

The radial intercepts method for measuring bronchial mucous gland volume

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Alli, A. F. (1975). *Thorax*, 30, 687–692 **The radial intercepts method for measuring bronchial mucous gland volume.** A new linear method, the radial intercepts method, has been introduced for quantitating the components of the bronchial wall. The method is an adaptation of Rosiwal's (1898) original linear measurement technique, but, unlike Rosiwal's, the radial intercepts method takes measurements along radii at regular sectoral intervals. The method is reproducible and, from its high correlation with the standard planimetric, cut-weigh, and point-count techniques for quantifying bronchial wall components (Bedrossian, Anderson, and Foraker, 1971), it is concluded that the method is reliable.

Reid (1960) measured the thickness of bronchial glands which lay between cartilage plates and the basement membrane, and also measured the wall thickness, which she defined as the distance between the basement membrane and the inner perichondrium, at the same site. She expressed the values as a ratio of gland thickness to wall thickness (G/W ratio). This is now known as the Reid Index.

Reid made only one or two such measurements per bronchial transection, but it was later shown (Restrepo and Heard 1963a, b; Thurlbeck and Angus, 1967) that since glands are normally present between cartilage plates and in the membranous and outer perichondrial tissues of the bronchi, measurements confined to sites where glands are present between cartilage plates and the basement membrane may not represent the true Reid Index for the case. Thurlbeck and Angus (1963, 1964) and Hayes (1969) made multiple measurements, but again only glands lying between cartilage plates and the basement membrane were used. Though multiple measurements improve the correlation slightly (personal observation), it has been shown (Bedrossian, Anderson, and Foraker, 1971) that differences still exist between the Reid Index determined only for the glands lying between cartilage plates and the basement membrane, and measurements which take account of all the glands in the bronchial transection.

Bedrossian *et al.* (1971) compared the methods for quantifying bronchial morphology, and in the

process showed that the point-count method (Dunnill, Massarella, and Anderson, 1969) and the determination of gland area (cut-weigh method of Restrepo and Heard, 1963b) correlated highly with a standard planimetric area measurement of bronchial glands, bronchial wall, and connective tissue. On the other hand, as shown by these authors, the Reid Index correlated poorly. Bedrossian *et al.* (1971) gave the reason for the poor correlation of Reid Index with planimetric measurements as the fact that Reid's method (a unidimensional measurement) did not take account of all the glands in the bronchial wall, but planimetry, the point-count, and cut-weigh methods, which are two-dimensional, did take account of all the glands and the whole of the wall.

The method described below is designed to show that linear measurements somewhat similar to that of Reid (1960) but differing from Reid's in that they are based on consistent cyclical sampling, can take account of the entire wall and all the glands in the bronchial wall, and can correlate well with cut-weigh, point-count or planimetric measurements. This new technique is called the radial intercepts method. It is an adaptation of Rosiwal's technique (1898) and, as explained by that author, is an area measure.

ESTABLISHMENT OF THE NEW METHOD

In order to establish the reliability of a new method, it must be shown to correlate highly with accepted standard methods.

In the course of establishing the radial intercepts method as an accurate technique for determining bronchial gland volume, the cut-weigh and the point-count methods were simultaneously carried out for comparison. It was justifiable to use only the cut-weigh and the point-count methods as controls in place of planimetry because these methods have been shown to be closely comparable to planimetry (Bedrossian *et al.*, 1971). However, in one case planimetric measurements were carried out along with the other methods as a test case.

MATERIALS Fifty bronchial transections used in this experiment were obtained from 50 random necropsies which formed part of the 359 cases studied in Nigeria for the survey of the prevalence of chronic bronchitis and emphysema. A fresh bronchial ring 5 to 10 mm long was carefully dissected free of connective tissue. It was obtained at necropsy from the main bronchus about 25 mm below its origin at the carina, and was fixed in buffered 10% formalin for at least 72 hours. Complete 5 μ bronchial transections were cut and were stained with haematoxylin-eosin, Alcian blue, and periodic acid-Schiff (PAS). In the process of cutting sections, some of the bronchial rings inevitably opened up at the membranous parts but this did not interfere with the measurements which followed.

The whole bronchial transection was projected on to a sheet of white drawing paper fixed to a vertical wall, at a magnification of about 10 diameters, and the whole thickness of the bronchial wall and basement membrane were traced (Fig. 1). The outer limit of the bronchial wall was taken as the junction of the outer border of a bronchial cartilage and the inner aspect of the outer perichondrium. The tracing was started at a point

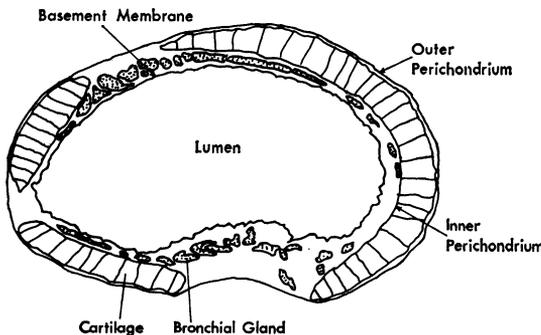


FIG. 1. *Tracing of bronchial transection.*

where there was a cartilage plate and was then carried along the inner aspect of the outer perichondrium, through parts where there were no cartilages, and the tracing was continued through the membranous posterior wall and along the wall at its junction with the loose areola tissue which was thus excluded from the wall thickness. All the glands were outlined on the paper and so also were the cartilage plates. Each tracing of the bronchial transection with its glands and cartilages was photocopied on to a 3 M brand transparency film dry photo automatic, and the transparency film copy was used in all the measurements.

THE METHOD The radial intercepts measurer is shown in Figure 2.

A systematic sampling, that is, sampling at equal sectoral distances, of the glands and the bronchial wall for measurement was carried out radially; hence the name of the method.

The measurer is constructed of a 300 mm Perspex ruler riveted at one end to the centre of a large circle drawn on a Perspex sheet 375×375 mm. The circumference of the circle was divided into 5° segments so that there were 72 sectors numbered 1 to 72. Seventy-two measurements could be carried out, but intermediate 10° intervals for 36 measurements were also included.

MEASUREMENTS BY RADIAL INTERCEPTS MEASURER

The bronchial tracing on the transparency film was placed on a table and the radial intercepts measurer was placed on it so that the centre of the circle was within the lumen of the bronchial transection. The centre of the circle could be made roughly to coincide with the visually estimated geometric centre of the bronchial lumen, but this was not essential, and neither was it essential for the bronchial wall to be a complete ring since the few transections that opened up during processing were equally satisfactorily measured. The weight of the grid upon the transparency film ensured that there was no displacement in the relative positions of the grid and the film. It was essential not to allow relative displacement in positions before all the measurements had been made.

The riveted ruler was then rotated round the circle along each of the 72 radii in turn, starting from number 1. The thickness of the wall, that is, the intercept between the basement membrane

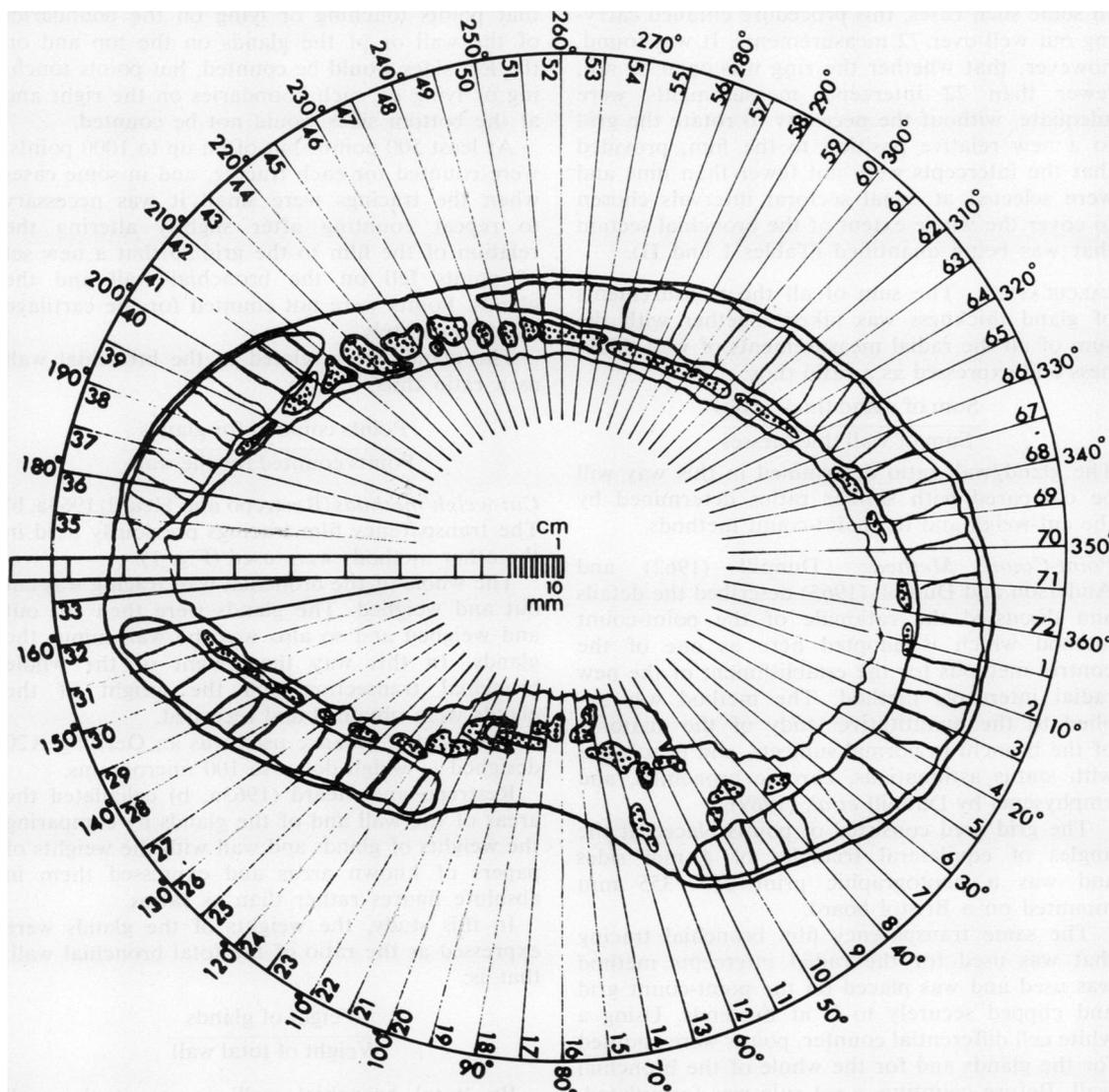


FIG. 2. *The radial intercepts measurer.*

and the outer boundary of the wall as traced, was read off from the ruler. The thicknesses of all the glands, that is, the intercepts made by all the gland tracings along each radius, were read off at the same time and recorded. In this systematic statistical sampling zero values for glands, that is, 'no glands' were recorded since they now had significant zero values, and the corresponding wall thicknesses were measured and included in the calculations.

Where the bronchial transections were complete rings, 72 operations were carried out, but where the transections had opened up, all the radial intercepts on the intact portions of the tracing were read and recorded and then the whole of the radial measurer was slightly rotated over the film so that a new set of intercepts on the tracing could be measured. It was essential to measure all the intercepts in a particular position of the grid before rotating it to the new position.

In some such cases, this procedure entailed carrying out well over 72 measurements. It was found, however, that whether the ring was open or not, fewer than 72 intercepts measurements were adequate, without the necessity to rotate the grid to a new relative position to the film, provided that the intercepts were not fewer than nine and were selected at equal sectoral intervals chosen to cover the whole extent of the bronchial section that was being quantified (Tables I and II).

CALCULATION The sum of all the measurements of gland thickness was taken together with the sum of all the radial measurements of wall thickness and expressed as a ratio thus:

$$\frac{\text{Sum of gland thicknesses}}{\text{Sum of wall thicknesses}}$$

The gland/wall ratio determined in this way will be compared with similar ratios determined by the cut-weigh and the point-count methods.

Point-Count Methods Dunnill (1962) and Anderson and Dunnill (1965) described the details and discussed the rationale of the point-count method which is adopted here as one of the control methods for the establishment of the new radial intercepts method. The method was applied to the quantitative study of the anatomy of the bronchi in normal subjects, and in subjects with status asthmaticus, chronic bronchitis, and emphysema by Dunnill *et al.* (1969).

The grid used consisted of points placed at the angles of equilateral triangles of 4 mm sides and was a photographic print 355×355 mm mounted on a Bristol board.

The same transparency film bronchial tracing that was used for the radial intercepts method was used and was placed on the point-count grid and clipped securely to it at the ends. Using a white cell differential counter, points were counted for the glands and for the whole of the bronchial wall. Before counting, a set rule was formulated,

that points touching or lying on the boundaries of the wall or of the glands on the top and on the left sides would be counted, but points touching or lying on such boundaries on the right and at the bottom sides would not be counted.

At least 500 points, but often up to 1000 points, were counted for each tracing, and in some cases when the tracings were small it was necessary to repeat counting after slightly altering the relation of the film to the grid so that a new set of points fell on the bronchial wall and the glands. Points were not counted for the cartilage plates separately.

The glands were related to the bronchial wall as a ratio thus:

$$\frac{\text{Points counted for glands}}{\text{Points counted for the wall}}$$

Cut-weigh methods (Restrepo and Heard, 1963a, b) The transparency film tracings previously used in the other methods were used (Fig. 1).

The whole of the bronchial wall tracing was cut out and weighed. The glands were then cut out and weighed and so also was the wall minus the glands. In this way the weight of the whole bronchial transection and the weight of the glands were obtained and recorded.

The electric balance used was an Oertling R20 designed to weigh down to 100 micrograms.

Restrepo and Heard (1963a, b) calculated the areas of the wall and of the glands by comparing the weights of glands and wall with the weights of papers of known areas and expressed them in absolute figures rather than as ratios.

In this study, the weights of the glands were expressed as the ratio of the total bronchial wall that is:

$$\frac{\text{Weight of glands}}{\text{Weight of total wall}}$$

By 'total bronchial wall' is meant the wall

TABLE I
VARIATION IN GLAND/WALL RATIO WITH FREQUENCY AND NUMBER OF RADIAL INTERCEPTS MEASUREMENT

Case	No. of Measurements					
	72 at 5° apart	36 at 10° apart	24 at 15° apart	18 at 20° apart	12 at 30° apart	9 at 40° apart
1	0.085	0.086	0.085	0.089	0.086	0.089
2	0.075	0.072	0.072	0.055	0.062	0.061
3	0.108	0.099	0.131	0.101	0.130	0.114
4	0.093	0.076	0.126	0.078	0.076	0.098
5	0.039	0.045	0.050	0.055	0.067	0.042
6	0.084	0.091	0.092	0.091	0.093	0.097
7	0.047	0.056	0.055	0.055	0.097	0.065
8	0.128	0.136	0.125	0.137	0.134	0.145
9	0.057	0.060	0.033	0.033	0.042	0.040
10	0.087	0.086	0.086	0.061	0.058	0.045

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TABLE II
 STATISTICAL EVALUATION OF VARIATION IN
 GLAND/WALL RATIO WITH FREQUENCY AND NUMBER
 OF RADIAL INTERCEPTS MEASUREMENTS

72 measurements at 5° v 36 measurements at 10°; $t = 0.15$ NS
72 measurements at 5° v 24 measurements at 15°; $t = 1.06$ NS
72 measurements at 5° v 15 measurements at 20°; $t = 0.97$ NS
72 measurements at 5° v 12 measurements at 30°; $t = 0.56$ NS
72 measurements at 5° v 9 measurements at 40°; $t = 0.07$ NS

t = correlation coefficient; NS = no significant difference.

tracing from which the glands had not been cut out.

RESULTS

Detailed individual results for the three different methods are available on request to the author. The results for the single case in which planimetry was carried out are shown in Table III.

TABLE III
 COMPARISON OF GLAND/WALL RATIO (G/W) BY
 PLANIMETRY WITH G/W BY OTHER METHODS
 (ONE CASE ONLY)

Methods	G/W
Point-counting (Dunnill, 1962)	0.1113
Cut-and-weigh (Restrepo and Heard, 1963)	0.1077
Radial intercepts (this paper)	0.1114
Planimetry	0.0895

The results obtained by the various methods have been correlated statistically (Table IV) and, as can be seen, the radial intercepts, the cut-weigh, and the point-count methods are highly correlated with each other ($P < 0.001$). In the one case in which planimetric area measurements were carried out, there was good correlation between planimetry, radial intercepts, point-count, and cut-weigh methods (Table III).

DISCUSSION

The imperceptible blending of the peribronchial connective tissue with the outer perichondrium makes it difficult to decide precisely the line of cleavage between the outer perichondrium and the

areola connective tissue. However, a close study of the outer perichondrium will show that in a well-processed bronchial transection free from artefact, there is a definable, even though potential line of cleavage between the cartilage and the inner aspect of the outer perichondrium. This line can be projected easily along the bronchial wall, including the membranous part, to define a reproducible bronchial wall boundary. The advantage of doing this is that in most cases all the bronchial glands are included in the wall so demarcated. This principle has been employed in this study, and it was easy to trace the wall in this way when projected on a screen or when viewed with the camera lucida.

Bedrossian *et al.* (1971) thought that the sources of error in Reid's original method were the variation in gland sizes and the presence of glands between and external to cartilage plates which the method did not include in the measurements used to calculate the Reid Index. The radial intercepts method is designed to overcome this criticism as it takes account of the variation in gland sizes and of all the glands present in the bronchial wall.

Using standard planimetric measurements as controls, Bedrossian *et al.* (1971) showed that the point-count and the cut-weigh methods are the methods of choice for the accurate determination of the relative proportions of bronchial wall components.

The high correlation which the radial intercepts method shows with the cut-weigh and the point-count methods is proof of its reliability and accuracy.

The radial intercepts method is not only reliable but it is also convenient to use and it may be employed, where applicable, to quantify the components of biological materials other than those of the bronchial wall.

The radial measurer can be fixed to a wall or to a vertical stand and the bronchial transection projected on to it. For ease in measurement and

TABLE IV
 DEGREE OF CORRELATION BETWEEN THE DIFFERENT METHODS FOR DETERMINATION OF GLAND/WALL
 RATIO (G/W)

Method	Mean G/W	Standard Deviation
Point-counting	0.0920	0.0447
Cut-and-weigh	0.0922	0.0460
Radial intercepts	0.0921	0.0439
Methods Compared	r	P
Point-counting v cut-and-weigh	0.9567	< 0.001
Point-counting v radial intercepts	0.9492	< 0.001
Cut-and-weigh v radial intercepts	0.9396	< 0.001

to reduce doubts about the outer boundary of the wall during measurement, only the outer bronchial wall need first be traced out, as described earlier, and then with the rotating ruler riveted to the dial, the thickness of the glands and the corresponding wall thicknesses are read off along the radial intercepts. The method is also convenient to use with the camera lucida and it can be adapted for microscopical measurements by incorporating the radial measurer as an eyepiece graticle. Since not every laboratory, particularly in the developing countries, is equipped with an electric balance, this method commends itself to a wide use and it is less tedious than cutting the glands out of paper or point-counting them.

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