Anorexic activity was evaluated in mice which were trained to consume their daily food intake over an 8-hr period. The amount of food intake for a group of mice treated with a test compound was determined and compared to that of a nontreated control group. A reduction of 30% in the test group was considered significant.

Local anesthetic activity was determined by instilling a solution of the test compound into a rabbit eye and determining the presence or absence of the corneal reflex. Absence of the corneal reflex is taken as an indication of local anesthetic activity. The lowest concentration (per cent) preventing the corneal reflex is taken as the minimal effective dose for local anesthetic activity.

Microbiological Testing.—Test compounds were evaluated for antimicrobial activity employing a series of gram-negative and gram-positive organisms. In addition, these compounds were also tested for antitrichomonal and antifungal activity. All of these tests were performed *in vitro*.

Phenothiazines Exhibiting Lesser Extrapyramidal Manifestations

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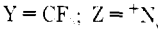
Since alkylaminoalkylphenothiazines are valuable agents in psychiatry, control of their extrapyramidal side effects offers an important challenge. Several intriguing views regarding the relationship of tranquilizing potency and extrapyramidal reactions are reported in the literature. Freyhan,[1] Haase,[2] and Brune, *et al.*,[3] are of the opinion that the ability of a drug to induce a Parkinson-like syndrome bears a direct correlation to its therapeutic efficacy. Cole and Clyde,[4] Brooks,[5] and Hollister,[6] on the other hand, believe that the suppression of parkinsonism does not lead to the impairment of therapeutic efficacy of tranquilizers and that the clinical apperance of extrapyramidal syndromes in no way predicts the therapeutic response of a tranquilizer.

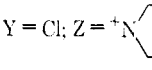
An endeavor to find out the relationship between parkinsonism and antipsychotic activity of a tranquilizer can be made profitably on the basis of the hypothesis of McGeer,[7] *et al.* According to this hypothesis, parkinsonism may be brought about by a disturbance in normal brain equilibrium between serotonin and catecholamines on one hand, and histamine as well as acetylcholine on the other. Since the therapeutic efficacy of the phenothiazine group of tranquilizers bears a close correlation to their antiadrenergic[8] and antiserotonin[9] activity, the two possible ways to inhibit parkinsonism without interfering with antipsychotic activity is to enhance antihistaminic or anti-acetylcholine action. But potent antihistaminics among phenothiazines like promethazine or ethopropazine possess little tranquilizing activity, while perphenazine and trifluoperazine which are weak antihistaminics are potent tranquilizers; therefore the only way to reduce parkinsonism liability of phenothiazine tranquilizers is to increase their antiacetylcholine activity. Since oximes are well known as antidotes for organophosphorus poisoning (anti-DFP), we could speculate that anticholinergic quaternary oximes of phenothiazine drugs might provide a tranquilizer with less liability for Parkinson disease, and that the study of these compounds would lead to certain clues for the relationship between these two actions.

Three well-established phenothiazine tranquilizers, *viz.* chlorpromazine hydrochloride (CPZ), trifluoperazine dihydrochloride (TFP), and perphenazine dihydrochloride (PER), were selected for the present study. These hydrochlorides, on treatment with 40% sodium hydroxide solution, provided their respective free bases, which were treated with phenacyl bromide oxime. This resulted in the precipitation of the quaternary oxime of each drug, *viz.*, phenacyloxime chlorpromazine bromide (I), diphenacyloxime trifluoperazine dibromide (II), and diphenacyloxime perphenazine dibromide (III).

$(CH_2)_3Z[CH_2C(=NOH)C_6H_5]_n$

I. Y = Cl; Z = $N^+(CH_3)_2 \cdot Br^-$; $n = 1$

II. Y = CF_3; Z =  $\cdot 2Br^-$; $n = 2$

III. Y = Cl; Z =  $\cdot 2Br^-$; $n = 2$

These compounds were assayed for their liability to induce catatonia in rats, a method which is believed to produce symptoms similar to those of parkinsonism.[10,11] The effects were compared to those of their parent drugs. Compound I at 10 mg/kg produced no catatonia while CPZ at this dose produced 62.5% catatonic reaction; II at 3 mg/kg produced only insignificant (20% catatonia) imbalance of posture, but TFP exhibited 100% catatonia at the same dose level. Similarly III at 3 mg/kg exhibited only 16.66% catatonia while PER at an equivalent dose produced 100% catatonic reactions.

Since these derivatives (I–III) offered a marked reduction in parkinsonism-like reactions over their parent drugs, it became of interest to find out to what extent these derivatives were devoid of tranquilizing property. The tests employed were as follows.

(i) Qualitative assessment of spontaneous motor activity (SMA), ptosis, and the influence of tactile and auditory stimuli on the mobility of mice by following a double blind observational method.[12]—In general, these derivatives affected the SMA to a lesser extent as compared to their parent drugs. III at 3 mg/kg compared well with 1 mg/kg of PER. The case was similar

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(2) H. J. Haase, ref 1, p 329.

(3) G. G. Brune, C. Morpurgo, A. Bielkus, T. Kobayashi, T. T. Tourlentes, and H. E. Himwich, *Comprehensive Psychiat.*, **3**, 227 (1962).

(4) J. O. Cole and D. J. Clyde, *Rev. Can. Biol.*, **20**, 565 (1961).

(5) G. W. Brooks, *New Engl. J. Med.*, **254**, 1119 (1956).

(6) L. E. Hollister, *Clin. Pharmacol. Therap.*, **5**, 321 (1964).

(7) P. L. McGeer, J. E. Boulding, W. C. Gibson, and R. G. Foulkes, *J. Am. Med. Assoc.*, **177**, 665 (1961).

(8) R. A. Webster, *Brit. J. Pharmacol.*, **25**, 566 (1965).

(9) D. H. Tedeschi, R. E. Tedeschi, and E. J. Fellows, *Arch. Intern. Pharmacodyn.*, **132**, 172 (1961).

(10) W. Wirth, R. Gosswald, U. Horlein, K. L. H. Risse, and H. Kreiskott, *ibid.*, **115**, 1 (1958).

(11) C. Morpurgo, *Progr. Brain Res.*, **16**, 121 (1965).

(12) P. C. Dandiya and M. K. Menon, *J. Pharmacol. Exptl. Therap.*, **145**, 42 (1964).

with II and TFP. Significant loss of SMA and ptosis could be observed at 10 mg/kg of I, which compared well with 3 mg/kg of CPZ.

(ii) Their potencies to potentiate pentobarbital-induced hypnosis.[13]—At an equal dose (3 mg/kg), PER (70%) and III (58%) potentiated the sleeping time to approximately the same extent. Similarly TFP (64%) and II (63%) at 3 mg/kg, were equipotent in this regard. At 10 mg/kg CPZ exhibited a 119% increase in sleeping time while I at this dose could increase the sleeping time to only 35%.

(iii) Their ability to abolish fighting behavior in paired mice.[14]—III and II were more or less equipotent to their parent drugs, *viz.* PER and TFP, respectively. At an equal dose (10 mg/kg) these compounds could block the fighting behavior to 100, 91.66, 100, and 100%, respectively. CPZ at this dose blocked the fighting response 100%, while I did this only to the extent of 42%.

(iv) Their effects on blocking the conditioned avoidance response (CAR) in trained rats.[15]—III and PER at 3 mg/kg blocked the CAR to the extent of 92 and 100%, respectively. II and TFP at this dose exhibited 83.3 and 100% block of CAR, respectively. Thus II and III are only slightly less potent as compared to their parent drugs. I (10 mg/kg) compared well with 3 mg/kg of CPZ (50% block by both) showing thereby that I is one-third as potent as CPZ.

(v) Their protective effects against the toxicity of amphetamine in aggregated mice.[16]—III and PER at 3 mg/kg were equipotent in offering protection (100%) to the toxicity. II provided 80% protection while TFP afforded 100% protection at a dose of 3 mg/kg. CPZ at 3 mg/kg, offered 80% protection while I could provide only 20% protection at this dose.

(vi) Besides this, the solubilities of I–III, CPZ, TFP, and PER were also determined spectrophotometrically at pH 7.4 by following the procedure reported earlier[17] with slight modifications. Their relative solubilities at this pH were thus calculated,[18] keeping the solubility of CPZ as standard, *i.e.*, 1. These values were 1, 0.4, 0.38, 4.58, 0.83, and 0.60 for CPZ, TFP, PER, I, II, and III, respectively.

From these tests it was concluded that the tranquilizing potency of I was about one-third that of CPZ; II was about half as potent as TFP, and III was slightly less potent then PER. Therefore, it may be concluded that tranquilization potency is also decreased along with the decrease of parkinsonism liability.[19] But this decrease is not proportional because, although both II and III exhibit little decrease in tranquilizing potency, there is a marked reduction in the catatonic liability as compared to TFP and PER, respectively. Thus it seems that it is possible to decrease parkinsonism liability greatly, with a little loss of antipsychotic action; probably a positive correlation between extrapyramidal effects and antipsychotic activity does not exist. Perhaps slight disturbances of the extrapyramidal tract are, however, always present with tranquilization. Finally, these studies indicate that II and III may prove to be promising therapeutic agents.

(13) C. Winter, *J. Pharmacol. Exptl. Therap.*, **94**, 7 (1948).

(14) R. A. Turner, "Screening Methods in Pharmacology," Academic Press Inc., London, 1965, p 96.

(15) (a) M. Sidman, *Science*, **118**, 157 (1953); (b) L. Cook and E. Weidley, *Ann. N. Y. Acad. Sci.*, **66**, 740 (1957).

(16) (a) J. H. Burn and R. Hobbs, *Arch. Intern. Pharmacodyn.*, **113**, 290 (1958); (b) L. Lasagna and W. P. McCann, *Science*, **125**, 1241 (1957).

(17) A. L. Green, *J. Pharm. Pharmacol.*, **19**, 10 (1967).

(18) Such a study was undertaken because Green[17] has recently shown that the potent tranquilizing drugs in this series all have relative solubilities of one or less, with CPZ as standard. These findings are thus expected to support the pharmacological data qualitatively if not quantitatively.

(19) The concept that quaternary compounds do not cross blood–brain barrier was largely based on lack of evidence to the contrary and the known resistance of blood–brain barrier to passage of many ionized molecules. During the past decade, reports have been made with increasing frequency and persistence that many quaternaries do reach the CNS to a sufficient degree to induce responses. Some quaternary phenothiazines have been reported to possess an antitremorine activity which makes one suspect the validity of the rule that quaternaries are devoid of direct CNS action because of their inability to pass the blood–brain barrier; see, L. Albanus, E. Hansson, and C. G. Schmiterlow, *Acta Pharmacol. Toxicol.*, **18**, 105 (1961); R. Nowak and K. Femmer, *Naturwissenschaften*, **49**, 470 (1962); also refer to E. J. Ariëns and A. M. Simonis, *Mol. Pharmacol.*, **1**, 53 (1964) (particularly p 97 and the references cited therein); also see, C. J. Cavallito and A. P. Gray, *Progr. Drug Res.*, **2**, 135 (1960) (particularly refer to pp 138–139, 216–218, and 226, and the references cited therein). Our findings also seem to invalidate this general rule and indicate that quaternary compounds of certain types do penetrate the blood–brain barrier to a sufficient degree to induce responses.

Experimental Section[20]

Phenacyl Bromide Oxime.[21]—A mixture of phenacyl bromide[22] (9.95 g, 0.05 mole) and $HONH_2 \cdot HCl$ (12.16 g, 0.175 mole) in 100 ml of MeOH containing 0.5 ml of AcOH was refluxed for 4 hr on a steam bath. The mixture was then poured on crushed ice and the solid which separated was filtered, dried, and crystallized from petroleum ether (bp 60–80°), white needles (75%), mp 88–89° (lit.[21] mp 89.5°).

Phenacyloxime Chlorpromazine Bromide (I).—An aqueous solution of chlorpromazine hydrochloride (5.325 g, 0.015 mole) was treated with 40% aqueous NaOH to liberate the free base which was then taken up in ether. The ethereal layer was then removed and kept over anhydrous Na_2SO_4 overnight and decanted, and the solvent was removed under reduced pressure. The remaining oil (3.185 g, 0.01 mole) was then dissolved in anhydrous Et_2O (*ca.* 40 ml) and to it was added a solution of phenacyl bromide oxime (2.46 g, 0.0115 mole) in absolute Et_2O (*ca.* 40 ml), drop by drop with constant stirring at room temperature. The reaction, being spontaneous, resulted into an immediate precipitation of a yellowish white solid (80%). It was filtered, washed (Et_2O), and dried at 50°. It showed partial swelling at 102° and then decomposed as it melted at 132°; crystallized from EtOH–C_6H_{14} (1:3); mp 132–135° dec; λ_{max} (aqueous EtOH, 10%) 255 mμ (log ε 4.51), 304 mμ (log ε 3.80); ir (principal peaks, cm^{-1}), 3350, 3080, 2890, 2800, 1590, 1565–1570 (doublet), 940, 898, 865, 855, 810, 742, 758, and 707. *Anal.* ($C_{25}H_{27}BrClN_3OS$) C, H, S.

Diphenacyloxime Trifluoperazine Dibromide (II).—From 2.035 g (0.005 mole) of trifluoperazine base and 2.461 g (0.0115 mole) of phenacyl bromide oxime, a light yellow solid (82%) was formed from EtOH–C_6H_{14} (1:3); it exhibited partial swelling at 127° and then decomposed as it melted at 137–39°; λ_{max} (aqueous EtOH, 10%) 258 mμ (log ε 4.62) and 310 mμ (log ε 3.57); ir (principal peaks, cm^{-1}), 3350, 3080, 2890, 2800, 1590, 1565–1570 (doublet), 1320, 1175, 1145, 940, 890, 828, and 765. *Anal.* ($C_{37}H_{40}Br_2F_3N_5O_2S$) C, H, N.

Diphenacyloxime Perphenazine Dibromide (III).—From 1.98 g (0.005 mole) of perphenazine base and 2.461 g (0.0115 mole) of phenacyl bromide oxime, a pale white product (89%) formed from EtOH–C_6H_{14} (1:3); it exhibited partial swelling at 103° and then decomposed as it melted at 120–122°; λ_{max} (aqueous EtOH, 10%) 255 mμ (log ε 4.75) and 310 mμ (log ε 3.765); ir (principal peaks, cm^{-1}), 3350, 3080, 2890, 2800, 945, 855, 810, 770, 755, 740, and 707. *Anal.* ($C_{37}H_{42}Br_2ClN_3O_3S$) C, H, N.

(20) Melting points are by capillary tube method and are uncorrected. Ir and uv absorption spectra were measured on Perkin-Elmer Model 137 and Beckmann Quartz spectrophotometer Model D.U., respectively. Corex cells (1 cm) were used to run uv spectra in aqueous ethanol (10%) while ir spectra were run in KBr disks. These spectra were used in conjunction with microanalyses to ascertain the structures of all products. Zeromatic pH meter was used for pH measurements for finding solubilities at pH 7.4.

(21) On account of the early nature of the work,[21a,b] the modified optimal conditions providing better yields of this compound had to be studied: (a) H. Korten and R. Scholl, *Ber.*, **34**, 1907 (1901); (b) "Dictionary of Organic Compounds," Vol. IV, I. Heilborn and H. M. Bunbury, Ed., Eyre and Spottiswood, 1953, p 81.

(22) J. B. Rather and E. E. Reid, *J. Am. Chem. Soc.*, **41**, 75 (1919).