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The radial alveolar count method of Emery and Mithal: a reappraisal 1—Postnatal lung growth

THOMAS P COONEY, WILLIAM M THURLBECK

From the Department of Pathology, University of British Columbia, Vancouver, Canada

ABSTRACT The radial alveolar count method of Emery and Mithal has been re-evaluated on 76 normal postnatal lungs. Results of reproducibility assessments suggest that each observer should establish normal control values when beginning with the method, and should subsequently use control cases to maintain strict reproducibility. The use of 10 fields per case was found to be inadequate to obtain satisfactory reproducibility, even for a single observer. Prior inflation of the lungs significantly increased the radial counts, and this factor may help to explain the large discrepancy between the results of this study and that of Emery and Mithal. The radial counts correlated well with the chronological age of the child ($r = +0{\cdot}76$; $p < 0{\cdot}001$).

Alveolarisation of the acinus occurs primarily between birth and 2 years; significant but slower growth is seen up to 8 years, after which the results plateau, suggesting that alveolarisation is complete. The radial count method appears to provide a relatively simple and reasonably satisfactory assessment of alveolar development, as originally proposed by Emery and Mithal.

There is a wide variation in estimations by different observers of the number of alveoli in the human lung at a particular age and of the time at which alveolar multiplication ceases. These discrepancies have been attributed to several causes[1]—different methods of preparation of the lungs for analysis; variation in results between different observers when the same sections are examined; difficulty in recognition of alveoli; and the small number of cases from which current data have been derived. Additional theoretical limitations are imposed by the alveolar shape constant (β) and the assumption that the characteristic linear dimensions of alveoli are normally distributed.[2] The paucity of cases examined is due to the difficulties inherent in the counting technique. Most workers use the method of Weibel and Gomez.[3] This method is both time consuming and tedious; and results derived from small numbers of cases are unsatisfactory as prediction data because of the large biological variation seen in adults.[4]

In 1960 Emery and Mithal[5] introduced the radial count method, which assesses the complexity of the terminal respiratory unit (acinus). Measurements in that study were made from a respiratory bronchiole to the edge of the acinus. A respiratory bronchiole was defined as a bronchiole lined by epithelium in one part of the wall. From the centre of such a bronchiole a perpendicular was dropped to the edge of the acinus (connective tissue septum or pleura), and the number of alveoli cut by this line was then counted. Ten such counts were made from each of 309 cases and the mean for each case was estimated. Results were expressed as average figures in different age groups, and an increase in the radial alveolar count throughout the whole of childhood was documented. By the use of this method of choosing bronchioles adequate reproducibility of results was said to have been achieved.

Although the information derived by this method differs from (and is less than) that obtained by conventional alveolar counting, the technique has obvious merits. Large numbers of cases may be rapidly examined. It is claimed that the method overcomes the effects of varying degrees of collapse, so that sections from uninflated lungs may be examined. The method is also independent of tissue shrinkage in the course of processing. Because of these advantages the method has been widely applied in assessment of the development of the terminal respiratory unit in such diverse conditions as congenital diaphragmatic hernia,[6–9] anencephaly,[7,9] renal anomalies,[7,10] congenital heart disease,[11] severe

Address for reprint requests: Dr WM Thurlbeck, Department of Pathology, University of British Columbia Health Sciences Centre Hospital, 2211 Wesbrook Mall, Vancouver, BC, V6T 1W5, Canada.

rhesus isoimmunisation,[12] polyalveolar lobe with emphysema,[13] kyphoscoliosis,[14] phrenic nerve agenesis,[15] and primary pulmonary hypoplasia.[16]

Examination of the method both in its original description and in its subsequent application has revealed several problems. Emery and Mithal achieved adequate reproducibility by making their measurements from a respiratory bronchiole; the degree of reproducibility, however, was not specified. They observed great variability between children of the same age. Only 10 counts, however, were made from each case, and this number may not be adequate in view of the fact that the primary measurement may be made in any of three orders of respiratory bronchioles: if only 10 counts are made, the random inclusion of even a couple of additional readings from a first-order or a third-order respiratory bronchiole may drastically alter the radial count, which appears best represented by a mean value derived from all three respiratory bronchioles. The use of only 10 readings per case may also adversely affect reproducibility of measurements, for the reasons just outlined. With regard to the examination of collapsed (uninflated) lungs, it is not clear whether comparable results might be obtained by looking at inflated lungs. Finally, no attempt was made to relate the radial count estimations to conventional alveolar counts of even a small group of cases.

Important inconsistencies are evident in the application of the method. Of the 11 case reports cited above, in only three was the method applied precisely as described by Emery and Mithal. In six instances the investigators made the measurements from a terminal bronchiole[8 16] or counted alveolar septa rather than alveoli.[7–9 12 15 16] Two reports did not provide a precise description of the method.[10 14] Because of the very small spread of results in the series of Emery and Mithal (2·2 to 9·3) such differences in technique may introduce a significant variation in radial counts. In all but two instances[7 9] the authors used the data of Emery and Mithal as normal controls. One of these reports, by Reale and Esterly,[7] provided a control group of 24 normal infants of gestational age 32–44 weeks. The mean radial count for that group was 6·2. The mean radial count for the equivalent group in the series of Emery and Mithal is about 4. According to the latter count the cases of Reale and Esterly are normal; using their inbuilt control group the authors felt that the lungs they examined were hypoplastic.[7]

The present study is a re-evaluation of the radial alveolar count method of Emery and Mithal in the light of the problems raised above. A range of normal values is established and compared with the original data. Both intraobserver and interobserver reproducibility are scrutinised. The effects of inflation versus non-inflation on the radial count estimation are examined. Finally, the results are compared with conventional alveolar counts in the same cases.

Methods

Lungs from 76 children were examined (48 boys and 28 girls), ranging in age from 6 weeks to 17 years. The cases were selected to illustrate normal lung growth. Cases of sudden accidental death comprised 54% of the group; in the remainder the terminal illness was of short duration (less than two weeks) in children previously well. All cases of congenital anomaly or chronic disease were excluded from the study. Fifty-seven of these cases (37 male, 20 female) had complete morphometric evaluation, and the results of that examination are available for comparison with the radial count data (see paper by Thurlbeck, pp 564–71).

At necropsy the lungs were separated from the heart and thymus, weighed, and inflated at a constant pressure of 25 cm of formalin for at least 24 hours. Representative sampling of the mid-sagittal slice of each lung was carried out.[17] Tissue blocks were routinely processed and sections stained with haematoxylin and eosin.

The radial count method was applied strictly according to the description of Emery and Mithal.[5] To ensure reproducibility the following additional criteria were used: (1) When the respiratory bronchiole was symmetrical the geometric centre was used as a starting point (fig 1). When the bronchiole was irregular the starting point was mid-way between the most proximal part (that is, the wall lined by conducting epithelium) and the first branch point (fig 2). (2) No count was made if the respiratory bronchiole was nearer to the edge of the slide than to the nearest connective tissue septum. (A closer septum might have been removed in taking the block.) (3) All alveoli traversed by the perpendicular were counted. Where the perpendicular traversed the common wall of adjoining alveoli a count of one was made (the alveolus on the right of the perpendicular). (4) When more than 20 alveoli were noted between respiratory bronchiole and the nearest septum or pleura, the field was discarded on the assumption that the nearest septum was in a plane other than that of the section.

The age and sex of the patient were withheld from the observers when the counts were made. All suitable areas in all sections available were counted. In the 57 cases that form the core of the study the mean number of sections examined per case was 10, and the mean number of fields counted per case was 32.

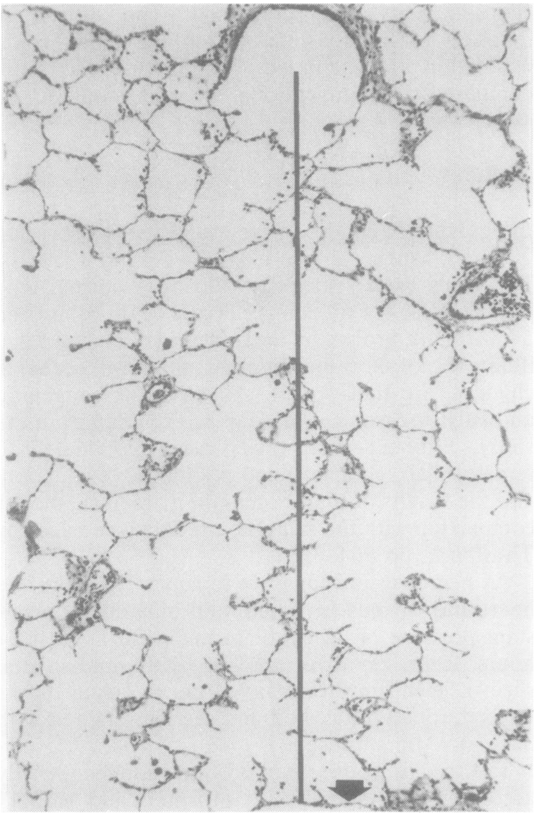

Fig 1 *Radial count estimation in a symmetrical respiratory bronchiole. The perpendicular is placed from the geometric centre of the bronchiole to the nearest connective tissue septum (arrow). (Haematoxylin and eosin; original magnification ×25.)*

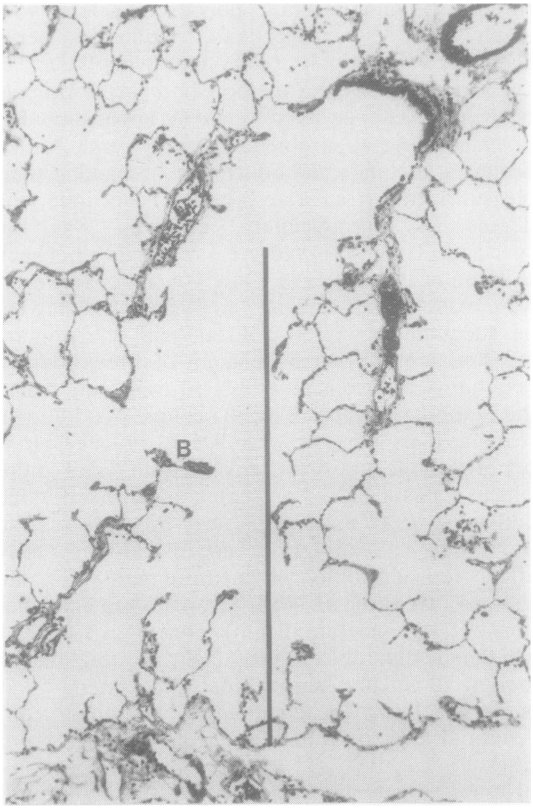

Fig 2 *Radial count estimation in an irregular respiratory bronchiole. The perpendicular begins at the midpoint between the conducting epithelium and the first branch point (B), and ends at the nearest connective tissue septum. (Haematoxylin and eosin; original magnification ×25.)*

Testing the hypothesis that 10 counts per case are inadequate

To test the hypothesis that 10 counts per case are inadequate, 25 cases were randomly selected from the group. In each case 10 radial counts were made and a mean value per case obtained (count 1). The cases were recounted at a later time by the same observer (TC), the first 10 suitable fields encountered being used (count 2), and a mean radial count per case was again estimated. On two subsequent separate occasions (counts 3 and 4) the same observer carried out radial counts on all suitable fields in all available sections of the same 25 cases. This resulted in a mean value of 40 suitable fields per case. The results from the four separate counts were used to assess intraobserver reproducibility and to test the hypothesis that 10 counts per case is inadequate.

Interobserver variation

To assess interobserver variation 25 cases were evaluated by two observers. In each case all suitable areas of all available sections were examined.

Testing the hypothesis that inflation affects radial count

To assess the effect of inflation on radial count examination, seven additional cases were selected. One lung from each case was inflated and the other fixed uninflated. Representative sections were obtained from each lung as described above. Radial counts were carried out on all sections and the results of inflated versus uninflated compared.

Pleural sections versus random sections

Specific (non-random) sections which included the pleura were available in 28 cases. Radial counts

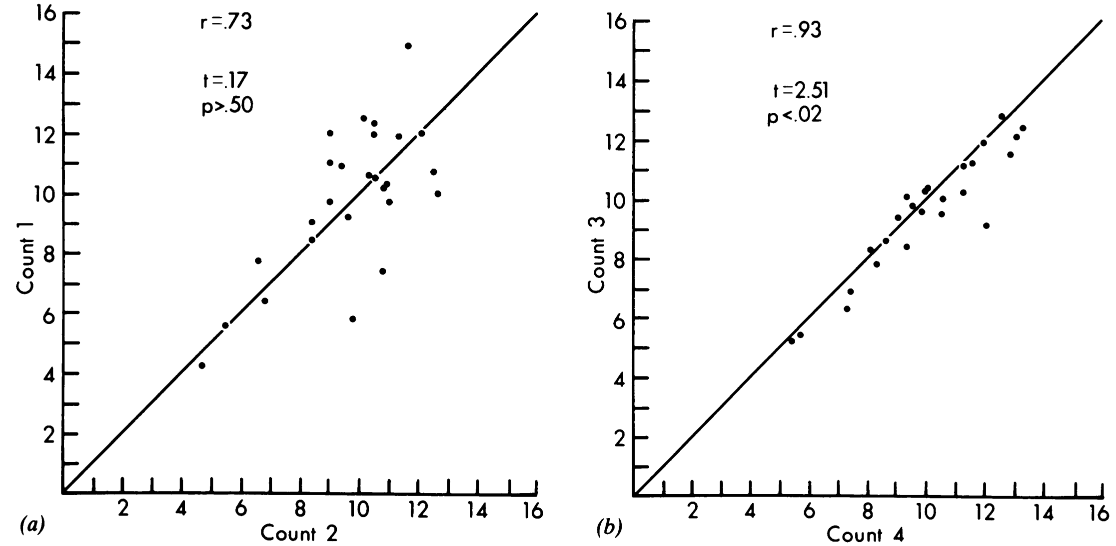

Fig 3 *(a) Intraobserver reproducibility of radial counts with 10 fields per case. (b) Intraobserver reproducibility of radial counts with a mean of 40 fields per case.*

were carried out on all sections from these cases and the radial counts from the specific pleural sections were compared with those from the random sections.

Testing the hypothesis that alveolar multiplication is complete by 8 years

To investigate the point during growth at which acinar alveolar multiplication ceases, lungs from children 1–2 years, 6–8, and 11–13 were selected. The mean radial count of each age group was estimated and the differences between the means were examined statistically.

Statistical evaluation of the results was by means of correlation coefficient (r) and Student's unpaired and paired *t* test.

Results

The results presented are those relating to radial count estimations in the 57 cases for which complete morphometric data were available. The addition of results from the 19 cases for which other morphometric data were not available did not significantly alter the correlation of radial count with age (r_{57} = +0·77; r_{76} = +0·80).

Ten counts per case inadequte

Counts of 40 fields per case (counts 3 and 4) provided a much closer correlation of results than when 10 fields per case were used (figs 3*a* and 3*b*). Student's paired *t* test, however, showed a significant difference between counts 3 and 4 (t = 2·51; $p < 0·02$) but no difference between counts 1 and 2 (t = 0·17, $p > 0·50$). This apparent discrepancy between results of the correlation coefficients and the Student's paired *t* test is discussed further below.

Reproducibility

Intraobserver It is evident from fig 3*b* that although a close correlation was obtained by the observer when repeating the readings (r = +0·93) a significant difference was detected between the populations ($p < 0·02$). The error detected in counts 3 and 4, however (fig 3*b*), was only 4% of the mean (0·18 standard deviation).

Interobserver There was a highly significant difference between the two observers (t = 3·18; $p < 0·01$). The correlation coefficient (r) was +0·72 ($p < 0·001$) and the error detected was 9% of the mean (0·56 SD).

Radial count affected by inflation

A highly significant difference was found between the radial counts from inflated and uninflated lungs (fig 4). The mean radial count of inflated lungs was 22% higher than that of the uninflated lungs. The two counts produced a high correlation coefficient but the counts of inflated lungs were higher than those of the uninflated in every case.

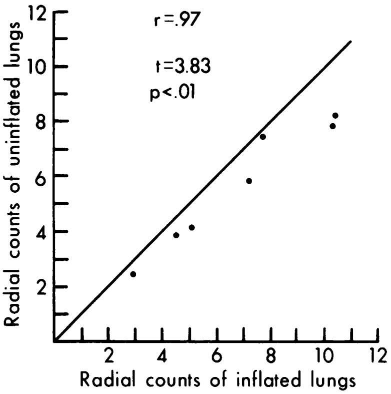

Fig 4 *Comparison of radial counts from inflated and uninflated lungs.*

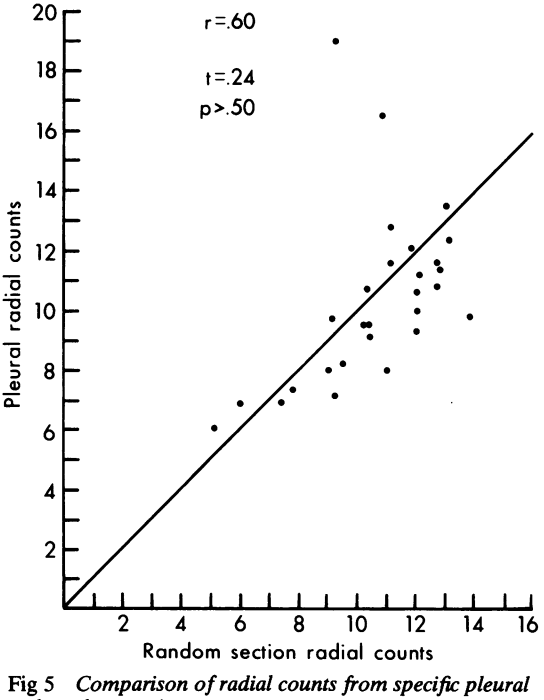

Fig 5 *Comparison of radial counts from specific pleural and random sections.*

Pleural sections versus random sections

Radial counts of specific pleural sections correlated poorly ($r = +0{\cdot}60$) but significantly ($p < 0{\cdot}001$) with those of the random sections from each case (fig 5). The divergence of results was greatest in those cases in which fewer than five radial counts were possible in the pleural sections available, supporting the idea that adequate numbers of fields are necessary for a reliable mean count for each case.

Alveolar multiplication completed by 8 years

Figure 6 depicts the correlation of radial count estimations with age ($r = +0{\cdot}76$; $p < 0{\cdot}001$) in the 57 cases examined. The logarithmic curve fit with $\pm 1{\cdot}74$ standard deviations is shown. Examination of mean radial counts at 1–2 years, 6–8 years, and 11–13 years showed a statistically significant difference between the means at 1–2 years and 6–8 years ($t = 2{\cdot}303$; $p < 0{\cdot}02$). There was no significant difference between the means at 6–8 years and 11–13 years ($t = 0{\cdot}949$; $p < 0{\cdot}10$).

Other correlations examined

The correlation coefficients of radial count (RC) measurements with age, bone age, body length, body weight, fixed lung volume, and internal surface area are set out in the table. The correlation of the number of alveoli per unit area (N_A), the number of alveoli per unit volume (N_V), and the total number of alveoli (N_{AT}) with these same parameters in this population is also given for comparison with the radial count correlations (table). The correlation coefficient of radial count with N_{AT} was $+0{\cdot}59$. The ratio N_{AT}/RC was estimated for each case, but correlation of this ratio with age was poor ($r = +0{\cdot}31$). The correlation of N_{AT}/RC with age between 6 weeks and 2 years was, however, closer ($r = +0{\cdot}43$); after 2 years no correlation was found ($r = +0{\cdot}05$).

No difference was detected between boys and girls when radial counts for each sex were correlated with age. Regression lines were drawn for the groups and were closely apposed.

Twenty native American Indian children were included in the group (35%). No difference was detected when correlation of radial count with age was estimated for Indian and non-Indian subgroups. The regression lines for the groups were almost identical.

Discussion

Our results lend support to the method of radial alveolar counting proposed by Emery and Mithal both in regard to method and also as a measure of the progressive development of the acinus postnatally.

In the application of the method as many suitable areas as possible should be counted per case. Ten or fewer than 10 areas yield a radial count which is unreliable and difficult to reproduce. If suitable areas are available, 40 counts per case are desirable. As mentioned above, this number of fields is required to offset bias due to the considerable difference between counts made from first-order and from third-order respiratory bronchioles. If enough

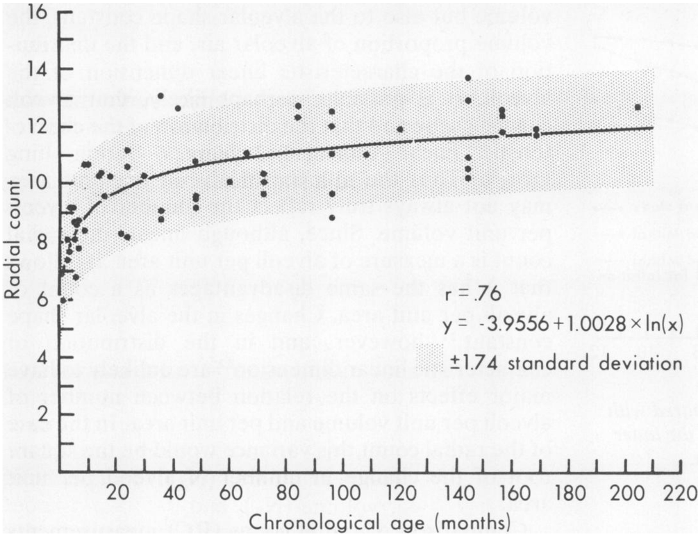

Fig 6 *Correlation of radial counts with chronological age in normal children more than 1 month of age. The logarithmic curve fit with ±1·74 standard deviation is shown.*

Comparison of correlation coefficients for the different morphometric estimations

Parameters	*Radial count*	N_A	N_V	N_{AT}
Age	r = +0·76 (log)	r = −0·74 (exp)	r = −0·76 (exp)	r = +0·61 (log)
Bone age	r = +0·78 (log)	r = −0·76 (exp)	r = −0·78 (exp)	r = +0·61 (log)
Body length	r = +0·73 (exp)	r = −0·76 (exp)	r = −0·78 (exp)	r = +0·58 (exp)
Fixed lung volume	r = +0·68 (log)	r = −0·78 (exp)	r = −0·80 (exp)	r = +0·68 (log)
Internal surface area	r = +0·71 (log)	r = −0·65 (exp)	r = −0·68 (exp)	r = +0·78 (log)
Body weight	r = +0·74 (log)	r = −0·73 (exp)	r = −0·75 (exp)	r = +0·57 (log)

N_A – number of alveoli per unit area (cm^2); N_V – number of alveoli per unit volume (ml); N_{AT} – total number of alveoli (both lungs, expressed in millions). The best fit is given in each instance.

suitable areas are not found in the available sections it is worth cutting deeper in the blocks, as hitherto unseen septa may thereby become evident. Emery and Mithal commented on the difficulty of obtaining adequate numbers of countable fields in some cases. We have examined as many as 14 sections in some cases to obtain 11 suitable areas for counting. The problem seems more common in older children, in whom septa are further apart; but fortunately this difficulty is offset in these cases by the ease with which a larger number of sections may be obtained.

A significant intraobserver error was detected in assessing reproducibility. For strict reproducibility each observer should intermittently use reference control cases. The interobserver error was highly significant, so that different observers need to establish normal controls in each instance. This is borne out in the study of Reale and Esterly,[7] who examined uninflated lungs and found the control mean radial count to be significantly higher than that of Emery and Mithal, even after due allowance is made for the fact that the former workers counted alveolar septa while Emery and Mithal counted alveoli. It seems appropriate that studies of abnormal lung growth carried out by the radial count method should in each study incorporate a small series of normal controls. The essential simplicity of the method renders this a feasible procedure.

The apparent discrepancy between correlation coefficients and Student's paired *t* test in the examination of intraobserver reproducibility (figs 3*a* and 3*b*) is explicable on the basis of the statistical test used. Use of 40 fields per case was more reproducible (r = +0·93) than 10 fields per case (r = +0·73). Visual evidence of this is seen in figure 3, with the proximity of the data points to the line of identity. Student's paired *t* test showed a significant difference between counts 3 and 4 (t = 2·51; $p < 0·02$) because the observer counted consistently higher during count 4. No statistical difference was seen between counts 1 and 2 (t = 0·17; $p > 0·50$). This occurred because the correlation between the counts was less strict (r = +0·73), and the results were thus randomly scattered about the mean.

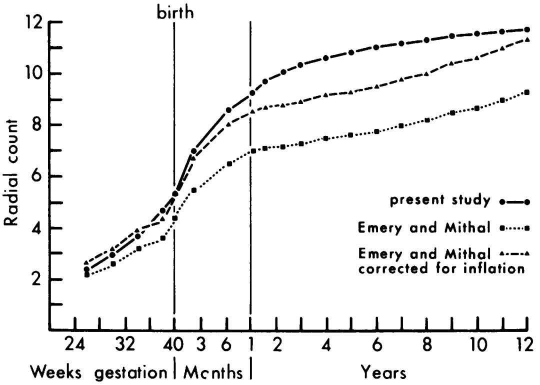

Fig 7 *Radial counts of the present study compared with those of Emery and Mithal,[5] with correction of the latter counts for the effects of inflation before fixation.*

Inflation significantly affects the radial count estimation. The range of normal postnatal values documented here is consistently two to three units higher at all ages than the values noted by Emery and Mithal (fig 7). This may be explained by the observation of Short that inflation of the lungs by fixative before sectioning may result in a completely different appearance of the lung.[18] Gil and Weibel[19] have also suggested that alveolar recruitment occurs on inflation of the lung. The radical difference between our cases and those of Emery and Mithal is considerably offset by correcting the latters' results for the effects of inflation by the use of the factor of +22% derived in this study (fig 7).

The discrepancy between radial counts for random sections and those for specific pleural sections is difficult to evaluate, as in many cases within the latter group fewer than five areas were counted. No statistically significant difference was seen, however.

Our results indicate a progressive increase in complexity (alveolarisation) of the terminal respiratory unit postnatally (fig 6). The most rapid growth occurs between birth and 2 years. Appreciable but slower growth is seen between 2 and 8 years ($p < 0{\cdot}02$). After this the results plateau, suggesting that alveolarisation of the acinus is reaching completion. The timescale is similar to that documented by Dunnill.[20] As noted by Emery and Mithal, the radial count varies considerably among children of the same age (fig 6).

There are theoretical objections to counting alveoli. The most important number to know is the number of alveoli per unit volume. This determines the total number of alveoli in the lung, and the number of alveoli seen per unit area. The latter is related not only to the number of alveoli per unit volume but also to the alveolar shape constant, the volume proportion of alveolar air, and the distribution of the characteristic linear dimension of the alveoli. It is possible, perhaps likely, that alveoli change shape and that the distribution of the characteristic linear dimension changes during lung growth. Thus the number of alveoli per unit area may not always truly reflect the number of alveoli per unit volume. Since, although linear, the radial count is a measure of alveoli per unit area, it follows that it has the same disadvantages as a count of alveoli per unit area. Changes in the alveolar shape constant,[21] however, and in the distribution of characteristic linear dimension[22] are unlikely to have major effects on the relation between number of alveoli per unit volume and per unit area. In the case of the radial count this variance would be the square root of the change in number of alveoli per unit area.

Comparison of radial count (RC) measurements with the number of alveoli per unit area (N_A) is of interest, as these morphometric variables are closely related. As growth proceeds RC rises with increasing complexity of the acinus. N_A remains the same in the first two to three years of life (see Thurlbeck, pp 564–71) and decreases thereafter. N_A and RC could be closely related and it might be possible to predict N_A from RC if the relationship were close enough. The correlation of (increasing) RC with age, bone age, body weight, and length parallels the correlation of (decreasing) N_A with the same growth parameters (table). N_A and N_V, however, are as closely related to growth parameters as RC is but the relation between RC and N_A ($r = +0{\cdot}34$) and between RC and N_V ($r = +0{\cdot}32$) is insufficiently close to predict N_A and N_V from RC. In retrospective studies, or where conventional morphometry is not feasible, it appears that RC may validly be used as a substitute for N_A in assessing the adequacy of acinar alveolarisation. If both RC and N_A are available more precise deductions are possible; if, for example, N_A is found to be normal and RC reduced then too many units are present per unit area, indicating that alveolarisation of the distal bronchiolar tree has not proceeded adequately.

Unexpectedly, RC correlated more closely with N_{AT} ($r = +0{\cdot}59$) than with N_A or N_V. This is apparently related to the introduction of another variable, lung volume, in the calculation of N_{AT}. The relationship between RC and N_{AT}, however, was still insufficiently close to predict N_{AT} from RC. The relation between RC and N_{AT} is of interest. The ratio of N_{AT}/RC is a factor of the number of respiratory bronchiolar units in the lung. This ratio correlates poorly with age ($r = +0{\cdot}31$, logarithmic regression). The correlation is, however, closer between 6 weeks and 2 years (r = +0·43); but after this age there is no correlation (r = +0·05). This indicates an increase in respiratory bronchiolar units up to the age of 2 years, with no significant change thereafter.

The radial alveolar count method of Emery and Mithal appears a simple and satisfactory method of assessing lung growth. It should, however, be applied with attention to the limitations which have emerged from this study.

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References

[1] Thurlbeck WM. Structure of the lungs. In: Widdicombe JG, ed. *International review of physiology.* Vol 14. *Respiratory physiology II.* Baltimore: University Park Press, 1977:1–36.

[2] Holmes C, Thurlbeck WM. Normal lung growth and response after pneumonectomy in the rat at various ages. *Am Rev Respir Dis* 1979;**120**:1125–36.

[3] Weibel ER, Gomez DM. A principle for counting tissue structure on random sections. *J Appl Physiol* 1962;**17**:343–8.

[4] Angus GE, Thurlbeck WM. Number of alveoli in the human lung. *J Appl Physiol* 1972;**32**:483–5.

[5] Emery JL, Mithal A. The number of alveoli in the terminal respiratory unit of man during late intrauterine life and childhood. *Arch Dis Child* 1960;**35**:544–7.

[6] Kitagawa M, Hislop A, Boyden EA, Reid L. Lung hypoplasia in congenital diaphragmatic hernia. A quantitative study of airway, artery and alveolar development. *Br J Surg* 1971;**58**:342–6.

[7] Reale FR, Esterly JR. Pulmonary hypoplasia: a morphometric study of the lungs of infants with diaphragmatic hernia, anencephaly, and renal malformations. *Pediatrics* 1973;**51**:91–6.

[8] Areechon W, Reid L. Hypoplasia of lung with congenital diaphragmatic hernia. *Br Med J* 1963;i:230–3.

[9] Askenazi SS, Perlman M. Pulmonary hypoplasia: lung weight and alveolar count as criteria of diagnosis. *Arch Dis Child* 1979;**54**:614–8.

[10] Hislop A, Hey E, Reid L. The lungs in congenital bilateral renal agenesis and dysplasia. *Arch Dis Child* 1979;**54**:32–8.

[11] Rabinovitch M, Haworth SG, Vance A, *et al.* Early pulmonary vascular changes in congenital heart disease studied in biopsy tissue. *Hum Pathol* 1980;**11**, suppl:499–509.

[12] Chamberlain D, Hislop A, Hey E, Reid L. Pulmonary hypoplasia in babies with severe rhesus isiommunisation: a quantitative study. *J Pathol* 1977;**122**:43–52.

[13] Hislop A, Reid L. New pathological findings in emphysema of childhood: 1. Polyalveolar lobe with emphysema. *Thorax* 1970;**25**:682–90.

[14] Berend N, Marlin GE. Arrest of alveolar multiplication in kyphoscoliosis. *Pathology* 1979;**11**:485–91.

[15] Goldstein JD, Reid LM. Pulmonary hypoplasia resulting from phrenic nerve agenesis and diaphragmatic amyoplasia. *J Pediatr* 1980;**97**:282–7.

[16] Swischuk LE, Richardson CJ, Nichols MM, Ingman MJ. Primary pulmonary hypoplasia in the neonate. *J Pediatr* 1979;**95**:573–7.

[17] Langston C, Waszkiewicz E, Thurlbeck WM. A simple method for the representative sampling of lungs of diverse size. *Thorax* 1979;**34**:527–30.

[18] Short RHD. Alveolar epithelium in relation to growth of the lung. *Philos Trans R Soc Lond (Biol Sci)* 1951;**235**:35.

[19] Gil J, Weibel ER. Morphological study of pressure-volume hysteresis in rat lungs fixed by vascular perfusion. *Respir Physiol* 1972;**15**:190–213.

[20] Dunnill MS. Postnatal growth of the lung. *Thorax* 1962;**17**:329–33.

[21] Hansen JE, Ampaya EP. Lung morphometry: a fallacy in the use of the counting principle. *J Appl Physiol* 1974;**37**:951–4.

[22] Weibel ER. *Stereological methods.* Vol 1. *Practical methods for biological morphometry.* London: Academic Press, 1979.