	165	161	122
8	50.00	44.10	266	268	184	190
9	47.77	48.98	274	120	169	135
10	32.30	43.39	241	170	191	161
11	46.85	48.15	237	160	178	157
	Not significant*		P < 0.01*		P < 0.05*	

*Wilcoxon test for pair differences.

The results of fetal decapitation in rabbits and rats including lung enlargement and delayed maturation (Blackburn *et al.*, 1973; Chiswick *et al.*, 1973) have been interpreted as evidence that fetal respiratory activity is of little importance in prenatal lung development (Jost and Policard, 1948). As fetal decapitation causes tracheal occlusion the growth-promoting action of retained lung liquid (Alcorn *et al.*, 1977) may counteract any effects of fetal respiratory standstill. A similar result occurred in one fetus with an obstructed larynx in our series. This indicates that cervical cord transection does not prevent lung liquid secretion.

If our interpretation is correct, cessation of fetal respiratory movements should have a more severe effect in man than in the rabbit, as the fetal period occupies a far larger proportion of total gestation. Impairment of fetal respiratory activity by oligohydramnios could offer a satisfactory explanation for the hypoplastic lungs seen in renal agenesis. The hypoplastic lungs seen with diaphragmatic aplasia could be interpreted similarly on the basis of impairment of lung function rather than by a mechanical compression effect. Diminished fetal breathing movements caused by weakness of the respiratory muscles may also explain the low lung volumes and enlarged airways recently found in infants with Werdnig-Hoffmann disease of intrauterine onset, as compared with normal pulmonary function in cases of post-natal onset (Cunningham and Stocks, 1977). We conclude that our experiment provides good evidence for an important role of the central nervous system in control of mammalian fetal lung development and supports the hypothesis that this control is exerted through fetal respiratory movements.

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