therefore that the portion of rabbit gamma globulin that combines with gp C′(3) resides in fragment III. The decrease in rate of agglutination by treatment of rabbit antisheep erythrocyte gamma globulin with pepsin implicates to some extent the role of fragment III in another immune phenomenon.

Summary. Fragment III derived from papain digestion of rabbit gamma globulin inhibits immune hemolysis primarily by its ability to interact with gp C′ and to some extent by interacting with sheep erythrocytes. Peptic digests of normal and antipertussis rabbit gamma globulins do not inhibit immune hemolysis. Peptic digests of rabbit antisheep erythrocyte gamma globulins are capable of agglutinating sheep erythrocytes at a slower rate when compared to that of the intact antibodies. The latter digests will not lyse sheep erythrocytes in the presence of gp C′, because they fix less complement than intact antibody.

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Effects of Psychotropic Compounds on Enzyme Systems, I. Inhibition of Human Plasma and Erythrocyte Cholinesterases.* (26964)

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Although there is some literature on enzyme inhibition by psychotropic compounds, good quantitative data, particularly relating *in vivo* with *in vitro* effects in the same species are relatively rare. Some enzyme systems, for example choline acetylase, have been largely ignored, probably because of difficulty of assay rather than lack of significance of this enzyme. We propose to report investigations on *in vivo* and *in vitro* quantitative inhibitory effects of a large number of compounds with psychotropic activity on various enzyme systems and to attempt to correlate psychotropic action with effects on enzyme systems.

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Most of the published inhibition studies have been either in qualitative terms ("inhibits", "enhances") or in semi-quantitative terms (50% inhibition = I_{50}). The I_{50} value, however, is dependent upon substrate concentration, so that it is frequently difficult to compare numerical values obtained by different assay methods. Moreover, determination of I_{50} values does not necessarily entail an analysis of the kinetics of inhibition. It has therefore been found desirable to determine K_i values (K_i = inhibitor constant), as defined by Dixon and Webb(1).

Tranquilizers of the phenothiazine type have been found to be *in vitro* inhibitors of cholinesterases(2,3). It has also been suggested(2) that the effectiveness of phenothiazine tranquilizers might be related to their cholinesterase inhibitory activity.

Material and methods. The tranquilizers

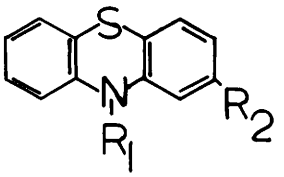

FIG. 1. Phenothiazines used.

Compound	R_1	R_2
Promazine	$-(CH_2)_3-N(CH_3)_2$	-H
Chlorpromazine	$-(CH_2)_3-N(CH_3)_2$	-Cl
Trifluprom azine	$-(CH_2)_3-N(CH_3)_2$	$-CF_3$
Thiopropazate	$-(CH_2)_3-N$ $N-(CH_2)_3-$ $O-C-CH_3$ $\|\|$ O	-Cl
Secergan	$-C-CH-N(CH_3)_3$ $\|\|\ \ \|\ \ \ +$ $O\ CH_3\ \ Br^-$	-H

used in these experiments were kindly supplied as follows: chlorpromazine, Smith Kline and French Labs; promazine, Wyeth Labs; secergan, Astra Pharmaceutical Products, Inc.; thiopropazate, G. D. Searle and Co.; trifluprom azine, The Squibb Inst. for Medical Research. (The structures of these compounds are given in Fig. 1.) Two of the substrates used, acetyl-β-methyl-choline chloride (MeCh) and acetylcholine iodide (AcCh), were commercial preparations. The third substrate, butyrylcholine iodide (BuCh), was prepared in this laboratory according to the method of Tammelin(4). The human blood used, obtained from healthy adult volunteers, was collected directly into chilled flasks containing ethylene diamine tetraacetic acid. It was centrifuged immediately after collection at 3,000 rpm for 10 minutes at 4°C. Plasma was diluted to twice its original volume with Krebs Ringer solution and served as the source of plasma cholinesterase (ChE). Erythrocytes were washed twice with isotonic saline then diluted to 3 times the original volume with 0.001% saponin solution and served as the source of erythrocyte acetylcholinesterase (AcChE). Both plasma and the erythrocyte preparations were stored at 4°C, and were used without further purification. No loss in activity was observed over a period of 10 days.

Cholinesterase activities were measured by the manometric method of Ammon(5) at pH 7.4, 37°C, using 5% CO_2-95% N_2 as the gas phase. All inhibitors were dissolved in Krebs Ringer solution immediately before use, and were incubated with the enzyme preparation for 40 minutes before addition of substrates. Manometers were read every 5 minutes for a total of 30 minutes. All manometer readings were converted to μl CO_2 and all data expressed as velocity = μl CO_2/min. All data given are averages of at least 3 different runs, each in duplicate.

Inhibitor constants were calculated either by a direct graphical method, plotting 1/V vs. [i] for at least 2 different substrate concentrations, or by plotting 1/V vs. 1/S for control and inhibited runs. In the latter method, the ratios of the slopes of the inhibited and control lines were used to calculate K_i. For purely competitive inhibition, $\frac{\text{slope inhibited line } (s_i)}{\text{slope control line } (s_o)}$ plotted vs. [i] gives a straight line. For partially competitive inhibition, $\frac{s_i}{s_o} = \frac{1 + i/K_i}{1 + (i/K_i)(K_m/K_{m'})}$; a plot of the ratios of the slopes of the lines vs. [i] will not yield a straight line, and therefore several inhibitor concentrations are needed to calculate K_i. When the lines obtained in a plot of 1/V vs. 1/S for different inhibitor concentrations do not meet on either the 1/V or the 1/S axis, inhibition is of the mixed type and K_i can be calculated from the ratios of the slopes, as in the case of competitive inhibition.

Results. Erythrocyte AcChE with MeCh as substrate was inhibited to varying extents by the 5 phenothiazines used, with K_i ranging from 1.5×10^{-4} M to 1.2×10^{-5} M (Table 1). Although the differences in K_i values were considerable, all followed the mixed type kinetics as is illustrated graphically for thiopropazate (Fig. 2).

The inhibition of plasma ChE with AcCh as substrate was generally more pronounced than the inhibition of AcChE. Competitive kinetics were found with secergan, promazine, chlorpromazine and trifluprom azine, while

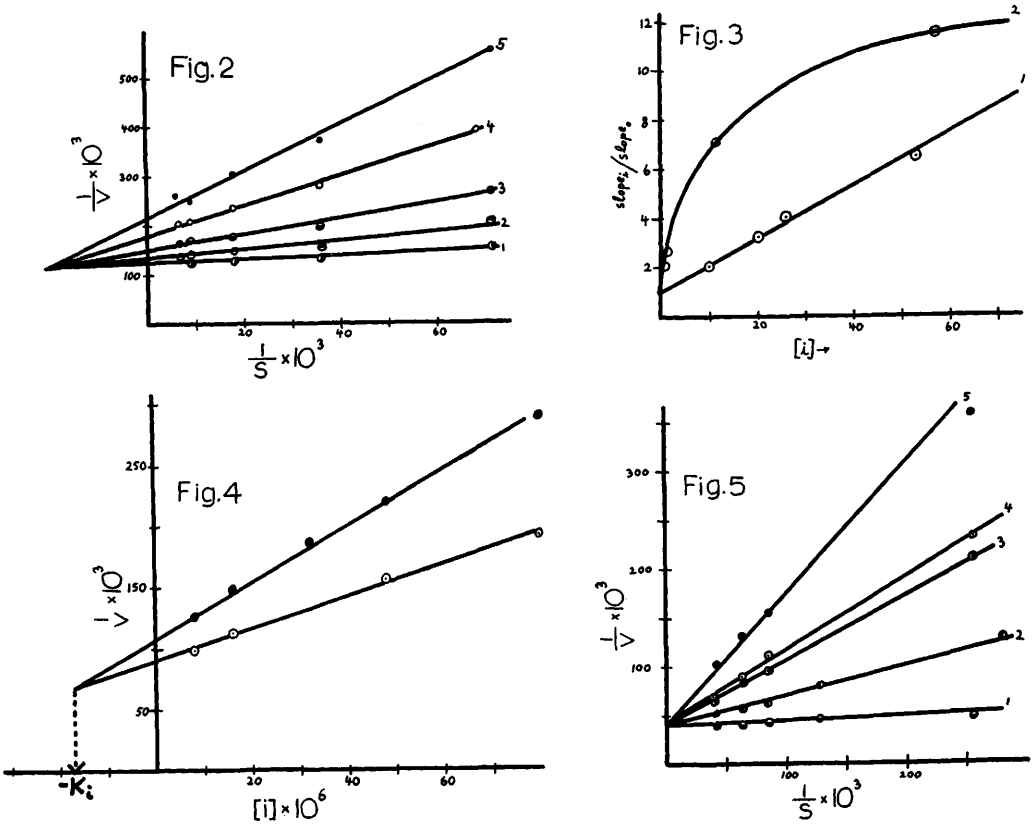

FIG. 2. Inhibition of AcChE by thiopropazate, MeCh as substrate
1. No inhibitor
2. Inhibitor conc. 1.14×10^{-3} M
3. " " 3.46×10^{-3} M
4. " " 6.96×10^{-3} M
5. " " 10.4×10^{-3} M

FIG. 3. Ratios of slopes for chlorpromazine and for thiopropazate

FIG. 4. Inhibition of ChE by secergan, AcCh as substrate
1. S = 8.0×10^{-3} M
2. S = 16×10^{-3} M

FIG. 5. Inhibition of ChE by promazine, BuCh as substrate
1. No inhibitor
2. Inhibitor conc. 1.2×10^{-5} M
3. " " 3.1×10^{-6} M
4. " " 3.8×10^{-5} M
5. " " 7.5×10^{-5} M

thiopropazate exhibited the partially competitive type of reaction (Fig. 3). A Dixon and Webb type plot is shown for secergan in Fig. 4. When BuCh was used as substrate instead of AcCh, similar results were obtained except in the case of secergan, where the competitive inhibition with BuCh was considerably greater than with AcCh. The competitive inhibition with BuCh as substrate is shown graphically for promazine in Fig. 5.

Discussion. Four of the phenothiazines used are tranquilizers, whereas the fifth, secergan, is primarily an antispasmodic(6). Secergan was included in this study since it was expected that it would be a stronger ChE inhibitor than the other phenothiazines because of its quaternary ammonium nitrogen. This was not found to be true in the case of the ChE-AcCh system. Possibly, the close proximity of the carbonyl group to the charged

TABLE I. K_i Values and Type of Inhibition.

Compound	Rec. dose (mg)	AcChE-MeCh K_i	AcChE-MeCh Type	ChE-AcCh K_i	ChE-AcCh Type	ChE-BuCh K_i	ChE-BuCh Type
Thiopropazate	15	$(9.8 \pm .6) \times 10^{-4}$	mixed	$(6.7 \pm .3) \times 10^{-5}$	partially competitive	$(4.4 \pm .3) \times 10^{-6}$	partially competitive
Trifluprom azine	50	$(5.1 \pm .1) \times 10^{-5}$	"	$(3.5 \pm .4) \times 10^{-5}$	competitive	$(2.5 \pm .2) \times 10^{-5}$	competitive
Chlorpromazine	10-100	$(7.0 \pm .7) \times 10^{-5}$	"	$(9.1 \pm .2) \times 10^{-6}$	"	$(1.2 \pm .2) \times 10^{-5}$	"
Promazine	150	$(1.5 \pm .2) \times 10^{-4}$	"	$(1.9 \pm .1) \times 10^{-6}$	"	$(2.9 \pm .2) \times 10^{-6}$	"
Secergan		$(1.2 \pm .1) \times 10^{-5}$	"	$(1.7 \pm .2) \times 10^{-5}$	"	$(1.5 \pm .2) \times 10^{-6}$	"

nitrogen, or the methyl group on the carbon α to $-\overset{+}{N}-(CH_3)_3$ group, modifies the interaction with the anionic site of ChE. Secergan was the strongest of the inhibitors for the AcChE-MeCh and the ChE-BuCh systems.

In Table 1 the compounds are listed in order of increasing recommended dosages. In general, inhibition of erythrocyte AcChE has little relationship to psychotropic activity. Inhibition of plasma ChE, on the other hand, appears to be inversely related to activity, if the recommended dosage is taken as an index to such activity. Thiopropazate, with the lowest suggested dosage, had the highest numerical value for K_i, indicating that, under the conditions used, it is a weaker inhibitor than the other compounds. The mechanism of inhibition by thiopropazate of plasma ChE, however, was found to be different from that of the other compounds tested (partially competitive rather than competitive), possibly because of the presence of the piperazine ring in the side chain of thiopropazate which could interact with a site different from the esteratic site.

Summary. Inhibition constants for 5 phenothiazines, chlorpromazine, promazine, secergan, thiopropazate and trifluprom azine, using the systems human erythrocyte acetylcholinesterase with acetyl-β-methylcholine chloride as substrate and human plasma cholinesterase with both acetylcholine and butyrylcholine as substrates were determined. The mechanism of inhibition was of the mixed type for acetylcholinesterase, and of the competitive or partially competitive type for plasma cholinesterase. *In vitro* inhibition appeared to be unrelated to activity in the case of acetylcholine esterase and inversely related in the case of plasma cholinesterase.

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