BPD and no longer give rise to fibril formation upon routine passage. It is possible to induce a surface type growth by scraping the cells from the glass surface and transplanting them into new BPD media with a minimum of agitation. Under these conditions the floating cells never produce the long strands shown in Fig. 3, but grow and expand as cell sheets with a morphology similar to, but not as compact as, the attached cell sheet.

Early passages of the L cell line(4) in the BPD medium produce floating cell sheets similar to those seen with the mouse lung line. The discrete fibrils seen in the case of the mouse lung cells have not been seen with the L cells.

Discussion. As a result of the above investigation certain facts concerning fibril formation can be stated. It is certain that fibrils can arise without agitation of the medium, and are therefore not the result of entwining of single strands of material. From the photomicrographs it appears that the fibrils arise from the cell body *per se* and are not the result of entrapment of cells in fibrous material released by the degenerated cells. The fact that the fibrils are no longer seen once the cells become adapted to a rapid growth in the BPD medium points up the fact that they may be the result of unfavorable growth conditions as previously hypothesized(1). Attempts to stimulate a similar response by other sub-optimal conditions in the absence of BPD have failed. It is probable therefore that the Bactopeptone itself is at least partly responsible for formation of this material.

Summary. The presence of fibril-like material in a strain of mouse lung cells grown in a serum-free medium is described. This material was resistant to digestion by hyaluronidase and trypsin and susceptible to digestion with collagenase. The period of appearance and disappearance is discussed and photographic evidence of the cell body-fibril association is presented.

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Elastin Content of the Human Lung.*†‡ (24280)

A. M. BRISCOE AND W. E. LORING

Departments of Biochemistry and Medicine, Columbia University College of Physicians and Surgeons, and Chest Service, Bellevue Hospital; and the Department of Pathology, New York University College of Medicine, New York City

Elastic tissue is readily demonstrated histologically in the parenchymal tissue of the human lung but no quantitative chemical studies of it have been reported in this tissue. Such investigations are now in progress in this laboratory. Chemical estimation of elastic tissue at present must be based on its content of the fibrous protein, elastin. The purpose of this communication is to report the amount of elastin in the human lung, and the correlation between pulmonary elastin concentration and age. In addition, the normal concentrations of elastin have been compared with those obtained in lung parenchyma subjacent to areas of bulla formation. It has been suggested that elastic tissue plays a role in the pathogenesis of such lesions.

Of several methods of elastin determination which have been reported(1,2,3), the method of Lowry *et al.* was chosen for study of the

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lung, and adapted to exclude insoluble substances other than elastin which may be encountered in lung tissue.

Materials and methods. The lungs examined were fresh specimens obtained at the time of autopsy. Causes of death were either traumatic or of extrapulmonary nature. Tissue was selected which was ostensibly free of pulmonary disease.

Elastin was determined by the following modification of Lowry's method(1). The samples were minced, ground to a paste, suspended in 0.1N NaOH and digested in a boiling water bath for 10 minutes. The insoluble material was separated by centrifugation, resuspended in NaOH and digested similarly twice more. The residue was then washed, dried and weighed. It consisted of elastin and insoluble foreign material and was designated W-1. Elastin was then removed by autoclaving in 6% KOH for 45 minutes. The residue was again washed, dried and weighed to give W-2. Difference in weight between W-1 and W-2 was assumed to be the weight of elastin in the sample. The amount of soot and other insoluble foreign matter in the lung could also be calculated from the difference between W-2 and the weight of the empty tube.

For each lung, 4 contiguous pieces weighing between 1.0 and 1.7 g were selected for quadruplicate analyses. These specimens were taken from the left upper lobe in nearly all cases. Pleura and large blood vessels and bronchi were excluded. Water content was determined in other specimens from the same area. In addition, selected specimens were taken for routine histological examination using Weigert's resorcin-fuchsin elastica stain and Golder's modification of Masson's trichrome stain, respectively. In 4 lungs with bullae, specimens for analysis were taken from the tissue directly beneath the bullae. The results are reported as per cent of dry weight of the tissue.

Results. Analyses of normal pulmonary parenchyma from men and women between 16 and 93 years of age revealed that elastin constituted an average of 8.28 ± 0.6% of the solids of the lung. The range of values was from 1.75 to 13.1%. These results are summarized in Table I.

TABLE I. Concentration of Elastin in the Human Lung.

Group	No.	Elastin, % dry wt
Normal men	17	8.08 ± 1.4
" women	12	9.48 ± 1.2
Men with bullae	4	4.61 ± .9

TABLE II. Comparison of Concentrations of Insoluble Residue and Elastin in Lung.

Age	Insol. residue, % dry wt	Elastin, % dry wt
25	.1	7.23
22	1.65	3.65
42	1.53	11.2
65	4.70	11.5
60	18.1	7.5
66	12.6	11.2

The last step in the revised method of analysis described above was added to correct for insoluble residue in the lung. Data calculated from 6 experiments are presented in Table II to illustrate the necessity for this correction. The accumulation of this residue increases with age and is quite evident on gross inspection of the lungs.

In Fig. 1, results of analyses of lungs from 29 apparently normal men and women are plotted against age at time of death. Data

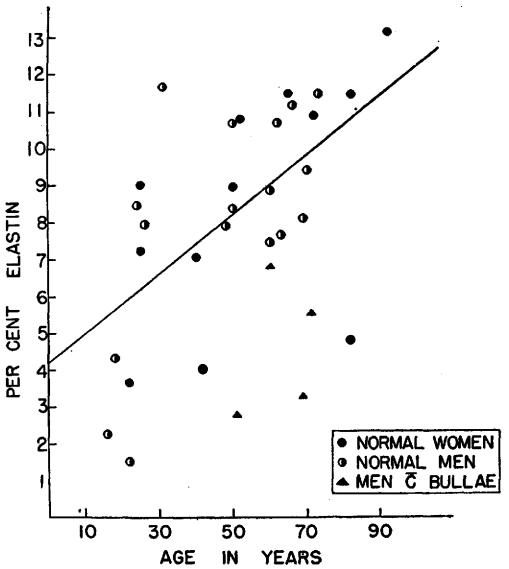

FIG. 1. Elastin concentration plotted against age: % of dry wt of tissue.

obtained from tissue under bullae in lungs of 4 older men are also included. Statistical analysis of this graph shows a highly significant increase in relative amount of elastin with age ($t = 3.87$, $p>0.001$). The regression slope shows an increase in elastin content of the lung of 0.816% per decade of life. On superficial examination of the graph, women over 50 appeared to have more pulmonary elastin than men in this age group. However, when subjected to statistical analysis, the difference in this small series was found to be insignificant ($t = 0.75$, $p = 0.4$). Comparison of the elastin content of sub-bullous tissue with that of normal lung parenchyma of older men revealed a highly significant decrease in the former ($t = 5.09$, $p>0.001$).

Histological studies of the elastin isolated by our method showed that the end-product was composed only of elastic tissue. However, one preparation contained traces of collagen deep within the walls of a pulmonary arteriole.

Discussion. It has been generally believed that the elastic tissue content of the aging lung is reduced. Our measurements must be viewed critically and are simply a prelude to further investigation. Although the method was well standardized and corrected for errors due to foreign material in the lung, results obtained from lungs of different ages may not be entirely comparable. Pulmonary elastin may change physically and chemically with age as has been demonstrated in elastin from the aorta(4), and these changes may affect the efficiency of isolation and separation. Confirmatory studies of elastin content by other methods are needed. Simultaneous measurement of pulmonary collagen is contemplated in view of the close relationship between collagen and elastin. It is of considerable interest that concentration of elastin in the lung is relatively high. Of the tissues thus far analyzed, only the aorta and the ligamentum nuchae contain more elastin than the lung(1,2).

Summary. 1. An alkaline digestion method for quantitative determination of elastin has been adapted for study of lung parenchyma. 2. Concentration of elastin in the adult human lung has been determined over a wide age span, and has been found to increase with age. 3. Concentration of elastin in lung parenchyma subjacent to areas of bulla formation is significantly reduced.

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Effect of 6-Mercaptopurine on Antibody Production.* (24281)

ROBERT SCHWARTZ, JANICE STACK AND WILLIAM DAMESHEK
Blood Research Laboratory, New England Center Hospital, and the Department of Medicine, Tufts University School of Medicine

Since immunologic mechanisms participate in the rejection of homografts by experimental animals(1), some modification of the immune response is necessary in order to achieve a "take" of the grafted tissue. Alteration of antibody production may occur naturally, as in agammaglobulinemia(2), or may be induced by means of X-radiation(3) or cortisone(4). Protein(5) or vitamin(6) deficiencies obtained by means of special diets (7) or by the action of amino acid analogues (8) may lead to suppression of antibody formation and to prolonged survival of skin homografts(9). In the special case of tumor

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