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γ-GLUTAMYL TRANSPEPTIDASE: A NOVEL BIOCHEMICAL MARKER IN INFLAMMATION

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Abstract—γ-Glutamyl transpeptidase (γ-GT) plays an important role in the turnover of glutathione and protein biosynthesis. Because in inflammation both catabolic and anabolic steps are activated together with migration of cells, an alteration in γ-GT activity was postulated to occur at the site of inflammation with the development of the inflammatory process. We discovered that γ-GT activity was increased markedly at sites of inflammation produced in several ways in rats. A significant increase in enzyme activity appeared soon after the induction of inflammation. In carrageenin-induced acute inflammation, the paw tissue attained a 3- to 4-fold increase in enzyme activity within 4 hr; in established adjuvant arthritis, a 20- to 24-fold increase over its basal activity occurred in the rat paw tissue. The specific enzyme activity was 20–22 nmoles/min/mg protein in the cellular sediment of carrageenin-induced pleural exudate. In cotton granulomatous tissue it was 5- to 6-fold higher compared to the enzyme activity of the skeletal muscles. The *in vivo* increase in γ-GT activity was prevented from occurring in proportion to the anti-inflammatory potencies of the test drugs given orally. The prevention of enzyme activity was observed with indomethacin in carrageenin-induced edematous paw tissue and with phenylbutazone in both adjuvant arthritis and carrageenin-induced pleural exudate. Prednisolone was observed to be the most potent drug against cotton granuloma. Nonsteroidal anti-inflammatory drugs (NSAIDs) were not found to affect enzyme activity *in vitro* when incubated with cellular infiltrate from a cotton pellet granuloma. Differences in certain physico-chemical characteristics, viz. stability at 50°, pH dependency and effects of solvents, were not discernible in between the enzyme activities of the untreated and edematous paw tissues. The studies thus suggest that measurement of γ-GT in inflammation may prove to be a valuable biochemical marker for the assessment of anti-inflammatory activity of drugs *in vivo*.

γ-Glutamyl transpeptidase (γ-GT) plays an important role in the metabolic turnover of glutathione by catalyzing the conversion of glutathione thioethers to mercapturic acid [1–3]. Presently, a role in the transport of amino acids and peptides across cell membrane [4] and, also, as a marker of neoplastic transformation [5–7] has been suggested.

In inflammation, both catabolic and anabolic processes are activated with attendant migration of leucocytes and may require greater turnover and transport of amino acids at the site of inflammation in an attempt to replace the damaged tissue. There is, therefore, likelihood that the enzymatic activity of γ-glutamyl transpeptidase may increase in inflamed tissue or at the site of inflammation because of its role in amino acid transport and protein biosynthesis [4]. Further, the enzyme may be of considerable significance in inflammation because of its proposed role in the formation of leukotrienes (slow reacting substances, SRS). Samuelsson and associates [8, 9] discovered the leukotriene (LT) pathway and proposed that the so-called "SRS" are lipoxygenase metabolites of arachidonic acids that contain peptidyl groups and are derived from LTA_4 via the action of glutathione-*S*-transferase(s) and γ-GT. Other workers [10] also simultaneously confirmed the presence of Cys-Gly, a dipeptide at C-6. Thus, it has been identified that LTE_4 contains cysteine, whereas LTD_4 and LTF_4 contain Cys-Gly and Cys-Glu at C-6 of these leukotrienes respectively. The enzyme responsible for the conversion of LTA_4 to peptidyl leukotrienes is reported to be associated with plasma membrane and has been found to be similar to γ-glutamyl transpeptidase [11].

In light of the above background we considered it important to examine the levels of enzyme activities in tissues obtained during various inflammatory conditions. An attempt was also made to find out the possibilities and limitations of this biochemical marker for the assessment of anti-inflammatory activities of drugs *in vivo*.

MATERIALS AND METHODS

Materials

γ-Glutamyl-*p*-nitroanilide, glycylglycine and indomethacin were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). All other reagents used were of analytical grade and were available locally.

Animals and drug treatment

Adult, male albino, Charles Foster rats weighing 130–150 g were used. The animals were fed a standard pellet diet (Hindustan Lever, Bombay, India) and water *ad lib*. Drugs were prepared as microfine suspensions in 2% Arabic Gum Acacia in normal saline. The drugs were administered orally by gavage

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in a volume of 1 ml/100 g body wt. The control animals received only the vehicle. Doses of drugs and schedules of administration are given under their respective experiments.

Carrageenin edema. Acute inflammation was induced by injecting the phlogistic agent, carrageenin (Marine Colloids, Inc., F.M.C. Corp., Springfield, NJ 07081, U.S.A.), into the plantar surface of the hind paw of rats according to Winter *et al.* [12]. Drugs were administered orally 1 hr prior to carrageenin injection. At timed intervals, the animals were killed by cervical dislocation and the edematous tissue was removed from the inflamed paw. The paw volume of the rat was measured [12] before and following drug treatment using a volume differential meter (model 7101 Ugo Basile, Biological Research Apparatus, Comerio—Italy).

Cotton pellet granuloma. Two autoclaved cotton balls weighing 50 ± 1 mg were implanted under the skin of each rat, one each on the dorso-lateral sides, according to the method of Winter and Porter [13]. Drugs were administered orally just before the induction of inflammation and then daily for 7 days in a volume of 1 ml/100 g body wt. On day 8, the rats were killed, and the granuloma pellets were removed. Each pellet was dissected, and the cotton mass was removed to cold saline. The granulomatous tissue around the cotton was rinsed free of cells in saline. The cotton ball in saline was stretched loose to disperse all the cells. The cells (total leukocyte count) were counted in the cell suspension. The cell suspension was then centrifuged at 2000 *g* for 5 min at 4°. The cell pellet and the supernatant fraction were stored at −20° for enzymatic assay. The granulomatous tissue was homogenized and stored at −20°. A portion of normal skeletal muscle was also removed from the ventral region of the same rat for comparison of enzyme activity with that of granulomatous tissue.

Adjuvant arthritis. Arthritis was produced in rats according to Newbould [14]. The animals were injected with 0.05 ml of a 0.5% (w/v) suspension of killed *Mycobacterium tuberculosis* (Difco) (homogenized into liquid paraffin) into the plantar surface of the left hind paw. The drugs were administered 1 hr prior to the injection of adjuvant. At timed intervals, the animals were killed, and the edematous tissue of the paw was obtained for biochemical studies.

Carrageenin-induced rat pleurisy. Pleurisy was induced according to Meacock and Kitchen [15]. Carrageenin (0.5 ml of 1%, w/v in saline) was injected into the pleural cavity. Phenylbutazone was given orally 1 hr prior to the injection of carrageenin. Twenty-four hours after the carrageenin injection, the pleural exudate was collected in tubes containing 10 units of heparin in 0.1 ml saline. The fluid was diluted with an equal volume of normal saline. Total leukocyte counts were made using a Neubaur Chamber. The cells were centrifuged at 2000 *g* for 5 min at 4°, rewashed, and centrifuged; the supernatant fraction and the sediment were stored at −20° for enzymatic study.

Biochemical studies

Whole homogenates (10%, w/v) of edematous, granulomatous and skeletal tissues were made in 150 mM KCl–50 mM Tris–HCl, pH 7.6, using a Potter–Elvehjem type glass homogenizer at 4°. The whole homogenate was passed through a double layer of nylon mesh (pore size 0.25 × 0.25 mm) and stored at −20° in a freezer. Frozen cell pellets were also homogenized in a suitable volume of homogenizing medium and used directly for enzyme assay within a week.

γ-GT activity was determined in the biological samples as described by Boelsterli and Zbinden [16]. The assay was performed under linear reaction conditions using γ-glutamyl-*p*-nitroanilide as the donor substrate. Briefly, 50-μl samples of the biological probes were incubated at 37° for 15–30 min with 450 μl of buffer substrate solution (100 mM Tris–HCl, pH 7.6; 75 mM glycylglycine; 10 mM $MgCl_2$, 4 mM γ-glutamyl-*p*-nitroanilide · H_2O). The reaction was terminated with 1.5 ml of 10% acetic acid. The tubes were cooled in ice-cold water, centrifuged, and read at 405 nm in a Uvikon 810 spectrophotometer (Kontron Ltd., Zurich, Switzerland) against a blank. Protein was determined according to Lowry *et al.* [17] using bovine serum albumin as standard.

RESULTS

Alteration in γ-GT activity in the edematous tissue of rat hind paw during the development of carrageenin edema

Changes in the activity of γ-GT during the progression of acute inflammation are shown in Table 1. γ-GT registered a remarkable increase of 150% 2 hr after carrageenin injection, which progressed to a maximum increase of 250% after 4 hr of inflammation. When we simultaneously measured the paw volume, a close relationship between γ-GT activity and paw volume was observed.

Developmental pattern of γ-GT activity in adjuvant arthritis

It may be seen (Fig. 1) that the γ-GT activity of the edematous paw tissue increased about 7-fold over its basal enzymatic activity during the initial sensitization phase of arthritis, and the level increased further to 22- to 24-fold when the arthritis condition was fully established (days 21–28). The increase in activity was curvilinear and did not appear to acquire a saturable plateau until day 28 of arthritis. It appears that the enzyme activity might increase further with severity or aggravation of the disease condition.

This suggested that γ-GT activity *in vivo* in inflammation might prove to be a valuable biochemical marker with which to assess the alleviation of the disease during a course of drug therapy. In the following, the levels of γ-GT activity in various inflammatory conditions are presented and the responses to anti-inflammatory drugs are described.

Response of γ-GT activity to steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) in various conditions of inflammation in rats

Carrageenin edema. The effect of indomethacin pretreatment on γ-GT activity during edema development in tissue is shown in Table 2. The enzyme

Table 1. Correlation of γ-GT activity and paw volume in the hind paw of the rat during the development of carrageenin-induced acute inflammation

Time after carrageenin injection	γ-GT activity (nmoles/min/mg protein)	Paw volume (ml)
Normal control (without carrageenin)	0.8 ± 0.10	1.0 ± 0.2
Carregeenin		
2 hr	2.0 ± 0.42* (150%)	2.3 ± 0.3* (130%)
4 hr	2.8 ± 0.34* (250%)	3.0 ± 0.5* (200%)
6 hr	2.7 ± 0.26* (237%)	2.8 ± 0.4* (180%)

Data are mean ± S.D. from five animals for each group. Procedures for the determination of paw volume and γ-GT activity are described in Materials and Methods. Values given in parentheses indicate per cent increase over basal enzyme activity (normal control) taken as 100.

* Significance of the difference from control was estimated by Student's *t*-test ($P < 0.001$).

activity was dose- and time-related to the indomethacin pretreatment, showing a diminished rise of enzyme activity and a diminished rise in paw volume measured simultaneously, in comparison with the rise in activity with inflammation alone. Indomethacin at a 5-mg dose reduced the enzyme activity with respect to the non-treated edema more than 50% during the first 4 hr of acute inflammation. In comparison, the antipyretic and analgesic, but not antiinflammatory, drug acetaminophen (250 mg/kg body wt, p.o.) was without any effect on either γ-GT activity of paw tissue or paw swelling after 4 hr of acute inflammation due to carrageenin in rat hind paw (data not shown).

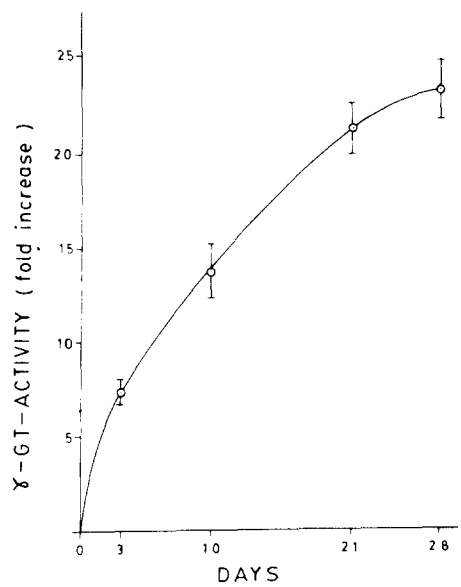

Fig. 1. Developmental pattern of γ-GT in adjuvant arthritis. The procedures for the induction of adjuvant arthritis and the determination of γ-GT activity are described in Materials and Methods. The basal enzyme activity in the normal control animals was the same as in Table 1. The data represent the mean ± S.D. of eight animals.

Developing adjuvant arthritis. Dose-related effects of phenylbutazone on the γ-GT activity of edematous tissue and edema volume of the rat paw are shown in Fig. 2. A close correspondence between edema volume and γ-GT activity of the paw tissue was observed, with a dose-related diminution of inflammation, compared with control rats, by phenylbutazone. Phenylbutazone given orally at doses of 25, 50 and 100 mg was followed by 12, 25 and 35% less enzymatic activity respectively; a similar effect was seen on edema volume.

Effects of phenylbutazone on γ-GT activity and cellular response in carrageenin-induced pleurisy in rats. γ-GT activity and total leukocyte count of pleural exudate are given in Table 3. Phenylbutazone decreased significantly both γ-GT activity and total leukocyte count in almost a dose-related fashion. The maximum inhibition of γ-GT activity was observed at a dose of 50 mg of phenylbutazone.

Effects of steroidal and nonsteroidal anti-inflammatory drugs on the γ-GT activity of granuloma. γ-GT activity of granulomatous tissue surrounding the cotton pellet was increased by 5-fold over the enzymatic activity obtained from the skeletal muscles (Table 4). The specific enzyme activities in skeletal muscle and tissue from rat hind paw were found to be similar. The γ-GT activity responded differently to indomethacin, phenylbutazone, ibuprofen and prednisolone treatment. Among these drugs only prednisolone significantly suppressed (84%) the rise in enzyme activity caused by inflammation in the controls. A similar pattern of enzyme activity was observed when the absolute activity based on per gram wet weight of the granulomatous tissue was calculated.

When the enzyme activity and protein content were determined in the cellular sediment of the cotton infiltrate and the sediment-free supernatant fraction (Table 5), a different picture emerged. The

Table 2. Effect of indomethacin on γ-GT activity and paw volume during acute inflammation in rats

Indomethacin (mg/kg body wt. p.o.)	γ-GT (nmoles/min/mg protein)	% Reduction from control (γ-GT)	Paw volume (ml)	% Reduction from control (Vol)
2 hr				
2.5 mg	1.75 ± 0.19	21	2.0 ± 0.22	23
5.0 mg	1.15 ± 0.12*	70	1.4 ± 0.10*	69
4 hr				
2.5 mg	2.22 ± 0.15*	30	2.0 ± 0.18*	45
5.0 mg	1.53 ± 0.13*	65	1.8 ± 0.11*	60
6 hr				
2.5 mg	2.0 ± 0.21*	36	2.4 ± 0.11	22
5.0 mg	1.7 ± 0.16*	57	2.3 ± 0.20	25

Animals were killed at 2, 4 and 6 hr after induction of carregeenin edema. Indomethacin was administered 1 hr prior to carrageenin injection. Percent reduction from control was calculated from the normal control (without carrageenin) and carrageenin-induced (experimental) values taken from Table 1 using:

$$\frac{100}{\text{Exptl.} - \text{normal contl.}} \times (\text{Exptl.} - \text{drug-treated exptl.})$$

Each data point is the mean ± S.D. of four animals.
*† Significance of difference was measured by Student's *t*-test: *$P < 0.02$ and †$P < 0.01$.

protein in the supernatant fraction was almost the same in all groups, whereas in the sediment the protein was 2- to 3-fold higher in all the NSAID-treated groups. The specific γ-GT activity, when expressed on a per mg protein basis, would have decreased normally if there were no quantitative increase in γ-GT activity, but, on the contrary, the enzyme activity was significantly increased in all the NSAID-treated groups. Similarly, 2- to 4-fold increases in transpeptidase activity were observed in the supernatant fraction of these groups. Accordingly, the total enzymatic activity of the cellular infiltrate was enhanced by the NSAIDs used. Similarly, an increase in total leukocyte count was observed with NSAIDs. With prednisolone, however, the enzyme activity, protein content, or total cell count was not increased in comparison to the untreated control.

In vitro *effect of various NSAIDs on the γ-GT activity of cellular infiltrate of the cotton pellet granuloma.* The cellular infiltrate of the cotton pellet granuloma was separated into cell sediment and supernatant fractions to represent apparently the membrane bound and soluble forms of γ-GT. The

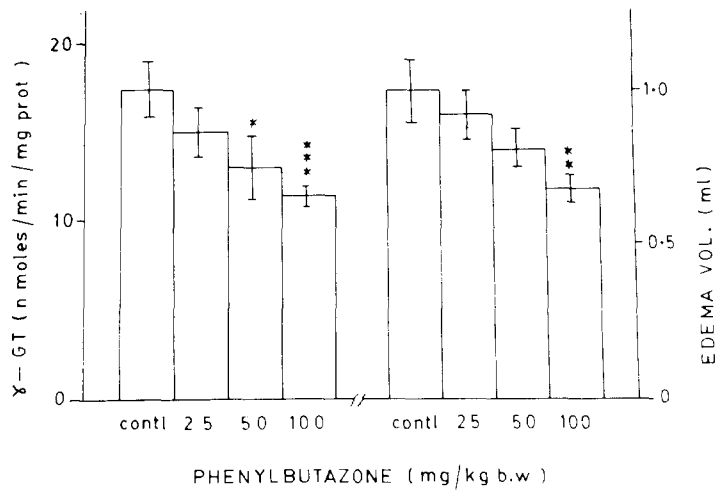

Fig. 2. Dose-dependent inhibition of γ-GT and edema volume by phenylbutazone in developing adjuvant arthritis of hind paw of rat. The rats were given phenylbutazone at the indicated doses 1 hr before the injection of adjuvant. The control group received only saline instead of drug before and after the injection of adjuvant. The experiment was continued until day 13; the animals were killed the next day. The enzyme activities and the edema values were determined as described in Materials and Methods. The data are the mean ± S.D. of four animals for each group. The significance of change was measured by Student's *t*-test: (*)$P < 0.05$, (**)$P < 0.02$; and (***)$P < 0.01$.

Table 3. Effect of pretreatment with phenylbutazone on γ-GT activity and cellular response in carrageenin-induced pleurisy in rats

Drug treatment (mg/kg body wt, p.o.)	γ-GT activity (nmoles/min/mg protein)	% Inhibition	Cell count	
			TLC/ml	% Inhibition
Control	21.5 ± 2.4		6.9×10^7	
Phenylbutazone				
25	16.4 ± 1.1*	25	5.0×10^7	27
50	13.1 ± 1.5†	39	4.2×10^7	39
100	14.2 ± 2.0†	34	4.1×10^7	40

Procedures for the treatment of animals with phenylbutazone and the separation of pleural exudate into cellular sediment and supernatant fraction are given in Materials and Methods. The control group represents carrageenin-induced pleurisy in animals without drug treatment. TLC = total leukocyte count. The activity presented in the table is from the sediment. The specific activity in the supernatant fraction was less than 0.4 nmole/min/mg protein and was consistent in drug-treated and untreated sediment-free supernatant fractions. For each point, the data represent mean ± S.D. of four animals.

*† Significance of changes was measured by Student's *t*-test: *$P < 0.02$; and †$P < 0.01$.

Table 4. Effect of steroidal and nonsteroidal anti-inflammatory drugs on the γ-GT activity of the cotton granulomatous tissue

Drug treatment (mg/kg body wt)	γ-Glutamyl transpeptidase activity			
	nmoles/min/mg protein	Recovery (%)	nmoles/min/g wet wt.	Recovery (%)
Control	4.58 ± 1.90		184	
Indomethacin, 5 mg	4.80 ± 1.11		226	
Phenylbutazone, 50 mg	3.79 ± 1.39	22	174	10
Ibuprofen, 1 mg	4.17 ± 1.55	11	185	
Prednisolone, 2.5 mg	1.58 ± 0.78*	84	50	120

Procedures for drug treatment and preparation of whole homogenate of the granulomatous tissue for the determination of γ-GT activity are described in Materials and Methods. The control group represents cotton granuloma animals without any drug treatment. The basal γ-GT activity of the skeletal muscle obtained was 1.0 ± 0.3 nmoles/min/mg protein and this value was taken as 100 for calculation of "percent recovery". The enzyme activity per g wet wt. was calculated from the protein content/g wet wt. For each point the data are the mean ± S.D. of four animals.

* Significance of changes was measured by Student's *t*-test ($P < 0.001$).

Table 5. Effects of steroidal and nonsteroidal anti-inflammatory drugs on the recovery of cellular protein and γ-GT activity in the cotton exudate of the cotton granuloma in rats

Drug treatment (mg/kg body wt)	Protein content (mg)		γ-GT (nmoles/min/mg protein)			Total cell count
	Supernatant	Sediment	Supernatant	Sediment	Total*	
Control	31 ± 3.6	3.5 ± 0.72	1.88 ± 0.38	8.6 ± 0.47	88	6.8×10^7
Indomethacin, 5 mg	34 ± 1.6	6.2 ± 0.50†	8.0 ± 0.11†	13.9 ± 0.50†	354	10.6×10^7
Phenylbutazone, 50 mg	39 ± 3.2‡	9.5 ± 0.85†	6.94 ± 0.8†	11.6 ± 2.3‡	392	12.4×10^7
Ibuprofen, 1 mg	33 ± 3.8	6.5 ± 1.50§	8.56 ± 0.72†	18.5 ± 3.8‖	391	8.5×10^7
Prednisolone, 2.5 mg	28 ± 1.6‡	3.5 ± 0.8	1.85 ± 0.50	7.4 ± 1.5	80	6.7×10^7

Procedure for the determination of enzyme activity in the supernatant fraction and sediment of the cotton pellet cellular infiltrate of the granuloma is described in Materials and Methods. The data for each point is the mean ± S.D. from four animals. The control group represents cotton granuloma animals without any drug treatment.

* Total γ-GT activity was obtained by summing the enzyme activities in the supernatant fraction and sediment of each group and multiplying by the respective total protein recovered.

†–‖ Significance of changes was measured by Student's *t*-test: ‡$P < 0.05$; §$P < 0.02$, ‖$P < 0.01$ and †$P < 0.001$.

Table 6. *In vitro* effect of various NSAIDs and prednisolone on the γ-GT activity of cellular infiltrate of the cotton pellet granuloma of rats

Drugs (mg/ml of γ-GT assay)	γ-GT (nmoles/min/mg protein)	
	Supernatant	Sediment
None	2.42	7.00
DMSO	2.37	6.31
Aspirin		
0.05	2.10	6.04
0.20	2.01	6.56
0.50	2.40	5.82
Ibuprofen		
0.01	2.15	6.25
0.05	1.97	6.04
0.10	2.01	6.14
Prednisolone		
0.01	2.24	6.04
0.05	2.10	5.73
0.10	2.19	5.73

Values are the means of replicate assays. Drugs were dissolved in DMSO so that the final concentration of DMSO in the tubes was 2.5%. The γ-GT assay was performed at 37° for 20 min. In the case of reagent blanks, the substrate was added after the termination of the reaction. Source of the enzyme preparation is the same as described in Table 5.

effects of various NSAIDs at several concentrations on γ-GT activity *in vitro* are seen in Table 6. None of the drug concentrations was found to inhibit the enzyme activity directly.

Some characteristics of γ-GT in edema tissue from control and arthritic rat paw

Some preliminary studies were conducted (our unpublished results) to observe if the γ-GT activities in normal paw and in edematous tissue (14-days adjuvant arthritis) belong to the same form of transpeptidase measured in the present studies. Optimal pH for γ-GT activity determined in both the tissues was found to be 7.6. Dimethyl sulfoxide (DMSO) and methanol at 10, 20, 30, 40 and 50 μl per ml assay system decreased the enzyme activity by 5–20% in both tissues with increasing solvent concentration in the assay. When whole homogenates from both control and experimental tissues were incubated at 50° in homogenizing medium for 0, 5, 10, 15 and 20 min, and aliquots were taken for γ-GT assay under standard assay conditions, we did not find any significant change in enzyme activity of samples subjected to high temperature for variable time periods (0–20 min) of incubation. The enzyme activity in the absence of glycylglycine was only 10–15% of the maximum activity when determined in the presence of glycylglycine which showed that the reaction rate measured under the present assay conditions was mainly due to transpeptidation.

DISCUSSION

Our studies demonstrate that the γ-glutamyl transpeptidase activity level determined in inflamed tissues is raised significantly in various experimental models of inflammation, e.g. carrageenin hind paw edema, adjuvant arthritis, carrageenin pleurisy and cotton pellet granuloma. A significant increase appeared soon after the onset of inflammation. This was evidenced from experiments on carrageenin edema and adjuvant-induced arthritis conditions. A parallel was observed between paw edema and γ-GT activity, for with alleviation by drugs of the inflammatory conditions both γ-GT activity and paw volume decreased towards their basal values.

Various biochemical and pharmacological methods have been in use for the assessment of anti-inflammatory activities of drugs [18, 19]. Among these, prevention of prostaglandin synthesis by drugs inhibiting cyclooxygenase [20] is presently recommended as a standard criterion for the assessment of anti-inflammatory activity, although some discrepancies have been observed in literature [19, 21].

We report in the present study that γ-GT activity increased greatly in inflammation *in vivo*, and that this enzyme activity remained near its basal level during drug therapy. The maintenance of normal enzyme activity was related to the nature and dose of drug administered and to the experimental model used. The basal level of enzyme activity was not more than 1 nmole/min/mg protein (whole homogenate) in paw tissue or skeletal muscle: this level increased about 24-fold in 28 days of adjuvant arthritis, 4-fold in carrageenin edema, and 5-fold in granulomatous tissue. Further, the enzyme activity was 20 times higher in the cells of carrageenin-induced pleural exudate when compared to γ-GT activity of paw tissue or skeletal muscle. This elevation in γ-GT activity in inflammation offers a wide working scale in which the measurement of enzyme as a reflection of drug potency can be monitored satisfactorily with improvement in inflammatory condition. Based on these investigations using standard

anti-inflammatory drugs and corresponding inhibition of γ-GT *in vivo*, we arrived at almost the same conclusion as observed by others using different techniques, discussed at length in a volume edited by Vane and Ferreira [22]. For instance, indomethacin showed dose-dependent restoration of γ-GT activity in paw tissue in acute inflammation, and phenylbutazone did in adjuvant arthritis. Similarly, phenylbutazone caused a parallel decrease in γ-GT activity and total leukocyte count in carrageenin-induced pleural exudate. Though our studies were limited to a few drugs at present, it was, however, deduced that inhibition of γ-GT *in vivo* may prove to be a valuable biochemical marker in screening and predicting the anti-inflammatory potencies of drugs and never compounds.

Generally, assessment of the anti-inflammatory activities of drugs in cotton pellet granuloma has been related to the decrease in total dry weight of the latter. We, instead, measured the enzyme activity in both the granulomatous tissue and in the cotton exudate. Measurement of γ-GT activity in the granulomatous tissue appeared to be a better approximation of anti-inflammatory potency of drugs, because NSAIDs were found to increase the total leukocyte count in cotton infiltrate in our studies and this may affect the dry weight determination. In granulomatous tissue, prednisolone restored strongly the γ-GT (84%) activity, which was unaffected by NSAIDs. Such corticosteroids have been reported to be very potent against granuloma [23]. We do not know the reason for the increase in enzyme activity with NSAIDs in cotton exudate; it was unchanged in the prednisolone-treated group.

The present data thus suggest that the increase in γ-GT activity is a feature common to all the inflammatory conditions and that the *in vivo* lowering in enzyme activity relates to the anti-inflammatory activity of drugs rather than the direct effect of drugs on the γ-GT activity. Further, the activity did not distinguish between the steroidal and non-steroidal drugs. Cell migration at the site of inflammation is a common feature of inflammation, and high γ-GT activity is found associated with these cells. This is evidenced from our results on carrageenin-induced pleural exudate, in cotton granuloma, and from results of others in lymphoid cells and macrophages [4, 22]. Anti-inflammatory drugs inhibit cell migration [22], and it is understandable that a decrease in γ-GT activity may be a consequence of impaired cell migration. Further, γ-GT has been implicated in the synthesis of peptidyl leukotrienes (slow reacting substances, SRS) [11], the level of which is increased in inflammation that is enhanced further on treatment with NSAIDs [24]. Obviously then, the level of γ-GT should have increased upon NSAID treatment in our studies. We did not observe such an effect, however, except in exudate of cotton granuloma. It may also be argued that the turnover of peptides and amino acids may be high in inflammation because both anabolic and catabolic processes occur simultaneously. This might require greater participation of γ-GT, with transport of amino acids and protein synthesis at the site of inflammation.

It can be concluded that measurement of γ-GT in inflammation may offer a useful procedure in the assessment and screening of drugs and newer compounds for their anti-inflammatory activity *in vivo*. The low level of basal γ-GT activity in paw tissue or skeletal muscle and the dramatic increase in inflammation adds to the sensitivity and precision of the assay. The simplicity and ease of measurement, the stability of enzyme at high temperature, and the low cost of the assay offer additional advantages of the procedure for routine primary screening of newer compounds for their anti-inflammatory activities *in vivo*.

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