J. Physiol. (1967), **192**, *pp.* 53–62
With 9 text-figures
Printed in Great Britain

EFFECTS OF GESTATION AND PRENATAL ASPHYXIA ON PULMONARY SURFACE PROPERTIES OF THE FOETAL RABBIT*

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(*Received* 3 *February* 1967)

SUMMARY

1. The pulmonary surface properties of foetal rabbits were studied after delivery by Caesarean section at 26–30 days and after normal delivery at term (31 days).

2. Quasi-static pressure–volume curves were defined for the lungs and the surface properties of the same lungs were further studied both by the bubble-stability method and by means of a surface balance and trough. Results given by each method are compared and discussed.

3. At the 26th day of gestation the pulmonary surface properties, characteristic of the animal at term, were not yet present. These properties were first evident on the 28th day.

4. A period of 16 min intense prenatal asphyxia on the 28th day of gestation failed to produce a change in pulmonary surface properties.

INTRODUCTION

The low surface tension, characteristic of normal lung extracts, appears to develop in the last 15% of gestation in the mouse (Buckingham & Avery, 1962), guinea-pig, rat (Pattle, 1961) and lamb (Howatt, Avery, Humphreys, Normand, Reid & Strang, 1965; Orzalesi, Motoyama, Jacobson, Kikkawa, Reynolds & Cook, 1965). In these studies gestational age was usually calculated indirectly from the histological appearance of the lungs or from the length and weight of the foetus. Only in the experiments of Howatt *et al.* (1965) from this laboratory was gestational age known from tupping dates, and even here there must be some uncertainty as the tuppings were recorded in the field by the farmer and not in the laboratory.

* These studies have been described as part of a thesis by P.W.H. for the degree of Ph.D. of the University of London.

In the present experiments the time of appearance of pulmonary surfactant in gestation was studied in foetal rabbits in whom the time of copulation was known to within 2 hr. Surface properties of the lungs were measured by the bubble-stability technique of Pattle (1958), the surface balance technique of Clements (1957), and from the deflation limb of static pressure–volume curves. The results of these techniques were compared. Since prenatal asphyxia has been suggested as a cause of depletion or inactivation of surfactant (e.g. Reynolds, Jacobson, Motoyama, Kikkawa, Craig, Orzalesi & Cook, 1965), and of respiratory distress in new-born infants (Cohen, Weintraub & Lilienfeld, 1960) and animals (Dawes, Jacobson, Mott, Shelley & Stafford, 1963; Davis & Stafford, 1964), experiments were performed to test the effect of an episode of prenatal asphyxia on the surface properties of rabbits' lungs at a stage of gestation when the surface properties of the lungs appeared to have begun to mature.

METHODS

Fifty-three New Zealand White doe rabbits were mated in the laboratory, the doe being introduced into the buck's cage for only 2 hr. Does were killed at 26, 27, 28, 29 and 30 days gestation, except for two which were allowed to deliver their young spontaneously at term (31 days). The does were killed by stunning and exsanguination and the young swiftly delivered by Caesarean section and killed by the intracranial injection of saturated KCl solution.

Ten does at 28 days were anaesthetized with urethane (average dose 7 ml./kg of 25 % solution). A tracheostomy was performed and a cannula placed in a carotid artery from which blood samples were withdrawn and blood pressure and heart rate recorded. Arterial oxygen and carbon dioxide tension P_{a,O_2} and P_{a,CO_2} were measured with Clark & Severinghaus electrodes and pH_a with a Beckmann micro blood pH assembly. The does breathed spontaneously throughout the experiment. After a control period they were given a prepared gas to breathe for 16 ± 1 min. This consisted of 8·3–10·5 % O_2, 0–6 % CO_2, and nitrogen to 100 % (see Table 1). At the end of the asphyxial period the foetuses were swiftly delivered by Caesarean section. In both control and asphyxiated groups it was always confirmed that the animals were alive by observation of heart beat before they were killed.

In the initial experiments the surfactant of these lungs was immediately assessed by means of the bubble-stability technique. In the later experiments lung pressure–volume curves were first determined (attempts were made on at least four foetuses in each of twenty-three control and ten asphyxiated litters), and then the bubble-stability measurements were made on 3–4 foetuses of each litter (litter-size permitting). The lungs were then inflated, frozen and stored for up to 3 days when measurements were made by the surface balance technique on the pooled lungs of the four foetuses. (This was performed on twenty-seven control and nine asphyxiated litters.)

The bubble technique was carried out as described by Pattle (1958), except that here the bubbles were photographed and their diameters measured from a negative. Results were expressed as stability ratio (S.R.) derived as the ratio d_1^2/d_2^2 where d_1 is the initial diameter of a bubble and d_2 the diameter of that bubble 20 min later. The mean value of S.R. from about twenty bubbles for each animal was calculated.

The surface balance and trough technique followed that of Clements, Hustead, Johnson & Gribetz (1961). The lungs were first inflated and then 3 g were minced as finely as possible

with scissors in 25 ml. of 0·9 % NaCl and filtered through gauze into a thoroughly cleaned trough. Surface tension (dyn/cm) of the film formed on the trough was measured by a strain gauge as the downward traction on a fine plate of platinum. The surface area of the trough was cyclically compressed and expanded between 13 and 65 cm^2, the duration of the cycle being 14 min. Measurements were taken after 10 cycles when repeated values of the minimum surface tension (γ_{min}) recorded were similar.

The surface balance used was supplied by the Research and Development Laboratory of the University of California. It was necessary to increase the output signal of the instrument by increasing the lever arm connecting the transducer to the platinum plate; thermal effects were reduced by the insertion of a compensatory thermistor and resistor network in one arm of the transducer bridge network.

For defining pressure–volume (P.V.) curves the chest was opened and a fine plastic cannula sealed into the trachea. The animal was placed in an evacuating jar and the ambient pressure reduced 3 times to less than 2 mm Hg in order to render the lungs airless. Quasi-static inflation and deflation P.V. curves were performed by the displacement of air from a bottle by water run in from a burette. By means of a transducer the pressure changes were displayed on an electrical recorder. The animals were immersed in saline so that air leaks were readily detected. The pressure–volume curves were corrected for the compression characteristics of the gas volume external to the lungs: these were determined with a clamp across the tracheal cannula. After an initial rapid change in volume a small very slow change could be observed at some pressures in the absence of leaks. In order to standardize the procedure, readings were taken at 2 min after each change in pressure. During inflation, volumes were measured at 0 and 8 cm H_2O, then at 5 cm steps to a maximum of 38 cm H_2O. During deflation, volumes were measured at 5 cm steps to 3 cm H_2O and at 0 cm H_2O. Lower maximum pressures were used to inflate the more mature lungs, as these would rupture at 38 cm H_2O.

RESULTS

Unasphyxiated animals

Pressure-volume curves. Figure 1 shows individual pressure–volume curves at 27, 28 and 30 days gestation. During this period a large increase is evident in maximum volume and in the proportion of this volume retained in the lungs at low pressures during deflation. A progressive decrease in opening pressure is also evident. Figure 2 shows the change in maximum lung volume for gestation for the unasphyxiated animals. Figure 3 shows the increase with gestation in the volume of air retained at 5 cm H_2O (V_5). Figure 4 shows the air retained at low pressures as a proportion of total lung volume in terms of L (eqn. (1)), the index of Gruenwald, Johnson, Hustead & Clements (1962).

$$L = \frac{V_5 - V_0}{V_M - V_0} + 0{\cdot}5\frac{V_{10} - V_0}{V_M - V_0}, \qquad (1)$$

where V_{10}, V_5 and V_0 are volumes at 10, 5 and 0 cm H_2O during deflation and V_M is maximum volume.

Bubble-stability ratio. The mean difference in S.R. in duplicate samples of thirty-three lungs was 0·03 with a standard deviation of these differences of 0·11 (P that 0·03 does not differ significantly from 0·00 = 0·85).

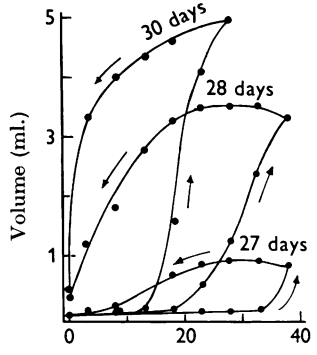

Fig. 1

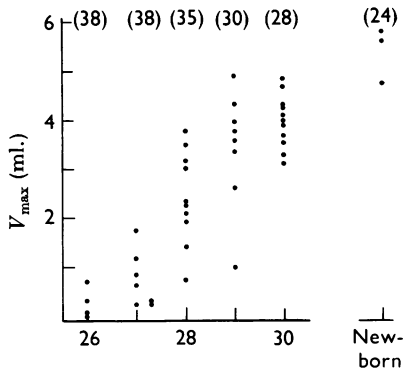

Fig. 2

Fig. 1. Pressure–volume curves of lungs from three premature, unasphyxiated rabbits at 27, 28 and 30 days during their first inflation and deflation following evacuation.

Fig. 2. The maximum lung volumes of unasphyxiated rabbits at different ages after a single inflation subsequent to evacuation of the lungs. The figures in brackets at the top of each column represent the mean maximum pressure of inflation in cm H_2O used for that age. Each point represents the result from an individual rabbit.

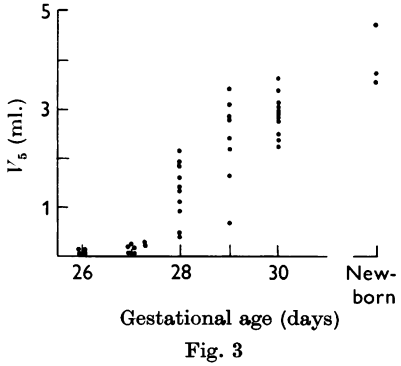

Fig. 3

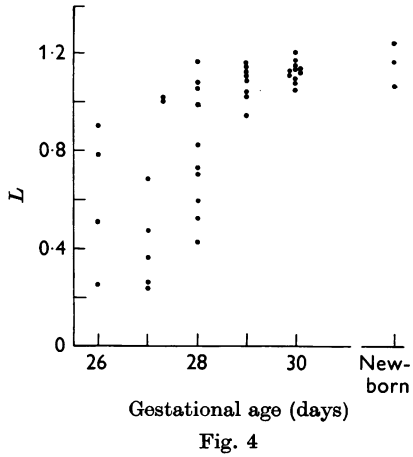

Fig. 4

Fig. 3. The lung volume (V_5) in ml. of premature and new-born unasphyxiated rabbits on deflation to a pressure of 5 cm H_2O subsequent to evacuation and a single maximum inflation. Each point is the result from an individual rabbit.

Fig. 4. The air-retention index L. of Gruenwald, Johnson, Hustead & Clements (1962) of unasphyxiated rabbits at different gestational ages. Each point is the result from an individual rabbit.

Figure 5 shows S.R. for gestational age: a striking increase took place between the 27th and 29th days of gestation, with a marked scatter in the results at 28 days; very little change in S.R. occurred after 29 days.

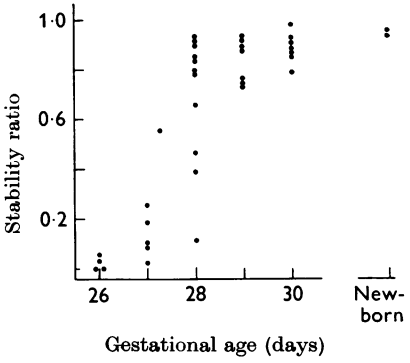

Fig. 5

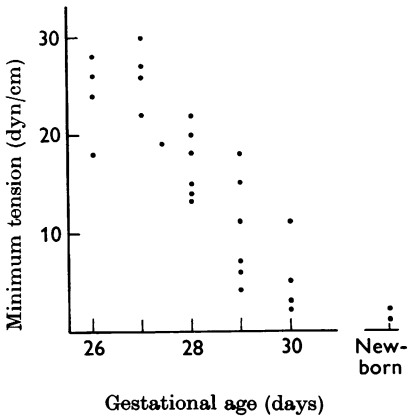

Fig. 6

Fig. 5. The mean stability ratios of bubbles obtained from the lungs of premature unasphyxiated rabbits and two litters of new-born rabbits. Each point is the mean value from an individual rabbit.

Fig. 6. The minimum surface tension, measured on the surface balance, of lung extracts of unasphyxiated litters of premature and new-born rabbits. Each point is the result from a litter of rabbits.

Minimum surface tension. Duplicate measurements of minimum surface tension (γ_{min}) on the five litters gave results in dyn/cm of 17 and 15, 5 and 6, 18 and 18, 16 and 16, and 12 and 14. Figure 6 shows that γ_{min} fell steadily with increasing gestational age from 27 days until term, with no obvious step change as for S.R.

Asphyxiated animals

Table 1 gives blood gas results and details of the asphyxia in 28-day foetuses. Table 2 gives S.R., γ_{min}, and V_5 values for these animals and the control animals of the same gestational age. The asphyxiated animals tended to have higher γ_{min} ($P = 0{\cdot}06$) and lower V_5 ($P = 0{\cdot}20$) values than the controls. However, the control and asphyxia groups were poorly matched for foetal weight, the mean weight of the control animals being 41·0 g and that of the asphyxiated animals 36·7 g. To allow for any bias due to the heavier foetuses being at a more advanced stage in development, the asphyxiated animals were matched with controls of similar weight by exclusion of the three litters of the lightest mean weight (all asphyxiated)

and the two heaviest litters (both controls). Matched litters are marked with an asterisk in Table 2. These two groups gave similar results for V_5, S.R. and γ_{min} (minimum P of any difference = 0·3).

TABLE 1. Gases inhaled and blood sample analyses of doe rabbits asphyxiated when 28 days pregnant: the young were delivered alive

Rabbit no.	P_{a,O_2} at 10 ± 1 min (mm Hg)	P_{a,CO_2} at 10 ± 1 min	pH_a	Gas inspired	
				% O_2	% CO_2
44	35	23	7·62 at 9 min	10	0
48	40	18	—	10	0
53	41	47	7·31 at 9 min	10·5	2
54	36	62	7·17 at 11 min	10·5	6
55	—	72	7·17 at 11 min	10·5	6
56	49	69	7·22 at 5 min	10·5	6
57	57	70	7·18 at 5 min	9·5	6
64	42	57	7·30 at 10 min	8·3	6
66	33	58	7·22 at 10 min	8·3	6
67	10	62	7·08 at 16 min	8·3	6

TABLE 2. V_5, S.R. and γ_{min} of control and asphyxiated 28-day foetuses. Pairs of litters matched for weight are shown on the same line and marked with an asterisk

	Control					Asphyxiated animals			
Doe no.	Mean foetal wt. (g)	V_5 (ml.)	S.R.	γ_{min} (dyn/cm)	Doe no.	Mean foetal wt. (g)	V_5 (ml.)	S.R.	γ_{min} (dyn/cm)
*46	35·5	1·35	0·83	13	*54	35·0	0·92	0·80	22
*50	36·8	1·84	0·84	—	*55	37·5	0·89	0·80	20
*24	38·1	—	0·10	—	*66	38·2	1·17	0·87	21
*58	38·4	0·40	0·65	19	*44	38·5	0·69	0·56	25
*60	39·2	1·25	0·90	20	*64	40·4	0·40	0·86	15
*37	41·2	1·43	0·79	14	*53	42·3	1·77	0·82	18
*27	41·8	—	0·78	22	*48	43·0	2·54	0·88	—
40	47·3	2·15	0·91	15	57	30·6	0·25	0·52	21
38	51·3	1·60	0·89	10	67	30·8	0·76	0·64	23
					56	30·9	0·68	0·60	17
Means	41·0	1·43	0·74	16		36·7	1·01	0·74	20
Means of matched litters	38·7	1·30	0·70	18		39·3	1·20	0·79	20

Comparison of γ_{min}, S.R. *and* V_5

Figure 7 shows a more or less linear correlation between mean V_5 and γ_{min} of the litters (r = 0·5). When S.R. of each animal is plotted against V_5 (Fig. 8) a step function is apparent, similar to that shown in the plot of S.R. with gestation (Fig. 5). A similar step change is apparent in the plot of mean S.R. of each litter against γ_{min} (Fig. 9).

DISCUSSION

Comparison of techniques

Whether stability ratio is plotted against gestational age, γ_{min} or V_5, its value is altered by a large part of its total range for a small change in the other measurements. Thus the bubbles appear saturated with

relatively low levels of lung surfactant. In the bubble method there must always be a tendency for the sample of bubbles examined under the microscope to be biased in favour of the more stable bubbles, as the

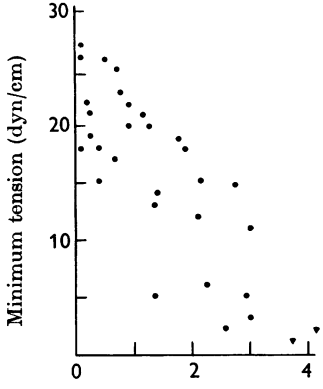

Fig. 7

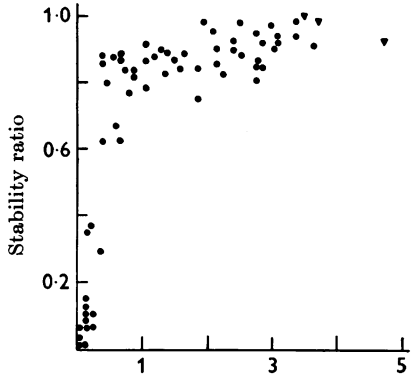

Fig. 8

Fig. 7. Results of minimum surface tension (γ_{min}) plotted against mean V_5. ● Delivered prematurely, ▼ normal new-born.

Fig. 8. Results of stability ratio (S.R.) plotted against V_5. ● Delivered prematurely, ▼ normal new-born.

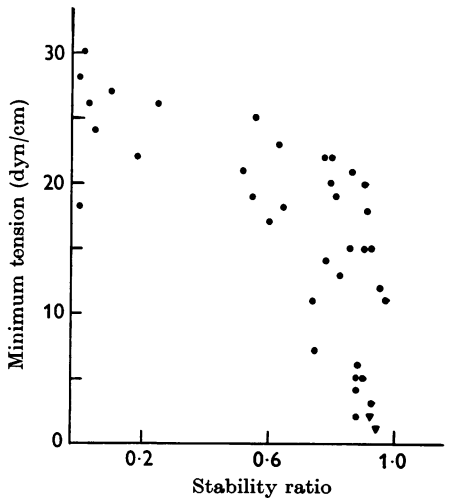

Fig. 9. Results of mean stability ratio (S.R.) of litter plotted against minimum surface tension (γ_{min}). ● Delivered prematurely, ▼ normal new-born.

unstable bubbles must have more of a tendency to disappear before the microscopic field is examined. There must also be a tendency for bubbles in the size range examined to be saturated with surfactant,

as some of them will be derived from the constriction of larger bubbles. Another explanation could arise owing to the fact that the stable bubbles of mature alveoli are far more easily produced in this method than the unstable bubbles from immature alveoli. If a lung is at a stage of development where, amongst neighbouring alveoli, some have surfactant, whilst others lack it, then bubbles will be readily obtained from the mature alveoli and will give a high value for stability ratio: immature alveoli will produce very few bubbles of low stability, and their sheer lack of numbers will affect the mean stability ratio of the lung but little. The distribution of stability ratios within any single sample of lung which gives an intermediate value of mean stability ratio tends to be bimodal, which lends support to any or all of the hypotheses discussed above.

The minimum surface tension of lung extracts decreases continuously at the same time as the capacity of the lung to retain air at low pressures increases. A correlation has already been shown between pressure–volume deflation curves and surface balance results in the lungs of babies (Gruenwald *et al.* 1962) and in lambs (Howatt & Strang, 1965).

In the past normal lungs have often been arbitrarily divided from abnormal according to whether their minimum surface tension was above or below some fixed value. Since there is no discontinuity in the results here, whether plotted against V_5 or gestational age, such division is indeed arbitrary.

The analysis of the deflation limbs of pressure–volume curves gives the most easily interpreted information about lung stability. Although the index L of Gruenwald *et al.* (1962) provided a convenient means of expressing the shape of the curve at low pressure, in some very immature lungs high values of L may be obtained when the lung can accommodate only an insignificant amount of air (see Figs. 2 and 4); consequently the unqualified use of the index L is considered unwise.

Surface properties and gestation

The ability of the lung to expand increases progressively from 26 days until term. The capacity of the lungs to retain air at low pressure is first evident at 28 days and almost fully developed at 29 days. As emphasized already, the minimum surface tension of lung extracts continues to fall until term at 31 days.

In the rabbit, as in the lamb (Howatt *et al.* 1965), surface properties develop very late in gestation. Similar results have been obtained by indirect estimation of gestation for mice (Buckingham & Avery, 1962), rats and guinea-pigs (Pattle, 1961). In the human, Avery & Mead (1959) estimated the time of appearance of surfactant at about 30–32 weeks, relatively earlier than in the animal species studied.

As in lambs (Howatt *et al.* 1965), no close relationship appears to exist in the gestation of rabbits between the loss of cuboidal alveolar epithelium and the appearance of surfactant as reported in guinea-pigs and rats by Pattle (1961). Short (1951) showed that cuboidal epithelium disappears in the rabbit between the 24th and 26th days of gestation, whereas surfactant does not appear in functional amounts until the 28th day.

Pulmonary surface properties and prenatal asphyxia

It was apparent that a change in the pulmonary surface properties could not be produced by means of a relatively short acute period of intense prenatal asphyxia. This result would be expected if an effect of asphyxia was to prevent the formation of surfactant for this short period rather than to destroy surfactant already formed. Longer periods of asphyxia could not be studied since they killed the foetuses of this age. In three such dead litters, not included in Results, pulmonary surface properties were virtually identical to those of live control litters matched for weight. Indeed, there is still no decisive evidence that foetal asphyxia affects pulmonary surface properties. The respiratory distress observed by Dawes *et al.* (1963) might have been due to hyperventilation during recovery from the acidosis of asphyxia. The respiratory distress observed by Davis & Stafford (1964) in the rabbit at 28 days gestation might be similarly explained, and further could have been caused, at least in some of the animals, by surfactant deficiency due to prematurity: a considerable scatter exists in surface properties at this stage of gestation.

The most suggestive evidence in favour of foetal asphyxia as a factor in surfactant deficiency comes from the experiments of Reynolds *et al.* (1965), where prolonged severe asphyxia appeared to have an effect on the pulmonary surface properties of some of their lambs. Since the gestation of these lambs was determined indirectly from crown–rump length, it is difficult to be sure that the control and asphyxiated lambs were of comparable gestational ages. The present experiments show that in the rabbit small differences in gestation can make a large difference to surface properties.

The help of Professor H. O. Schild is gratefully acknowledged. We wish to thank Mr F. Wise for mating and caring for the rabbits, and Mr C. M. J. Bright, Miss E. Kifford and Mrs P. Tanti for technical help. This work was supported by a grant from the Medical Research Council.

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