Atypical contractile responses to beta adrenoreceptor agonists were described by us in coronary arteries[9], uterus[10] and vas deferens[11]. However, no currently available studies have described a similar situation regarding alpha adrenoreceptor agonists.

The fact that inhibitors of cyclo-oxygenase, namely indomethacin and ASA[12], abolished the vasodilating effect of methoxamine in abdominal aorta, suggests the possibility that this agonist produces its action by influencing initial reactions involved in the biosynthesis of vasodilator prostaglandins. Prostacyclin (PGI_2), synthesized by vascular endothelial cells, is a product of arachidonic acid metabolism with potent dilating effects on blood vessels of a variety of mammalian species[7,13,14]. The fact that tranylcypromine, a nonspecific inhibitor of PGI_2 synthetase[15], abolished the vasodilation induced by methoxamine, suggests that PGI_2 may participate in this influence.

The additional finding demonstrating that abdominal aortic strips to generate an unstable product with antiaggregatory capacity which is increased in the presence of methoxamine, supports the notion advanced.

The finding that all the inhibitors of PG synthesis tested, as well as the alpha adrenoreceptor blockade, abolished the vasodilating effect of methoxamine, suggests that the mechanism by which this agonist stimulates the output of PGI_2, could be mediated by an activation of alpha adrenoreceptors similar to that observed in the rabbit spleen[16]. It is therefore plausible that methoxamine decreases arterial tone, interacting with alpha adrenoreceptors and producing subsequently the release of prostacyclin.

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The effect of propylthiouracil on glutathione S-transferase activity of rat spleen in vitro and in vivo

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Summary. Propylthiouracil (PTU) inhibited glutathione (GSH) S-transferase (EC 2.5.1.18) activity of rat spleens in a concentration dependent manner in vitro. PTU (1.5 mmoles/kg) treatment of rats for 1 or 2 weeks caused a decrease in leukocyte number and spleen weight. Nevertheless, GSH S-transferase activity was not affected by the same treatment.

GSH S-transferases are present in various organs of many species[1,2], and the purification[3,4] and characteristics[5] of these enzymes have been well established. These enzymes catalyze the conjugation of a wide variety of electrophilic foreign compounds with GSH[1,6,7]. It is established that the role of these enzymes is to protect physiological nucleophiles by conjugating foreign compounds, including pesticides and carcinogens. Recently, Yamada and Kaplowitz have reported that PTU inhibited GSH S-transferase activity by competing with GSH as a substrate in reactions catalyzed by the enzymes[8].

On the other hand, it is recognized that thiono-sulfur-containing antithyroid drugs such as PTU and methimazole cause low level agranulocytosis and granulocytopenia during the treatment of hyperthyroidism[9,10]. The postulated mechanism of PTU action is that it inhibits the synthesis of thyroid hormones by preventing thyroid peroxidase activity[11-13]. However, the mechanism by which PTU induces these adverse reactions is not yet known.

In relation to studies of the mechanism producing these effects by PTU, we found a decrease in spleen weight of rats treated with PTU for 1 or 2 weeks, but a recovery of that weight 2 weeks after the treatment. This paper describes the relationship between PTU and GSH S-transferase activity in rat spleens, with regard to hematopoiesis.

Materials and methods. PTU and GSH were purchased from Sigma Chemical Co. 1-Chloro-2,4-dinitrobenzene (CDNB) was from Aldrich Chemical Co. All other reagents were of analytical grade, and available in our laboratory. Isolated spleens of rats were homogenized with 9 vols of 0.25 M sucrose using a Potter-Elvehjem homogenizer with a Teflon pestle at 4 °C. A preliminary experiment revealed significant activity of GSH S-transferase in the various subcellular fractions, especially the supernatant produced at 105,000 × g for 60 min, referred to as the soluble fraction, where more than 60% of the total enzyme activity occurred (data not shown). Therefore, in subsequent experiments, the soluble fraction was used as an enzyme source for GSH S-transferase activity. The standard assay medium for measurement of GSH S-transferase consists of 100 mM potassium phosphate buffer (pH 6.5), 1 mM GSH, 1 mM CDNB as the substrate, and the enzyme source (about 100 µg

Effect of the administration of PTU on weight, blood cell counts and GSH S-transferase activity

	Weights Body (g)	Spleen (g/100 g b.wt)	Leukocytes ($\times 10^3$ cells/mm^3)	Erythrocytes ($\times 10^6$ cells/mm^3)	Enzyme activity[e] (nmoles/mg protein/min)
1 week					
Control	129±2	0.38±0.02	14.4±0.5	5.8±0.1	25.7±0.6
PTU	107±2*	0.27±0.03**	12.1±0.6*	6.0±0.1	26.8±2.3
2 weeks					
Control	189±4	0.30±0.04	13.8±0.2	6.1±0.5	30.8±3.7
PTU	146±3*	0.19±0.02**	10.3±0.8*	5.8±0.3	30.4±3.0
2 weeks after the treatment					
Control	315±9	0.24±0.02	14.2±0.2	ND	31.7±2.7
PTU	242±2*	0.22±0.01	14.5±0.6	ND	30.0±1.4

PTU (1.5 mmoles/kg) was administered as described in 'Materials and methods'. In some experiments, after the administration of PTU for 2 weeks, the animals were kept for 2 weeks to restore the leukocyte numbers. Values are the mean ±SE of 5 to 7 experiments. *$p<0.05$, compared with control; **$p<0.01$, compared with control. ND, Not determined.

protein) in a total volume of 1.0 ml. The reaction was started by adding CDNB, and the enzyme activity was determined by monitoring the changes in absorbance at 340 nm for 3 min at 25 °C[3]. Under these conditions, the enzyme activity was linear with respect to reaction time and protein concentration. Protein was measured by the method of Lowry et al.[14].

It has been reported that repeated treatment with 0.5–1.5 mmoles/kg of PTU caused a dose-dependent decrease of leukocytes with decreasing spleen weight in rats[15]. PTU (1.5 mmoles/kg) dissolved in propylene glycol was administered i.p. to male SD rats, weighing about 80 g, once a day for 1 or 2 weeks. Control animals received only propylene glycol. In some in vivo experiments, rats were sacrificed at 24 h after the final administration of PTU, and blood and spleen were removed.

Results and discussion. Figure 1 shows that PTU inhibited the activity of GSH S-transferase in a concentration-dependent manner. A maximum inhibition of the enzyme activity by PTU was observed at a concentration of 4 mM, showing a decreased activity of the enzyme by 30%. In the presence of 1.5 mM PTU, the K_m-value for GSH was increased (control, 0.40 mM; PTU, 0.67 mM), however, the K_m-value for CDNB was decreased (control, 0.68 mM; PTU, 0.30 mM), indicating that PTU decreased the enzyme activity competitively with GSH, but uncompetitively with CDNB. These observations are compatible with the data of Yamada and Kaplowitz[8]. As shown in figure 1, a Ki-value of 0.83 mM was obtained. This value is in good agreement with a previous report for rat liver cytosol[8]. Thus, PTU inhibited the activity of GSH S-transferase in the soluble fraction in vitro.

To clarify the effect of PTU on blood cell counts, the erythrocyte and leukocyte number in rats treated with PTU was determined. The table summarizes the toxic effects of PTU administration. The treatment with PTU for 1 week significantly decreased the weights of body and spleen with a concomitant decrease in leukocyte counts but not in erythrocytes. These actions of PTU became remarkable after treatment for 2 weeks. The toxic effects of PTU such as the decrease of both spleen weight and leukocyte numbers were reversed, and the normal condition restored, by 2 weeks after withdrawal of this drug (table). Therefore, these findings indicate that leukopenia induced by repeated administration of PTU is associated with the decrease in spleen weight.

Since PTU had an inhibitory effect on GSH S-transferase activity of the spleen in vitro (fig. 1), the relationship between the enzyme activity and the adverse reaction of PTU in the spleen was studied. The table also shows that the activity of GSH S-transferase in the spleen was not influenced by the PTU treatment for 1 or 2 weeks. In addition, the K_m-values for both GSH and CDNB were unchanged by the PTU administration for 2 weeks (fig. 2). Moreover, the acute administration of PTU (1.5 mmoles/kg) had no effect on the enzyme activity of the spleen (control, 28.6±0.6 nmoles/mg protein/min; PTU, 30.7±1.5 nmoles/mg protein/min; n=5–7). It has been demonstrated that the competitive inhibitory kinetics between PTU and GSH, in studies of GSH S-transferase in the liver[8], are also observable in the spleen (fig. 1). However, PTU treatment does not affect GSH S-transferase in

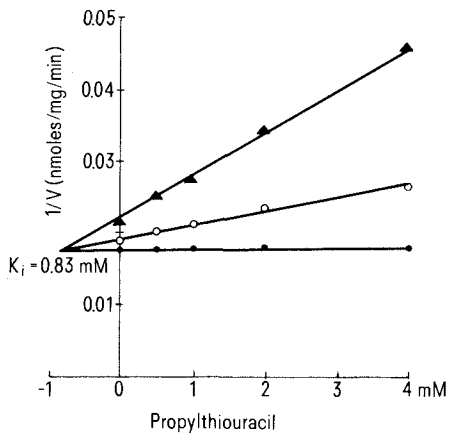

Figure 1. Dixon plot of the inhibition of GSH S-transferase. The reactions were performed at 0.5 (▲), 1.0 (○), and 2.0 (●) mM GSH and 1.0 mM CDNB in the presence of varying concentrations of PTU. The results are the means of values from 5 experiments. The SE is less than 10%.

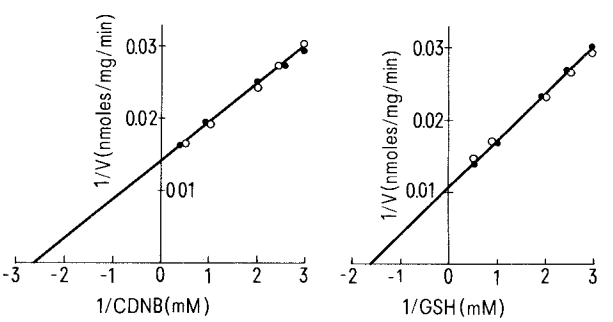

Figure 2. Lineweaver-Burk plot of GSH S-transferase from PTU-treated rats. Propylene glycol and PTU (1.5 mmoles/kg) were administered to control (○) and PTU-treated (●) rats, respectively, for 2 weeks. The results are the means of values from 4 experiments. The SE is less than 10%.

vivo. Thus, the findings obtained in vitro did not apply to the mechanism of PTU toxicity.

The evidence that PTU treatment caused the marked decrease of spleen weight with a concomitant loss of leukocyte numbers seems interesting. The spleen weight returned to normal, and the leukopenia induced by PTU was removed when its administration was discontinued. Neal and Halpert[16] proposed that the toxicity of thinosulfur-compounds such as PTU is most likely to be the result of the covalent binding of the electrophilic S-oxides or S-dioxides or cardene derivatives of these S-oxides or S-dioxides to tissue macromolecules; the decrease of rat spleen weight treated with PTU may be due to these metabolites.

This study suggests the participation of the spleen in the adverse effect of PTU. However, little is explained as to the function of the spleen's influence on granulopoiesis. At present, we propose that leukopenia induced by PTU treatment may be due to a decrease in the biosynthesis by the spleen of a colony-stimulating factor, which regulates granulopoiesis in the bone marrow[17]. Experiments are in progress to clarify the mechanism of induction of leukopenia by PTU.

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A herbicidal principle from *Caesulia axillaris* L., a weed of paddy fields

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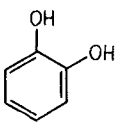
Summary. Screening of extracts from weeds of cultivated fields for their herbicidal activity was undertaken. The crude from alkaline aqueous extracts from leaves and stems of *Caesulia axillaris* (family Asteraceae), an aquatic weed from paddy fields, gave inhibitory effects in the seed germination test. Separation of the active principle by differential solubility method, and its identification by determination of m.p., b.p., UV- and mass-spectra established it as catechol (1,2-dihydroxybenzene). It was found to have selective herbicidal activity on *Ocimum* sp., *Ammannia* sp. and *Fimbrystylis* sp. against *Oryza sativa.*

Reports on plant products to be used as herbicidal[2], fungicidal[3,4] and insecticidal[5] agents are rapidly appearing in literature. Rizvi et al.[6] have reported that caffeine from seeds of *Coffea arabica* has herbicidal property and may be called a 'natural herbicide'. Hence, it was considered desirable to further screen plants for isolation of such active principles.

Experimental procedure and results. 1 kg dried shoots of *Caesulia axillaris* were crushed in 2 l of distilled water at room temperature (30±2 °C). The extract was filtered through cheese cloth and an equal volume of ethyl acetate was added to the filtrate. The ethyl acetate layer was separated with a separatory funnel to which 500 ml aqueous solution of 10% (w/v) NaOH was added. The mixture was again separated and the ethyl acetate layer was discarded. Concentrated HCl was then added to make the alkaline solution neutral. This was treated with ether or ethyl acetate, and the organic layer containing phenol, was preserved. The aqueous layer was discarded.

Identification of the active principle. The organic layer was evaporated to dryness at 30 °C in vacuo. The dry powder was dissolved in ethyl acetate and was subjected to TLC using acetic acid : HCl : Water (6 : 3 : 1) as solvent system and gave 5 spots. All the 5 spots were eluted separately and were bioassayed by the seed germination test. One of them was found to be most effective in inhibiting germination of the 3 test weeds (*Ammannia* sp., *Ocimum* sp., and *Fimbrystylis* sp.), but no such inhibition was noted on germination of seeds of *Oryza sativa.* However, it was interesting to note that the unknown compound promoted the growth of root hairs and caused branching in the primary root. Co-TLC with known phenolic compounds and the R_f resembled that of catechol. This was confirmed by its m.p. 105 °C, b.p. 240 °C, UV- and the mass-spectra. The compound was found to have mol.wt of 110 with structural formula as given in the figure.



Catechol (1,2-dihydroxybenzene)

Germination experiments. Experiments on germination of seeds of *O. sativa* and the test weeds were carried out both