Begg[14]. The protein content was determined by the method of Lowry et al.[15] using bovine serum albumin as a standard. The activity of acetylcholinesterase (EC 3.1.1.7) was measured by the method of Fonnum[16], slightly modified. For all the biochemical analyses, the brains were immediately frozen (−30 °C) and thawed directly prior to analysis.

Results. The number of nuclei in the ependyma of the diencephalon from 1-day-old mice is significantly reduced when the animals have absorbed PTC via the placenta during the whole intrauterine development (table 1). The brains of 1-day-old mice having absorbed PTC during the whole intrauterine development contain less protein than those of control animals. This is valid for the fresh weight as well as DNA or RNA content (table 2). The content of DNA or RNA of the brains from new-born mice treated in this manner remained unchanged compared with control animals (table 2). The activity of acetylcholinesterase is significantly reduced in the brains of likewise pretreated new-born mice (table 3).

Discussion. The central nervous system of new-born mammals is not fully differentiated and biochemically immature. For the completion of brain maturation, euthyreotic conditions are necessary. If, during this critical period, the function of the thyroid gland is impaired, further brain development is retarded. For instance, hypothyreotic young rats show a reduced brain weight[17] and in addition fewer synapses are formed in their cerebellum[18].

The reduction of the number of nuclei in the ependyma of the diencephalon in new-born mice treated with PTC indicates an impaired brain development. However, the histological structure of the thyroid of such mice scarcely differs from that of control animals (unpublished results). Possibly PTC in the concentration used is not strong enough to cause visible changes in the thyroid structure.

The suspected inhibitory effect of PTC on protein synthesis in the brain[2] is confirmed by our demonstration of a reduced protein content after PTC-treatment. A similar effect was described in juvenile rat brains after treatment with propylthiouracil[19]. A change in total DNA or RNA in the whole brain of PTC-pretreated mice was not observed in our experiments, suggesting a relatively weak toxicity of this substance.

Thyroxine also controls the biochemical maturation of neurotransmitter systems. For instance, the content of acetylcholine in 1-day-old rat brains is only 73% of the values from adult brain, referred to g fresh weight[12]. Furthermore, the activity of acetylcholinesterase in young rat brains is reduced under hypothyreotic conditions[7,20]. PTC also causes a reduction of activity of this enzyme in the brain of new-born mice. This is a further indication that PTC causes an inhibition in the brain development by affecting the thyroid gland.

The described influence of PTC on the brain of new-born mice, possibly via an inhibition of thyroxine, is also supported by the observation, that PTC inhibits the metamorphosis of Xenopus larvae (unpublished results).

Table 3. The effect of PTC on the activity of acetylcholinesterase in brains of 1-day-old mice

Treatment	Experiment A	Experiment B
Controls	2.86±0.08 (12)	3.48±0.16 (10)
PTC-treated	1.99±0.05* (10)	2.54±0.09* (10)

The values are presented as μmoles acetylcholine hydrolyzed per min per g brain (mean±SEM). *p=0.02 (Man-Whitney-test), in brackets: number of brains.

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Interaction between azapropazone and warfarin

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Summary. In vitro studies, using 2 separate techniques, have shown that the anti-inflammatory agent azapropazone caused displacement of warfarin from its plasma albumin binding and it is therefore suggested that such a displacement mechanism may be involved in the reported clinical interaction between these 2 drugs.

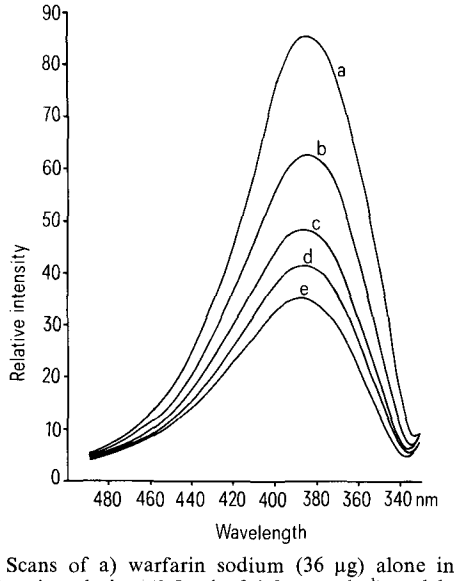
A number of non-steroidal anti-inflammatory agents, notably phenylbutazone[1], have been shown to potentiate the anticoagulant effect of warfarin. This type of drug interaction is particularly dangerous since fatal haemorrhage may occur when a patient previously stabilized on warfarin is treated with a drug having phenylbutazone-like properties.

2 possible mechanisms may be implicated in such an interaction: a) a displacement of warfarin from plasma albumin binding sites[2] and b) an effect on warfarin R and S isomer metabolism and elimination[3]. Both mechanisms probably play a role. Preliminary findings[4] from in vitro experiments have suggested that binding of warfarin to

human serum albumin was decreased in the presence of azapropazone, a relatively new non-steroidal anti-inflammatory drug. In additional studies the involvement of plasma protein displacement in this interaction has been further investigated.

Materials and methods. 2 different experimental techniques were used; firstly, a serial ultrafiltration technique[5] gave values for free and bound warfarin over a range of concentrations. The 2nd method was based on the enhancement of warfarin fluorescence by its binding to human serum albumin, and the subsequent quenching of this fluorescence when it is displaced from its plasma albumin binding sites[6]. With the serial ultrafiltration technique, 0.2% human serum albumin (AB Kabi, Stockholm) was used in phosphate buffer at pH 7.4 as the binding medium and serial 2-ml additions of solutions of warfarin alone and mixtures of warfarin and azapropazone were made to 40 ml of this albumin solution. These additions (15 in all) were alternated with the taking of 15×2 ml ultrafiltrate samples. This allowed warfarin concentration to steadily increase within the filtration cell. As 2 differing warfarin solutions were used (50 µg ml^{-1} and 100 µg ml^{-1} warfarin sodium in buffer) the binding of warfarin was characterized over 2 concentration ranges; a narrow range using the former solution and a wider range using the more concentrated solution. In interaction studies, the same warfarin stock solutions were used but both solutions now contained 100 µg ml^{-1} of azapropazone. Computer evaluation of the bound and free drug concentration data, using the plot described by Sellers and Koch-Weser[7], gave n,K_a and nK_a values for primary warfarin binding sites. Sellers and Koch-Weser[7] have suggested that the product nK_a is a useful index of total binding affinity of protein for warfarin. The results from this part of the study are summarized in the table.

With the fluorescence technique, scans were run using a fluorescence spectrophotometer of a warfarin-albumin mixture both before and after addition of azapropazone. This involved adding warfarin sodium (0.02 ml of 1.8 mg ml^{-1}) to human serum albumin (3.5 ml of 0.5 mg ml^{-1}) in a cuvette, measuring the fluorescence and then subsequently making further scans after each of 4 additions of azapropazone (0.02 ml of 0.4 mg ml^{-1}) to the system. This allowed examination of the peak enhancement of warfarin fluorescence when bound to albumin and any subsequent quenching of this fluorescence when azapropazone was added to the system in increasing concentration. Control experiments established that azapropazone did not interfere with this fluorescence technique. The results from this study are illustrated in the figure.



Analyser Scans of a) warfarin sodium (36 µg) alone in human serum albumin solution (3.5 ml of 0.5 mg ml^{-1}) and b-e) after additions of 0.02, 0.04, 0.06 and 0.08 ml of azapropazone solution (0.4 mg ml^{-1}) respectively. Exciter wavelength used was 320 nm. The scans show relative fluorescence intensities (ordinate) at various emission wavelengths (abscissa).

Results. The serial ultrafiltration technique showed that over both warfarin concentration ranges there was a dramatic fall-off in binding capacity (seen by a decrease in n) and in total affinity (nK_a) when azapropazone was added to the system (table). This represented a displacement of warfarin from its albumin binding sites by azapropazone.

Binding parameters obtained for warfarin alone and while in combination with azapropazone using the ultrafiltration technique. Parameters were obtained using graphical treatment of data obtained over 2 warfarin concentration ranges: a) narrow range $= 0\text{-}1.2 \times 10^{-4}$ M and b) wide range $= 0\text{-}2 \times 10^{-4}$ M. Numbers of primary binding sites per molecule of albumin (n), association constants (K_a) and total affinity values (nK_a) are given for the 4 different experimental conditions

	n	$K_a, 10^4 M^{-1}$	$nK_a, 10^4 M^{-1}$
Narrow concentration range			
Warfarin alone	1.836	12.288	22.561
Warfarin + azapropazone	0.491	13.089	6.428
Wide concentration range			
Warfarin alone	2.194	10.296	22.589
Warfarin + azapropazone	1.051	8.869	9.321

The fluorescence technique also provided data which indicated that warfarin was displaced from its binding sites when combined with azapropazone. This was demonstrated (figure) by the reduction of the intensity of the fluorescence peak in the presence of azapropazone; this intensity became progressively reduced as the concentration of azapropazone was increased.

Discussion. Collectively, data from these 2 in vitro studies suggest that, if the 2 drugs are used concomitantly, then warfarin may be markedly displaced from its albumin binding by the anti-inflammatory agent. This might be expected to produce increased anticoagulation due to a rise in the level of unbound warfarin in the plasma. This does not, however, exclude the possibility of other mechanisms being involved in this interaction, e.g., changed metabolism of warfarin isomers. Powell-Jackson has already reported increased anticoagulation when these drugs are used together in the clinic[8]; we would therefore advise that concomitant administration of azapropazone and warfarin should be avoided.

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