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The effect of *in utero* decapitation on the morphological and physiological development of the fetal rabbit lung

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INTRODUCTION

In 1929, von Neergard described a surface tension-reducing substance in the lung. The presence of this lining substance (surfactant) was later verified by Pattle (1955) and Mead, Whittenberger & Radford (1957). The precise nature of surfactant is not clear but its main component is now known to be a phospholipid, dipalmitoyl lecithin.

The Type II pneumonocyte (synonyms – great alveolar cell, Type A cell, niche cell) of the alveolar epithelial lining is thought to be the site of production and storage of surfactant, although the evidence for this is mainly circumstantial (Sorokin, 1967; Askin & Kuhn, 1971; Meyrick & Reid, 1973; Wang, Kotas, Avery & Thurlbeck, 1971). Two facts are of particular interest. Lamellated (? surfactant) bodies are first identified in the fetal rabbit lung at the time of a fall in alveolar surface tension (Humphreys & Strang, 1967; Reid & Meyrick, 1969; Kikkawa, Motoyama & Gluck, 1968). In hyaline membrane disease or idiopathic respiratory distress syndrome of newborn infants, the lamellated bodies are sparse (Gandy, Jacobson & Gairdner, 1970) and alveolar surface tension abnormally high.

It has been said that *in utero* decapitation interferes with lung development. In the rat, Blackburn, Travers & Potter (1972) found that cytodifferentiation of Type II pneumonocytes was impaired and the surface lining layer inactive, while in the rabbit Chiswick, Ahmed, Jack & Milner (1973) described a reduction in the number of osmiophilic inclusion bodies although surface tension, measured by the Pattle (1958) Bubble Stability method, was normal. Conversely it has been shown that maturation of the lung can be hastened, and surfactant demonstrated earlier, after stimulation with 9-fluoro-prednisolone (Wang *et al.* 1971), or cortisol (Kikkawa *et al.* 1971), or thyroxine (Redding, Douglas & Stein, 1972).

This report deals with the morphological development of the fetal rabbit lung and its physiological properties after *in utero* decapitation at 22 days, which of course removes the fetus' pituitary gland.

MATERIAL AND METHODS

The rabbit does used in these experiments were mated between 10 a.m. and 12 noon and then housed in individual cages. The following day was designated as day 1 of fetal life.

At 22 days laparotomy was performed on the mother under light halothane anaesthesia and the uterus exposed through a midline incision. The second fetus from the ovarian end of each uterine horn was decapitated by the 'intra-amniotic' method (Bearn & Pilkington, 1963) to avoid loss of amniotic fluid. Fetuses were delivered by Caesarian section at various ages, after the mother had been killed by a blow to the back of the head.

Electron microscopy

Twelve fetal rabbits were examined in all – 6 decapitated fetuses and 6 control littermates. One decapitated fetus and one control fetus were taken from each mother and altogether two fetuses from each group were studied at 27, 28 and 30 days of gestational age. Each fetus was weighed and in the case of the controls – with and without the head. Immediately after removal from the amniotic sac the lungs were dissected from the fetus and 1 mm cubes of tissue were taken from the right lower lobe and fixed in 2 % glutaraldehyde in cacodylate buffer (pH 7·2) for 90 minutes. After fixation the blocks were transferred to cacodylate buffer at 4 °C overnight, post-fixed in 1 % osmium tetroxide in cacodylate buffer for 60 minutes, washed well in distilled water, dehydrated through alcohols, impregnated with epoxy propane and embedded in Araldite. Using glass knives and an LKB Ultratome III, pale gold sections were cut, stained with uranyl acetate and Reynolds' lead citrate (1963) and examined with an AEI EM6B.

Quantification. From the control and decapitated fetuses electron micrographs of 10 Type II pneumonocytes were taken at each age, from each of two fetuses. Only one cell was photographed from any alveolus, this being the first Type II that had a nucleus and a free alveolar surface. The number of lamellated and dense bodies per cell was counted and the mean for the 20 cells at each age calculated. The significance of differences between control and experimental material was determined by applying the Student's t-test.

The amount of glycogen in the cell was assessed subjectively. Normal amounts were recorded as ±, large pools of glycogen as + + +, moderate amounts as + + and little as +.

Light microscopy

The remainder of the right lower lobe was fixed in 10 % formalin, processed through graded alcohols and chloroform and then embedded in wax for light microscopic examination. Sections were cut at 4 μm and stained with haematoxylin and eosin. The number of air sacs in 10 microscopic fields was assessed and the mean obtained for both the decapitated and control fetuses. Other blocks from the lung and liver were fixed in Gendre's fixative at 4 °C, processed as above and stained with the periodic acid Schiff technique for glycogen.

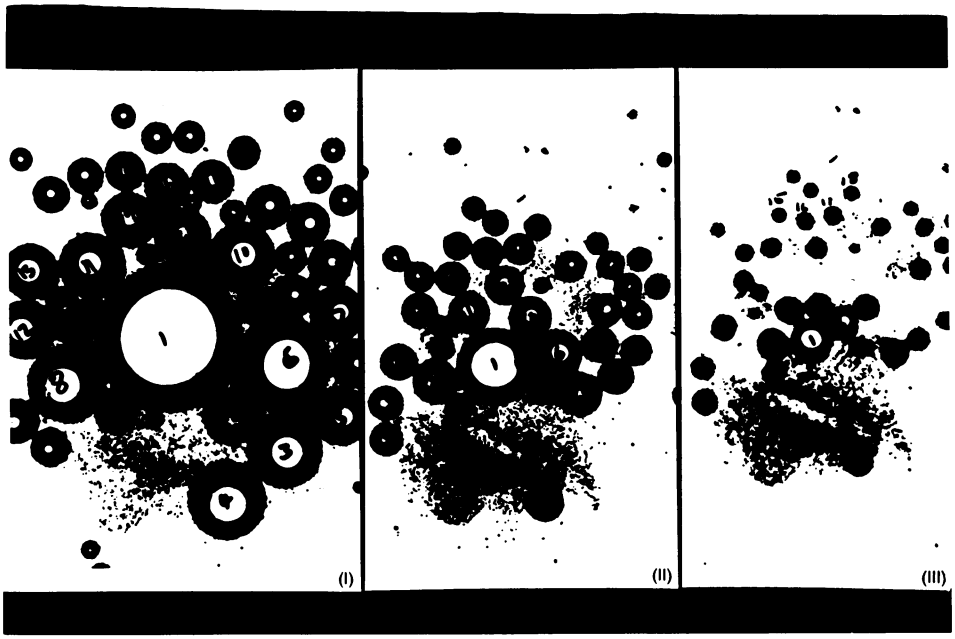

Fig. 1. Field of bubbles photographed (i) immediately, (ii) at 20 minutes and (iii) at 40 minutes, under standard conditions of temperature and humidity, from a control fetus at 27 days.

Physiological methods

Both decapitated and litter mate control fetuses were studied from 25 to 30 days of gestation.

Each fetus was killed by a blow on the back of the head either immediately before a breath had been taken, or following 30 minutes breathing in a humidicrib at 38–40 °C. Lungs from decapitated fetuses, and from fetuses which had not been allowed to breathe, were inflated by cannulating the trachea and then injecting air with a 2 ml syringe.

Bubble stability

Surface activity was measured by the Bubble Stability Method of Pattle (1958) as modified by Humphreys & Strang (1967). One lung lobe from each fetus was cut into several small pieces, which were then squeezed and froth was obtained from the cut edges. The lung fluid was transferred to a drop of aerated tap water hanging from a coverslip which was placed over a welled glass slide and sealed with a drop of tap water to prevent evaporation. Five slides of bubbles expressed from the lungs of each fetus studied were prepared and a suitable field showing a collection of bubbles was photographed at once, at 20 minutes and at 40 minutes under standard conditions of temperature and humidity (Fig. 1). The diameters of 20 bubbles from each slide were measured and the Stability Ratio of each bubble was calculated, using the formula:

$$\text{Stability Ratio} = \frac{(\text{Bubble diameter after 20 min period})^2}{(\text{Bubble diameter before 20 min period})^2}.$$

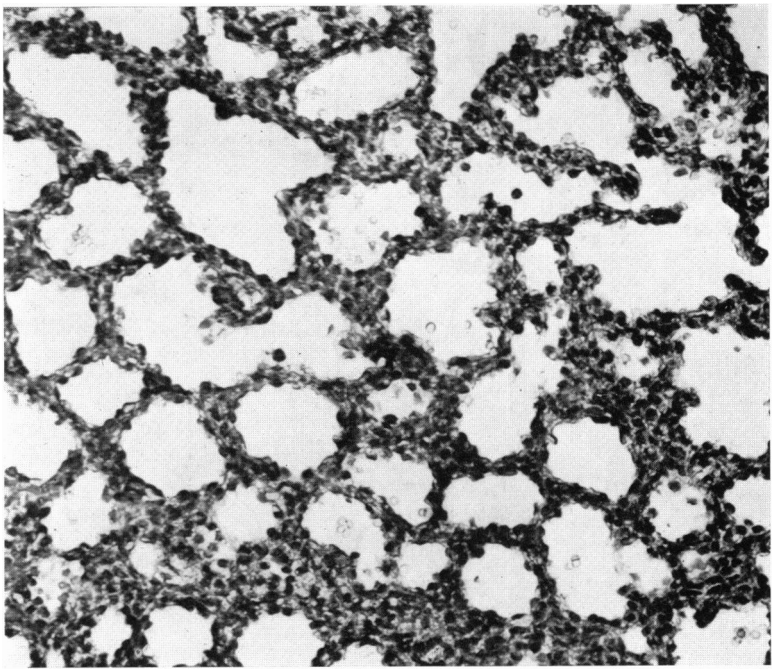

Fig. 2. Control fetal rabbit lung at 27 days, showing late canalicular/early terminal sac phase H & E. × 235.

For each fetus studied the Stability Ratio was calculated for the period 0–20 minutes and 20–40 minutes and the mean Stability Ratio for each age was determined.

Chromatography

Several rabbit fetuses between 26 and 30 days were killed within their amniotic sacs and the trachea exposed.

Three separate procedures were carried out on each fetus:

(1) A syringe needle was introduced into the lumen of the trachea and 0·5 ml of fluid was aspirated.

(2) 0·2 ml normal saline was then injected into the trachea and primary bronchi, and aspirated immediately.

(3) 0·2 ml of fluid from the stomach lumen was aspirated.

Corresponding samples from fetuses of the same age were pooled; that is, lung fluid (1), lung washings (2) and stomach fluid (3).

Lecithin and sphingomyelin were extracted by the method of Gluck *et al.* (1971), and separated chromatographically on Silica gel H coated glass plates.

RESULTS

No significant difference was found between the body weights of control fetuses decapitated after birth and those of the fetuses decapitated *in utero* (see Bearn, 1971).

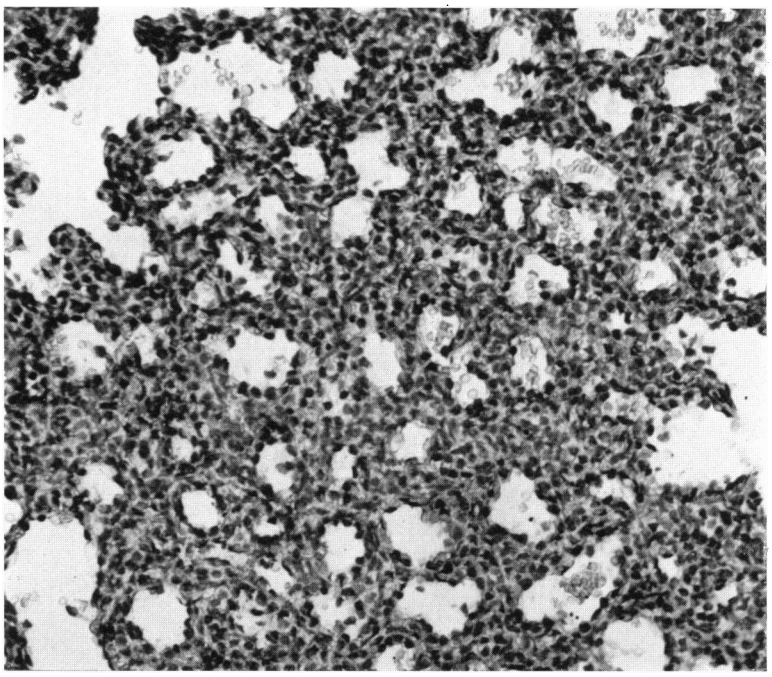

Fig. 3. Lung of decapitated littermate at 27 days, showing increased cellularity and thickened saccular walls. H & E. × 235.

Light microscopy

At the time of decapitation (22 days) the fetal lungs are normally in the pseudo-glandular phase with loose mesenchyme surrounding 'glandular' buds lined by columnar epithelium.

Between 24 and 27 days the lungs are in the canalicular phase and it is during this period that the thinning of the saccular (alveolar) walls occurs.

At 27 days, the first age examined in the present experiments, the control lung was in the late canalicular, early terminal sac or alveolar phase (Boyden, 1972) (Fig. 2). The decapitated fetal lungs were more cellular and showed thicker saccule walls (Fig. 3). There appeared to be a slight decrease in the number of air spaces (saccules) per field in the decapitated fetuses and these seemed smaller, but the differences from the control were not statistically significant.

At 28 days a similar picture to that at 27 days was found.

At 30 days the control lungs showed further differentiation and thinning of the saccular walls (Fig. 4). In the decapitated fetuses saccules appeared smaller and fewer than in the control animals but this was not statistically significant. The saccular walls remained thicker and more cellular than the controls (Fig. 5). This would suggest that the saccules were forming in the decapitated fetuses but the walls had failed to thin.

Liver glycogen. Decapitated fetuses were found to exhibit strikingly less glycogen in their livers than the controls, at each age studied.

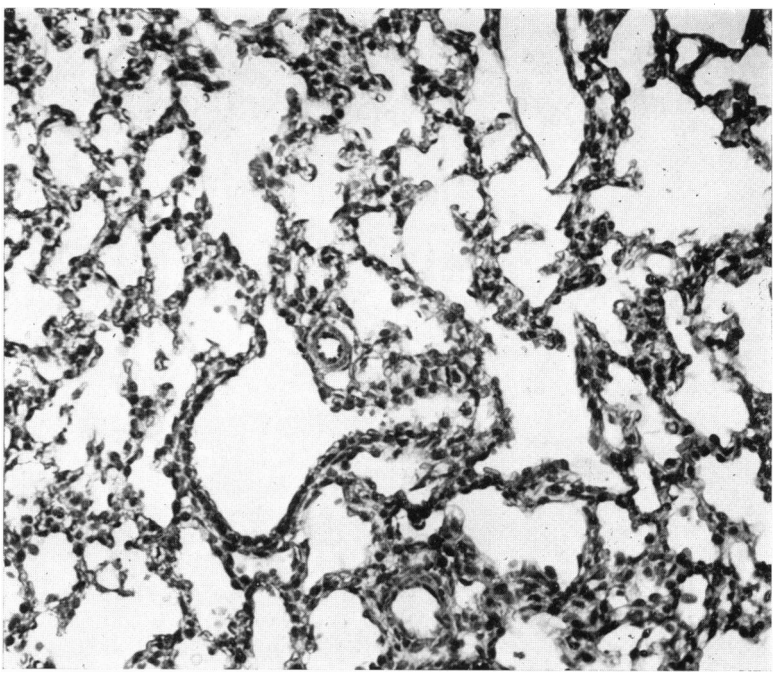

Fig. 4. Control fetal rabbit lung at 30 days, showing increased complexity of terminal saccules. H & E. × 235.

Ultrastructure

The ultrastructure of the control developing fetal rabbit lungs followed a similar pattern at 27, 28 and 30 days to that previously reported by Kikkawa *et al.* (1968) and Reid & Meyrick (1969). The development of the lungs in the decapitated fetuses, however, showed a markedly different pattern of development at all the times examined.

The percentages of Type I and Type II pneumonocytes present in both control and decapitated fetuses were similar (approximately 50 % each), although at 27 days, particularly in the decapitated fetuses, assessment was difficult as the lamellated bodies were rarely found and identification was mainly based on the cuboidal shape of the Type II pneumonocyte. The number of lamellated bodies per Type II pneumonocyte was reduced in the decapitated fetuses (Figs. 6, 7, and 8).

(1). *Lamellated bodies.* At 27 days a 60 % reduction in the number of lamellated bodies was found, at 28 days a 70 % decrease and at 30 days a 50 % decrease ($P = > 0{\cdot}001$) was observed. The number of lamellated bodies at 30 days in the decapitated fetuses was similar to that found in the controls at 27 days. The sizes of the lamellated bodies in the control and decapitated fetuses were similar, with a mean diameter of 0·6 μm (0·47–0·87 μm) (Figs. 6 and 7).

(2). *Dense bodies.* Dense (homogeneous, electron-dense) bodies, measuring approximately 0·38 μm (0·27–0·47 μm) diameter, were found in some Type II pneumonocytes (Fig. 8) and these were assessed in a similar manner to the lamellated bodies (Fig. 6).

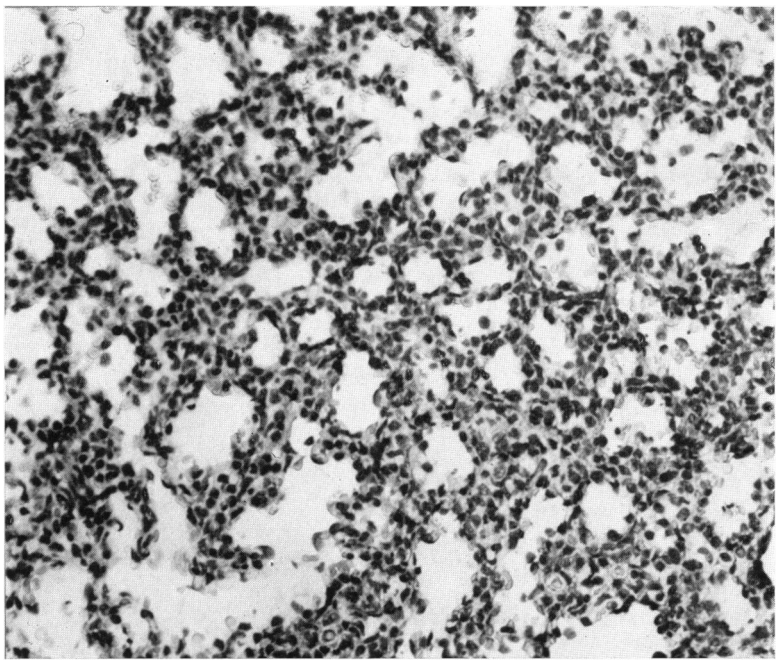

Fig. 5. Lung of decapitated littermate at 30 days. Formation of saccules has occurred, although the walls are still markedly thickened. H & E. × 235.

The number of these dense bodies per Type II pneumonocyte was found to be less in the control than in the decapitated animals at all ages examined ($P = < 0{\cdot}05$ in two cases – 27 and 30 days; $P = < 0{\cdot}6$ at 28 days). These dense bodies are most probably immature or abortive lamellated bodies. When the numbers of dense and lamellated bodies were combined, the numbers in the decapitated fetuses were still significantly less than in the controls ($P = < 0{\cdot}001$ at 27 and 30 days, $< 0{\cdot}02$ at 28 days).

Osmiophilic material was observed in saccules of control fetuses from day 28, and in the alveoli of the decapitated fetuses from 30 days.

(3) *Glycogen.* The amount of glycogen was found to be dramatically less in the control animals than in the decapitated ones at each stage studied (Table 1; Figs. 7 and 8). The decapitated fetuses at 30 days showed similar amounts of glycogen to the control fetuses at 28 days.

(4) *Interstitium.* In the decapitated fetuses the alveolar wall was thicker than in the controls, and lung maturation was retarded. The walls contained large numbers of fibroblast-like cells (Fig. 9), and capillaries were more centrally placed within the alveolar walls. A little oedema fluid was found in the walls of the decapitated fetuses.

In the few terminal bronchioles examined from the decapitated fetuses, the non-ciliated cells or 'Clara' cells of the terminal bronchioles appeared similar to those of the controls, that is, they did not contain secretory granules or smooth endoplasmic reticulum.

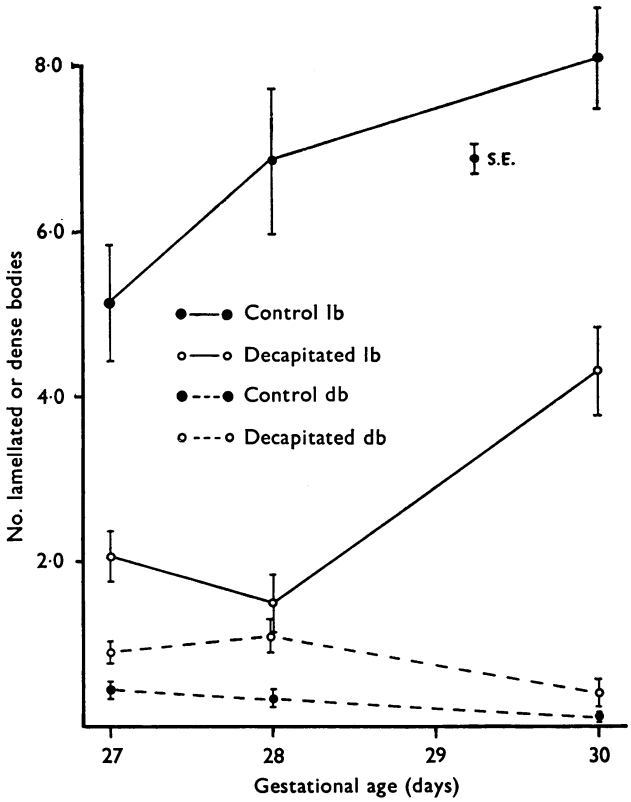

Fig. 6. Mean number of lamellated (lb) and dense bodies (db) per Type II pneumonocyte at 27, 28, and 30 days, for control and decapitated fetal rabbits.

Bubble stability

The Stability Ratio (SR) of bubbles from lungs of control fetal rabbits increased most rapidly between 26 and 28 days of gestation; at 28 days it reached a level that was maintained until term (Table 2, Fig. 10).

No bubbles could be obtained from the lungs of either 25 day old breathing fetuses or 27 day old decapitated fetuses (Fig. 10). On release of pressure after artificial inflation, lungs at these stages collapsed quickly and completely, being unable to retain any air. The 25 day fetuses which 'breathed' for 30 minutes could only make very feeble respiratory movements towards the end of that time. The blood of these fetuses was very dark in colour, indicating hypoxia. Lung bubbles from breathing fetuses under 26 days' gestational age were considerably less stable than those from non-breathing fetuses of similar ages (Fig. 10).

The increase in bubble stability of fetuses decapitated at 22 days took place between 28 and 29 days (Fig. 10). The maximum SR attained by decapitated fetuses was reached by day 29, but the mean SR of 29 and 30 day groups was significantly lower ($P = < 0{\cdot}01$) than that of comparable non-breathing control animals.

In all fetuses studied, the SR of lung bubbles was greater in the second 20 minute period after the preparation of the slides than in the first (Figs. 11 and 12).

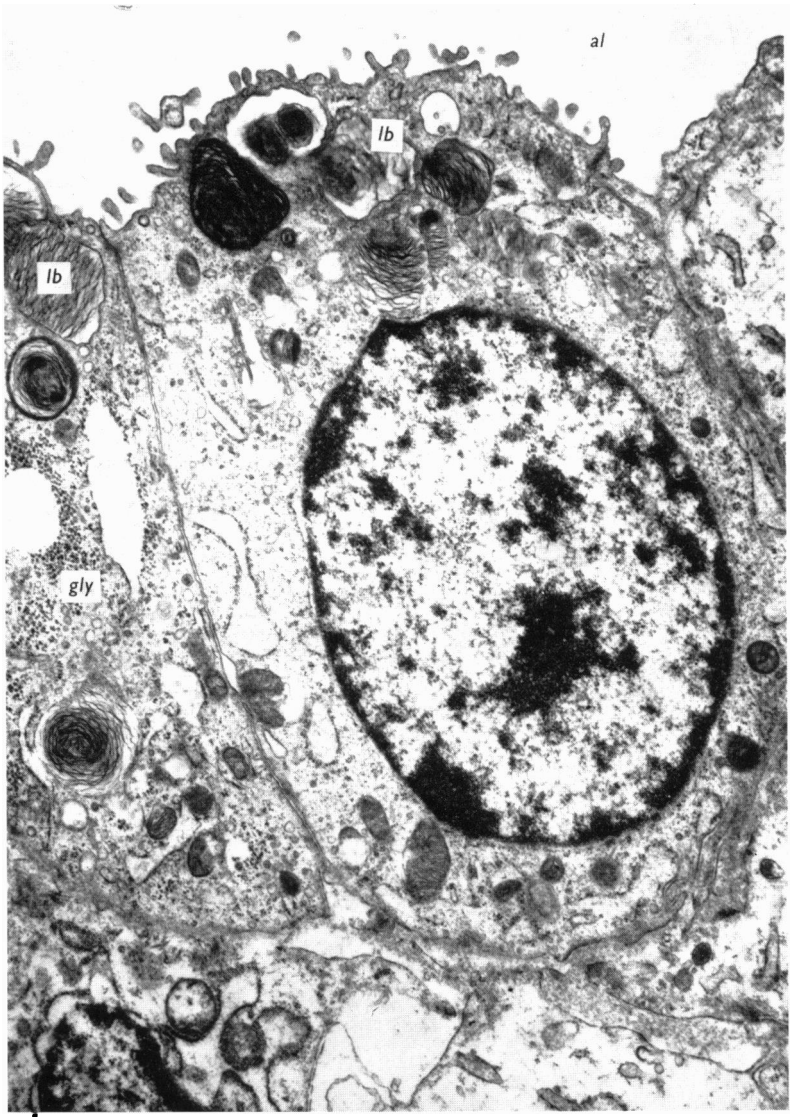

Fig. 7. Type II pneumonocytes from control rabbit fetus at 27 days, showing lamellated bodies (*lb*) and little glycogen (*gly*). Alveolus (*al*). × 13000.

Chromatography

Lecithin was detected in the samples of lung fluid aspirated from 27 day fetal lungs and, in increasing concentration, from the lungs of 28, 29 and 30 day fetuses. Traces of sphingomyelin were also detected on the chromatograms of lung fluid from 26–30 day fetuses.

In the lung washing, lecithin was present at 28 days' gestational age and increased markedly between 29 and 30 days; no sphingomyelin was detected.

The rabbit fetus swallows amniotic fluid as well as fluid overflowing from its

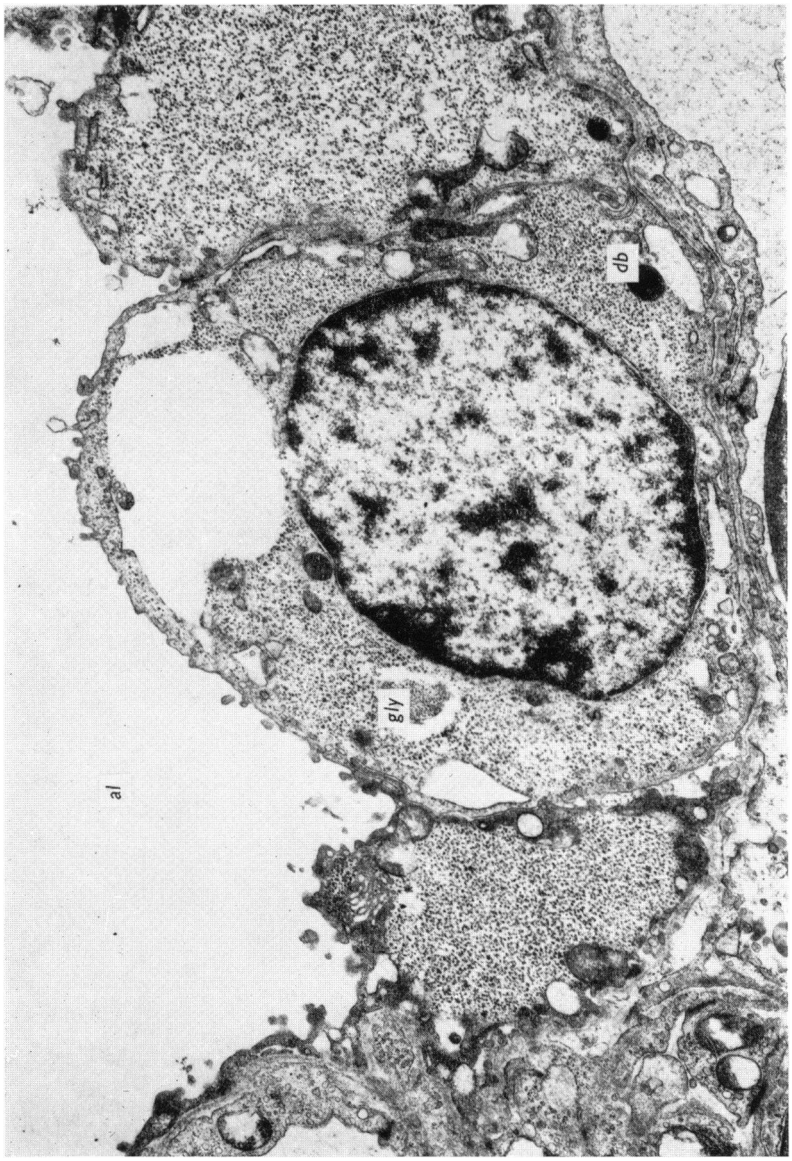

Fig. 8. Type II pneumonocyte from a decapitated fetus at 27 days. Lamellated bodies are sparse; dense bodies (*db*) and large amounts of glycogen (*gly*) are found. × 11500.

Table 1. *Glycogen per Type II pneumonocyte in fetal rabbit lung (control, – C; decapitated fetus, – D)*

(± = normal amounts; + + + = large pools of glycogen; + + = moderate amounts; + = little glycogen.)

Fetal age (days)		±	+	+ +	+ + +
27	C	0	5	*10*	5
	D	0	3	7	*10*
28	C	*12*	6	2	0
	D	0	1	9	*10*
30	C	*18*	0	2	0
	D	*11*	6	3	0

trachea. In the stomach fluid, lecithin was present in fetuses of 29 and 30 days, while the concentration of sphingomyelin increased steadily in the stomach fluid of fetuses of increasing gestational ages (Fig. 13).

DISCUSSION

It is well established that hypoplasia of the adrenal cortex follows early intra-uterine decapitation (Bearn, 1971).

Blackburn *et al.* (1972), after intra-uterine decapitation of rat fetuses, reported a decrease in the number of lamellated bodies present in the lung and a decrease in lung surfactant. In the present study it has been shown, for the first time, that if the fetal rabbit is decapitated at as early as 22 days' gestation when the lung is in the pseudoglandular phase, the appearance of surfactant activity is delayed as well as the appearance of the lamellated bodies in the Type II pneumonocyte. Chiswick *et al.* (1973), after decapitation of the fetal rabbit at 24 days' gestation, were able to show a decrease in number of osmiophilic inclusion bodies in the lungs of decapitated fetuses using 1 μm light microscope sections, although the bubble stability and pressure volume studies showed surfactant activity to be normal. The date of decapitation would thus appear to be critical and earlier decapitation, as in the present experiments, seems to have a profound effect on lung function.

Morphological examination of lungs of the decapitated fetus showed that the walls of the saccules were thicker than normal and the capillaries more centrally placed within them. The increased thickness of the wall was due, in part, to oedema but mainly to increased cellularity. The cells found in the walls closely resembled fibroblasts, but their function is obscure since collagen and elastin were not increased. These findings suggest that canalization of the lungs, known to occur in the fetal rabbit lung between 24 and 27 days, had not taken place in the accepted manner. The number of saccules in the decapitated fetus was not significantly reduced, which agrees with the finding of Blackburn *et al.* (1972). It would seem that continuity of the tracheal lumen with the exterior is not necessary for saccule formation: rather it depends on appropriate mitotic activity (Alescio & Cassini, 1962).

Blackburn *et al.* (1972) suggested that the developing lung contributes to the

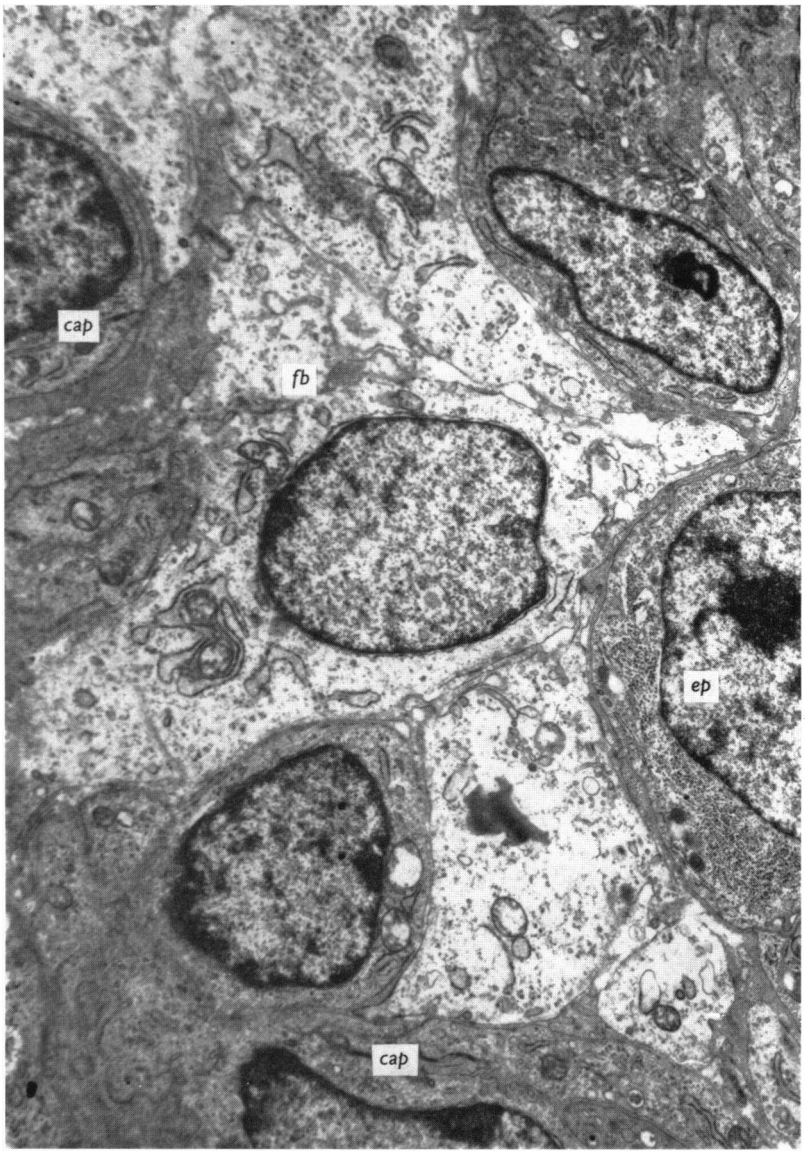

Fig. 9. Thickened alveolar wall from a decapitated fetus at 30 days, showing fibroblast-like cells (*fb*). Capillaries (*cap*), alveolar epithelium (*ep*). × 8500.

formation of the amniotic fluid, since *in utero* decapitation of rats resulted in over-distension of saccules, the tracheal occlusion preventing outflow of lung fluid. In the present study, however, the saccules of the decapitated fetuses appeared slightly smaller than those of the controls.

The presence of lecithin in the stomach of the control fetuses demonstrated that even in normal animals at least some lung fluid is swallowed. Liggins & Tooley (1969)

Table 2. *Stability ratios of bubbles obtained from the lungs of experimental and control rabbit fetuses aged between* 25 *and* 30 *days*

Age (days)	Time (min)	Breathing	Non-breathing	Decapitated
25	0 – 20		0·492 ± 0·022 (67)	
	20 – 40		0·540 ± 0·069 (16)	
26	0 – 20	0·365 ± 0·017 (64)	0·529 ± 0·028 (35)	
	20 – 40	0·400 ± 0·027 (49)	0·633 ± 0·039 (35)	
27	0 – 20	0·605 ± 0·025 (48)	0·591 ± 0·028 (76)	
	20 – 40	0·623 ± 0·025 (53)	0·675 ± 0·022 (89)	
28	0 – 20	0·909 ± 0·010 (100)	0·851 ± 0·035 (39)	0·629 ± 0·028 (94)
	20 – 40	0·938 ± 0·010 (100)	0·870 ± 0·027 (20)	0·756 ± 0·022 (82)
29	0 – 20	0·851 ± 0·017 (120)	0·868 ± 0·022 (92)	0·768 ± 0·025 (75)
	20 – 40	0·926 ± 0·010 (120)	0·967 ± 0·014 (94)	0·864 ± 0·020 (88)
30	0 – 20	0·894 ± 0·010 (281)	0·863 ± 0·017 (100)	0·758 ± 0·020 (80)
	20 – 40	0·922 ± 0·010 (281)	0·941 ± 0·010 (100)	0·866 ± 0·017 (60)

Mean ± standard error.
( ) = number of bubbles

have shown that in the fetal lamb the entire output from the lung is swallowed and none enters the amniotic fluid.

The sudden appearance of lamellated bodies in the fetal rabbit lung at 26 days (Kikkawa *et al.* 1968; Reid & Meyrick, 1969), and dramatic rise in their number between 26 and 28 days, correlates well with the functional maturity of the lung as measured by the bubble stability method. This agrees with the findings of Humphreys & Strang (1967). The chromatographic estimation of lecithin in lung washing described here indicated that this basic constituent of surfactant was detectable from 27 days.

The reduction in the stability ratio of lung bubbles shown by fetuses younger than 27 days which had been allowed to breathe for 30 minutes, probably reflects a lack of stored surfactant. Kikkawa *et al.* (1968) described a threefold increase in surfactant production during the first hour of breathing. From 27 days, when the lamellated bodies are present in fairly large numbers, the ability to survive the first few breaths seems to be established, and it is evident that pools of stored surfactant must be available to re-line the alveoli continuously.

In the decapitated fetus the decrease in the number, and the late appearance, of the lamellated bodies in Type II pneumonocytes, and the parallel change in surface tension lend support to the concept that the Type II pneumonocyte is the source of pulmonary surfactant (Askin & Kuhn, 1971; Wang *et al.* 1971; Meyrick and Reid, 1973).

In the decapitated fetus the lamellated bodies were not completely absent: rather, they were later in appearing. This suggests that there may be a second mechanism responsible for lung maturation and that the lung is not completely dependent on the pituitary-adrenal axis; the thyroid may also be involved (Blackburn *et al.* 1972).

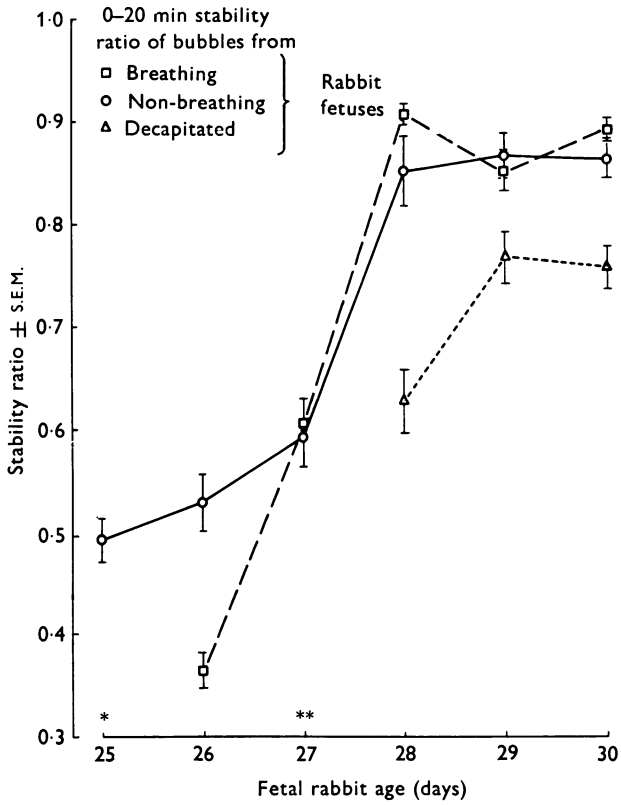

Fig. 10. Bubble stability ratio of lungs from decapitated fetuses and control non-breathing and breathing fetuses at various ages. Bubble stability could not be produced by either control breathing fetuses before 26 days (*) or decapitated fetuses before 28 days (**).

Alternatively, it may be that the mother's pituitary-adrenal axis contributes to the maturation of the fetal lung.

Dense bodies have previously been described in the fetal rabbit lung (Kikkawa *et al.* 1968). They were of various shapes, situated close to the Golgi apparatus and were thought to be precursors of the lamellated bodies. In the present study the dense bodies were frequent in the decapitated fetus; they were round or ovoid, usually situated at the cell's apical edge and rarely present in the Golgi region. This suggests that the bodies are abortive lamellated bodies, in which the enzymes responsible for surfactant synthesis are lacking. In 1972, Farell & Zackman showed that 9-fluoroprednisolone administered to fetal rabbits increased the concentration of pulmonary phosphyl-choline transferase, an enzyme necessary for the synthesis of dipalmitoyl lecithin. This supports the concept that a stimulatory effect of adrenocortical secretions on surfactant production is mediated by activation of essential enzymes. In the control fetuses these bodies were occasionally encountered, as were a few transitional between dense and lamellated bodies, suggesting that the dense body may alternatively be an immature lamellated body.

In the decapitated fetus the disappearance of glycogen was delayed. The relation-

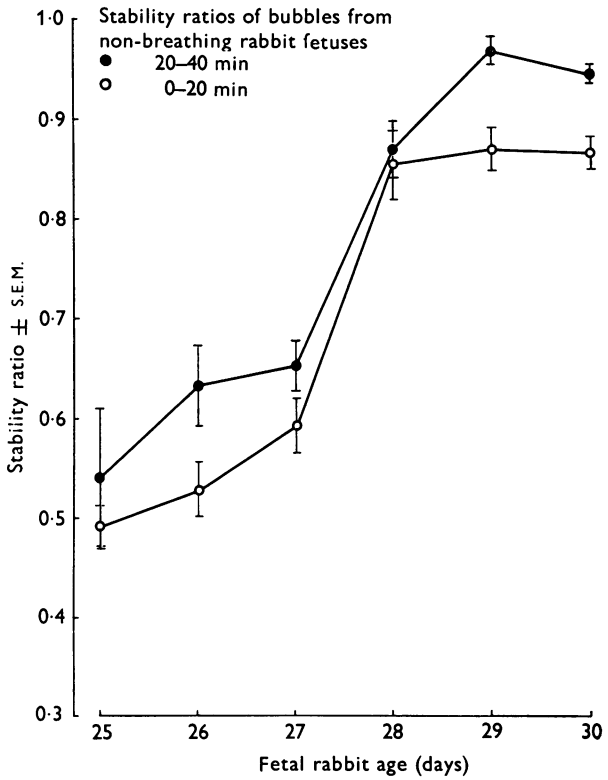

Fig. 11. Bubble stability ratio (SR) of control non-breathing fetal lung at 0–20 minutes and 20–40 minutes. Gestational age 25 to 30 days. At all times the SR of the 0–20 minute period was less than for the 20–40 minute period.

ship between glycogen disappearance and the appearance of the lamellated bodies has previously been noted (Kikkawa *et al.* 1971; Blackburn *et al.* 1970). The present study supports the concept that glycogenesis is associated with the formation of surface active phospholipids known to be found in the lamellated bodies (Askin & Kuhn, 1971; Chevalier & Colet, 1972). It is now recognized that two pathways exist for the formation of surface active phospholipids within the lung. *In utero*, the CDP-choline pathway is active between 23 and 25 days, when glycogen is present (Gluck, Sribney & Kulovich, 1967), while from 27 days, when glycogen is no longer present in the lung, the three-step methylation pathway is predominant (Kikkawa *et al.* 1971).

Whether, in the rabbit, any maternal glucocorticoids cross the placenta is not known. Jost & Jacquot (1955) showed that fetal decapitation at 22 days' gestation prevented the normal deposition of glycogen in the fetal rabbit liver, but that, after administration of ACTH to these fetuses, glycogen was increased to near normal levels. If maternal glucocorticoids, in the rabbit, were able to diffuse freely across the placenta, then interference with the fetal adrenal gland would not be expected to produce such profound changes.

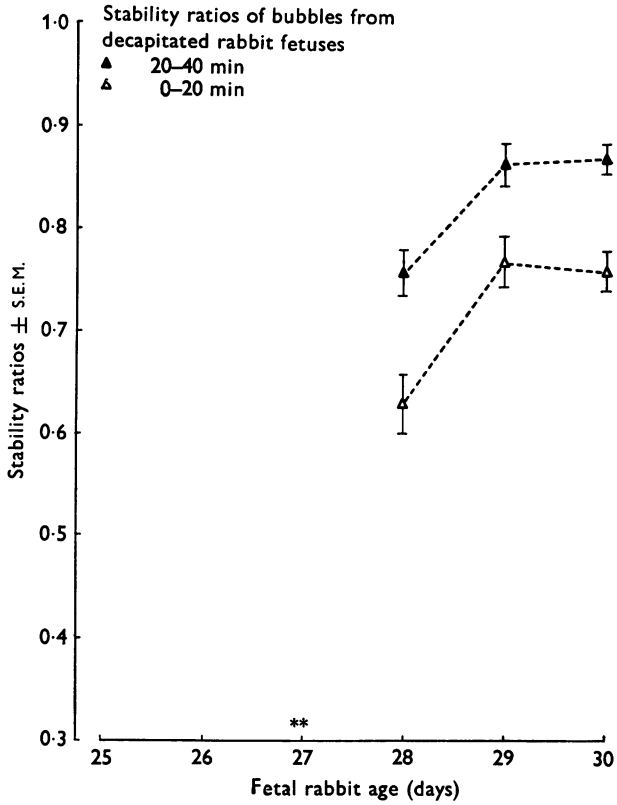

Fig. 12. Bubble stability ratio for the lung from the decapitated fetuses was less from 0–20 minutes than from 20–40 minutes at 28, 29 and 30 days gestational age. No bubbles could be produced at day 27 (**).

In this study a profound reduction in liver glycogen content in the decapitated fetuses was taken as verification that the removal of the pituitary gland had been effective in inhibiting the development of adrenocortical function.

It has been shown that the administration of thyroxine hastens lung maturation while the removal of the thyroid retards the development of the lamellated bodies (Redding *et al.* 1972). Therefore any effect of decapitation may be due, at least in part, to the removal of the fetal thyroid.

Naeye, Harcke & Blanc (1971) studied 387 consecutive autopsies of newborn and stillborn infants dying between 7–72 hours after birth and, using 1 μm light microscope sections, reported that anencephalics had only 45 % of the mass of osmiophilic bodies found in the 'control' infants. Using point-counting techniques, the anencephalic infants were found to have little or no fetal type adrenal cortex. It has also been reported that infants dying from respiratory distress syndrome, in which the number of lamellated bodies is reduced (Gandy *et al.* 1970), had adrenal glands on average 19 % lighter than those of the 'controls', due again to a lesser amount of fetal cortical tissue (Naeye *et al.* 1971).

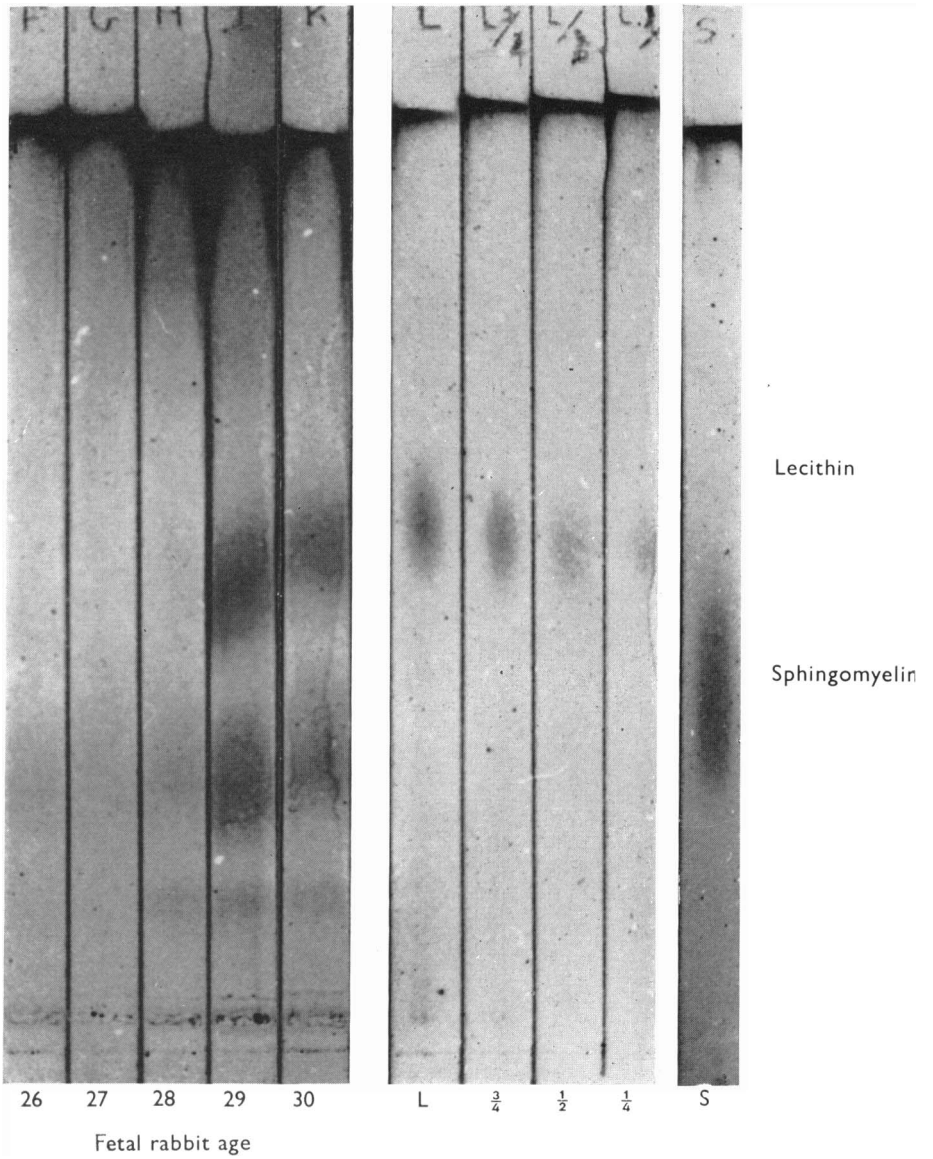

Fig. 13. Chromatograms of lecithin and sphingomyelin in stomach fluid from 26–30 days rabbit fetuses (left), as compared with standards (right).

Recent evidence that steroids hasten the maturation of Type II pneumonocytes and the appearance of alveolar surfactant (Wang *et al.* 1971; Kikkawa *et al.* 1971) points to the probable importance of these hormones in normal lung maturation. The present results, both morphological and functional, lend further support to the hypothesis that the fetal pituitary–adrenal axis is involved in lung maturation.

SUMMARY

A study has been made of the consequences of *in utero* decapitation on the morphological and physiological development of the fetal lung. Fetal rabbits were decapitated *in situ* at 22 days, without losing any amniotic fluid, and allowed to continue their development with their undamaged littermates as controls. Such decapitation, of course, removes the pituitary and so interferes with adrenal cortical development.

Morphological studies showed an interference with lung development in that, although the number of alveolar saccules increased normally, their walls failed to thin. In the decapitated fetuses, a reduction in the number of lamellated bodies per Type II pneumonocyte was found at each age studied; while dense, homogeneous bodies were more numerous. The normal disappearance of glycogen in the Type II pneumonocytes of the decapitated fetuses was retarded.

Physiological studies supported these findings. In control fetuses allowed to breathe for a while the Bubble Stability Ratio increased rapidly from day 26 to reach a maximum at 28 days; whereas, in the decapitated ones, bubble stability was not apparent before day 28 and by the 29th day had reached a maximum which was lower than that of the controls.

In the control fetuses, lecithin was detected in lung fluid from 26 days on, and in stomach fluid from 29 days.

It is argued that lung development must be, at least in part, under the control of the fetus' own pituitary–adrenal axis.

REFERENCES

ALESCIO, T. & CASSINI, A. (1962). Induction *in vitro* of tracheal buds by pulmonary mesenchyme grafted on tracheal epithelium. *Journal of Experimental Zoology* **150**, 83–94.

ASKIN, R. O. & KUHN, C. (1971). The cellular origin of pulmonary surfactant. *Laboratory Investigation* **25**, 260–268.

BEARN, J. G. (1971). The role of the foetal pituitary in organogenesis. In *Hormones in Development* (Ed. M. Hamburgh and E. J. W. Barrington), pp. 121–134. New York: Appleton – Century – Crofts, Educational Division, Meredith Corporation.

BEARN, J. G. & PILKINGTON, T. R. E. (1963). The hormonal control of the metabolism of cholesterol in the rabbit foetus. *Nature (London)* **198**, 1005–1006.

BLACKBURN, W. R., TRAVERS, H. & POTTER, M. (1972). The role of the pituitary-adrenal-thyroid axes in lung differentiation. I. Studies of the cytological and physical properties of anencephalic fetal rat lung. *Laboratory Investigation* **26**, 306–318.

BOYDEN, E. A. (1972). Development of the human lung. In Brennerman's *Practice of Pediatrics*, vol. 4, chap. 64. Hagerstown: Harper and Row.

CHEVALIER, G. & COLLET, A. J. (1972). *In vivo* incorporation of choline-^{3}H and galactose-^{3}H in alveolar Type II pneumonocytes in relation to surfactant synthesis. A quantitative radioautographic study in mouse by electron microscopy. *Anatomical Record* **174**, 289–310.

CHISWICK, M. L., AHMED, A., JACK, P. M. B. & MILNER, R. D. G. (1973). Control of fetal lung development in the rabbit. *Archives of Disease in Childhood* **48**, 709–713.

FARRELL, P. M. & ZACHMAN, R. D. (1972). Enhancement of lecithin synthesis and phosphoryl choline glyceride transferase activity in the fetal rabbit lung after corticosteroid administration. *Pediatric Research* **6**, 337.

GANDY, G., JACOBSON, W. & GAIRDNER, D. (1970). Hyaline membrane disease. I. Cellular changes. *Archives of Disease in Childhood* **45**, 289–310.

GLUCK, L., SRIBNEY, M. & KULOVICH, M. V. (1967). The biochemical development of surface activity in mammalian lung. II. The biosynthesis of phospholipids in the lung of developing rabbit fetus and newborn. *Pediatric Research* **1**, 247–265.

GLUCK, L., KULOVICH, M. V., BORER, R. C., BRENNER, P. H., ANDERSON, G. L. & SPELLACY, W. N. (1971). Diagnosis of the respiratory distress syndrome by amniocentesis. *American Journal of Obstetrics and Gynecology* **109**, 440–445.

HUMPHREYS, P. W. & STRANG, L. B. (1967). Effects of gestation and prenatal asphyxia on pulmonary surface properties of the foetal rabbit. *Journal of Physiology* **192**, 53–62.

JOST, A. & JACQUOT, R. (1955). Recherches sur les facteurs endocriniens de la charge en glycogène du foie foetal chez le lapin. *Les Annales d'endocrinologie* **16**, 849–872.

KIKKAWA, Y., MOTOYAMA, E. K. & GLUCK, L. (1968). Study of the lungs of fetal and newborn rabbits. *American Journal of Pathology* **52**, 177–210

KIKKAWA, Y., KAIBARA, M., MOTOYAMA, E. K., ORZALESI, M. M. & COOK, C. D. (1971). Morphologic development of fetal rabbit lung and its acceleration with cortisol. *American Journal of Pathology* **64**, 423–442.

LIGGINS, G. C. & TOOLEY, W. H. (1969). In *Foetal Autonomy* (Ed. G. E. D. Wolstenholme and M. O'Connor), p. 144. London: J. and A. Churchill Ltd.

MEAD, J., WHITTENBERGER, J. L. & RADFORD, E. P. Jr. (1957). Surface tension as a factor in pulmonary volume-pressure hysteresis. *Journal of Applied Physiology* **10**, 191–196.

MEYRICK, B. & REID, L. (1973) Electron microscopic aspects of surfactant secretion. *Proceedings of the Royal Society of Medicine* **66**, 386–387.

NAEYE, R.L., HARCKE, H. T. & BLANC, W. A. (1971). Adrenal gland structure and the development of hyaline membrane disease. *Pediatrics* **47**, 650–657.

VON NEERGAARD, K. (1929). Neue Auffassungen über einen Grundbegriff der Atem-mechanik. Die Retraktionskraft der Lunge, abhängig von der Oberflachenspannung in den Alveolen. *Zeitschrift für gesamte experimentalle Medizin, zugleich Fortsetzung der Zeitschrift für experimentalle Pathologie und Therapie* **66**, 373–394.

PATTLE, R. E. (1955). Properties, function and origin of the alveolar lining layer. *Nature* (*London*) **175**, 1125–1126.

PATTLE, R. E. (1958). Properties, function and origin of the alveolar lining layer. *Proceedings of the Royal Society, Series* B **148**, 217–240.

REDDING, R. A., DOUGLAS, W. H. J. & STEIN, M. (1972). Thyroid hormone influence upon lung surfactant metabolism. *Science* **185**, 994–996.

REID, L. & MEYRICK, B. (1969). Étude au microscope électronique du poumon foetale de lapin. *Le Poumon et la Coeur* **25**, 201–206

REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an opaque stain in electron microscopy. *Journal of Cell Biology* **19**, 209–212.

SOROKIN, S. (1967). A morphologic and cytochemical study on the great alveolar cell. *Journal of Histochemistry and Cytochemistry* **14**, 884–897.

WANG, N. S., KOTAS, R. V., AVERY, M. E. & THURLBECK, W. M. (1971). Accelerated appearance of osmiophilic bodies in fetal lungs following steroid injections. *Journal of Applied Physiology* **30**, 362–365.