triester isomers (48% axial (ax.) and 52% equatorial (eq.) as shown in chromatogram (fig. 1a)). This equilibrium of the diastereoisomers was to be expected, as the large di-phenyl-phosphorochloridate substituent forces the 6-membered 1,3,2-dioxaphosphorinane ring to form a 'chaise longue' conformation with a significant loss of preferential stability of one of the 2 isomer configurations[20]. A 1:1 ax./eq. ratio was also obtained (results not shown) when we reacted the di-adenosinemonophosphate ester, ApA, with NEU under the standard conditions described for 3', 5' cAMP.

The amount of ax. (30%) and eq. (70%) conformers of the neutral P-O-ethyl ester of 3', 5' cAMP which resulted from repeated incubations with NEU is shown in the CLC chromatogram (fig. 1c).

This finding was in complete contradiction to the results of Engels and Pfleiderer[23]. In keeping with the greater thermodynamic stability of axial O-alkyl in 2-substituted 1,3,2-dioxaphosphorinanes[20] as a consequence of the gauche-effect[24] they presented evidence for a 7:3 ax./eq. ratio from the analysis of data obtained from NMR spectra of the phosphotriesters. This ratio corresponds exactly to the values we found (fig. 1b), when we reacted the 2-ethyl ether 3', 5' cAMP (which we had previously synthesized by alkylation with ethyl-iodate accordingly to Tazawa et al.[25]) and 3', 5'c-d2'-AMP (results not shown) with NEU according to our standard procedure.

Our results are straightforward and, in our view, their explanation seems to be obvious. In the SN_1-type reaction of diazoalkane and NEU the carbonium ion reacts with the P→O nucleophile which in the case of the 2-substituted 1,3,2-dioxaphosphorinane shows a greater thermodynamic stability in the $P\lesssim{}^{O}_{O}{}^{ax.}$ than in the $P\lesssim{}^{O}_{O}{}_{eq.}$ position and favors the formation of P-O-ethyl$_{ax.}$ stereoisomer. It can be seen from the molecular model shown in figure 2 that the hydrogen bond between the 2'-OH group of the ribose moiety and the N→O group of NEU reacting with 3', 5' cAMP will induce a labilization of the N-alkyl bond in NEU and thus favor the formation of the ethyl carbenium. This carbonium will then react preferentially with the closest nucleophilic site which is the equatorially positioned P→O. When neighboring group catalysis of the 2'-OH cannot possibly occur as the hydrogen is either substituted by an alkyl residue (3', 5'c(2' O-C_2H_5)AMP) or missing (3', 5'c d2'-AMP), then oxygen substitution in the phosphate moiety of cyclophosphate nucleotide leads to the thermodynamically favored equilibrium of ax.: eq. = 7:3.

At present it may be difficult to prove hydrogen-bond-catalysis in chromatin alkylations by N-nitroso compounds. But in theory, it can be expected that histones and other nuclear proteins determining tertiary DNA structure contain a variety of appropriate hydrogen-bond donator groups. In hyperreactive genome regions their effect on alkyl transfer to nucleophilic DNA sites might be compared to the catalysis of active centers from transferase enzymes.

Despite tremendous research effort in this field our knowledge of the qualitative and quantitative relationships between the tumorigenicity of N-nitroso compounds and DNA alkylations[26] and also of the persistence of promutagenic DNA lesions[27] remains unsatisfactory. It may yet be possible to relate tumorigenicity to DNA alkylations but only when the genotopic selectivity of the promutagenic lesions can be consistently analyzed and better understood.

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Collagenolytic activity from circulating polymorphonuclear leucocytes of patients with asbestosis[1]

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Summary. Levels of collagenolytic activity produced by circulating polymorphonuclear leucocytes (PMN) of patients exposed to asbestos and patients with asbestosis were found to be similar to those of normal controls.

Asbestosis is an occupational lung disorder characterized by chronic inflammation of the alveolar structures and progressive diffuse interstitial fibrosis[2]. The asbestotic lung displays extensive fibrosis with emphysematous changes[3] sug-

Collagenolytic activity from PMN of controls and 3 groups of patients exposed to asbestos

Groups	n	Asbestos exposure (years)	(index)[a]	X-Ray opacifications	Pressure-volume curve	Asbestosis	PMN collagenolytic activity (cpm × 10^{-3}/10^6 cells[b])	(%)
Controls	8	–	–	none	normal	no	24.8 ± 3.7	100
A	12	35 ± 1	66 ± 7	none	normal	no	21.3 ± 2.8	85.9
B	8	36 ± 2	71 ± 5	none	rigid	no	24.7 ± 3.0	99.3
C	7	32 ± 3	76 ± 11	+	rigid	+	19.2 ± 2.3	77.2

[a] Calculated as the number of years of exposure X degree of exposure (low = 1, moderate = 2, high = 3); [b] Assays were done in triplicate and values represent means ± SEM. Data were analyzed for statistical significance using Student's t-test ($p < 0.05$).

gesting that the fibrotic process of this disease may be associated with alterations in collagen degradation as well as in collagen synthesis.

Extracellular collagen degradation is carried out by collagenase, a specific enzyme which splits the collagen molecule into 2 distinct fragments[4]. Cells that may be responsible for the production of collagenase in lung include alveolar macrophages[5], fibroblasts[6] and blood borne PMN[4] which produce collagenase in both active and latent forms[7]. Recently we have shown that PMN accumulate in the lungs of sheep exposed to asbestos[8]. Furthermore, there have been reports that collagenolytic levels in bronchial aspirates were elevated in idiopathic pulmonary fibrosis[9] and following intratracheal instillation of bleomycin, a fibrogenic drug[10]. Since circulating PMN produce collagenase and are attracted to the lungs before the appearance of the fibrotic lesions in experimental asbestosis, we investigated whether circulating PMN of patients exposed to asbestos produced altered levels of collagenolytic activity. Collection of blood samples is a much less invasive technique than bronchoalveolar lavage and we questioned whether circulating PMN collagenolytic levels may be useful as a clinical index for diagnosis and staging of asbestosis.

Methods. Venous blood from 27 patients exposed to asbestos and 8 normal controls was collected in a fasting state. None of the patients was under medication. Of the 27 patients, 18 were smokers. All were workers in the mining and milling industry and had been exposed to asbestos for an average of 34 years. They were classified in 3 groups according to standard clinical, roentgenographic and physiologic criteria[11]. Polymorphonuclear leucocytes were obtained by centrifugation of heparinized (10 U/ml) blood on a Ficoll-Hypaque gradient[12]. The final yield of PMN was suspended in Dulbecco's modified Eagle's medium at a concentration of 1.5×10^6 per ml and incubated for 48 h. The collagenolytic activity was assayed in culture media as described by Labrosse et al.[13] with slight modifications. The radioactive collagen (9.9 μCi/mg) used in this assay was prepared by the methylation of Type I calf skin collagen (Calbiochem) with formaldehyde and ^{14}[C] sodium cyanoborohydride[14] (NEN). ^{14}[C] collagen was not appreciably degraded by trypsin, chymotrypsin and elastase whereas EDTA, which is an inhibitor of collagenase[15], caused 100% loss of activity. The assay lasted 2 h and was carried out at 37°C. Nonspecific background radioactivity from appropriate control incubation media was substracted from all of the test samples. Collagenolytic activity is expressed as the number of counts per minute released into the reaction mixture in relation to the number of PMN.

Results. As shown in the table, 12 patients exposed to asbestos for an average of 35 years were found to be normal by all criteria (Group A). Levels of collagenolytic activity produced by PMN of these patients were similar to those of PMN from controls. A second group of patients with comparable asbestos exposure had lung function abnormalities characterized by a rigid pressure volume curve but had normal chest roentgenograms (Group B). The PMN collagenolytic levels of these patients were also similar to control values. The last group of patients (Group C) had asbestosis as evidenced by irregular X-ray opacifications, bibasilar fine rales, restrictive lung functions and dypsnea. Polymorphonuclear leucocytes from this group of patients produced lower levels of collagenolytic activity (77.2%) compared to PMN of controls (100%) and PMN of patients in Group A (85.9%) and B (99.3%). These differences, however, did not reach statistical significance.

Discussion. Our study indicates that the collagenolytic activity produced by circulating PMN does not correlate with asbestos exposure and asbestosis and could not be helpful for monitoring the course of the disease. These results however do not exclude a possible role of collagenase in the pathogenesis of asbestosis since PMN attracted to the lung may differ from circulating PMN in their state of activation and enzyme production. Similarly, enhanced pulmonary levels of collagenase may be the result of higher concentrations of a proteolytic enzyme known to activate collagenase, reduced levels of collagenase inhibitors, or a higher number of collagenase producing cells rather than increased production of collagenase by these cells.

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