bation with muscle. In early experiments each individual slice was weighed and counts/mg wet weight/cc/minute determined. However, it became apparent that weight variation was insignificant, and this was discontinued.

Results. Table I represents accumulated data from 5 experiments. Numerical values are the mean of 3 aliquots from each flask, and measured amount of radioactivity remaining after incubation. It is apparent that addition of PTE to the incubating medium consistently increased amount of radioactivity remaining and consequently must have inhibited uptake of Ca^{45} by muscle, or altered degree of absorption of Ca^{45} on the muscle cell membrane. Addition of PTE to the incubating medium in absence of muscle did not affect the final activity of Ca^{45}. The data were analyzed by Student-Fisher t test for significance of mean differences, and mean difference was significantly different from zero (P value $<.001$).

Summary and conclusions. Administration of parathyroid extract (PTE) elevates serum calcium(5). The present experiment indicates that PTE consistently inhibited *in vitro* uptake of calcium by rat voluntary muscle. This observation is compatible with the known hypercalcemic effect of parathyroid hormone and provides evidence for the hypothesis that parathyroid glands regulate movement of calcium between extracellular and intracellular fluid.

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Changes in Human Lung Collagen and Lipids with Age. (25147)

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Preliminary investigations indicate that collagen in human lung undergoes both quantitative and qualitative changes with increasing age(1). It is already known that a quantitative change occurs in animals; collagen content of guinea pig lung(2) and of rat lung(3, 4) increases with age. A qualitative change with advancing age has been demonstrated in collagen isolated from animal tissues other than lung. Collagen becomes increasingly stable with age when subjected to chemical and enzymatic degradation and to physical stress(5,6,7,8). While our investigations were in progress, it was reported that shrinking temperature (T_s) of collagen isolated from human skin increases with age(9). Differences in T_s among collagens from fish and bovine sources were reported by Gustavson (10). Imino acid analyses of these collagens led him to postulate that T_s is related to hydroxyproline content of the molecule, because collagens with the highest hydroxyproline content had the highest T_s(11). Hydroxyproline is thought to provide crosslinks between peptide chains by means of hydrogen bonds and thus to stabilize the structure of the protein and increase its resistance to stress. Our results concern both these quantitative and qualitative changes in collagen in human lung parenchyma and pleura. Concentration of collagen in lung parenchyma, the T_s of collagen of pleura, and the hydroxyproline content of collagen isolated from pleura have been studied and correlated with age. Observations of the lipid content of the lung are also included.

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Materials and methods. Samples of normal human lung parenchyma and pleura were ob-

tained at autopsy in Bellevue Hospital. The term pleura includes the serous membrane, subpleural connective tissue and probably small amounts of attached underlying parenchyma. Parenchyma was selected from peripheral areas of the left upper lobe. These specimens were analyzed as follows: 1. Lung parenchyma from 8 women and 18 men, aged at time of death from 28 to 84 years, was analyzed for total lipids. Lipids were determined by weighing ether soluble extract obtained from an aliquot of whole dried tissue by the Soxhlet method. 2. Lung parenchyma from 8 women and 19 men varying in age from 27 to 77 years was analyzed for hydroxyproline by the colorimetric method of Stegemann(12). Quadruplicate samples of fresh tissue weighing 200 to 300 mg were placed in vials with 6 N HCl and sealed. The vials were heated at 110°C for 48 hours. Hydroxyproline was determined in aliquots of neutralized, diluted hydrolysate thus obtained. Results are reported either as per cent hydroxyproline or as per cent collagen of dry weight of tissue. Percentage dry weight was determined in another specimen of each lung by drying to constant weight in oven at 110°C. 3. The T_s of strips of pleura from 7 women and 20 men 20 to 81 years old was measured in distilled water. Strips of pleura were prepared as described by Gustavson for fish and bovine skin (10). For measurement, the strip was attached under slight tension with thread to a lever and submerged in beaker of water heated at the rate of about 1°C/min. The T_s was recorded as that temperature at which the lever began to move. 4. Hydroxyproline content of collagen, isolated as gelatin from pleura of 14 males and 10 females, from 10 weeks to 77 years old, was determined. Gelatin was isolated by a method essentially similar to that of Neuman and Logan(13). Hydrolysis of gelatin and determination of hydroxyproline was done as described above. The results are reported as grams of hydroxyproline/100 g of gelatin.

Results. Lipid concentration of dried lung parenchyma, plotted as a function of age, is presented in Fig. 1. The hydroxyproline concentration of lung parenchyma is similarly plotted against age in Fig. 2, and expressed as per cent of hydroxyproline or collagen of the dry weight, assuming former to be 13.5% of the latter(13). The average values of lipid, collagen and elastin/decade of adult life used to calculate these graphs are shown in Table I. Correlation between T_s of pleural strips and age is illustrated in Fig. 3 ($t = 7.24$; $p < 0.01$). Results of analyses of collagen isolated as gelatin from these pleural strips are presented in Fig. 4.

TABLE I. Average Concentrations in Human Lung of Collagen, Lipid and Elastin per Decade of Adult Life.

	Collagen*		Lipid	Elastin†
Age, yr	% dry wt	% fat-free dry wt	% dry wt	% dry wt
25	8.7	9.7	10.1	6.2
35	9.7	10.7	9.2	7.0
45	10.8	11.8	8.3	7.8
55	11.9	12.9	7.4	8.6
65	12.9	13.8	6.5	9.5
75	14.0	14.8	5.5	10.3
85	15.0	15.7	4.3	11.1

* Values corrected for elastin.
† Taken from Fig. 1 in previous report(14).

Discussion. Hydroxyproline concentration of human lung increases with age both on the basis of total dry weight and of fat-free dry weight ($t = 4.33$; $p < 0.01$). A further correction for the inhaled foreign material found in increasing amounts in aging lungs would increase steepness of slope of the regression line. The question arises, whether or not this increase in hydroxyproline content represents increase in collagen concentration. If the amount of hydroxyproline due to lung elastin is subtracted (Fig. 2, dotted line), using values for elastin obtained in an earlier series of experiments(14), presumably the remainder is present only in collagen. This assumption is based on the hypotheses that no other protein contains hydroxyproline, and that hydroxyproline is not free in the tissues but arises within the protein molecule from proline(15, 16). If the hydroxyproline content of collagen in lung parenchyma increases with age, it is conceivable that an increasing hydroxyproline content of lung parenchyma (Fig. 2) could be compatible with a constant collagen content at all ages. This could not occur, however, unless the hydroxyproline content of collagen increased with age about 6 times as

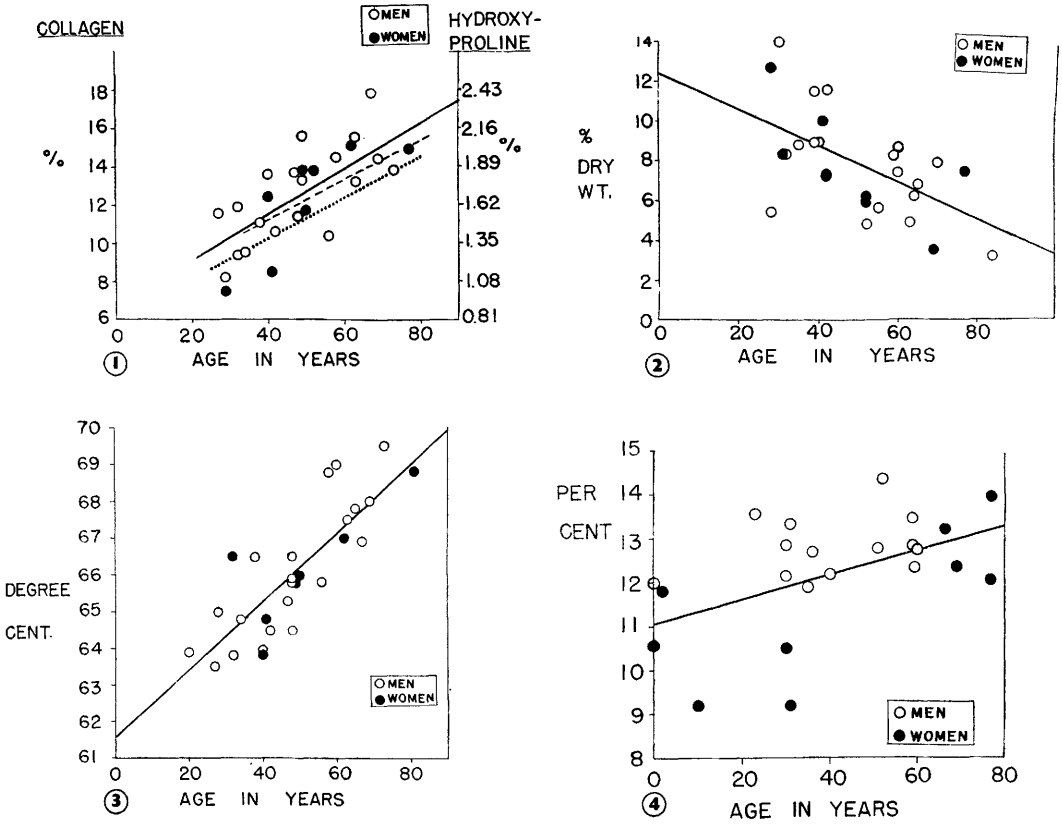

FIG. 1. Total lipids of lung plotted *vs* age: % of dry wt of tissue.

FIG. 2. Collagen or hydroxyproline concentration of lung plotted *vs* age. Solid line: total hydroxyproline as % of dry wt of tissue. Broken line: collagen, corrected for elastin, as % of fat-free dry wt. Dotted line: collagen, corrected for elastin, as % of dry wt.

FIG. 3. Shrinking temperature of pleura plotted *vs* age.

FIG. 4. Hydroxyproline concentration of pleural collagen plotted *vs* age: % of dry wt of collagen isolated as gelatin.

much as in pleura. Other workers whose results were based on alkali extraction method rather than amount of hydroxyproline found that the collagen content of rat and guinea pig lungs increased similarly with age(2,3,4) and the magnitude of their values was of the same order as that reported here. The data of Fig. 4 obtained from collagen from pleural strips were not applied to the data in Fig. 2 which concerns lung parenchyma. Pleura and parenchyma have different structures and might contain collagens of different hydroxyproline concentrations. A study of collagen isolated from lung parenchyma should precede the assumption that it is identical to that of pleura.

Lipid in the lung is not present in the form of adipose tissue. Thus, the practice of defatting tissue as a preface to analysis for other substances, which may be justified in the case of tissues containing variable amounts of adipose tissue, is not valid here. Results obtained with defatted tissue do not reveal the proportion of lung which is collagen. Other workers reported that collagen and elastin content of defatted, dried lung remains constant through life(17). Our data indicate, on the contrary, that even in fat-free lung, the increase in hydroxyproline concentration with age still obtains (Fig. 2, broken line). In addition, our results correlate lipid content of lung with age ($t = 3.42$; $p < 0.01$). The amount of lipid in the lung is apparently not a function of body size or nutritional state since the specimens were obtained from a random selection of individuals with regard to these physical

and physiological characteristics.

Analogous to work with skins, the assumption has been made here that T_s of a pleural strip is due to contraction of its collagen fibers. Contraction of collagen fibers on heating is sharp and characteristic under identical circumstances. Thus values obtained here for collagen under a slight tension are higher than those reported for isolated fibers from human skin which were allowed to float free(9). This is consistent with Gustavson's comparison of freely suspended fish skins with those fastened to a shrinkage measuring device like ours(10) Our data agree with those of Brown and Consden on human skin(9) insofar as age change is concerned and are in contrast to those of Hall and Reed(18) who found no age difference in T_s of isolated human collagen in water. However, the measurement of T_s of collagen in tissue may be more valid because the isolation procedure may alter the collagen molecule.

Correlation between increasing hydroxyproline concentration and increasing age was significant ($t = 2.44$; $p<0.025$). If the 2 lowest values of 9.2% each are considered questionable and prejudicial, they may be excluded; the statistical significance of the data is not impaired thereby. The average value of hydroxyproline concentration in collagen isolated from 21 adult specimens was 12.4%. The range was from 9.2 to 14.4%. The data in Fig. 2 and Fig. 3 suggest that collagen of women has a lower shrinking temperature and lower hydroxyproline concentration than that of men, but a larger number of observations is needed to confirm this.

Although some correlation between T_s of collagen and its hydroxyproline content has been demonstrated, there are collagens from widely different sources which do not conform to the scheme(19,20). That T_s of invertebrate collagen is higher than that of vertebrate collagen of similar hydroxyproline concentration suggests that some other feature of collagen structure contributes to its hydrothermal stability. It may be invalid to compare collagens of invertebrates with those of vertebrates. A study of aging invertebrate collagen may show a relation between the 2 properties. Among collagens of similar composition, variations in hydroxyproline concentration appear to produce corresponding changes in T_s.

Summary. 1) Hydroxyproline content of human lung increases with age. 2) The collagen content of human lung increases with age. 3) The lipid content of human lung decreases with age. 4) The shrinking temperature of human pleura increases with age. 5) The hydroxyproline content of collagen isolated from human pleura increases with age.

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