1.5×10^{-3}M in 0·05 M Tris–HCl buffer pH 7·6 containing 0·1% Trition X-100 were incubated at 37° with 0·5–2·0% mouse liver homogenate. Aliquots of the digest were extracted at 5-min intervals up to 60 min with an equal volume of *iso*butanol–benzene (1:1), and the residual malaoxon was assayed after evaporation of the solvent as described previously [6]. It was found that the hydrolysis of malaoxon under these conditions was negligible. Malaoxon is obviously only an inhibitor of non-specific B-esterases.

In 0·05 M Tris–HCl buffer pH 8·8. the activity was low (about 4 μmoles of malaoxon hydrolysed/g of wet liver per hr) when no SH group activator was added. In the presence of 10^{-3}M 2-mercaptoethanol in the buffer and 10^{-3}M reduced glutathione in the digest the activity was 26·1 ± 6·1 (S.D.) μmoles of malaoxon/g liver per hr (8 determinations). The value was not significantly affected when the assay system contained 10^{-5}M DFP. It is therefore concluded that malaoxon esterase is an A-type esterase and the B-type of esterase is without effect on malaoxon.

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The effect of allylisothiocyanate and other antithyroid compounds on blood coagulation in rats

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The seeds of various plants of the Brassica family are goitrogenic when fed to rats [1, 2]. The active component is considered to be a mixture of esters of isothiocyanic acid in the form of glucosides. These esters commonly called essential oil of mustard, have a high content of allylisothiocyanate (AITC) which is also goitrogenic [3]. Ahmad *et al.* [4] showed that AITC reduces blood coagulation time in rats. Since blood phospholipids are involved in blood coagulation [5], it was of interest to see if there were any changes in the plasma phospholipids following the administration of allylisothiocyanate associated with reduced blood coagulation time. As AITC is an antithyroid compound we studied the effect of other antithyroid compounds such as thiourea and thiouracil on blood coagulation time and plasma phospholipids and observed their effect in combination with thyroxine (T4).

MATERIALS AND METHODS

Female Norwegian rats, 8–12 weeks old and weighing 110–180 g, were kept on a basal diet [4] for 1 week before the experiment. On the eighth day, the animals were divided into eight groups. One of the groups served as a control and the remaining groups were treated for 7 days with 2 mg AITC, 23·3 μg thyroxine, 0·5 mg thiourea or 0·5 mg thiouracil per 100 g body weight administered intraperitoneally in 0·5 ml of water, alone or in combination.

Blood was collected from the tail of the rats of each group and clotting time was determined according to Sabraze's capillary tube method [6].

Anaesthetized animals were bled to death. The blood was centrifuged for 10 min at 3000 rev/min. The plasma was extracted [7] using the Folch reagent [8]. An aliquot

Table 1. Effect of thyroxine and antithyroid compounds on blood coagulation time seven days after treatment

Groups of animals	No. of animals	Blood coagulation time (sec)	*P ≤
Control	18	98·0 ± 5·0	
Thyroxine treated	18	89·0 ± 3·0	NS
AITC treated	18	55·5 ± 6·0	0·001
Thiourea treated	18	68·0 ± 5·0	0·001
Thiouracil treated	18	60·5 ± 7·0	0·001
Thyroxine and AITC treated	18	78·0 ± 4·0	0·05
Thyroxine and Thiourea treated	18	85·0 ± 5·0	0·05
Thyroxine and Thiouracil treated	18	83·0 ± 3·0	NS

* P is the average of two tail areas for the values of *t*.

Table 2. Changes in the total phospholipid content of plasma seven days after administration of thyroxine and antithyroid compounds

Groups of animals	No. of experiments*	Total phospholipid content of plasma	†P ⩽
Control	6	99·0 ± 4·0	
Thyroxine treated	6	98·5 ± 0·9	NS
AITC treated	6	133·7 ± 5·0	0·001
Thiourea treated	6	129·5 ± 4·2	0·001
Thiouracil treated	6	132·5 ± 5·5	0·001
Thyroxine and AITC treated	6	99·9 ± 3·2	0·05
Thyroxine and Thioura treated	6	100·06 ± 0·2	0·10
Thyroxine and Thiouracil treated	6	99·5 ± 0·5	0·05

Results are expressed as mg/100 ml plasma.
* Three rats were used in each experiment.
† P is the average of two tail areas for the values of *t*.

was reserved for total plasma phosphorus estimation and the rest was chromatographed. Plasma from the animals of each group was pooled to obtain sufficient material.

Total plasma lipid phosphorus was estimated by a modified Fiske and Subba Row method [9]. Values of phospholipids [10] were obtained by multiplying the phosphorus content by 25. The phospholipid fractions were separated and identified by t.l.c. The developing system was chloroform–methanol–acetic acid–water (25:15:4:2 v/v). Three reference phospholipids used for identification were obtained from Koch Light Laboratories, Colnbrook, Bucks, England: Cephalin ex sheep brain; phosphatidyl choline, lecithin ex egg; sphingomyelin, ex bovine brain, pure CHR.

To obtain phosphorus from phospholipids in the form of inorganic phosphate the developed and dried chromatograms were sprayed with 50% (w/w) sulphuric acid. Mineralization was done by direct heating of the plates at 180° for 60 min. Inorganic orthophosphate thus obtained after direct mineralization was determined quantitatively using the Hahn and Luckhaus reagent [11, 12].

RESULTS

Blood coagulation time. Table 1 shows the effect of thyroxine and antithyroid compounds on blood coagulation time. The coagulation time of the rats treated with thyroxine was similar to that of the control animals. Administration of allylisothiocyanate (AITC), thiourea and thiouracil caused a marked decrease in the blood coagulation time of 43·3, 30·6 and 38·2 per cent. This effect was reversed to a great extent by simultaneous administration of thyroxine.

Plasma phospholipids. The total phospholipid content of plasma of the different groups is shown in Table 2. Administration of AITC, thiourea or thiouracil increased the total phospholipid concentration of plasma by 30–35 per cent. In the thyroxine treated group the concentration remained almost the same as that in the control group. When the animals received both thyroxine together with the antithyroid compounds, the increase was not significant.

Changes in the individual phospholipid fractions of each group after treatment are shown in Table 3. Administration of AITC, thiourea, thiouracil caused an increase in the concentration of all phospholipids, especially phosphatidyl ethanolamine. The per cent increase was 25–30, 20–28 and 55–60 for phosphatidyl choline, sphingomyelin and phosphatidyl ethanolamine, respectively. In the groups receiving thyroxine alone or in combination with the antithyroid compounds the phospholipid levels did not differ significantly from the control.

Table 3. Changes in the phospholipid fractions of plasma seven days after administration of thyroxine and antithyroid compounds

	No. of experiments*	Phospholipids			†P ⩽		
		PE	PC	SP	PE	PC	SP
Control	6	6·3 ± 1·0	80·5 ± 2·5	10·5 ± 2·0			
Thyroxine treated	6	6·0 ± 2·0	79·5 ± 3·0	9·0 ± 2·0	NS	NS	NS
AITC treated	6	10·0 ± 2·0	104·7 ± 5·7	13·1 ± 2·5	0·001	0·05	0·05
Thiourea treated	6	9·5 ± 2·5	.00·5 ± 2·8	12·5 ± 2·8	0·001	0·05	0·05
Thiouracil treated	6	10·1 ± 2·0	103·2 ± 5·0	11·8 ± 3·0	0·001	0·05	0·05
Thyroxine and AITC treated	6	6·5 ± 0·5	82·5 ± 2·0	11·0 ± 0·6	0·001	NS	0·05
Thyroxine and thiourea treated	6	6·7 ± 1·0	82·8 ± 4·0	11·0 ± 0·6	0·05	NS	NS
Thyroxine and thiouracil treated	6	6·6 ± 0·8	81·5 ± 3·0	11·5 ± 0·6	0·05	NS	0·05

Results are expressed as mg 1/100 ml plasma.
* Three rats were used in each experiment.
† P is the average of two tail areas for the values of *t*.
PE, Phosphatidyl ethanolamine; PC, Phosphatidyl choline; SP, Sphingomyelin.

DISCUSSION

The administration of AITC, thiourea and thiouracil reduced blood coagulation time by 30–40 per cent confirming previous results [4]. It appears that goitrogenic compounds in general are powerful agents for shortening the blood coagulation time. When thyroxine was administered with the antithyroid compounds, this effect on blood coagulation time is largely reversed.

The total phospholipid concentration of plasma was raised by 30–35 per cent in the groups treated with AITC, thiourea and thiouracil. Analysis of the individual phospholipid showed that the greatest increase was in the phosphatidyl ethanolamine fraction (55–60%). As blood platelets contain a considerable amount of phospholipids which are essential for the coagulation process [5], this increase in the concentration of plasma phospholipids may accelerate the coagulation process. In the thyroxine treated animals no such change occurred and the effects of antithyroid compounds were greatly reduced by simultaneous administration of thyroxine.

These results might explain why blood coagulation in rats is accelerated by administration of antithyroid compounds. It was shown previously [13] that the effect of feeding different fats on blood coagulation was related to changes in the phospholipid content of the plasma.

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Harmaline inhibits the $(Na^+ + K^+)$-dependent ATPase by affecting both Na^+ and K^+ activation

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The judicious use of inhibitors is frequently of enormous assistance in efforts to understand enzymatic processes. For the $(Na^+ + K^+)$-dependent ATPase, which is the enzymatic representation of the sodium/potassium pump of the cell membrane [1], further assistance in such efforts is greatly needed, in light of the overall complexity of the reaction mechanism [2]. This is particularly evident in terms of defining the cation sites that activate the enzyme and that are involved in the actual translocation process, since conflicting formulations describing such sites await resolution [3]. Currently, there are available several agents that modify affinities of the cation sites, including (1) phlorizin [4] and (2) deuterium oxide [5], both of which increase the apparent affinity for K^+ but decrease it for Na^+, and (3) dimethylsulfoxide [6–8], which increases the apparent affinity for K^+ of the associated K^+-dependent phosphatase reaction, but also acts as a "competitive" activator toward some substrates of the reaction and, in addition, is an uncompetitive inhibitor toward Na^+. Beyond these, a reagent that affected only activation by one cation would be particularly desirable. Canessa *et al.* [9] recently reported inhibition of this ATPase by harmaline (3,4-dihydro-7-methoxy-1-methyl-9-pyrid [3,4-bis]indole) that was competitive toward Na^+ but not toward K^+, an observation deserving further exploration.

The enzyme preparation used here was obtained from rat brain microsomes after treatment with deoxycholate and NaI, as described previously [10]. To determine ATPase activity, the enzyme preparation (0·5 mg/ml final concentration) was first incubated for 10 min at 37° in 30 mM histidine·HCl–Tris (pH 7·8) in the absence or presence of harmaline (obtained from Sigma Chemical Co.); the assay incubation was then initiated by adding 4 vol. of media to bring the final concentrations, under control conditions, to 30 mM histidine·HCl–Tris (pH 7·8), 3 mM Tris–ATP, 3 mM $MgCl_2$, 90 mM NaCl, and 10 mM KCl (and, where harmaline was originally present, maintaining its concentration). ATPase activity was measured in terms of inorganic phosphate production [10]. Although the maximal harmaline concentration present in these analyses, 60 μM, did not affect the phosphate method of Lowry and Lopez [11] measurably, all determinations were rou-