Urinary hydroxyproline excretion in coalworkers' pneumoconiosis

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Urinary hydroxyproline excretion in coalworkers' pneumoconiosis. As there is an increased
formation of one or all the fibrous proteins in coalworkers' pneumoconiosis, it was felt that
a study of the turnover rate of these proteins might shed some light on the pathogenesis of the
disease. Urinary hydroxyproline excretion was therefore studied in a group of miners, of
whom 19 had simple pneumoconiosis and five had progressive massive fibrosis. No significant
deviation from the normal was found.

Simple coalworkers' pneumoconiosis is characterized pathologically by focal emphysema which on occasion may be fairly extensive. Surrounding the dilatation is a mantle of coal dust and occasionally a little fibrosis. The latter, when present, can be shown to consist of some reticulin fibres and a very few collagen fibrils (Heppleston, 1954). It has been shown that the reticulin collagen mesh in simple dust lesions may be denser than was originally believed (Heppleston, 1947). In contrast, the fibrosis of progressive massive fibrosis is primarily of the collagenous variety (Gough, 1965). Large conglomerate masses appear in the lung, some of which subsequently undergo collagenous necrosis and cavitation. The cause of the transition of simple pneumoconiosis to progressive massive fibrosis remains uncertain in most subjects, as do the reasons for the breakdown of the confluent masses.

Since there is an increased formation of one or all of the fibrous proteins, *viz.*, collagen, elastin, and reticulin, in coalworkers' pneumoconiosis, it seemed desirable to study the possibility of an increased turnover rate of these proteins as a reflection of the pathogenesis of this disease.

Although the metabolic turnover of connective tissue proteins in the lung is low, the increased rate of deposition and degradation of these substances might serve as an index of the development of the disease state (Pierce and Hocott, 1960).

In recent years, the urinary excretion of hydroxyproline-containing peptides has been used as a measure of collagen metabolism. The validity of this technique is based on the unusual distribution of the amino-acid hydroxyproline. Virtually all the hydroxyproline found in animal tissues is present in the connective tissue protein, collagen, much smaller quantities being found in elastin. Furthermore, no significant amounts of free or peptide-containing hydroxyproline arise during collagen biosynthesis since free hydroxyproline is not incorporated into the synthesis of this connective tissue protein. Therefore, the urinary excretion of hydroxyproline-containing peptides must arise from either the synthesis or degradation of collagen (Prockop and Kivirikko, 1967). In view of the involvement of connective tissue proteins in coalworkers' pneumoconiosis, it was felt that some understanding of these processes might result from

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a study of the urinary hydroxyproline excretion in subjects with this disease.

Materials and methods

Three groups of subjects were used in this study. One group consisted of 24 miners who were admitted to West Virginia University Medical Center for detailed assessment of their cardio-pulmonary function. Most had been seen and investigated at other hospitals on account of shortness of breath or because of an abnormal chest film. Nineteen had simple pneumoconiosis and five had progressive massive fibrosis. Those with simple pneumoconiosis were subdivided by means of their chest films into categories 1, 2, or 3 simple pneumoconiosis (International Labour Office, 1959). Though some had easily demonstrable respiratory impairment, others had none despite the use of the most complete and sensitive methods of testing currently available. All the subjects included in this series had spirometry, arterial blood studies at rest and exercise, static and dynamic compliance measurements, the determination of airways resistance by means of the body plethysmograph, and most also had their diffusing capacity determined by the steady state method.

The control group of nine subjects was made up of non-miners, either normal subjects or patients with diseases in which no increase in collagen turnover would be expected, *viz.*, cardiac arrythmias (paroxysmal tachycardia), angina pectoris, asthma in remission, and established rheumatic heart disease. A third group of eight patients was selected in which an increased excretion of hydroxyproline was highly probable. Included in the latter were subjects with hyperparathyroidism, thyrotoxicosis, active rheumatoid arthritis, and multiple myeloma.

Two 24-hour specimens of urine were collected from all groups. Urinary hydroxyproline was measured in all subjects according to the method of Prockop and Udenfriend (1960), as modified by Kivirikko, Laitinen, and Prockop (1967). Standard curves were run with each series of tubes for samples in duplicate containing known amounts of *d*-hydroxyproline. As a further check on recoveries, internal standards containing known quantities of *d*-hydroxyproline were added to selected urine samples. In all cases, recoveries were 92% or better. Further checks on the 24-hour urine specimens were made by

measuring creatinine excretions by means of the alkaline sodium-picrate method as modified by Bonsnes and Taussky (1945). Normal values for hydroxyproline excretion are shown in Table 1.

TABLE 1

URINARY EXCRETION OF HYDROXYPROLINE IN NORMAL CHILDREN AND ADULT SUBJECTS (KIVIRIKKO AND LAITINEN, 1965; LAITINEN *et al.*, 1966)

No. of subjects	Age (years)	Hydroxyproline excretion (1) mg./24 hr	(2) mg./24 hr/m².
24	0–1	19–56	40–191
22	2–5	20–65	
21	6–10	35–99	37–95
25	11–14	63–180	40–113
22	18–21	20–55	
48	>21	15–43	9–24

(1) Range.
(2) Limits for mean ± 2 S.D.

For each of the 24 miners the hydroxyproline content of two 24-hour specimens of urine was determined. Collection of the first specimen was started soon after admission to hospital; collection of the second was delayed until the patient had been placed on a gelatin-free diet for 24 to 36 hours. Whereas the first 24-hour specimen would reflect the diet of the subject for the day or so prior to admission, the second would be more reliable since the diet would by then have been controlled for 24 hours or more. In practice, no significant difference in hydroxyproline content was found between the two specimens. Nonetheless, in the results (Table 2), the values for the miners relate to the second specimen.

Results

There were six subjects who had category 1 simple pneumoconiosis. Their 24-hour urinary hydroxy-

TABLE 2
24-HOUR URINARY HYDROXYPROLINE IN MINERS AND CONTROLS

Category of pneumoconiosis	No. of subjects	Range of 24-hour urinary OH proline (mg.)	Mean 24-hour OH proline (mg.)	Mean age (yrs)
Simple 1	6	32 to 46	37	50
2	6	23 to 47	36	52
3	7	26 to 62	42	51
Progressive massive fibrosis	5	15 to 65	34	57
Controls	9	22 to 51	39	42

TABLE 3

24-HOUR URINARY HYDROXYPROLINE IN SUBJECTS WITH ENDOCRINE AND BONE DISEASE

Subject	Sex	Diagnosis	Urinary[1] 24-hour OH proline (mg.)	Age (yrs)
P.A.	M	Myeloma and renal failure	22·1	52
G.M.	M	Active tuberculous pleural effusion	73·2	18
E.S.	F	Hyperparathyroidism	47·5	50
E.H.	M	Active rheumatoid arthritis and rheumatoid pleurisy; on steroids	71·9	55
E.S.	F	Hyperparathyroidism; no osteoporosis on radiograph	71·4	56
M.G.	F	Myeloma and spontaneous fractures	123·7	51
Q.J.	F	Thyrotoxicosis	108·8	27
J.S.	M	Burnt-out acromegaly	32·4	76

[1]Predicted values for normal subjects of 18 and over, 15-55 mg. in 24 hours.

proline excretion ranged between 32 and 46 with a mean of 37 mg. A further six subjects had category 2 pneumoconiosis and their 24-hour hydroxyproline excretion ranged between 23 and 47 with a mean of 36 mg. There were seven subjects with radiological changes indicative of category 3 and their hydroxyproline excretion ranged from 26 to 62 with a mean of 42 mg. The five subjects with progressive massive fibrosis had levels that ranged from 15 to 65 with a mean of 34 mg. (Table 2).

From this study it appears that the urinary hydroxyproline excretion of coal miners with simple pneumoconiosis falls within the normal range. The few subjects we studied with progressive massive fibrosis also had relatively normal figures, but we did not have enough subjects to justify our drawing any conclusions about this condition. The range of hydroxyproline excretion for our controls was similar to that found by other workers (Laitinen, Nikkilä, and Kivirikko, 1966). On the other hand, several of the subjects in whom an increased excretion might reasonably have been expected were indeed shown to have an abnormally high urinary hydroxyproline content (Prockop and Kivirikko, 1967) (Table 3).

Discussion

Apart from the small quantity that is found in elastin, all the hydroxyproline that is present in the tissues of mammals occurs in collagen. Other animal proteins do not contain this amino-acid. Furthermore, free hydroxyproline is not utilized in the synthesis of collagen fibres. It is only after the inclusion of proline residues into the polypeptide chains, with their subsequent hydroxylation, that hydroxyproline can be demonstrated in collagen fibres. Thus the excretion in urine of hydroxyproline-containing peptides has been shown to be a reliable indication

of collagen turnover. The presence of free hydroxyproline or hydroxyproline-containing peptides in tissue fluids or urine must therefore originate from either the synthesis or degradation of collagen or elastin fibres.

Tissues that contain appreciable amounts of collagen, when involved in a destructive process, might be expected to give rise to an increased excretion of urinary hydroxyproline. Nonetheless, in this connexion it is important to remember that normal subjects do excrete some hydroxyproline polypeptides and that the normal 24-hour excretion of hydroxyproline has a wide range which is related to age (Laitinen *et al.*, 1966; Kivirikko and Laitinen, 1965). Higher values are found during childhood and adolescence, times at which collagen turnover is greatest. Moreover, the total amount of hydroxyproline excreted in the urine represents only about 5 to 10% of the hydroxyproline released in the degradation of collagen. The remainder is metabolized first to other amino-acids, and ultimately to carbon dioxide and urea (Prockop and Kivirikko, 1967). This suggests that large changes in the collagen turnover are necessary before the rate of hydroxyproline excretion is significantly altered.

In view of the above, it is perhaps not surprising that the urinary hydroxyproline excretion was found to be normal in our subjects with coalworkers' pneumoconiosis. This is an insidious disease that is associated with minimal parenchymal destruction and little, if any, fibrosis. It is somewhat more surprising that no increase in hydroxyproline excretion was found in the subjects with progressive massive fibrosis. Nonetheless, this condition, despite its impressive radiological findings, is also of insidious onset and has a slowly progressive course. Although one of our subjects with progressive massive fibrosis had cavitation present in the conglomerate mass, this had been present for four

to five years and the radiological appearance of the chest has been unchanged for the same time. An increased excretion of hydroxyproline would be more probable at the time the cavitation is developing or when the conglomerate lesions are extending. Further, and perhaps serial, determinations of hydroxyproline excretion need to be carried out in a larger group of subjects with progressive massive fibrosis. Whether the test has application to the study of other industrial lung diseases, *viz.*, those characterized by more rapid destruction of pulmonary parenchyma, such as cadmium inhalation, berylliosis, farmer's lung, and silo-filler's disease, remains conjectural but seems more likely.

References

Bonsnes, R. W., and Taussky, H. H. (1945). On the colorimetric determination of creatinine by the Jaffe reaction. *J. biol. Chem.*, 158, 581-591.

Gough, J. (1965). Pathological changes in the lungs associated with cor pulmonale. *Bull. N.Y. Acad. Med.*, 41, 927-941.

Heppleston, A. G. (1947). The essential lesion of pneumokoniosis in Welsh coal workers. *J. Path. Bact.*, 59, 453-460.

—— (1954). The pathogenesis of simple pneumokoniosis in coal-workers. *Ibid.*, 67, 51-63.

International Labour Office (1959). Classification of radiographs in pneumoconiosis. *Occup. Safety Hlth.*, 9, 63.

Kivirikko, K. I., and Laitinen, O. (1965). Clinical significance of urinary hydroxyproline determinations in children. *Ann. Paediat. Fenn.*, 11, 148-153.

——, ——, and Prockop, D. J. (1967). Modifications of a specific assay for hydroxyproline in urine. *Analyt. Biochem.*, 19, 249-255.

Laitinen, O., Nikkilä, E. A., and Kivirikko, K. I. (1966). Hydroxyproline in the serum and urine. Normal values and clinical significance. *Acta med. scand.*, 179, 275-284.

Pierce, J. A., and Hocott, J. B. (1960). Studies on the collagen and elastin content of the human lung. *J. clin. Invest.*, 39, 8-14.

Prockop, D. J., and Kivirikko, K. I. (1967). Relationship of hydroxyproline excretion in urine to collagen metabolism. *Ann. intern. Med.*, 66, 1243-1266.

——, and Udenfriend, S. (1960). A specific method for the analysis of hydroxyproline in tissues and urine. *Analyt. Biochem.*, 1, 228-239.