Quantitative assessment of chronic non-specific lung disease at necropsy

Report by Panel on Pathology of the Medical Research Council Committee on Research into Chronic Bronchitis, April 1972

I INTRODUCTION
The Panel on the Pathology of Chronic Bronchitis met for the first time in June 1970 and the terms of reference were discussed. It was agreed that the aim of the Panel should be the production of a brief memorandum which would help to standardize the techniques presently used in the preparation of the respiratory tract and thoracic organs at necropsy by macroscopic and light microscope methods in cases of chronic non-specific lung disease (Ciba Guest Symposium, 1959). In any scheme for the quantitative examination of the respiratory and cardiovascular systems it is useful to distinguish between:

1. methods recommended for use by pathologists during their routine hospital duties, where accurate diagnosis of lung disease is required for certification, epidemiological studies or for correlation with radiographs, and
2. methods used by pathologists, working in close collaboration with clinical physiologists, interested in detailed research into pathophysiological correlation.

In the pages that follow the methods recommended for routine use are marked with an asterisk.

In all cases of chronic lung disease it is desirable to examine not only the lungs and airways but also the heart and carotid bodies. Since the methods described below are in some instances tedious and time-consuming, it is essential, if they are to prove worth while, that full clinical details should be made available, including not only the history and findings on physical and radiological examination but also details of pulmonary function tests.

II METHODS OF LUNG FIXATION
1. INTRODUCTION
The examination of the lungs post mortem is of interest to physiologists, clinicians, anatomists, and pathologists. The first two will normally require lungs to be as near the physiological state as possible, so that the time of removal after death is important and fixation may not be required, for instance in the preparation of plastic casts of the pulmonary blood vessels. However, resin diffusion into the air spaces may produce artefacts, so that fixation can be advantageous. The method by which lungs are treated depends almost entirely on the type of information which is being sought. No routine method is suitable for all purposes.

The position as far as quantitative morphological study is concerned is complicated, there being no satisfactory method of ascertaining at necropsy what the total lung capacity (TLC) or the functional residual capacity (FRC) were during life. Furthermore, no reliable and reproducible methods yet exist by which the lung can be fixed at any given volume. All the methods described below represent gross approximations to the ideal which is (i) to be able, from measurements made at necropsy, to estimate the values of TLC and FRC which were present before death, and (ii) to be able to fix the lung accurately at one or other of these volumes. It must be remembered that TLC and FRC represent volumes of gas in the lung and that the volume of the inflated lung at necropsy, whether measured by water displacement or any other method, is a measure of gas and lung tissue. This is likely to be at least half to one litre greater than TLC.

2. TOTAL OR SAMPLE EXAMINATION
If the form of the lungs as a total organ is to be studied, the lung must be treated as a whole, but if only a sample of the lung is to be fixed, information on organ structure will be incomplete, and data will be available only on local cellular conformation. Sample techniques refer only to fixation of a sample and not to the lung fixed in its entirety. Preservation or fixation of whole...
lungs is possible by perfusing fixative through either the pulmonary blood vessels or the airways.

3. GENERAL METHODS OF LUNG FIXATION
Lungs may be fixed by way of either the airways or blood vessels and using either liquid or gaseous methods. The selection of an appropriate combination must depend upon the information sought. Thus, if the nature of airways obstruction due to bronchial secretion is to be studied, fixation by liquid instilled into the airways will probably modify the results. Similarly, the examination of blood vessel obstruction might be modified if liquid fixation by the blood vessels is used.

4. WHOLE LUNG FIXATION BY THE AIRWAYS
*(a) Liquid Fixation
The common liquid fixative is 4% formaldehyde in normal saline (glutaraldehyde may be employed if electron microscopy is considered worth while). Zenker’s solution, either in combination or alone, has been used in order to harden the lung. The greatest difficulty in fixation is the determination of the appropriate lung volume and then measuring this volume during fixation. What is generally required is a volume between functional residual capacity and total lung volume, and there are four main ways of indicating this:

*(i) distension of the lungs until the pleural surface just loses its wrinkles and becomes smoothly stretched. This is a very subjective method and requires alterations in perfusion pressure as fixation proceeds. The method is unsuitable for careful morphometric work at alveolar level because it is often impossible to maintain this level of inflation once the perfusing cannula has been removed. It is satisfactory, for instance, in preparing an airway cast.

(ii) measuring the pressure of the perfusing liquid and relating this to lung volume. The perfusing pressure is gradually increased stepwise, and any resulting increase in lung volume is observed. When the plateau of the pressure-volume curve is reached an increment of pressure produces very little increment in volume; at that point the lung is almost fully distended and near to TLC. The pressure is then reduced a little to bring lung volume down to somewhere between FRC and TLC. After fixation the lung volume finally achieved is measured by liquid displacement (Horsfield, Cumming, and Hicken, 1966).

This method also has the drawback of subjectivity in determining the pressure change unless this is plotted against volume increments.

(iii) by inflating the lungs at the position of functional residual capacity using an artificial thoracic cavity. Chest radiographs recorded during life at FRC in the lateral and postero-anterior projection are obtained. From these, a plaster model of the thoracic cavity is made, comprising two halves for each lung and divided from apex to base through the mediastinal plane. From these four casts a plastic case is produced and bolted together, giving a thoracic cavity identical in size with that in life. The lung is then distended until the plastic case is completely filled (Cumming, personal communication). This method is tedious and the suspension of the lungs within the artificial cavity probably bears no relation to the conditions within the thorax. However, the volume of the lungs is precisely related to their volume in life.

In all these methods the great difficulty is in deciding what represents the ‘correct’ lung volume. The first three methods approximate to the value of TLC which is inappropriate for correlation with functional studies. The fourth method involves fixation near to FRC, and is more valid for functional correlations, but is extremely tedious and time consuming in practice.

Liquid fixation in general requires that the lung should be immersed in the same fixative as that which perfused the airways so that the liquid circulates. Since liquid is present both within and around the lungs, the influence of gravity on lung structures is virtually abolished and differences in morphometry due to this cause will be obscured.

(iv) instead of fixing the lungs at a predetermined volume they may be distended by a standard pressure (commonly 25 cm of water). This method has the merit of simplicity but does not allow for differing compliances in different parts of the lung. The method has been described in detail by Heard (1958).

(b) Vapour Fixation
(i) Blumenthal and Boren (1959) have described a method of fume fixation in which compressed air is bubbled through 40% formalin solution at room temperature. The resulting vapour is passed into the trachea, and lung inflation is judged by the
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6. Sample Fixation

One useful technique for preparation of the lungs, which involves a sample of the whole, is that of local rapid cooling of the inflated lung. The method has been described by Glazier et al. (1969). Freon is cooled in liquid nitrogen, after which it flows rapidly on to the pleural surface of the inflated lung through a metal tube having many side holes. The lung is thus frozen rapidly to a depth of a few millimetres and to a similar width. This frozen segment is rapidly resected and plunged into liquid nitrogen after which it is freeze-dried for microscopic examination. Alveolar size, capillary filling, and the nature of the interstitial space may then be studied.

Sample fixation may be carried out upon the inflated lung by applying the selected fixation method to a single lobe or bronchopulmonary segment.

III Preparation of Whole Lung Sections

The technique of cutting whole lung sections from formalin distended lungs, which has greatly facilitated the study of emphysema, was first described by Gough and Wentworth in 1948. They later published a revised version of the method, and for full details of the technique the reader should refer to this paper (Gough and Wentworth, 1960) and also to the review by Gough (1968). There are three disadvantages to the technique:

(a) The use of gelatine for embedding
The need to eliminate gelatine-splitting enzymes, derived from the tissues of the lung or from contaminating organisms, has always been recognized. To this end the period in the fixative should be extended and minute quantities (0.5%) of formalin introduced into the water which washes the lungs, before embedding. Trouble should arise only when the technique is kept going in an unbroken cycle which makes periodic cleaning of the glassware difficult. Dormer, Martinaglia, and Beimer (1951) recommended embedding the lung in concentrated soft-soap solution, but this method has not been adopted by others. MacNiven (1967) described the substitution of gelatine by Polycell cellulose paste. He claimed that with this material prolonged washing or fixing of the tissue was unnecessary. He has indicated that, although satisfactory for ‘normal’ lungs, penetration and support is inadequate for emphysematous lungs.

(b) The time interval between necropsy and the preparation of sections
Whimster (1969) has shortened the procedure to provide sections within 24 hours of necropsy. Although satisfactory for occasional lungs of special interest, this shortened schedule has been found to upset the sequence of a conventional long routine because of the contamination of glassware which the short schedule favours. The problem of contamination resulting

appearance of the pleural surface. After passing the vapour for three days the lung is fixed and is suitable for sectioning and staining.

If dry specimens are required, compressed air alone is blown into the trachea, leaving the lungs at a volume similar to TLC in life, but not fixed and unsuitable for histological examination.

(ii) The method of Weibel and Vidone (1961) using formalin steam has much to recommend it if detailed study of the pulmonary parenchyma is required. The advantages and disadvantages of this technique are given by Silverton (1964).

(iii) Wright et al. (1974) have described a method of vapour fixation in which the lungs are suspended in a Perspex box and made to rebreathe formalin vapour heated to 45°C. After a period of about six hours primary fixation is complete and the lungs remain in an inflated position, retaining their expanded contours. The expanded lungs are radiolucent and radiographs may be taken at this stage.

The lungs are then cut into slices 1 cm thick and radiographs of these are taken. Comparison of antemortem and post-mortem radiographs of the whole and sliced lungs permits accurate localization of lesions in the tissues. Blocks for histological examination are taken from these areas. All blocks are further fixed for 72 hours in 4% formaldehyde in saline.

It is important that the slicing of lung and selection of blocks are carried out promptly. Solid areas of lung may be incompletely fixed; delays in block selection and secondary liquid fixation may lead to deterioration in the histological appearances.

5. Fixation via the Pulmonary Arteries

This has a small place in preparing lungs where there is excessive exude present in the bronchi and in particular the lungs taken from patients dying in status asthmaticus.
in an enzyme attack on gelatine is particularly prone to occur in periods of warm weather and when lungs are being processed from necropsies carried out long after death.

(c) Artefacts due to the fixation pressures The 120 cm hydrostatic pressure used by Gough and Wentworth has not been found to damage the lung parenchyma. The only consequence of using lower pressures is that the lungs take longer to reach a stable volume.

PERMANENT RECORDS OF WHOLE LUNG STRUCTURE

Permanent records of whole lung appearances are desirable for analysis of collected series and essential for correlation of the findings of different workers. These records can take the form of either photographs or large lung sections. The choice will be determined by the facilities available and the type of material. Solid lung tissue (inflammation, fibrosis or neoplasm) is seen more easily in photographs whereas cysts and emphysematous spaces are better appreciated in large sections. Staining of large sections overcomes the low colour contrast of these preparations. Heard’s (1958) method of covering the surface of lung slices with a precipitate of barium sulphate facilitates gross photography although subsequent histological preparations are unsatisfactory because the precipitate vitiates photomicrography. Silverton (1964) claimed that the conventional Heard photographs are improved for the purpose of comparison with radiographs if the image is printed on to a fine grain positive film instead of on to paper.

IV MEASUREMENT OF LUNG VOLUME AT NECROPSY

1. The volume of the fresh unfixed lung is extremely difficult to measure at necropsy in terms which are comparable to physiological volumes. It is probably better to measure the lung volume from chest radiographs using the method of Barnhard et al. (1960) since radiographs are available in nearly all hospital cases. Postero-anterior and lateral views taken in inspiration are required. The results can be compared with the value for the TLC obtained during life. Measurements made on fixed or processed lungs can then be corrected to this value.

2. The volume of the fixed lung must be measured by one of two methods:

(a) Water displacement in a tank, which has the disadvantage that the lung may undergo some compression during immersion. The mean of at least three measurements should be taken.

(b) Simpson’s rule: the fixed lung is cut into slices of uniform thickness. The outline of each slice is traced on to paper and its area, A, measured by means of a planimeter. If there are n slices h cm thick, the volume of the fixed lung, \( V \), is given by:

\[
V = \frac{1}{3} \frac{h}{2} \left( A_0 + A_n + 4(A_1 + A_2 + \ldots + A_{n-1}) + 2(A_2 + A_4 \ldots + A_{n-2}) \right)
\]

Agreement between these two methods is surprisingly good: they seldom differ by more than 50 ml, but the second method is more time consuming.

V ASSESSMENT OF EMPHYSEMA

1. DEFINITION

Emphysema has been defined as ‘a condition of the lung characterized by increase beyond the normal in the size of air spaces distal to the terminal bronchiole either from dilatation or from destruction of their walls’ (Ciba Guest Symposium, 1959). Many workers now require destruction of the walls of the air spaces for the diagnosis of emphysema and would not include conditions in which dilatation alone is present. In reporting on emphysema the definition in terms of air space size and tissue destruction should be clearly stated.

*2. MACROSCOPIC ASSESSMENT OF WHOLE LUNG SECTIONS

Where there is only moderate generalized enlargement of air spaces throughout the entire lung, attempts at macroscopic quantitative analysis are subject to gross observer variation so that histological measurement is necessary (Thurlbeck et al., 1968). In many cases the emphysematous process may be circumscribed, although the affected areas themselves may be widely distributed throughout the lung. In these cases, the volume proportion of abnormal lung, conducting structures, and normal lung can be determined by the point counting method (Dunnill, 1962; Weibel, 1963).

If the whole fixed lung is available it should be cut into slices 1 cm thick and each slice should be sampled. The points on the grid may be spaced 2 or 2.5 cm apart, and this will give 500 or more points per lung. Details of this method with particulars of grid size are given by Anderson and Dunnill (1965). In general it is better to count a moderate number of points over a large number of slices than a large number of points over a small number of slices. This method can be used on whole lung paper sections.
A description of the distribution and type (e.g., panlobular, centrilobular, cicatricial, etc.) of emphysema within the lung should be included in each report.

Mention should be made here of methods other than point counting which have been used for assessing emphysema. These are of two main categories:

(i) methods whereby the sagittal slices of lung are divided into arbitrary areas and each area is assessed or graded according to the extent of the disease process. This procedure was developed by Heard and Izukawa (1964) using barium-impregnated slices of whole lung (Heard, 1958).

(ii) methods in which sample paper-mounted sagittal sections of whole lung are compared with 'standard' photographs of whole lung sections in which emphysema is present in agreed degrees of severity. This method was used by Thurlbeck et al. (1970) and has the advantage that it is very quick.

It should be noted that neither of these methods is measurement. They are merely grading procedures which attempt to assess both volume and distribution of disease. (There is an appreciable error both between readers and for the same reader at different times: Thurlbeck et al., 1969.) Point counting is a measurement of volume only.

3. MICROSCOPIC ASSESSMENT OF HISTOLOGICAL SECTIONS

This is of particular value when there appears to be a moderate generalized enlargement of air spaces, as in panacinar emphysema.

Special eyepieces are available for performing many of the measurements, such as point counting and intercept measurement, referred to below. However, projection methods whereby the histological section is projected on to a grid of points or lines drawn on white Bristol board is cheaper and often less time-consuming, allows more than one observer to count a given field, and causes less strain on the eyes.

(a) SAMPLING A method of stratified random sampling should be employed using a random number table in conjunction with a numbered sampling grid (Dunnill, 1964). This should ensure that the group of blocks taken for histology possesses the same characteristics as the entire lung, i.e., the same proportion in which each special feature is present or absent.

(b) POINT COUNTING This will give an estimate of the volume proportion of alveoli, ducts, tissue, small vessels, and abnormal air spaces.

(c) ALVEOLAR SURFACE AREA (Campbell and Tomkeieff, 1952) and NUMBERS OF ALVEOLI (Weibel and Gomez, 1962). These can be estimated using these histological sections.

VI ASSESSMENT OF BRONCHIAL PATHOLOGY

1. INTRODUCTION

Macroscopic examination of the bronchial tree can be carried out before fixation by dissection of the major branches at least to segmental level, but this precludes examination by other methods. The bronchi of the fresh or expanded lung should preferably be outlined with radio-opaque material. It is difficult to maintain fresh lung in the expanded condition for radiography. Dry powders of lead, tantalum or barium sulphate have all been insufflated into lungs to outline bronchial surfaces (Leopold and Gough, 1963). With fresh specimens the powder is introduced by artificially ventilating the lung. On the other hand, fixed lungs require preliminary drainage and 'forced ventilation' over a period of hours to remove fluid fixative from the bronchial surfaces, together with a compressed air supply to transport the powder to the periphery of the lung. Alternatively, liquid injections which coagulate on cooling may be preferred whenever bronchial blockage by exudate is expected, as in asthmatic cases. Many of the fluid injectants contain gelatine which must be washed out after cutting slices 2.5 cm thick from the lung before returning to formalin storage for the production of large lung sections. Radiography requires fine grain, industrial-type emulsions, x-ray sources with a small focal spot, and generators which work in the 40–50 kv range. Self-contained instruments are available which combine within a screened cabinet a suitable x-ray source and space for insertion of a lung specimen and film. These cabinets are self-adjusting in respect of voltage and duration of exposure for each individual specimen.

2. CHRONIC BRONCHITIS

It is generally agreed that this condition should be defined on a clinical basis, first proposed by Scadding (1959) and amplified by the Ciba Guest Symposium (1959) as persistent expectoration in the absence of other causes, in particular, localized disease of the lungs or bronchi or primary cardiovascular disease. The pathological counterpart of
this lies in hypertrophy of the mucous glands in the bronchi (Reid, 1960). Since approximately 96% of bronchial mucus is thought to be produced by the submucosal glands, and not from the goblet cells in the mucous membrane, an estimate of the proportional volume of these glands within the bronchial walls is needed for a pathological assessment of the severity of chronic bronchitis.

*(a) POINT COUNTING IN RELATION TO PATHOLOGY OF THE BRONCHIAL WALL Using the Zeiss I integrating eyepiece, or alternatively a projection method (Dunnill, Massarella, and Anderson, 1969), it is possible to estimate the proportion of mucous glands in the bronchial wall together with the proportion of cartilage muscle and connective tissue. Point counting has the advantage that:
(i) all constituents of the bronchial wall are assessed;
(ii) the method is independent of the shape and distribution of the various constituents and the crenated form of the bronchial mucous membrane and is not a source of error.

The main disadvantage is that the adventitia of the bronchus has to be defined and this can prove difficult. It is best overcome by deliberate selection of the bronchi to be measured. It is suggested that a main bronchus, the bronchus to the inferior segment of the lingula, and the bronchus to the basal segments of the lower lobe would be suitable for this purpose.

*(b) THE REID INDEX This is the ratio of the thickness of the mucous gland layer, as it lies between cartilage and epithelium, to the thickness of the bronchial wall at the same point, that is from the basement membrane of the epithelium to the inner aspect of the perichondrium. The measurement is made at a point where the cartilage and mucous membrane are parallel. The method presents certain difficulties:
(i) In many bronchi it is not easy to find suitable areas where the cartilage is parallel to the mucous membrane.
(ii) The point at which the measurement is made is arbitrary, and the ratio may vary considerably from place to place in the bronchial wall.
(iii) The method gives no indication of the total quantity of gland tissue, much of which in bronchitis may be between the cartilage plates.

It should be noted that Takizawa and Thurlbeck (1971), in comparing methods of assessing chronic bronchitis, found that the Reid Index and the point count correlated closely. There was little difference between them in separating patients with bronchitis from those without bronchitis. Bedrossian, Greenberg, and Duran (1973) in contrast have found a poor correlation between various methods of measuring bronchial gland area and the Reid Index.

*(c) MEASUREMENT OF BRONCHIAL CALIBRE
Measurement of bronchial diameter in a section of fixed lung is of little value unless the branching order of the observed airway can be defined. Hogg et al. (1969) used insufflation with lead powder to outline airways and the centrilobular space by radiography. It is possible, using radiographic techniques in inflated lungs, to identify the order of airways with a fair degree of certainty and hence to relate the order to bronchial calibre. Such techniques have been used by Nadel, Wolfe, and Graf (1968), who used tantalum powder, and Hughes, Hoppin, and Wilson (1972) who also employed stereoscopic pairs of films to improve the measurement.

Finally the bronchial tree can be cast as a whole (Horsfield et al., 1966), which simplifies ordering and diameter measurement, but the process is time-consuming. Observations on bronchial and bronchiolar diameter in chronic non-specific lung disease have also been made by MacKenzie, Glick, and Outhred (1969) and by Bignon, Andre-Bougaran, and Brouet (1970).

*(d) EXAMINATION OF BRONCHIOLES Matsuba and Thurlbeck (1973) have drawn attention to the importance of examining the diameter, number, and histology of airways of less than 2 mm diameter in cases of chronic non-specific lung disease. This can be performed on conventional histological sections by light microscopy.

VII EXAMINATION OF THE PULMONARY VASCULATURE
In chronic respiratory disease there are abnormalities of the airways, alveolar tissue, and the pulmonary vasculature. These should be considered together. A case of chronic respiratory disease has not been examined adequately unless the pulmonary vascular tree has been studied.

*1. FIXATION OF THE LUNGS
Lungs are fixed satisfactorily by distending them through the bronchi with 4% formaldehyde in saline until the pleural surfaces are smooth. This
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Technique avoids partial collapse of the lung parenchyma which may lead to a spurious impression of increased medial thickness in the small pulmonary blood vessels. Vacuum embedding is desirable.

In most instances more sophisticated methods of control of the distending pressure of the fixative are not required. Many of the features of pulmonary vascular disease are in any case qualitative rather than quantitative in nature. Quantitative differences of medial thickness of 'muscular pulmonary arteries' are unlikely to be significant if they are not apparent in preparations fixed by the simple method of intratracheal infusion of formalin fluid.

Neither formalin steam nor formalin vapour gives adequate fixation for subsequent histological examination of pulmonary blood vessels. In particular, fixation of the lung by the formalin-steam method of Weibel and Vidone (1961) is sometimes unsatisfactory for the study of the pulmonary vasculature.

Blocks of tissue should be selected from:
(i) the upper lobe;
(ii) the lower lobe to include the pleural surface with its bronchial and lymphatic vessels;
(iii) the hilum of the lung to include the major elastic pulmonary arteries and 'true' bronchial arteries.

*2. Classes of Pulmonary Blood Vessel to be Studied

Clear criteria for the recognition of various classes of pulmonary blood vessel were laid down by Brenner (1935) and the system of classification he devised has stood the test of time, being virtually unchallenged and widely used by workers in this field. In expressing results of studies on the pathology vasculature, it is essential to state which class of pulmonary blood vessel is being considered. Details of the structure of the different classes of pulmonary and bronchial blood vessel may be obtained from his original description or in greater detail from The Pathology of the Pulmonary Vasculature (Wagenvoort, Heath, and Edwards, 1964).

It should be noted that Reid (1968) does not entirely agree with this classification and has proposed a more complex one involving a system of muscular, partially muscular, and non-muscular pulmonary arteries. From the point of view of the practising pathologist, however, the following classes of pulmonary artery can be recognized:

the elastic pulmonary arteries, comprising the pulmonary trunk and the larger pulmonary arteries, over 1000 μm in diameter; the muscular pulmonary arteries, between 1000 μm and 100 μm in external diameter; and the pulmonary arterioles, usually less than 80 μm in diameter.

*3. Staining of Sections

The best method for demonstrating the structure of the pulmonary blood vessels in histological sections is to stain the elastic tissue by Verhoeff's method or by the Lawson modification of the Weigert-Sheridan method, and to counterstain the muscle and collagen by Van Gieson's reagent. Routine staining with haematoxylin and eosin should be employed. Other stains can be used for special studies, e.g., of fibrin, iron or acid mucopolysaccharides.

*4. Assessment of Pulmonary Trunk

Transverse blocks of tissue are cut from the pulmonary trunk and aorta 1 cm above the respective semilunar valves and fixed in 4% formaldehyde. Transverse sections of paraffin-embedded tissue are stained with an elastic-Van Gieson method to distinguish elastic fibres, smooth muscle, and collagen.

Ten measurements are made of the medial thickness of transverse histological sections of the pulmonary trunk and aorta using a calibrated eyepiece micrometer. From these 10 measurements the mean medial thickness of each vessel is calculated. The mean medial thickness of the pulmonary trunk (PT) is expressed as a ratio of the mean medial thickness of the aorta (A)—(PT/A ratio). The normal PT/A ratio lies in the range 0.4 to 0.7 (Heath et al., 1959).

*5. Assessment of Muscular Pulmonary Arteries and Arterioles

Histological sections stained by the elastic-Van Gieson method are examined with a microscope fitted with a calibrated eyepiece micrometer. Only vessels that are virtually circular in transverse section are measured: this reduces the number of vessels available for study but avoids errors in the measuring of the diameter.

The external diameter is taken as the mean of two measurements, at right angles to each other, of the distance between diametrically opposed points on the external elastic lamina.

The medial thickness is estimated as the mean of four measurements taken at approximately equally spaced points around the vessel wall. The
thickness of the media of each vessel can be expressed as a percentage of the external diameter.

The average percentage medial thickness of muscular pulmonary arteries in each subject is the sum of all the percentage medial thickness divided by the total number of vessels examined.

6. PULMONARY CAPILLARIES
Quantitative assessment of the pulmonary capillaries is fraught with a number of difficulties. It is known that the state of inflation and the perfusion gradient between arteries and veins have to be taken into account. Further, gravity affects the distribution of perfusate in different parts of the lung.

Nevertheless the lung capillaries can be adequately visualized in thick (200 μm) frozen sections of lungs injected with a suitable preparation such as India ink. (This can be used diluted and without the addition of gelatine.) Useful observations on lung capillaries have been made by this means (Butler and Kleinerman, 1970).

Penetration by injection masses often tends to be patchy, possibly because it is difficult to clear capillaries totally of red cells, and a preliminary perfusion of the lung by warm 2% sodium nitrite might help. An alternative procedure used by one of us has been to augment the capillary content of blood cells by the injection of out-of-date and undiluted human blood, cutting thick frozen sections and outlining the capillaries by peroxidase staining of the contained red blood corpuscles.

7. POSTMORTEM PULMONARY ANGIOGRAPHY
Methods have been described by Short (1956–57) and Doyle et al. (1957). The general configuration of the pulmonary vasculature may be demonstrated by injecting a radio-opaque medium into the pulmonary arterial tree of the fresh lung and subsequently taking radiographs of the inflated fixed lung. The method will indicate ‘tree-pruning’ effects due to obliterator disease of the pulmonary vasculature, and loss of parts of the normal arborescent pattern due to disease and loss of lung substance. It will demonstrate bronchopulmonary anastomoses. However, the method is of limited value and any abnormalities revealed must be subsequently investigated by histopathology frequently with the use of serial sections. The method is useful to identify areas of lung or particular vessels of interest for subsequent histological studies.

VIII EXAMINATION OF THE HEART
1. INTRODUCTION
The weight of the heart is usually recorded at necropsy and taken as an estimate of the mass of cardiac muscle, particularly of the left ventricle. It is often not realized what a poor relationship there is between the weight of the whole heart and that of the ventricular muscle. In a series of hearts (Lamb, 1973) with their chambers opened and antemortem thrombus and postmortem clot removed, the muscle mass of the left and right ventricles and the intraventricular septum ranged from 40% to 80% of the total heart weight. Hearts of small total weight tend to show a greater proportion of non-muscular tissue than those of large total weight. Assessment of right ventricular hypertrophy is of special importance in relation to chronic non-specific lung disease. Since a relatively small right ventricle makes little contribution to total heart weight, variations in right ventricular size cannot be assessed by weighing the whole heart.

*2. METHODS OF ESTIMATING RIGHT VENTRICULAR MUSCLE MASS
(a) WEIGHING THE RIGHT VENTRICULAR MUSCLE MASS To weigh the right ventricular muscle the muscular portion of the heart must be separated from non-muscular tissue. The major vessels and atria are dissected off the valve ring and discarded and the ventricles are freed from the coronary arteries and epicardial fat. The right and left ventricles are then separated and weighed individually.

Early workers (Muller, 1883; Lewis, 1913–14; Herrmann and Wilson, 1921–22) divided the septum between the left and right ventricle. This was a tedious procedure and open to error. In 1952 Fulton, Hutchinson, and Morgan Jones showed that, since the septum increased in parallel with left ventricular weight but showed no change with right ventricular hypertrophy, it should be included with the left ventricle. It was only necessary to separate the right ventricular muscle from the left ventricle and septum. The technique is to cut round the angle formed by right ventricle and septum with scissors and to trim off the chordae muscles flush with the septum. This was proposed as a standard for estimating right and left ventricular weights by the WHO Committee on Cor Pulmonale (World Health Organisation, 1961). Details are given by Fulton et al. (1952) and Lamb (1973).

The heart may be dissected fresh at necropsy or after fixation with formol saline. The latter has the advantage that complete removal of epicardial fat is easier. The greatest part of the epicardial fat is found in association with the right ventricle. Failure to remove this fat will significantly affect
the right ventricular weight. The proportion of fat overlying the left ventricle is relatively very small in relation to the total mass of the ventricle. A small percentage weight loss during fixation is probably insignificant over a week or 10 days. Dissection after fixation with formal saline is preferable, for any error in removal of epicardial fat is likely to affect the estimate of right ventricular weight more than the left ventricular weight.

(b) MEASUREMENT OF RIGHT VENTRICULAR THICKNESS It has been shown by several workers that this is an unacceptable method for assessing right ventricular hypertrophy (McPhie, 1957; Lamb, 1973). The usual site of measurement is the smooth wall of the outflow tract 1 or 2 cm below the pulmonary valve. There are two difficulties in the assessment of right ventricular muscle mass by measuring wall thickness. First, the thickness varies in different parts of the right ventricle, most of which has a trabeculated inner surface. Second, wall thickness is affected by ventricular dilatation.

(c) MEASUREMENT OF CARDIAC MUSCLE FIBRE DIAMETER If increase in muscle mass involves hypertrophy of cardiac muscle fibres, measurement of the muscle fibre size should relate to ventricular weight. There is, however, variation in fibre size between adjacent microscope fields and this is exaggerated in scarred areas. Sampling involves measuring large numbers of fibres: this is time-consuming and open to observer error. In a small group of cases a poor relationship between such measurement and ventricular weight has been found (Lamb, personal observation).

(d) OTHER TECHNIQUES OF ASSESSMENT OF RIGHT VENTRICULAR HYPERTROPHY Clinical estimates of right ventricular hypertrophy based on electrocardiographic or radiological findings are not really the province of the pathologist but it should be noted that neither gives consistently useful results. A more complex technique involving biplane angiocardiography described by Kennedy et al. (1967) gives estimates for ventricular weights during life which agree well with actual measurement after death. If it is desired to keep the heart intact and avoid the dissection necessary for separate weighing of left and right ventricles it is possible to derive values for ventricular weights from a series of measurements and calculations described by Rimoldi et al. (1971).

3. NORMAL RANGE OF VENTRICULAR WEIGHTS AND ASSESSMENT OF RIGHT VENTRICULAR HYPERTROPHY

The technique of weighing left and right ventricular muscle is simple to perform but interpretation is more difficult. There is a wide range of normal ventricular weights, probably related to body size and muscular activity. In the case of the right ventricle this extends from under 25 g to over 80 g. At present the normal range of ventricular weights by age and sex has not been established. A right ventricular weight of 80 g may represent a 150% increase for a small sedentary woman but only a 30 or 40% increase for an active man.

The most accurate way of identifying isolated right ventricular hypertrophy is to use the ratio between left and right ventricular weights, assuming that the left ventricle is normal. This method is discussed and criteria are suggested by Fulton et al. (1952) and Lamb (1973). More data are still needed in relation to ventricular weights and factors which influence the normal range.

IX THE CAROTID BODIES IN CARDIOPULMONARY DISEASE

Anatomically the carotid bodies are not thoracic organs but recent studies show that in states of chronic hypoxia associated with many cardiopulmonary diseases the carotid bodies enlarge (Heath, Edwards, and Harris, 1970; Edwards, Heath and Harris, 1971a) and show characteristic histological (Edwards et al., 1971b) and ultrastructural (Edwards, Heath, and Harris, 1972) changes. It seems likely that these anatomical and histological changes will prove to be associated with alterations in function. This organ should be examined routinely in necropsies on cases of cardiopulmonary disease, especially chronic respiratory disease associated with chronic hypoxia.

The carotid bodies should be dissected out, weighed, and examined histologically with a differential count of the different types of glomic cell, and any cytological abnormality should be noted (Heath et al., 1970).

The carotid body is a pedunculated ovoid structure lying just behind the bifurcation of the common carotid artery in close apposition to the carotid sinus; sometimes it is bilobed. Dissection of the carotid bodies is easy and takes 5 to 10 minutes. The pedunculated carotid body may be cut off with scissors, and after removal of associated adipose and fibrous tissue, it should be lightly blotted and weighed. The carotid body is commonly situated in a central position but sometimes
it is found on one side of the bifurcation, usually on the wall of the external carotid artery. Usually it is reddish-brown in colour but in hypoxic cases it is deeply congested and mauve in colour.

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