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Gamma Glutamyl Transpeptidase in Chronic Obstructive Pulmonary Disease[1] (38051)

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Chronic obstructive pulmonary disease involves gradual inflammatory alterations in the airways. These destructive alterations are insidious in onset, and after a certain period, relentless in progression. It has been suggested that the level of this inflammatory process can be judged from the increase in the DNA fibril content of the bronchial secretions (1), reflecting the influx of leukocytes, and from changes observed in the level and types of lactic dehydrogenase (1) whose precise significance in this connection has not been clarified. These measurements do not necessarily indicate the actual extent to which the lining of the airways is being damaged by the inflammatory reaction; a more direct means for assessing the status of this destructive process would be of interest. For example, a marker such as an enzyme normally bound to the epithelial membranes in the airways would be present at low levels in normal bronchial secretions. One would expect to find much higher levels in the secretions when tissue damage occurring in the airways resulted in fragmentation and shedding of the bronchial epithelium.

The gamma glutamyl transpeptidase (GGTP) that is normally bound in the bronchial mucosa (2) appears to fill this role as a marker for airway damage. In the present studies, this enzyme activity was found at low levels in bronchial secretions from normal subjects and at much higher levels in purulent secretions from patients with chronic bronchitis.

Materials and Methods. Sputum was obtained from 27 patients with chronic bronchitis (3), and was induced in 12 normal subjects by inhalation of 15% saline aerosol. Each donor was instructed to bring up secretions from the airways with minimal contamination by saliva. Each sputum specimen was lyophilized with concomitant determination of percent dry weight; DNA content was determined by the method of Burton (4).

Assay of γ-glutamyl transpeptidase activity was carried out in Tris–HCl buffer (0.1 *M*, pH 8.8) containing $MgCl_2$ (0.01 *M*), (5). For each assay, 10 ml of the buffer in which 6 μmoles of γ-glutamyl-*p*-nitroanilide (substrate) and 30 μmoles of glycylglycine (acceptor) had been dissolved, was added to the reaction flask containing 5 mg of lyophilized sputum. The mixture was incubated in a shaker bath at 37°. Seven minutes after mixing, and at three 15 min intervals thereafter, a 1 ml aliquot of the reaction mixture was removed and the reaction was stopped by addition of 2 ml of 1.5 *M* acetic acid. The liberation of *p*-nitroaniline (monitored by measuring absorption at 410 nm), was linear with time and amount of enzyme. Enzyme activity is expressed in terms of units, where one unit is defined as the amount of enzyme necessary to release 1 nmole of *p*-nitroaniline per min under the conditions described above. For determining the effect of added acceptors, the rate without acceptor was measured as described above (with glycylglycine omitted);

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a four-fold molar excess of acceptor was then added and measurements continued. Typically, the liberation of *p*-nitroaniline proceeded at a new linear rate after addition of an effective acceptor.

The presence of GGTP in the sputum was confirmed by subjecting the products of the enzyme reaction to thin layer chromatography on Gelman ITLC type SA, using *n*-butanol–acetic acid–water (4:1:1) for development. In order to confirm the fact that the GGTP is present in the airway lining (2), and to demonstrate that it is normally bound, and is released in quantity only under abnormal conditions, experiments on removal of the enzyme by lavage were performed on the lungs of adult male Sprague-Dawley rats.

The animals were killed by asphyxiation and the trachea was exposed and clamped to avoid introduction of blood into the airways. The lungs were removed, rinsed in saline, and lavage was performed by instilling 4 ml of isotonic saline via the trachea, and then allowing the fluid to run into a collecting tube. Lavage was continued by introducing 4 ml of isotonic saline containing 0.15% sodium deoxycholate; the deoxycholate lavage was repeated, and the lavage fluids were assayed for GGTP activity. Mechanical manipulation of the lungs was kept to a minimum.

Results. When subjected to thin-layer chromatography, the products of the enzymatic reaction (RM_2, Fig. 1) yielded a yellow spot corresponding to *p*-nitroaniline (1, Fig. 1) and one new ninhydrin-positive spot (5, Fig. 1), in addition to the three corresponding to the starting materials (2–4, RM_1, Fig. 1). No significant amount of free glutamic acid was detected in the reaction mixtures. Component 5, the principal reaction product in addition to *p*-nitroaniline, migrates like γ-glutamylglycylglycine.

After elution and hydrolysis, component 5 yielded spots corresponding to glycine and glutamic acid, the former being about twice as strong as the latter. Addition of authentic γ-glutamylglycylglycine to the reaction mixture produced enhancement but no blurring of component 5 in subsequent chromatography. Thus, the enzyme in sputum carries out a transpeptidation in which the γ-glutamyl group is transferred from γ-glutamyl-*p*-nitroanilide to glycylglycine, to form γ-glutamylglycylglycine and liberate *p*-nitroaniline.

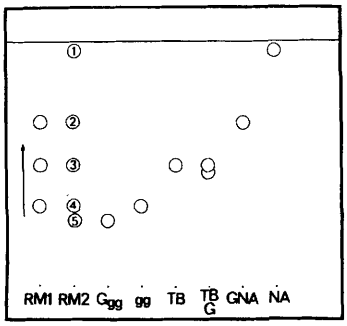

FIG. 1. Thin layer chromatography of enzyme reaction mixtures. In this example, lyophilized sputum (5 mg) was incubated in 10 ml of Tris–HCl buffer, containing 16 mg γ-glutamyl-*p*-nitroanilide and 40 mg glycylglycine, at 37° for 8 hr; 10 λ samples of the reaction mixture before incubation (RM1) and after incubation (RM2) were spotted on ITLC medium SA at the locations shown. Samples of test substances (5 λ each of solution containing 1 mg per ml) were spotted as shown: γ-glutamylglycylglycine (Ggg); glycylglycine (gg); Tris buffer (TB); glutamic acid (G); γ-glutamyl-*p*-nitroanilide (GNA); *p*-nitroaniline (NA). Development was by ascending chromatography, using *n*-butanol–acetic acid–water (4:1:1). The chromatogram was dried, sprayed with ninhydrin solution and heated to reveal the amino acid derivatives. The dots indicate the origin; the solid line, the solvent front.

Component 1 (*p*-nitroaniline) is yellow with or without ninhydrin. Component 2 is γ-glutamyl-*p*-nitroanilide (substrate). Component 3 (yellow) is due to Tris buffer. Component 4 is glycylglycine (acceptor). Component 5, the principal reaction product, behaves like γ-glutamylglycylglycine. No significant amount of glutamic acid was detected in the reaction mixture.

The effects of added acceptors are shown in Table I. Liberation of *p*-nitroaniline is more rapid in the presence of glycylglycine and methionine, and is slightly slower in the presence of glycine. The reaction with glycylglycine as acceptor was optimal near pH 8.8; it was not inhibited by *p*-chloromercuribenzoate, or by the protease inhibitor phenylmethylsulfonyl fluoride at 10^{-3} *M*. The sputum enzyme did not liberate

TABLE I. Modification of γ-Glutamyl Transpeptidase Activity in Sputum by Addition of Acceptors.[a]

Compound Added	Relative GGTP activity[b]	
None	100[c]	100[d]
Glycylglycine	363	155
Glycine	93	84
Glycine ethyl ester	93	—
Methionine	217	121
Glycylmethionine	100	—

[a] The reaction rate without added acceptor was measured as described in text; acceptor then was added in four-fold molar excess relative to substrate present, and measurements were continued to determine the reaction rate in presence of acceptor.

[b] GGTP, γ-glutamyl transpeptidase.

[c] Relative rate in absence of acceptor set at 100.

[d] Calculated from data of Orlowski and Meister (5).

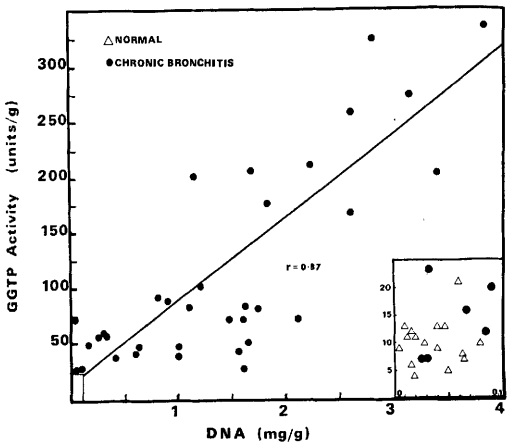

FIG. 2. Relationship between GGTP activity (units per gram of whole sputum) and DNA content (mg per gram of whole sputum). The inset shows the region in the vicinity of the origin in expanded scale.

p-nitroaniline from glycine-*p*-nitroanilide or lysine-*p*-nitroanilide in the presence of glycylglycine.

In Fig. 2, the GGTP activity is plotted against the DNA content of the sputum. As shown in the insert in Fig. 2, in which the region near the origin is expanded, the bronchial secretions induced in normal subjects contained little DNA and had low GGTP activity, with no correlation between the two ($r = 0.03$). In the sputum from patients with chronic bronchitis (CB), values ranged from those of normal subjects up to very high values, with a positive correlation between GGTP activity and DNA content ($r = 0.87$). The method of least squares was used to fit the solid line to the data for the patients.

In Fig. 3, the GGTP activity is plotted against percent dry weight of the sputum. Simple changes in water content of the secretions from the normal subjects would move the values for GGTP activity and percent dry weight along the dashed line, which is drawn from the origin through the

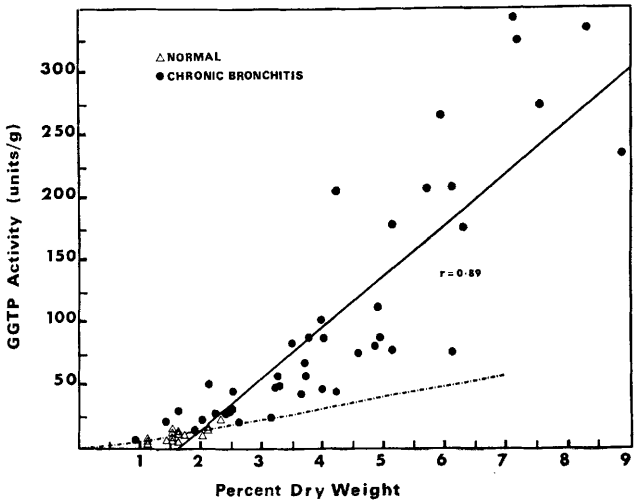

FIG. 3. Relationship between GGTP activity (units per gram of whole sputum) and dry weight of sputum.

mean values for their GGTP activity (10.1 units per gm wet weight) and percent dry weight (1.63). The high GGTP values for many of the patients with CB lie far above this line and cannot be due simply to loss of water from the secretions. In this case also, the solid line fitted to the data for the patient's secretions shows a high correlation between GGTP activity and percent dry weight ($r = 0.89$). Saliva samples from ten of the 27 patients with CB showed values for GGTP activity (6.0 ± 1.0 units per gm wet weight) and percent dry weight (1.0 ± 0.08), even lower than those for the sputum from normal subjects. The GGTP values for two leukocyte preparations from whole fresh blood were 0.05 and .01 units per mg dry weight, less than one tenth of the mean value (0.59 U/mg dry weight) for the 16 sputum samples from normal subjects. Many of the sputum samples from normal subjects were rich in alveolar macrophages but still showed low values for GGTP.

As shown in Fig. 4, lavage with isotonic saline removed little GGTP activity from rat lungs, whereas lavage with saline containing the detergent sodium deoxycholate was effective in removing GGTP from the airways.

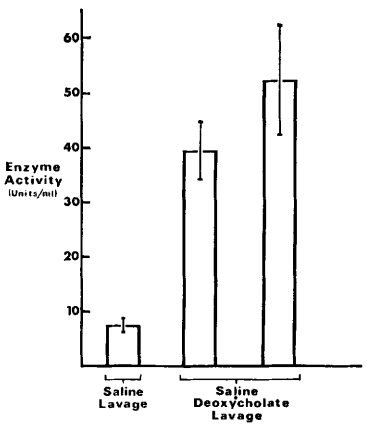

FIG. 4. Removal of GGTP from rat lungs by lavage: Lungs of 8 normal adult male Sprague-Dawley rats were lavaged first with isotonic saline and then twice with isotonic saline containing 0.15% sodium deoxycholate. The bars show the mean values ± the standard error for GGTP activity in the three washes.

Discussion. The present results demonstrate that the bronchial secretions contain a γ-glutamyl transpeptidase capable of cleaving γ-glutamyl-*p*-nitroanilide and transferring the γ-glutamyl group to glycylglycine, to yield γ-glutamylgycylglycine and liberate *p*-nitroaniline. This enzyme is present at low levels in induced sputum from normal individuals, and at much higher levels in purulent sputum from patients with chronic bronchitis. Our results indicate that there is very little GGTP activity in saliva, leukocytes, or alveolar macrophages, and therefore, these cannot be the source of the GGTP activity we find in the bronchial secretions.

This enzyme in the bronchial secretions resembles the GGTP isolated from hog kidney by Orlowski and Meister (5). It reacts with the same substrate and has the same pH optimum. For both, liberation of *p*-nitroaniline is more rapid in the presence of glycylglycine and methionine, and is slightly slower in the presence of glycine. Histochemical evidence indicates that in hog kidney this enzyme is associated with the epithelial membranes in the loops of Henle (6); and in the lung it is concentrated in the membranes of the epithelial mucosa lining the airways (2).

Liberation of GGTP from the membranes in the kidney, where it is firmly bound, required the use of a detergent, sodium deoxycholate (5, 7). Evidently, this enzyme is firmly bound in the epithelial membranes in the bronchi also, since we found only low levels in the isotonic saline lavage fluid from the lungs of the experimental animals (Fig. 4). The low levels of GGTP activity in the bronchial secretions induced in normal subjects apparently reflect some shedding of epithelial membranes that accompanies the normal degeneration and replacement of the epithelium lining the airways. Release of high levels of GGTP activity only on lavage with saline containing deoxycholate (Fig. 4) confirms that in the lung also, this enzyme is normally bound in the epithelial membranes. The presence of this enzyme at high levels in the purulent sputum of patients with chronic bronchitis suggests abnormal release due to large-scale destruction

of epithelial cells. Thus, whereas the DNA content reflects the inflammatory reaction (1), the levels of GGTP in the sputum may provide a direct indication of the extent to which the damaged airway epithelium is being shed into the bronchial secretions.

It is of interest to note that whereas many of the patients were producing purulent sputum, suggestive of extensive damage to the airways, some patients with chronic bronchitis were producing sputum with low DNA and low GGTP values similar to those for normal subjects. It appears that the chronic hypersecretion characteristic of chronic bronchitis can persist in some patients with little inflammatory reaction or damage occurring in the airways.

GGTP has been pictured as playing a key role in a gamma glutamyl cycle, of which the proposed function in the kidney is resorption of amino acids from the filtrate, thus avoiding the loss of these metabolites in the urine (8). Whether GGTP in the bronchial epithelium acts to prevent excessive loss of amino acids into the bronchial secretions, and whether there is an adaptive increase of this enzyme in the bronchial mucosa to compensate for the increased output of bronchial secretions in the airways of patients with chronic bronchitis remain to be determined.

Summary. A γ-glutamyl transpeptidase capable of transferring the γ-glutamyl group from γ-glutamyl-*p*-nitroanilide to glycylglycine has been found to be present at low levels in bronchial secretions from normal subjects and at much higher levels in purulent sputum from patients with chronic bronchitis.

This enzyme is concentrated in the bronchial mucosa where, in the absence of insult, it normally remains bound. Its presence at high levels in purulent sputum suggests that it will serve as a marker for assessing the existing level of destruction and shedding of airway epithelium into the bronchial secretions in patients with chronic bronchitis.

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