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CHLORPROMAZINE INDUCED TRACE METAL ALTERATIONS

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CHAPTER I

INTRODUCTION

In 1901 Sir William Osler (23) said: "One of the first duties of the physician is to educate the masses not to take medicine."

Never before has a society been exposed to such an extraordinary array of drugs which alter mood or perception. This has occurred at a time when the indiscriminate use of pharmacologic agents is the norm rather than aberration (226).

There is little doubt that drugs old and new, have reduced morbidity and mortality rates and continue to do so. It is disconcerting, however, to find that improper medication, polypharmacy, and adverse drug reactions are to the point where drugs, either because of their reactions, or economic impact on the family unit, constitute what is approaching a national health problem (219). In some reported series, adverse reactions occurred in 14 per cent of the hospitalized patients and 7 per cent of those reactions were fatal or life threatening (325). In 5 per cent of admissions to a general hospital, drug reactions were a major factor leading to hospitalization (325).

In an attempt to decelerate this trend, the Food and Drug Administration has been given "new teeth." But at this writing "package stuff-

ing" and legislation have not worked, and it is not implausible that the independent physician will likely continue to prescribe as he has done before in defiance of those edicts (248).

Only the most recalcitrant psychoanalyst would hold that disabling symptoms of anxiety should not be treated with medical as well as psychological measures. On the other hand, few responsible physicians would rely solely on drug therapy. Too much evidence indicates that anxiety may result from unresolved intrapsychic conflicts or environmental stresses to ignore these as etiological factors. To the extent that conflicts may be resolved or the environment improved, anxiety may be relieved in part or whole. Drug therapy, therefore, is most logically considered as adjunctive rather than primary in the management of anxiety reactions, psychophysiological disorders, or simply the nervousness of everyday life. Too much reliance on drugs for ameliorating symptoms may deprive patients of other types of treatment, perhaps resulting in more harm than benefit (171).

Various derivatives of phenothiazine, differing in the nature of the substituents in the 2- and 10- positions on the tricyclic structure, are in common clinical use as antipsychotic agents. These compounds have received wide acceptance despite a high incidence of side effects, including pseudoparkinsonism, neuroendocrine abnormalities and hepatic dysfunction. The author has examined chlorpromazine administration, attempting to correlate trace element alterations with metalloenzymes and undesirable effects.

CHAPTER II

LITERATURE REVIEW

In the past decade at least 50 million patients have received chlorpromazine and more than 10,000 publications have dealt with its actions (17, 137). Chlorpromazine and related phenothiazines derivatives are employed primarily in two situations: treatment of psychiatric patients and treatment of nausea and vomiting (137).

Historical Aspects

In 1951 research workers at Rhone Poulenc - Specia Research Laboratories in France discovered the diverse properties of chlorpromazine (thorazine) while searching for new drugs that would have a profound effect on the central nervous system (17, 137, 334).

The phenothiazine nucleus was already well known; phenothiazine had been used as an anthelminthic for livestock, a urinary antiseptic for man and was antihistaminic. Early animal studies of chlorpromazine, however, demonstrated that this derivative had more far-reaching and provocative effects on the central and autonomic nervous systems, the cardiovascular system and the skeletal-muscular system. The drug prevented apomorphine-induced vomiting; blocked conditioned response to stimuli; potentiated and prolonged the action of narcotics, sedatives and anesthetics; caused muscular flaccidity and helped to produce hypothermia.

It was clear that with a compound that possessed such a diversity of pharmacological properties, the scope of its possible clinical applications was extremely wide (334). According to Smith, Kline and French Laboratories (SKF) (334), Laborit and collaborators introduced the use of chlorpromazine in anesthesia and developed its use as an anesthetic potentiator and in producing the state of "artificial hibernation." This state was produced when the "lytic cocktail," (a combination of chlorpromazine, meperidine hydrochloride and promethazine hydrochloride) was administered in combination with external cooling to surgical patients, resulting in hypothermia, slowed metabolism and reduced oxygen requirements. The author reasoned that the patient in this state of physical and mental suspension would be more resistant to the stress of surgical trauma.

Further investigations showed that this hypothermic anesthesia also helped patients to resist psychic stress, and according to SK&F (334) two European psychiatrists, Delay and Deniken, demonstrated that chlorpromazine alone was effective in the treatment of psychiatric disorders (14).

Chlorpromazine proved to have an antipsychotic effect - it would dispel delusions and hallucinations. Secondly, it calmed hyperactive and agitated patients without clouding consciousness or dulling mental faculties (this is because phenothiazine derivatives do not block nerve impulses traveling upward to the cortex). Although at high dosages the drug would produce a sedative effect and even sleep (the result of inhibition of the reticular activating system), patients were easily aroused and, in the absence of their psychotic manifestations, were rational and

cooperative (334).

Further studies in the United States confirmed the clinical value of the drug and, in 1954, SKF released Thorazine (chlorpromazine, SK&F) as a tranquilizer, antipsychotic, potentiator and antiemetic (334).

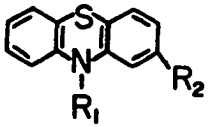
Chemistry

Phenothiazine has a three-ringed structure in which two benzene rings are linked by a sulfur and a nitrogen (Table 1). Usual substitutions are at positions 2 and 10. The addition of a substituent at position 2 increases the potency of phenothiazines for depressing motor activity and conditioned avoidance responses in animals and for altering psychotic behavior in humans. A CF_3 substitution in this position greatly increases antipsychotic and antiemetic potency as well as the tendency to produce extrapyramidal signs (137).

The nature of the substituent at position 10 also influences pharmacological activity (Table 1). The phenothiazines can be divided into three groups on the basis of substitution at this site. The group with an aliphatic side chain includes chlorpromazine, promazine, triflupromazine, and methoxypromazine. This is the least potent group of the phenothiazines, as can be seen by the dosage range. The next group, of approximately the same or somewhat lower order of clinical potency, contains a piperidine moiety in the side chain and includes mepazine and thioridazine. The most potent antipsychotic compounds in terms of effective dose levels are those with a piperazine (or piperazinyl) group, such as fluphenazine, perphenazine, trifluoperazine, etc. These have markedly augmented antiemetic properties and a greater tendency to produce parkinsonism, but diminished sedative effects; they are also the most active

TABLE 1

PROPERTIES OF SELECTED PHENOTHIAZINES

Drug Name	Formula	Usual Antipsychotic Dose (mg)	Extra-Pyramidal Effects	Sedative Effects
Chlorpromazine	 R_1 = $-CH_2-CH_2-CH_2-N(CH_3)_2$; R_2 = $-Cl$	300-800	Moderate	High
Promazine	R_1 = $-CH_2-CH_2-CH_2-N(CH_3)_2$; R_2 = $-H$	500-1000	Moderate	Moderate
Trifluprom azine	R_1 = $-CH_2-CH_2-CH_2-N(CH_3)_2$; R_2 = $-CF_3$	100-300	High	High
Fluphenazine	R_1 = $-CH_2-CH_2-CH_2-N$  $-CH_2-CH_2OH$; R_2 = $-CF_3$	2-10	High	Moderate
Perphenazine	R_1 = $-CH_2-CH_2-CH_2-N$  $-CH_2-CH_2OH$; R_2 = $-Cl$	16-32	High	Low
Trifluoperazine	R_1 = $-CH_2-CH_2-CH_2-N$  $-CH_3$; R_2 = $-CF_3$	3-15	High	Moderate
Thioridazine	R_1 = $-CH_2-CH_2-$  CH_3; R_2 = $-SCH_3$	100-500	Low	Moderate

in suppressing conditioned avoidance responses in animals (137).

All the phenothiazines used in psychiatry have a three-carbon bridge between the ring and side chain nitrogen atoms. This is in contrast to anticholinergic phenothiazines used in the treatment of parkinsonism (e.g., ethopropazide, diethazine) and to antihistaminic phenothiazines (promethazines, pyrathiazine), which have only two carbons separating the nitrogens. One exception should be noted: methdilazine, which produces sedation but lacks significant antipsychotic actions, has three carbons between the nitrogens (137).

In spite of their distinctive pharmacological and psychological effects, Sainsbury (307) maintains that the therapeutic efficacy of phenothiazines does not relate consistently to their chemical structure. According to him, the chemical structural features of the phenothiazines thus far have not provided for a sufficiently constant association between pharmacological, psychological and clinical effects to develop a theory of their mode of action.

Psychopharmacology and Effect on the Nervous System

The phenothiazines are almost unique in the variety of the pharmacological responses which they evoke (277). Chlorpromazine, for example, has activities, depending on dose, ranging from antibacterial and possibly antitumor activity to antihistaminic and tranquilizing properties.

A review of the variability seen in drug responses has been published by Irwin (188). He concluded that the art of predicition and treatment may be enhanced by due attention to at least three factors: (1) the baseline level, (2) the arousal-provoking quality of the environ-

ment, and (3) to the condition of behavioral arousal of the subject as important indicators of his response to drugs. The response to a drug is a complex drug - tissue - personality - environment interaction.

According to Summerfield (351) the most important central effect of chlorpromazine is its singular sedative effect. Animals become quiet and sleepy, remain in unnatural positions (so-called catalepsy), and at higher doses fall asleep. However, they are always readily awakened. Aggressive animals like rhesus monkeys and fighting fish become calm. Anxious animals like rabbits lose their fear.

The site and mechanism of action of chlorpromazine has been the object of many investigations, but no definitive decisions can be reached (139, 301). Some experiments suggested an action on the reticular activating system, but the consensus is that a tranquilizing action can occur at dose levels and in species in which the drug does not depress this system (139).

In the brain, chlorpromazine is said to influence the hypothalamus and the limbic system. The reticular apparatus of the brain stem is depressed with small doses of chlorpromazine; large doses cause a stimulation of the reticular formation. This stimulation results in the appearance of extrapyramidal symptoms, resembling the tremors of parkinsonism in man. Chlorpromazine also inhibits the "arousal reaction," a response of the electrical activity of the brain, recorded in the electroencephalogram (334, 59).

Chlorpromazine is a potent local anesthetic. It blocks epinephrine and is a potent serotonin antagonist. Chlorpromazine is a potent antiemetic, and a weak antihistaminic and ganglionic blocking agent. It

has anticonvulsant properties against nicotine and nikethamide convulsions, but not against strychnine or electroshock. Chlorpromazine has some antipyretic properties; it reduces blood pressure. Respiration is increased with small doses of chlorpromazine; with higher doses there is some depression. Vasomotor centers of the brain are depressed by chlorpromazine, and it potentiates the action of narcotics and analgetics at doses which are not in themselves hyponotic or analgetic. High concentrations of chlorpromazine decrease the conductivity of isolated nerve fibers (334, 17, 137, 139).

Disturbances of the central nervous system may be due in part to a deficiency of nutrient substances (294). This deficiency may be due to a diminished utilization of food by the brain or by an enhanced requirement of energy in consequence of stress phenomena. Furthermore, it has been known for many years that a marked reduction in blood sugar, reduced oxygen pressure in the air, or diminished cerebral blood flow can produce psychotic symptoms. In addition, it is known that a latent psychosis can be activated by sleep deprivation or by certain central stimulants.

In mice 1-5 mg/kg of chlorpromazine orally or subcutaneously produced a reduction in spontaneous motor activity proportional to the dose (65). Activity in mice was reduced about 50 per cent for the first hour after a dose of 2 mg/kg subcutaneously, and completely suppressed at 5 mg/kg. Even at the latter dose, however, the mice showed no ataxia or prostration and responded readily to stimuli with coordinated activity. This behavior is in marked contrast to the effects of barbiturates, which caused reduction in spontaneous motor activity only at doses which

produced ataxia or hypnosis. Some of the chlorpromazine effect was still evident 5 hours after small doses and it was partially antagonized by large doses of pipradrol (109).

Investigators have speculated whether chlorpromazine, by reducing motivational strength, would eliminate sterotyped behavior caused by pretrial submersion of rats swimming in a water T-maze. However, chlorpromazine produced severe behavioral disorganization and decreased learning and retention of both sterotyped and nonsterotyped responses. The results suggested that chlorpromazine may affect sensory input and effective integration of sensory data rather than motor incoordination or motivational strength (245).

In a study of the effect of reserpine, chlorpromazine, and meprobamate on learning in the newborn, the drugs were given to gravid rats and the offspring were measured for learning activity. Only the offspring of the meprobamate-treated mothers required significantly more trials to reach a control level of learning (368, 256).

The sexual behavior of male rats under the influence of chlorpromazine has been reported by Zimbardo and Barry (382) and by Gillett (128). A reduction in rate of copulation, the number of copulations and the latency period was observed, but there was a wide range of individual variation.

Chlorpromazine has the unusual ability to suppress responses to auditory or visual conditioned stimuli in doses which do not markedly alter responses to unconditioned stimuli. In rats conditioned to climb a pole at the sound of a bell, pretreatment with 2 mg/kg of chlorpromazine subcutaneously blocked response to the bell in about 40 per cent of

the trials (65, 6, 13, 112). Chlorpromazine doses of 10 mg/kg blocked the conditioned response in about 85 per cent of the rats, although the animals were capable of responding to an unconditioned stimulus--namely an electric shock to feet (65, 6, 10, 112). In a similar situation, 0.5 mg/kg of chlorpromazine reduced the response of rats to an auditory stimulus and higher doses abolished the response, without loss of muscular coordination (70).

Grossman and Miller (149) reported the evaluation of alcohol and chlorpromazine as fear-reducing drugs. A variable-length "telescope alley" apparatus was used, and the procedure involved approach training and avoidance training with and without drugs. Two testing conditions were measured, i.e., fear and fear-plus-pain (electroshock). Hungry rats tested under alcohol ran reliably faster and farther toward food than did controls in both fear and fear-plus-pain tests. Chlorpromazine-treated rats consistently ran farther and faster than controls in fear-plus-pain tests but slower than did controls in fear only test (19).

Davis et al. (80) studied the acquisition of a fear-motivated response to chlorpromazine. In their procedure training was sequential so that drug effects on the conditioning process could be analyzed. Chlorpromazine inhibited establishment of an association between conditioned stimulus and internal fear-motivated behavior and its drive and cue function. The data indicated that chlorpromazine might prevent progressive extension of psychoneurotic symptoms. Chlorpromazine also promoted persistent extinction of fear-conditioned response (271).

Irwin (187) showed that tolerance development to chlorpromazine was considerably accelerated by increasing the intensity of the condi-

tioned (buzzer) or the unconditioned (shock) stimulus in rats.

The effect of drugs on conditioning and habituation to arousal stimuli in animals was studied by Key and Bradley (203). Chlorpromazine increased threshold for both conditioned (behavior) and unconditioned (EEG) arousal response to auditory stimuli in cats and eventually blocked arousal response completely.

Pigeons trained to respond to a red light with a fixed ratio response (rewards given after a given number of pecks) and a blue light with a fixed interval response (reward after 5 minutes), still differentiated between the colored lights after 1.7 or 3 mg/kg of chlorpromazine i.m. (92). There was no interference with fixed rate performance, but long pauses developed during fixed interval periods.

In a study of temporal discrimination, pigeons were trained to peck a lighted key and were presented with the key alternately dark and lighted. The key was dark for interval of 3-30 seconds. Pecking of the lighted key was reinforced only after the shortest or, in a second experiment, longest interval that the key was dark. The pigeons were able to estimate the duration of dark interval. Chlorpromazine attentuated discrimination of duration of a stimulus but did not abolish pecking (299).

Thompson (354) studied the effects of chlorpromazine on "aggressive" responding in rats trained in lever pressing on a regular water-reinforcement schedule. The results showed that chlorpromazine-treated animals made fewer responses both before and after onset of extinction but exhibited relatively greater acceleration in rate of responding immediately after onset of extinction as compared with saline control group.

In schizophrenic patients and in monkeys with implanted cortical and subcortical electrodes, chlorpromazine produced a record resembling that of drowsiness and light sleep (250). Essig and Carter (105) have reported that chronic administration of high doses of chlorpromazine (44-77 mg/kg i.m.) produced major convulsions and behavior suggestive of hallucinations in monkeys (Macaca mulatta).

Electroencephalographic studies in normal human subjects, after intravenous or intramuscular administration of chlorpromazine, have shown patterns associated with fatigue, drowsiness, or sleep, depending on the doses used (95, 135). In patients given chlorpromazine, the changes induced by intravenous hexobarbital were intensified and occurred sooner (135). In epileptic patients with normal resting EEGs showed an activation of paroxysmal discharge while those with abnormal resting EEGs showed intensification of abnormal patterns (107).

Endocrine and Metabolic Effects of Chlorpromazine

Many of the chemical changes influenced by the administration of phenothiazines have been postulated as being responsible for tranquilizing actions, including depletion of epinephrine and its precursors, increased oxidation of epinephrine, chelation of epinephrine, combination with indole, and competitive inhibition of indoles (5).

Rats given single subcutaneous or oral doses of chlorpromazine from 10-50 mg/kg usually showed little change or a slight decrease in oxygen consumption, which appeared to be in proportion to the decrease in body temperature (36, 70). After administration of 20 mg/kg/day, this metabolic effect had disappeared. No antagonism to the metabolic effects of thyroxine was found (36).

In human subjects, basal metabolic rate changed little after chlorpromazine doses up to 2 mg/kg (95, 94, 350). Similarly, cerebral oxygen consumption was not changed by chlorpromazine in intravenous or intramuscular doses of 50-300 mg, unless the blood pressure dropped considerably (252).

Chlorpromazine (30 mg i.v.) increased serotonin in the spleen, decreased serotonin in the heart, and had little effect on serotonin in the visceral tissues of rabbits previously given 5-hydroxytrytophan-3-C^{14} (10 μg i.v.); LSD and BOL (bromolysergic acid diethylamine) (500 μg i.v.) increased serotonin levels in heart and BOL decreased them. Chlorpromazine and BOL decreased serotonin in all parts of brain (310).

In a study of drug induced alterations in the subcellular distribution of 5-hydroxytryptamine in rat's brain, Schanberg and Giarman (315) reported that CNS depressants (chlorpromazine, reserpine, phenobarbital, and methylparafynol) significantly increased the proportion of free 5-HT, regardless of whether they increased, decreased or had no effect on total 5-HT. LSD-25, imipramine, and β-phenylisopropylhydrazine increased the total 5-HT, accounted for by greater increase in bound than in free 5-HT.

The effect of drugs on amino acid levels in rat brain was studied by DeRopp and Snedeker (88). Convulsive agents and CNS stimulants, including amphetamine, significantly increased free alanine in brain. Chlorpromazine and methoxypromazine significantly increased brain glutamine and alanine. The authors concluded that there is correlation between the rise in brain alanine and convulsant or excitant activity.

Chlorpromazine (20 mg/kg i.p.), simultaneously with or 17 hours

after iproniazid, inhibited or reversed the effects of iproniazid on catecholamines and serotonin in rat brain. The authors concluded that the effects of chlorpromazine may either be due to a hypothermic or a direct-specific effect (42, 10, 237).

Chlorpromazine consistently increased epinephrine content of various areas of dog brain, whereas small doses increased and higher doses decreased norepinephrine levels (234, 89, 142).

Chlorpromazine was found by Burton and Salvador (44) to be bound to nicotinamide-methlpherase in vivo in mice, and in vitro. In the presence of this drug, enzyme activity was increased but the affinity of substrate (nicotinamide) was reduced. Competitive inhibition between chlorpromazine and nicotinamide may also be a factor. The relationship between the drug-enzyme complex and psychophysiology was speculated on.

Van Peenen and Way (362) noted that 3 mg/kg of chlorpromazine, given one hour earlier, blocked normal adrenaline-produced adrenal ascorbate depletion but not that of histamine or aspirin. Gold et al. (130) in a study on 20 psychiatric patients reported that the normal two-fold rise in urinary 17-ketogenic steroids following intravenous administration of mepyrapone was impaired by chlorpromazine (100 to 200 mg daily).

Holzbauer and Vogt (176) reported no effect of chlorpromazine on ACTH secretion in rats. Nasmyth (260) found that 2.5 mg/kg chlorpromazine in the rat did not, itself, stimulate ACTH release and failed to prevent ascorbate depletion after adrenaline, surgical stress, or high doses of histamine.

Egdahl and co-workers (100, 101) reported that 3.5 mg/kg of chlorpromazine in the dog produced significant adrenal response as meas-

sured by 17-OHCS levels in adrenal venous blood after i.v. administration of the drug. Saski (312), in guinea pigs, and Harwood and Mason (154) in monkeys, found increases in circulating adrenal steroids following chlorpromazine administration. Sapieka (311) reported that 50, 150, and 300 mg/kg of chlorpromazine in the rat in single oral doses increased ACTH release as measured by ascorbate depletion.

Smith et al. (338) and Buckley et al. (41) suggest that other investigators who found blockade of the stress-response following chlorpromazine administration may have been examining the system only after the pituitary ACTH stores had been exhausted.

Polishuk and Fligelman (284) reported that amenorrhea was found in six women out of eleven treated with chlorpromazine (150-200 mg i.v.) and in all four women treated with higher doses (350-400 mg i.v.). This amenorrhea was found to be of the low estrone, normal follicle stimulating hormone (FSH) type for the first group, but marked increases in FSH were reported for the higher dose group.

Chlorpromazine has been shown to increase blood glucose levels and to counteract insulin hypoglycemia in animals (194, 305) yet, it has also been noted that chlorpromazine causes a mild hypoglycemia and does not prevent the hypoglycemia produced by adrenaline in the rabbit (37).

Charatan and Bartlett (56) reported that chlorpromazine caused abnormal glucose tolerance curves in schizophrenic patients. Amdisen (7) performed glucose tolerance tests on a group of patients during long-term treatment with chlorpromazine. Very few patients showed glycosuria or abnormally high fasting blood sugar levels. However, glucose tolerance tests of the diabetic type were found in 40 per cent of the patients on

chlorpromazine.

Human subjects given 25-50 mg of chlorpromazine intramuscularly or 1.5 mg/kg intravenously showed a slight tendency to increased blood sugar one hour later (95). No change was noted in the response to intravenous insulin of patients pretreated with chlorpromazine (216), and no change in the hyperglycemic response to surgical stress was observed (16).

Insulin potentiates the hypothermia and prolonged the hexobarbital sleeping time normally caused by chlorpromazine; chlorpromazine does not potentiate the hypoglycemic effects of insulin (222).

Significant weight gain is commonly observed among patients taking phenothiazine (62). Physical inactivity and a high-calorie diet were considered the most likely contributing causes for the weight gain during hospitalization (138).

Chlorpromazine has been shown to produce uncoupling of oxidative phosphorylation in various preparations of liver and brain (27, 81). Like dinitrophenol, chlorpromazine also inhibits adenosinetriphosphatase activity (228, 85).

In mitochondria from the brains and livers of rats, chlorpromazine and imipramine both strongly inhibited oxygen uptake and phosphorylation, but on a molar basis chlorpromazine was twice as active as imipramine. The uncoupling effect was not very marked. No marked specificity for brain versus liver mitochondria was observed. The inhibitory effects were independent of the substrate (227, 1).

Oxygen uptake by slices of brain cortex showed no change during the first 0.5 hour after the addition of 20-251 ml/l of chlorpromazine,

remained steady for the next 0.5 hour and then decreased in proportion to the concentration of chlorpromazine (274).

Chlorpromazine reduced phospholipid turnover in brain according to Ansell and Dohmen (8) but Magee and Rossiter (233) and Grossi et al. (148) reported an increase for low levels of the drug.

Iriye and Simmonds (186) found that the effect of chlorpromazine on the phosphorylase level of rat brain differs according to the mode of sacrifice. When control rats are sacrificed by immersion in liquid nitrogen, the level of brain phosphorylase is lower than when the rats are sacrificed by inhalation of chloroform. Chlorpromazine depresses the phosphorylase level of rats immersed in liquid nitrogen but enhances the enzymatic activity of brains of rats sacrificed with chloroform. The enhancing effect produced by chloroform on the enzyme level as well as the further enhancing effect of a pretreatment with chlorpromazine are not peculiar to chloroform, since the same effects can be reproduced by nitrogen gas asphyxia.

According to Masurat et al. (239) chlorpromazine in concentrations from 8.5×10^{-6} M to 6.8×10^{-5} M, either greatly activated or inhibited hexokinase activity, determined by measuring spectrophotometrically the reduction of TPN (triphosphopyridine nucleotide). The biphasic action of chlorpromazine was a function of the relative concentration of drug and Mg-ATP substrate. The author indicated that chlorpromazine may function as metabolic regulator, primarily by interfering with utilization of ATP.

Burton and Salvador (44) studied the effect of chlorpromazine, nicotinamide, and nicotinic acid on pyridine nucleotide levels of human

blood. Nicotinic acid (0.5-0.8 g/day p.o.) increased blood pyridine nucleotide four- to six-fold in human subjects, whereas nicotinamide (0.5-1.1 g/day p.o.) did not. Chlorpromazine (50 mg/day p.o.) did not alter nicotinic acid-induced increase in blood pyridine nucleotide levels.

Glende and Cortnatzer (129) found that chlorpromazine and other phenothiazines inhibited the uptake of S^{35} into the sulfolipids.

Chlorpromazine significantly inhibited various types of mitochondial swelling in rat brain and liver preparations. The effects were noted at concentrations as low as 10^{-6} M for brain using triton as the swelling agent, or at 10^{-7} M for liver with thyrazine as the swelling agent. The authors stated that the concentrations of chlorpromazine were less than those necessary to produce changes in enzyme activities or oxidative phosphorylation; thus, chlorpromazine changes membrane permeability (341, 342, 335).

The effect of some phenothiazine derivative on the hemolysis of red blood cells in vitro was studied by Freeman and Spirtes (124). The compounds studied in human and dog erythrocytes were chlorpromazine, chlorpromazine sulfoxide, promazine, and promethazine. Chlorpromazine caused the greatest increase in hemolysis time; prochlorperazine was effective at the lowest concentration. The data suggest that phenothiazines decrease permeability of erythrocytes to water by altering cell membrane structure.

Chlorpromazine (5 or 40 mg/kg s.c.) produced a 13-19 per cent increase in survival time of leukemic mice. However, doses of 17 or 33 mg/kg had no effect (210).

It has been suggested that chlorpromazine alters the permeability of various membranes to substances like norepinephrine and serotonin, and the antipsychotic activity of chlorpromazine has been attributed to its membrane effects (190, 151, 384).

Serum alkaline phosphatase was determined at the start and during the course of treatment of patients with 350 mg chlorpromazine per day. During the first three weeks of treatment a significant elevation in enzyme activity was obtained in 2/3 of the subjects, which exceeded the upper limit of normal at times. In no instance was jaundice present. The elevation in enzyme activity spontaneously regressed in face of continuing treatment with the drug (165).

On the other hand, Caverns (54) in a similar study found that at 1-3 hours after parenteral administration of 0.025 mg chlorpromazine and of 0.100 mg orally the alkaline phosphatase activity decreased in the men and women. In ten mental patients who were treated for 1-10 months with 300 mg chlorpromazine daily no appreciable variation of the alkaline phosphatase occurred.

However, several other investigators (380, 46, 177, 241) in studying the activity of alkaline phosphatase in blood plasma, serum, or blood during treatment of schizophrenia with chlorpromazine found no change in alkaline phosphatase activity.

Kusch and Heinvich (211) reported, after a single dose of 5 mg of chlorpromazine/100 g body weight in rats, a marked increase in alkaline phosphatase in the liver within 18 hours. This increase was accompanied by a decrease of alkaline phosphatase in the plasma.

Dasgupta and Mukherjee (75) reported that chlorpromazine de-

presses the alkaline phosphatase activity of both cytoplasm and the nucleus of the brain cells, particularly those known to be normally rich in ribonucleic acid.

The effect of 10 compounds with psychotomimetic or tranquilizing activity on alkaline phosphatase and lactic and malic dehydrogenase activities of brain preparations was studied. Chlorpromazine significantly increased alkaline phosphatase. Malic and lactic dehydrogenase was relatively resistant to the compounds tested (63).

Phosphatase activity in the rat brain, measured at pH 7.2-7.5 with P-nitrophenol phosphate as a substrate, was markedly increased by chlorpromazine *in vitro* and *in vivo*. The results from the comparative *in vitro* studies with chlorpromazine and a detergent, Sterox, indicated that the increase by chlorpromazine was not due to its ability to solublize proteins from the membranes of subcellular structures, but probably due to its ability to reveal active sites of phosphatase which are normally masked under normal conditions (91).

Injection of chlorpromazine produced a 6-7-fold increase in the activity of the acid phosphatase inhibited by L-tartrate (type III), even after castration. There was no causal relation between serum phosphatase (type II) and weight changes of the prostate (212).

Ashby *et al.* (11) reported that chlorpromazine caused an increase in CNS carbonic anhydrase in the 3 parts of the CNS studied. Chlorpromazine caused large increases in the carbonic anhydrase concentration of the kidneys.

The influence of chlorpromazine on liver arginase was studied. A clear inhibitory action could be shown in the *in vitro* experiments up

to a concentration of 1 x 10^{-5} M, but no decrease of liver arginase activity was found in the *in vivo* experiments after 89 to 93 days of treatment (206).

Chlorpromazine has been reported to produce a decrease in aconitase in kidney particle preparations due to complexing of the drug and enzyme (276). In an interesting study, Khouw *et al.* (204) reported that chlorpromazine increases blood levels of alcohol by inhibition of the conversion of ethanol to acetaldehyde.

In rat brain homogenates chlorpromazine decreased the cytochrome C content, but only at high concentrations. A subcutaneous dose of 50 mg cpz/kg body weight only weakly affected the cytochrome C in the brain (126).

When fresh frozen sections from the brains of Sprague-Dawley rats were incubated in a medium containing 0.001-1 mM chlorpromazine or trifluoperazine, the cytochrome C oxidase of various areas of this tissue was inhibited. The histochemical technique employed permitted the degree of inhibition of cytochrome C oxidase to be related to the tissue concentration of the drug rather than to the concentration of drug in the incubating medium (251). However, when large doses of phenothiazines (150-200 mg/kg) were administered intraperitoneally to rats, no significant inhibition of cytochrome C oxidase was observed. The tissue concentration of the drug *in vivo* was less than the *in vitro* tissue concentration required to produce a minimum inhibition of cytochrome C oxidase. Brain tissue evidently was able to concentrate phenothiazines 100-150-fold (251).

Yamamoto *et al.* (377) reported that chlorpromazine inhibited

the activity of cytochrome c and oxygen uptake 55.8 per cent at concentrations of 5×10^{-4} M; but the S and N - oxides of chlorpromazine showed no effect on the activity, even in concentration of 2.5×10^{-3} M.

Inhibitory effect of chlorpromazine on cytochrome oxidase of rat liver mitochondria or rat brain homogenate was investigated (376, 82, 83, 84). The inhibitory effect of chlorpromazine on cytochrome oxidase was reversed by dialysis of chlorpromazine with enzyme preparations; decreased by $FeCl_2$, and not affected by glutathione. Chlorpromazine inhibited catalose, but not homogentisic case or ascorbic acid oxidase (376).

Chlorpromazine was reported to inhibit D-amino acid oxidase as a result of competition with flavin adenine dinucleotide (372, 373, 374, 375, 223, 227).

Chlorpromazine and other phenothiazine derivatives were found to inhibit the succinoxidase activity most strongly. In all cases, cytochrome oxidase was more sensitive and succinic dehydrogenase less sensitive to inhibition than was the complete succinoxidase system. Brain succinoxidase was less sensitive to inhibition than was liver succinoxidase (337, 159).

Johannesson and Lausen (191) studied the <u>in vitro</u> effect of chlorpromazine on brain cholinesterase. They found that chlorpromazine at 100 μg/g inhibited cholinesterase activity in brain tissue from an infant. The authors concluded that excitation seen as side effect of chlorpromazine (parodoxical action) is due to its inhibition of cholinesterase.

Chlorpromazine and its sulfoxide inhibited the cholinesterases of human plasma and red cells. The former drug was more active than the

latter and the plasma enzyme was more sensitive than that of the red cells. The inhibition was competitive in type (104).

Cholinesterase activity of 8 rats was determined following injections of 15 mg chlorpromazine per kg of body weight. The slight increase was not significant statistically (74).

Aminazine, (Russian name for chlorpromazine) 1 - 2 mg/kg, increases the level of cholinesterase activity in the blood of the animals. This level of the cholinesterase activity in the blood of the animals varies from individual to individual, remaining, however, the same in each animal. The changes in enzyme activity proceeds in phases. The dogs used for this experiment had permanently implanted electrodes in the rostal region of the reticular formation (150).

Chlorpromazine in a concentration below 10^{-3} M significantly inhibited the esterase activity of human blood plasma on acetylcholine and succinylcholine (327).

Chlorpromazine had no effect upon lactic dehydrogenase isolated from anaerobic yeast cultures (238). However, Chagovets and Lakhnol (55) reported that lactic, glutamic and citric dehydrogenase activity in cerebral cortex, cerebellum and skeletal muscles studied was greatly depressed (70-75% decrease).

Valdiguie et al. (359) reported that treatment with 600 mg chlorpromazine showed maximum serum lactic dehydrogenase value of 1207 units. These values occurred at 18 hours.

Some authors reported an increase in serum GOT and GPT after chlorpromazine treatment (275, 213).

Cats were given aminazine, 5 or 10 mg/kg wt. intramuscularly,

and killed 1 hour later. The activities of glutamic dehydrogenase and cytochrome oxidase in the homogenates of intervertebral ganglia and that of cytochrome oxidase in Gaulle and Burdach's nuclei were increased. On the other hand, succinate dehydrogenase and cytochrome oxidase activities were decreased in ventrolateral thalamic nuclei and the activities of all 3 enzymes in the motor area of the cerebral cortex. Similar but more pronounced effects were found in isolated mitochondria (98).

Results of experiments with rabbits and rats showed that the level of citric acid in blood serum of rabbits was relatively constant and did not change following injection of aminazine. Some rise in the citric acid content of serum occurred in rats which received toxic doses of aminazine and may have been caused by ensuing hypothermia (268).

Gielen (127) reported that intraperitoneal injections of 5 mg chlorpromazine increased serum ornithine carbamyl transferase, lactic dehydrogenase, oxyloacetic-glutamic transaminase, malic dehydrogenase and isocitric dehydrogenase within 12 hours after administration to rats, while serum pyruvic-glutamic transaminase and 1,6-diphosphofructoaldolase and hepatic lactic dehydrogenase showed no significant changes. Subcutaneous injections of chlorpromazine (0.5 mg 3 times/week for 8 weeks) increased the level of hepatic isoenzyme lactic dehydrogenase (fraction 5) during the 5th to 7th week of administration, although hepatic and serum lactic dehydrogenase and serum oxalacetic-glutamic transaminase, malic dehydrogenase, and ornithine carbamyl transferase were unchanged (127).

Serum total proteins were unchanged in patients undergoing chlorpromazine therapy; serum globulin tended to be increased and albumin

decreased (236, 257). Electrophoretic study of serum proteins in nine patients showed a double β-globulin peak in all of them. Such a "beta split" has been reported in various states involving tissue damage (infectious disease, acute rheumatism); its significance in relation to chlorpromazine is uncertain (355).

Nakajima (259) reported that the inactivation of serotonin by ceruloplasmin is not accelerated by lysergic acid diethlamide-25 (LSD), is accelerated by iproniazid, and is accelerated by chlorpromazine and EDTA but not to completion. The author concludes that iproniazid acts specifically on the serotonin-ceruloplasmin complex, and that chlorpromazine is a nonspecific inhibitor. From the action of EDTA, inactivation of serotonin depends primarily on protein portion of ceruloplasmin, whereas for inactivation of adrenaline by ceruloplasmin, Cu^{++} is most important (259).

A study was made of the effects of large doses of chlorpromazine and levomepromazine on the <u>in vitro</u> formation of noradrenolutin and noradrenochrome in the serum of seven mental patients. Variation in copper concentration in the seven sera was also determined. Before administration of the drugs the blood copper level was high; there was active production of noradrenolutin and a low rate of noradrenochrome product. Following drug administration, there was a decrease in blood copper level as well as noradrenolutin production whereas the noradrenochrome level increased. These results corresponded with clinical improvement of the patients (317).

Chlorpromazine at a concentration as high as 1×10^{-2} had no inhibitory effect on human ceruloplasmin activity <u>in vitro</u> (71).

Nunez (265) investigated the plasma copper level of 50 mental patients (27 women, 23 men, ages 14-73 years). After the administration of 0.025 g chlorpromazine intramuscularly to the male patients, and 0.050 g orally to the female patients, the value was determined in the men 1 hour, and in the women 1.5 hours later. In nearly all patients the copper level in the plasma decreased. In manic depressive patients it increased.

The copper concentration of the blood plasma was determined in 25 psychiatric patients before treatment with chlorpromazine and after 1, 3, and sometimes 5 and 7 weeks of treatment. In the majority there was a gradual rise in copper concentrations; in twelve patients definitely abnormal concentrations (above 160 mg/100 ml) were reached; five of these showed extrapyramidal signs. Similar determination in twenty-five patients without chlorpromazine showed no rise in copper concentrations (16).

Daily intramuscular injections of two tranquilizers (chlorpromazine or perphenazine) in guinea pigs caused increases in the copper in the caudate nuclei and cerebellar cortex in the absence of administered copper. Liver copper was also increased (168).

Chlorpromazine, 50 mg daily per orally, inhibited the diurnal variation in serum iron, but the inhibitory effect was not so evident in serum copper (353).

Chlorpromazine, 2 mg/kg intramuscularly, to thirty-six children caused some drop of basal blood iron and a lowering of gastro-intestinal absorption (iron gluconate administration). The curve after iron saccharate intravenous administration was higher if chlorpromazine was also

associated. The author ascribes the above action of chlorpromazine to a metabolic torpor of the main deposit organs, because of the macrobiotic action of the drug (340).

Effect on Body Temperature

Chlorpromazine lowers body temperature in various laboratory animals, the extent of the change depending on room temperature and chlorpromazine dose. This hypothermic action was initially attributed to inhibition of central thermoregulation, with resulting increased heat loss, and muscular relaxation, with decreased heat production. Later speculation involved effects on catecholamines (192). In mice kept at 25°C, 2 mg/kg of chlorpromazine subcutaneously produced an average minimum temperature of 32.8°C, 5 mg/kg a minimum of 31.9°C, and 10 mg/kg a minimum of 30.2°C (70). With the higher doses, temperatures remained below normal for over 24 hours. The hypothermic effect in dogs was less marked.

In another study in mice, the average maximum decrease in body temperature after 1 mg/kg was 3°C; after 3 mg/kg it was 5.7°C (208). When the high dose of 50 mg/kg was given intraperitoneally to mice kept at a room temperature of 27°C, body temperature fell to a low of 23.1°C within an hour and then gradually rose to normal (86). The same dose given to mice kept at 3°C reduced body temperature to an average of 11.7°C in 1.5 hours (86). With doses of 20 or 30 mg/kg, body temperature depended on ambient temperature, increasing slightly in a warm room and falling to 17.6°C after 6 hours exposure to 10°C. After single doses of chlorpromazine, body temperature effects in rats were about the same as those in mice, again depending on room temperature (86).

Fenters and Jeter (110) reported on the effect of variation in

body temperature on antibody production in rabbits and mice. Chlorpromazine, 5 mg/kg i.m. at 12-hour intervals or intra-abdominally, alone or with refrigeration, depressed body temperature of control rabbits and those immunized with bovine serum albumin or mice immunized with Diplococcus pneumoniae vaccine. Pyrogen treatment increased temperature. However, alterations in body temperature had no effect on antibody production in immunized animals.

Human subjects given ordinary doses of chlorpromazine show inconsistent temperature effects. When lightly anesthesized patients were given 50 mg intravenously and 100 mg intramuscularly, reinforced by doses of 50 mg intramuscularly every hour or two, body temperature was maintained at 92-94°F for long periods (299).

The antagonistic effect of cold acclimatization on chlorpromazine hypothermia is reported to be associated with increased sensitization of tissues to adrenaline and noradrenaline (218, 219, 220, 221). The acute toxicity of chlorpromazine in mice has been found to be eighteen times higher at a room temperature of 4°C than at 30°C (72). This may be due to vasodilatation and associated phenomena.

It is becoming more and more apparent that many of the previously reported effects of chlorpromazine <u>in vivo</u> were the result of hypothermia rather than a direct effect of the drug. For example, the increased incorporation of labeled amino acids into tissues, and the increased concentrations of NAD and nicotinamide which have been observed in chlorpromazine-treated animals are related to hypothermia (328), as is also the prevention by chlorpromazine of iproniazid acceleration of serotonin accumulation in the brain (18).

Fortunately, animals which have received chlorpromazine can be maintained in an isothermic condition if they are kept in an environment of elevated temperature, and most *in vivo* work is now done with animals so maintained.

Effects on Activity of Other Drugs

The duration of hypnosis after hexobarbital was more than doubled by 5 mg/kg of chlorpromazine (i.p.), but no alteration in the rate of metabolism of the barbiturate or its concentration in brain tissue could be demonstrated (39). When chlorpromazine was given subcutaneously a 0.5 hour before intravenous hexobarbital, a combination of 30 mg/kg of barbiturate and 15 mg/kg of chlorpromazine had an effect equivalent to 150 mg of hexobarbital alone in rats (70). Similar potentiation has also been observed in guinea pigs and dogs, as well as rabbits (306). Other investigators have reported potentiation of hexobarbital hypnosis in mice (12, 112) and rats (346).

The potentiating effect of chlorpromazine appears to extend to all sedative barbiturates, as well as ethinamate (231). In mice, doses of 2.5-5 mg/kg of chlorpromazine prolonged sleeping time after pentobarbital (208). In rats, doses of 10-50 mg/kg were effective in combination with thiopental, pentobarbital, and amobarbital, and 100 mg/kg of chlorpromazine moderately prolonged secobarbital sleeping time (334, 161).

Childs *et al.* (58) found that pretreatment with chlorpromazine decreases the induction time of barbital anesthesia without increasing the rate of brain penetration. The data suggested that reduced induction time does not necessarily imply increased permeability of the blood-brain barrier; it may have resulted from increased sensitivity of brain

cells to barbital or hypothermia produced by these drugs.

On the other hand, Kato and Chiesara (198) reported an increase of pentobarbital metabolism induced in rats by chlorpromazine and other drugs, with a decrease in sleeping time.

The subcutaneous administration of chlorpromazine in mice, rats, and guinea pigs shortened the time of onset of ether anesthesia, and its duration was considerably increased at doses of 1.25-20 mg/kg (70). The incidence of anesthesia in rats exposed to 80 per cent nitrous oxide was increased by chlorpromazine at 20 mg/kg. In rats exposed to 70 per cent nitrous oxide the incidence of anesthesia was increased by 50 mg/kg of chlorpromazine (s.c.) (50).

Chlorpromazine in doses of 0.25-10 mg/kg (s.c.) eliminated the excitement stage and significantly prolonged the hypnotic effect of intraperitoneal or oral alcohol in rats (70). A similar effect was seen in mice, although the rate of alcohol metabolism was not affected (39). The toxicity of alcohol in mice was slightly but significantly increased by doses of 0.5 mg/kg of chlorpromazine.

In mice pretreated with chlorpromazine there was a marked increase in the analgesic effects of morphine, meperidine, aspirin, salicylamide, and phenacetin (70). The rate of tolerance development to morphine was not retarded by chlorpromazine in rats (240). On the other hand, Carter and David (51) report that chlorpromazine in rats significantly decreased the rate of tolerance development to narcotic analgetics and hastened recovery during withdrawal.

The duration of action of the maximally effective dose of morphine, 10 mg/kg was markedly prolonged by 10 mg/kg of chlorpromazine

(319). On the other hand, a different group reported that minimally effective morphine dose 1.5 mg/kg plus chlorpromazine at 10 mg/kg gave a lower incidence of analgesia than chlorpromazine alone (208).

The centrally acting phenothiazines like chlorpromazine were found by Fink and Swinyard (112) to decrease amphetamine toxicity. The weight reducing properties of phenmetrazine were reported to be antagonized by chlorpromazine (297).

A study was done in rats by Stein and Seifter (344) to support the theory that drugs which are effective against agitation inhibit, whereas those effective in depression enhance, reward (self stimulation) activity. Imipramine, 5-15 mg/kg, augmented and prolonged d-methamphetamine-induced self stimulation; in contrast, chlorpromazine antagonized both methamphetamine-induced self stimulation and also the augmenting effect of imipramine on the same stimulation. The authors concluded that imipramine has a sensitizing effect on the central adrenergic synapses.

Chlorpromazine has been found by Elder and Dille (102) to antagonize the behavioral effects of LSD, although it inhibits the metabolism of it (14). Ray and Marrazzi (296) have studied quantitative relationships of LSD and chlorpromazine effects. LSD-25, in doses 0.19 mg/kg small enough to be devoid of gross effect, increased response latency in rats to a tone indicating the availability of water reward. This effect was greatly reduced by chlorpromazine in doses 0.30 mg/kg, that <u>per se</u> did not affect performance. On the other hand, larger but nondepressant doses (1 mg/kg i.p.) of chlorpromazine increased rather than reduced LSD-25 inhibition, whereas still larger doses produced depression of approach behavior.

Doses of LSD sufficient to dilate pupils, but producing no other physiological effect, 25 μg, could be recognized by human subjects (253). Recognition of the threshold dose could be blocked by 25 mg/kg of chlorpromazine or 10 mg/kg of phenoxybenzamine if given 30 minutes before the LSD (255).

The effects of chlorpromazine and lysergic acid diethylamide on the arousal response were reported by Key (201, 202). Chlorpromazine, 5 mg/kg or 10 mg/kg, increased the intensity of stimulation necessary to elicit arousal response in cats with permanently implanted electrodes. After LSD, 10-15 μg/kg i.p., presentation of an auditory stimulus was followed by abrupt arousal and long-lasting periods of alerting.

Chlorpromazine antagonized the EEG alerting produced by cholinergic agents, especially eserine salicylate and acetycholine in rabbits (346). Cholinesterase inhibitors potentiated the behavioral effects of chlorpromazine (132).

A study of the interaction of nicotinamide with chlorpromazine in the mouse showed that nicotinamide alone reduced spontaneous motor activity in mice, whereas chlorpromazine, 10 mg/kg, reduced, and chlorpromazine plus nicotinamide completely inhibited, activity. Nicotinamide alone had little effect on pentobarbital anesthesia but did potentiate the effect of chlorpromazine and reserpine on pentobarbital anesthesia (45).

Chlorpromazine was reported not to enhance the renal toxicity of edathamil sodium (a chelating agent, ethylenediamine-tetracetic acid calcium disodium salt) in rats (4).

Histamine catabolism during treatment with chlorpromazine was

studied by Johannesson et al. (191). Chlorpromazine, 600 and 750 mg, did not affect catabolism of labeled histamine in two psychiatric patients. These doses were twice those required for therapeutic improvement.

Levin (224) reported that cyanide intoxication is antagonized by chlorpromazine in rodents. The protective effect is thought to be due to the hypothermia induced.

Metabolism

Many thousands of chronic mental patients successfully rehabilitated by phenothiazine drugs, usually have to take these drugs for many years or even for life to maintain their remission. Long term toxicity studies and knowledge of the normal and abnormal metabolic pathways of drug metabolism are mandatory.

The metabolic fate of chlorpromazine is a difficult problem because various reports have suggested that more than 20 metabolites are formed, and there is the further complication that the bile is as important a channel of excretion as the urine. Numerous papers have been published on the metabolism of chlorpromazine, but relatively few of these will be quoted here.

Chlorpromazine is absorbed in 5 to 10 minutes after parenteral administration, and in 30 to 60 minutes after it is taken by mouth. Its immediate sedative action occurs within this period, to a noticeably greater extent with intramuscular than with oral administration. A hypothetical gastrointestinal blood brain barrier, which may prevent adequate absorption, is held responsible for this discrepancy (17).

The principal routes of chlorpromazine metabolism are sulfoxi-

dation, demethylation, N-oxidation, hydroxylation and conjugation (see Figure 1) (144, 122).

The oxidation of the heterocyclic sulphur was first reported by Salzman and Brodie (308) who found chlorpromazine 5-oxide as a urinary metabolite of the drug in man and the dog.

The use of S^{35}-chlorpromazine has shown that in rats 40-50 per cent of single doses (12 mg/kg) are excreted in the urine and an equivalent amount in the feces, the excretion of radioactivity being complete in 72 hours (103). About half of the urinary radioactivity is made up of four compounds, the main one (12 per cent of the dose) being unchanged chlorpromazine. The other three were the sulphoxides of chlorpromazine and its mono-demethylated and di-demethylated derivatives. The amount of these compounds excreted in the urine by rats after a single dose of chlorpromazine were 12.3, 5.0, 5.2, and 2.3 per cent, respectively (103). It has been claimed that man excretes six sulphoxides after chlorpromazine and three of these are the sulphoxides of chlorpromazine, and its mono-demethylated and di-methylated derivatives (134).

The proposed mechanism for sulfoxide formation is depicted in Figure 2. Haynes (156) has reported that 5.5 to 7 per cent of chlorpromazine is eliminated as free and bound chlorpromazine by patients receiving low daily doses (200 mg) of the drug. At higher levels of dosage (800 mg) somewhat greater amounts (7 to 9 per cent) are eliminated in these forms. Fishman and Goldenberg (113) have obtained evidence for the presence of at least six sulfoxide derivatives of chlorpromazine in the urine of patients receiving the drug.

Subsequently, demethylation of the terminal nitrogen of the

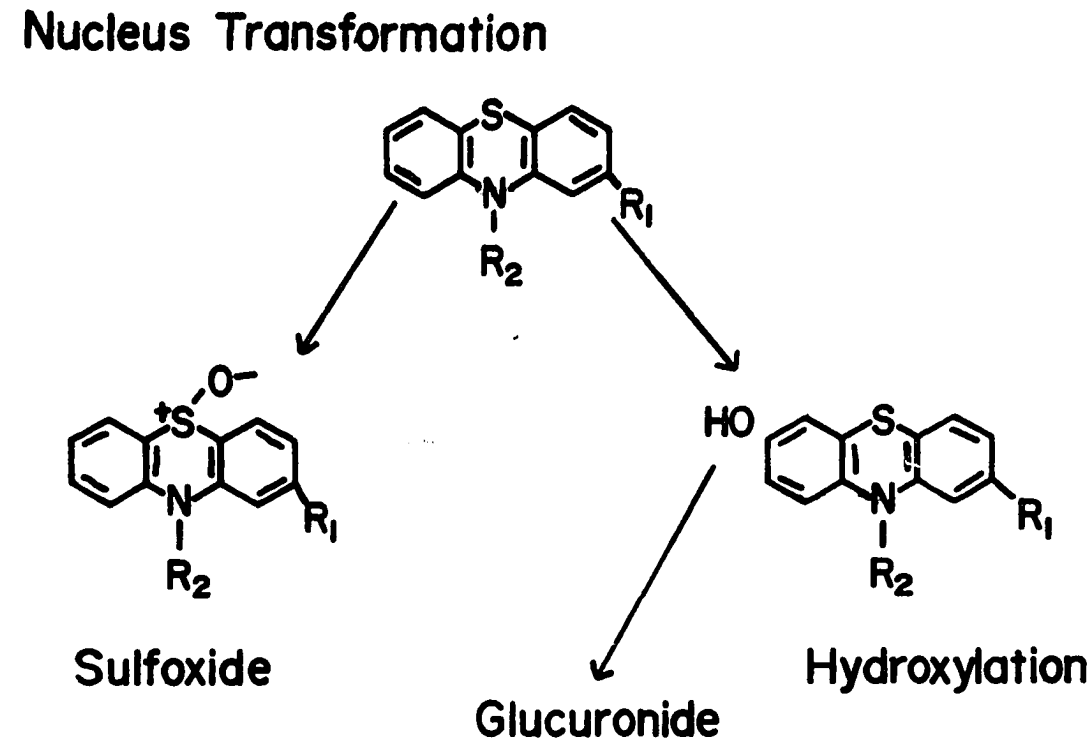


Fig. 1. Scheme of chlorpromazine metabolism

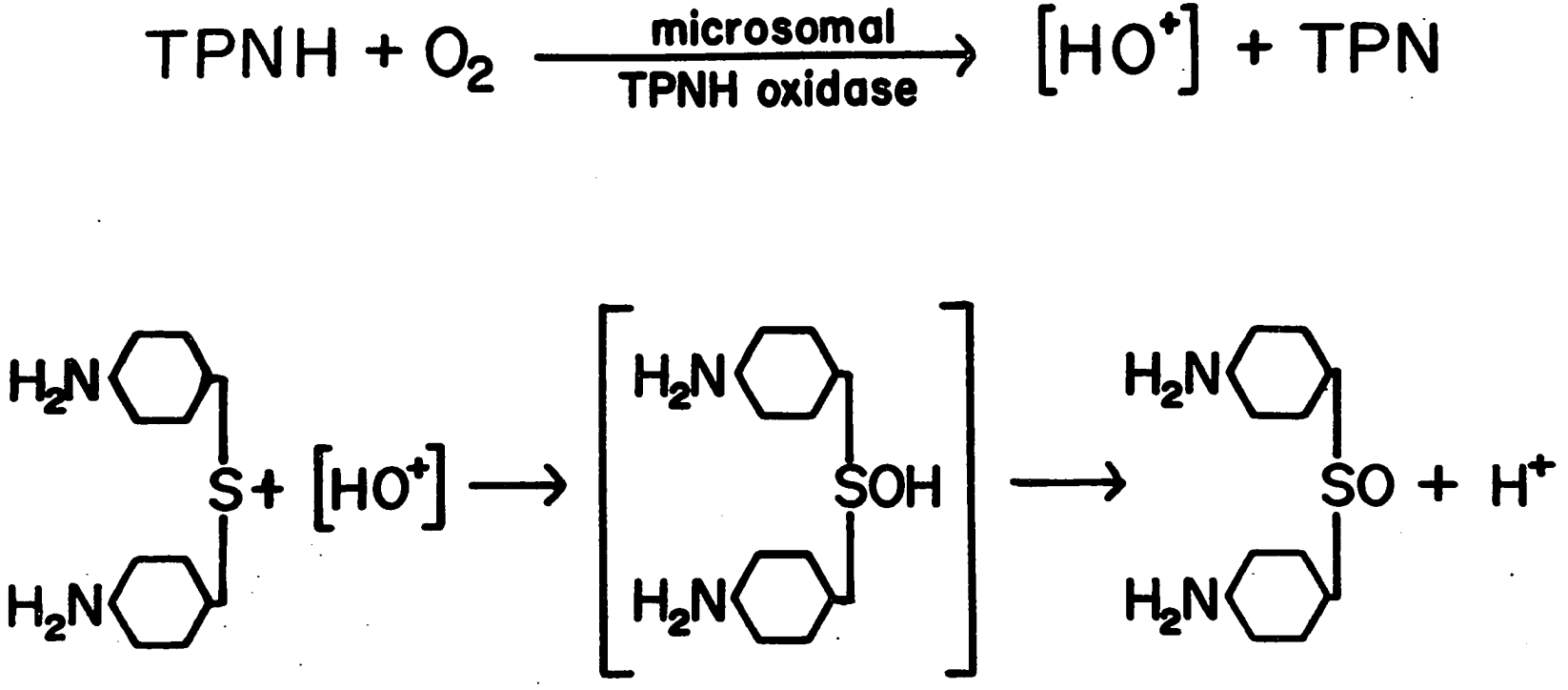


Fig. 2. Proposed mechanism for sulfoxide formation

dimethylchlopromazine and the aromatic hydroxylation and conjugation of chlorpromazine were reported (115, 304, 378).

The demethylated and hydroxylated compounds are more active in animals than the sulfoxide which has a weak sedative action in man. The hydroxylated metabolites are conjugated with glucuronic acid and to a lesser extent with sulfate (144).

The formation of the N-oxide of chlorpromazine has now been proved and the compound has been isolated from human and dog urine (115). This substance is excreted by humans in amounts equivalent to 0.7 per cent of the dose. In dogs the excretion of this metabolite is greater than in man, amounting to 2.0 to 3.5 per cent of the dose. On keeping this N-oxide for several weeks, it decomposes to give formaldehyde and desmonomethyl chlorpromazine. It is interesting to note that the N-oxide and its decomposition product, the desmonomethyl derivative are nearly as active pharmacologically as the parent drug (287).

There are several publications which suggest that the major urinary metabolites in man are polar and water soluble. Four of these metabolites have been claimed to be glucuronides of hydroxylated phenothiazine (225, 31). On analogy with phenothiazine itself hydroxylation could be expected to occur in positions 3 or 7 or both, but hydroxylation in other free positions, 1, 4, 6, 8 and 9, cannot be excluded. Since hydroxylation could be combined with sulfoxidation and demethylation, a relatively large number of glucuronides are possible. Four of these glucuronides have now been identified in dog and human urine, and since the aglycones of these glucuronides also occur in the free state in the urine, this means that eight more metabolites of chlorpromazine

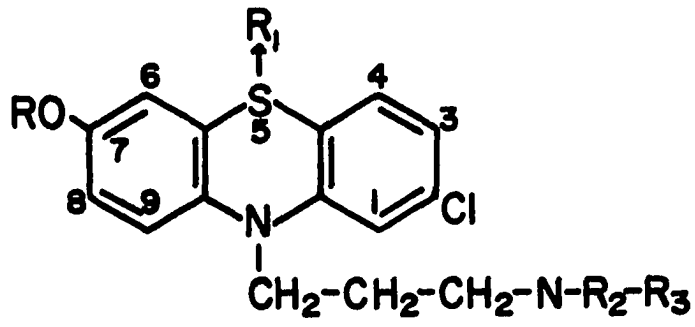
have been identified (114, 31) (Fig. 3). All of these compounds are derivatives of 7-hydroxychlorpromazine, and include the 5-oxide, the desmonomethyl and desdimethyl 7-hydroxychlorpromazine and their glucuronides. Fishman and Goldenberg (114) have stated that additional hydroxy derivatives occur in dog urine and still more in human urine. In fact, hydroxylation and glucuronide formation is a more important pathway of biotransformation in man than in dog. Whether or not these hydroxy metabolites have pharmacological activity is an open question (123).

Forrest and Green (144) stated that just on the basis of the above metabolic pathways, over 150 metabolites are possible.

In addition, some unusual but minor urinary metabolites have been described, such as 2-chlorphenothiazine and its sulfoxide (193). A chlorpromazine polymer with a hypothesized 1-2 linkage has been attributed to exposure to ultraviolet light and might be related to some photosensitivity reactions (179).

The metabolic pattern varies considerably among patients, so it has been tempting to relate patterns of metabolite excretion to clinical response. However, evidence for such a relationship is still tenuous. The rate of relapse of fifteen schizophrenics who were switched to placebo after chronic treatment with 600 mg daily doses, chlorpromazine was not related to the duration of excretion of glucuronide metabolites as the same investigators had previously thought (214).

Still, the marked variation in relapse times and the prolonged excretion of metabolites following discontinuation of treatment suggests that many patients maintain effective tissue levels of drug for considerable periods following cessation of treatment (214).



7- Hydroxychlorpromazine

Hydroxylated Metabolites of Chlorpromazine

	R	R_1	R_2	R_3
(a)	H	—	CH_3	CH_3
(b)	$C_6H_9O_6$	—	CH_3	CH_3
(c)	H	—	CH_3	H
(d)	$C_6H_9O_6$	—	CH_3	H
(e)	H	—	H	H
(f)	$C_6H_9O_6$	—	H	H
(g)	H	O	CH_3	CH_3
(h)	$C_6H_9O_6$	O	CH_3	CH_3

Fig. 3. Some chlorpromazine metabolites

Blood levels of drug have been difficult to measure and even more difficult to interpret. Plasma concentrations of chlorpromazine in patients chronically treated with 600 to 1200 mg daily doses usually range between 1 and 4 mg/ml, with small increases two to three hours after individual doses. Following an intramuscular dose of 100 mg, peak blood concentrations are attained in 30 minutes, and the drug has virtually disappeared from the blood after four hours (180, 214). Blood concentrations apparently bear little relationship to clinical pharmacologic effects.

A complete post-mortem analysis of drug distribution was done on a patient, who received daily oral doses of 900 mg of chlorpromazine up to death. The concentration of drug in various parts of the brain varied from 1.6 to 9.6 mg/gm wet tissue, as compared with concentration in hair of 124 mg/gm, lung 97.5 mg/gm, and liver 13.5 mg/gm (121).

Adverse and Side Effects

Definitions

Sir George Pickering (278) pointed out that the uncritical use of terms not only tends to make them meaningless but, what is worse, it also makes scientific communication difficult and imprecise.

Although they are to be found elsewhere, it will be helpful in this endeavor to bring together definitions of toxic effect, adverse effect, side effect, and drug allergy.

"Adverse effect" has broad and far reaching implications in that, as well as toxic effects as defined below, it also includes non-pharmacological consequences of drug use: e.g., the population explosion

in India resulting from effective antimicrobial therapy and the geriatric problem which we now have as a result of advances in drug therapy.

The "toxic effects" of a drug are the consequences of its pharmacologic actions, by whatever mechanism and for whatever reason, occurring promptly or after a latent period, directly or indirectly, as a result of one or more effects on tissue or functional activity by the drug. A toxic effect is a pharmacologic function, which has become undesirable and is a threat to normal physiologic function, if not a threat to life. Usually by being excessive, toxic effects may be attributed to the pharmacologic effects of a drug which also provide its therapeutic usefulness. Or, they may be associated with other facets of the pharmacologic properties of the drug. And, of course, toxic effects result from drugs which have no therapeutic value - industrial chemicals, chemicals which are used only for destructive purposes, and agents which are not intended for man or animal and affect them only by accident (247, 264).

The "allergic drug reaction" is an immunologic reaction to drugs which can only develop in subjects in whom the specific antibody is already present, usually as a result of previous exposure to the drug. The basic pharmacologic effect, in all the allergic reaction, is the formation of antigen from drug. Theoretically, therefore, an allergic drug reaction cannot occur the first time a subject receives a drug, whereas the toxic reaction can (247, 264).

A "side effect" is a pharmacologic effect other than the one sought for in the particular use of the drug. Thus, a side effect need not be adverse, non-therapeutic, or even unpleasant, or it may be a fatal

effect. Often a pharmacologic action leads to a side effect in one context and a therapeutic effect in another. Dryness of the mouth is a side effect of atropine when it is used to reduce intestinal mobility, while reduction of intestinal motility is a side effect of atropine when it is used by the anesthesiologist to dry the secretions of the mouth and respiratory tract. Thus, an effect of a drug becomes a side effect not because it is bad or unpleasant or because of what it does, but only because it is not the pharmacologic action on which the therapy is based (247, 249, 264).

According to Modell (247), "drug idiosyncrasy" has been defined by Rosenheim as uncharacteristic pharmacologic reactions in particular patients: e.g., excitement after barbiturate.

"Drug intolerance" is characterized by the production of typical pharmacologic effects by unusually small doses of drug (247).

"Drug-induced disease" implies a response to drug in which a disease results from its use, it does not imply any kind of pharmacologic effect. It is proposed to designate as drug-induced diseases only those consequences of the use of drugs which are characterized by functional disturbances which persist after the offending drug has been withdrawn and has been grossly eliminated (247).

Chlorpromazine-Induced Diseases

The administration of chlorpromazine leads to jaundice in 1-5 per cent of the recipients (257, 384, 90, 99, 302). This phenomenon has been considered a manifestation of individual host idiosyncrasy or hypersensitivity to the drug (90, 99). Accordingly the likelihood has been considered that the adverse effect chlorpromazine may have upon the liver

is aggravated by the coincidence of hypersensitivity. Some support for this view is provided by the demonstration that almost 50 per cent of patients who receive chlorpromazine develop some hepatic dysfunction (16, 93, 90).

Popper (286) stated that drugs which produce cholestatic jaundice in some persons cause a subicteric injury to the bile secretory apparatus of the liver cells in all persons. It is proposed that a superimposed hypersensitivity reaction to the same drug leading to nonspecific drug hepatitis results in the development of the commonly seen cholestatic drug hepatitis with jaundice. Nonspecific drug hepatitis and the hepatic reaction resembling viral hepatitis both possibly represent immunologic injuries. The difference between these two reactions may only be quantitative, and they should be considered as variants of the same group. Such an explanation might account for occasional transitional stages.

The most prominent and frequent of the neurological disorders complicating the use of the major tranquilizing drugs has been the dyskinesias which have mimicked the naturally occurring extrapyramidal diseases.

It has been known for several years that extrapyramidal disorders occur frequently in patients taking phenothiazines; however, a reduction in dosage or cessation of medication appeared to produce a return to the normal state (16). Hunter et al. (183) recently suggested that such extrapyramidal side effects are not always reversible. They found 13 of 250 chronically ill female patients in a mental hospital to have an irreversible syndrome of dyskinesias affecting the face and head. None

of the 200 men in the same hospital were affected.

The incidence of drug-induced parkinsonism varies with the drug, the dose, the duration of treatment and the age and sex of the patients. Ayd (15) found in an extensive survey that parkinsonism occurred in 15.4 per cent of 3,775 patients but mild akiesia was not recorded. On the other hand, clinicians who feel that the development of an extrapyramidal syndrome is necessary and look for very mild changes find an incidence of 90 per cent or more. In several small series of patients specifically examined for signs extrapyramidal reactions the incidence has been 100 per cent (332). Women seem to be more frequently affected than males. To some extent this may reflect the use of larger dosages in women, but hormonal factors may well play a role and the administration of an estrogenic agent has been reported to precipitate parkinsonism in patients on phenothiazines therapy (141). Although no age specific-rates are available, older patients are generally considered more apt than younger ones to develop extrapyramidal reactions. Ayd (15) stressed the similarity in age distribution of a large number of cases of drug-induced parkinsonism and the mean age of onset of Parkinson's disease. In any event the Parkinson syndrome resembling parkinsonism including the characteristic hand posture has been described in two infants born of a schizophrenic mother receiving large doses of phenothiazines derivatives (164).

In these circumstances, it is clear that the question of predisposition to the development of extrapyramidal reactions is extremely complex. Myriathopoulous and Kurland (258) suggested that hereditary factors may play a role but their statistical evidence is not fully convincing. Other authors, although noting marked individual differences

up to fifteen-fold in the dosage required to induce parkinsonism, were unable to relate the occurrence thereof or the dosage required to family history of parkinsonism (155).

Hollister and Glazener (173) found that eight of thirty-six patients with Parkinson's disease admitted to a mental hospital had concurrent schizophrenia and described one patient whose neurological disease developed during chronic treatment with a phenothiazine drug.

There have been several post-mortem reports of structural alteration of the basal ganglia in patients who had been on large doses of phenothiazine drugs (303, 289).

Ocular Reactions and Hyperpigmentation

The occurrence of ocular lesions following chlorpromazine ingestion was probably first noted by Bock (28) in 1962 who described changes in the lens in three patients who were on long-term chlorpromazine therapy. In two of these patients, the lens changes were of the typical senile variety and were of no significance. In the third patient the lens changes were described as "fine powdery white spots on the anterior surface of the central pupillary area."

Mackiewicz and Greshon (229) have demonstrated cerebellar lesions in guinea pigs treated with long-term oral doses of chlorpromazine. They believe these alterations are irreversible.

Greiner and Berry (146) described corneal and lens opacities as well as abnormal skin pigmentation in a group of seventy patients, all women, who had been receiving doses of chlorpromazine averaging 500 to 1,500 mg daily for three years or longer prior to the first evidence of the side effects. Of seventy patients described, twenty-one had very

marked bluish-gray pigmentation of the skin on exposed areas of the face, neck, and hands. Of the later group of twenty-one patients, twelve had grossly visible opacities affecting the cornea and lens.

Santanove (313) reports dermatological studies on seventy-eight patients receiving abnormally high doses of chlorpromazine and related phenothiazines derivatives for prolonged periods. These patients developed pigmentary changes in the exposed areas of the skin after exposure to specific bands of light. Histochemical studies suggested that this color was melanin or a melanin-like substance. The phototoxic action of sunlight acting on abnormal amounts of the phenothiazines in the skin produced an inflammatory response followed by increased production of melanin. The accumulation of melanin, or melanin plus chlorpromazine or its metabolites is thought to cause the purple color. The skin window technique demonstrated a sequence of inflammatory cells which transport the pigment to the blood stream. It is postulated that the cellular transportation of the pigment from the skin into the blood stream may produce the generalized melanosis found on autopsy.

Identical findings were reported by others and have come to be known as the "skin-eye syndrome" (20, 380, 108, 159, 319, 47).

Bolt and Forrest (33) reported that <u>in vitro</u> and <u>in vivo</u> studies strongly suggest a causual relationship between chlorpromazine induced hyperpigmentation of the skin and the drug metabolite 7-hydroxychlorpromazine rather than with chlorpromazine itself.

Most investigators have reported no significant reduction of visual acuity associated with lens or corneal opacities, but there have been a few exceptions (47, 369, 230, 329). There have also been sporadic

reports of partial resolution of eye lesions when chlorpromazine is withdrawn or partially replaced by other drugs (120).

Scott and Nading (324), in experiments with frogs have observed that several phenothiazine drugs including chlorpromazine effect the release of melanin-stimulating hormone from the hypophysis.

Of the many drugs currently used, the phenothiazines have been implicated in agranulocytosis with greatest frequency. According to the registry on drug induced blood dyscrasis of the American Medical Association, through June 1965 (347) chlorpromazine was implicated 83 times, promazine 30 times and mepazine 13 times as the only drug given to patients who developed agranulocytosis.

Agranulocytosis due to phenothiazines occurs most frequently in older people. In the compilation by the A.M.A. Committee on Drug Reactions (347), 70 per cent of the patients were women and 30 per cent were men. This peculiar sex distribution may not be significant since it has been estimated that 70 per cent of phenothiazines have been prescribed for women (261), thereby increasing the risk of reaction. The higher incidence of drug sensitivity in older age groups suggests greater susceptibility with age.

The blood cell count during phenothiazine induced agranulocytosis shows marked leukopenia with complete absence of neutrophils and monocytes. Lymphopenia is present as well. Early in the disease the bone marrow appears aplastic. It consists primarily of fat and contains no cells other than occasional nuclei, resembling lymphocytes. Anemia does not generally occur, despite the aplastic appearance of the marrow because the red cell survival time is 120 days. As long as recovery or

death occurs within a relatively short time, anemia never really gets the opportunity to develop (280).

If the drug is discontinued, and infection is avoided, the patient will recover within about two weeks. As recovery begins, the absolute lymphocyte count increases and if followed within 24 hours by a secondary decline, as the neutrophils suddenly increase in number (281).

The concept that a biochemical host defect could be responsible for chlorpromazine induced agranulocytosis has a precedent. For example, hemolytic anemia occurs whenever primaquine is given to a patient whose red cells are deficient in glucose-6-phosphate dehydrogenase (87).

Hollister (175, 174) concluded that the occurrence of agranulocytosis requires two elements which must be brought together, a drug capable of inducing a toxic reaction, and a patient who reacts abnormally and hence is unable to compensate for the compound's toxic effect. This may result from either an inability to detoxify or eliminate the compound so that it accumulates or remains in active form, or a limited function of a cellular system which is overcome by the drug.

Chlorpromazine affects many chemical, enzymatic and biologic functions, including inhibition of the enzymes that catalyze nucleic acid synthesis. This effect is felt chiefly in those tissues that turn over DNA rapidly; the hematopoietic cells (175, 174).

Toxicology

Acute Toxicity

According to Smith Kline and French Laboratory (334), in rats, the approximate 14-day LD_{50} was as follows:

Intraperitoneal	75 to 100 mg/kg
Subcutaneous	540 mg/kg
Oral	492 mg/kg
Intravenous	25 mg/kg

Chlorpromazine at 125 mg/kg i.m. produced death within 6 hours in guinea pigs (229).

Chronic Toxicity

In general, chronic toxicity studies have revealed no ill effects with chlorpromazine except for some depression of growth (due to lower food intake) at relatively high doses (334).

In guinea pigs, all organs and glands were unaffected by the drug, except that the male guinea pig thymus glands showed a decrease in weight (334).

Administration of 5 mg/kg of chlorpromazine per day subcutaneously to pregnant rabbits caused no significant difference from control animals in size or viability of litters. Likewise, administration of 10 mg/kg per day of chlorpromazine in food, to female rats, produced no ill effects on the mothers or litters; second generation studies showed no adverse effects (334).

Overdosage

Overdose is a frequent cause of drug reactions, especially among the antianxiety drugs. It may be due to mistaken prescribing by physicians or abuse of the drug by patients.

Several cases of intoxication due to overdose of chlorpromazine, taken either accidentally or intentionally, have been reported in the

literature (22, 152, 333, 111, 343, 97). Complete details are not available for all cases. The ingested amounts ranged from 250 to 9,800 mg (131, 136, 22, 152, 333, 111, 343, 97), presumably taken as a single dose, but it was so stated in only three instances.

It is evident that ingestion of about 10 to 50 mg/kg by humans resulted in deep sleep, stupor and coma. When an amount over 64 mg/kg was ingested, tremor and convulsion were also noted in most instances (22, 77, 349). This is in accordance with the observations of Salzman and co-workers (309) on dogs and Essig and Carter (105) on monkeys, regarding the effect of chlorpromazine on the central nervous system. Differences in the clinical picture of intoxication following large overdosages are explained either by the partial removal of the ingested material by gastric lavage or by the possibility that the amount actually was less than the alleged intake. The same reasons may account for the lack of coma and for the relatively short duration of the state of intoxication in spite of massive dosage such as seen in the case of Douglas and Bates (97).

Ingestion of 75 mg/kg of the drug by a 13-months-old child resulted in death (152). This case is the only published fatality attributed to intoxication by chlorpromazine. Death occurred during hospitalization on the fourth day after ingestion. Autopsy showed generalized cerebral edema. The blood contained 0.1 mg per cent of free chlorpromazine.

Reports of detection and quantitation of chlorpromazine are scant in the reported cases. Douglas and Bates (97) and Beal (22) emphasize that gastric lavage is indicated even after a considerable lapse

of time following ingestion since chlorpromazine diminishes the gastric motility, thus allowing the removal of some of the ingested material still remaining in the stomach.

When convulsions occur, the choice of an anticonvulsive drug is difficult since chlorpromazine may potentiate the action of many central nervous system depressants such as barbiturates and narcotics as well as anesthetics and alcohol (334). The makers of the drug are quite emphatic in their recommendations regarding the use of vasopressor drugs to combat hypotension. They state that non-epinephrine is the most suitable and that epinephrine should not be used since the chlorpromazine may reverse its action (334).

Haggerty (152) suggests that exchange transfusion may be beneficial in treating intoxication in children and that hemodialysis would be of no value.

Trace Elements

Associated with all vital processes and essential for all living cells are a few inorganic elements; most are classified in the periodic chart as being members of the transition series. They are: Mg, Mn, Fe, Ca, Co, Cu, Zn, Mo. Though present in only micrograms per gram of body tissue, and therefore called trace elements, they have profound effects on all cellular functions (125).

The term trace elements has been defined by many workers in this field, but it is generally agreed that any element that comprises less than 0.01 per cent of the organism is called a trace element (357). They are also known as micronutrients, oligonutrients, or minor elements as opposed to macro or bulk elements. The designation "trace," however,

often refers to the extent of our technical and analytical ability of detecting that particular element in biological material rather than its relative concentration. Hence, our concept of a "trace" keeps changing as we keep improving our method of detection. For example, according to Vallee (361), zinc has lost its standing as a "trace" element because of its broad base of biological importance that has been recently discovered.

The biological role of trace metals was not elucidated until the early thirties. Prior to this, tools for their study were not available and even more important the advent of newer tools of biochemistry to isolate and characterize metalloenzymes were lacking. Today trace elements are recognized as vital parts of the biochemical makeup of the organism.

Essential trace elements are interdependent, and are generally associated with enzymes. Metal-dependent enzymes fall into two classes, (1) metalloenzymes which have a fixed amount of specific metallic ions firmly bonded to enzymes and whose metal/enzyme ratio is constant, (2) metal-activated enzymes where the metal is reversibly bonded to enzyme with a variable metal/protein ratio. Metals may substitute for each other and may also be removed by dialysis in the latter type of bonding.

A metal associated with the enzymes may play a variety of roles. One popular concept is that metals act as a bridge between substrate and the protein which activates the metal by withdrawing electrons from it, hence becoming positively charged and thus enabling it to withdraw electrons from substrate. A chemical change is precipitated and hence the free energy of activation is lowered by the electronic alteration mediated by the metal (125).

In other instances the metal plays an important role in the tertiary folding of the protein which may have to bring together two or three amino acid residues that are located far apart on a polypeptide chain, but which may become active when brought into the proximity of each other. It is believed that metals are bound to proteins mainly by the histidine residues in the protein (125)

There are over 2,000 enzymes that regulate a vast number of chemical reactions within the cell. The metal coordinated to the enzyme, and in many cases with the substrate helps to regulate the speed of the reaction. Alteration of the metal environment alters the kinetics of the system.

According to Schroeder (322), four of the nine inorganic essential micronutrients cause deficiency disease and three cause disease of accumulation.

Schroeder (322) lists three types of trace metal deficiencies. These include simple deficiency such as iron and copper in man and Mo and Co in other mammals, and malabsorption or decreases in carriers of the blood such as zinc in Laennec's cirrhosis of the liver. Although overt toxicities of trace elements have been well established experimentally as well as clinically, small chronic exposure over long periods of time is not well defined. There are also excesses of trace elements, certain diseases such as Wilson disease and copper accumulation (24, 357), and parkinsonism and high manganese concentration (67). Other types of excesses may be due to other large dietary intakes, increased absorption or decreased excretion resulting from other disorder such as in biliary obstruction or renal insufficiency (322).

In spite of much speculation regarding essentiality of trace metals, several workers in this field (69, 273, 357) agree on the criteria for essentiality. First, it is present in all healthy tissues of all living things. Second, its concentration from one animal to the next is fairly constant if compared to nonessentials; even the variability of human organs as found by Perry et al. (273) was more alike with respect to the absolute concentration of essential than of nonessential metals. Third, its withdrawal from the body induces in a reproducible fashion the same structural and physiological abnormalities regardless of the species studied. Fourth, its addition either prevents or reverses these abnormalities. Fifth, the abnormalities manifested by the deficiency state are always accompanied by pertinent, specific biochemical changes. Sixth, these biochemical changes can be cured or prevented when the deficiency is corrected. Hence, by these criteria a beneficial effect on growth or reproduction does not qualify an element as essential.

According to Underwood (357), the tremendous progress made during the last 29 years in our understanding of metabolic movements of trace metals within the body and their mode of action within the tissues was greatly assisted and stimulated by concurrent developments in three major fields of study. The first of these involved the use of radioactive isotopes of suitable half-life of virtually every element of biological interest. In the case of trace elements, absorption, retention, excretion, and kinetic studies were facilitated that were tedious, difficult, or impossible with the stable elements. The second significant development was in the field of enzymology, especially the discovery of a number of metalloproteins with enzymic activity and of trace element-

catalyzed reactions in living tissues. These advances directed attention to the basic biochemical lesions associated with the diverse manifestations of trace element deficiencies and excesses in the body. Finally, the remarkable advances in analytical procedures such as emission spectroscopy, atomic absorption, and neutron activation, and more recently the laser and electron microprobes, which have enabled us to determine qualitatively and for some elements quantitatively, concentration at the cellular level. Table 2 shows selected enzymes and metal relationships.

Copper

It is estimated that the adult human body contains approximately 100-150 mg of copper, most of which is concentrated in the liver and the central nervous system (357).

Newborn and very young animals are normally much richer in copper per unit of body weight than adults of the same species. This is largely, but not entirely, a reflection of high liver copper storage at this stage (357).

Most of the copper present in the plasma is firmly bound to α-2-globulin called ceruloplasmin which according to Markowitz (235) was discovered first by Holmberg and Laurell. A small fraction, about 5 per cent is bound to albumin. The important metalloenzymes containing copper are: ascorbic acid oxidase, uricase, cytochrome oxidase, and S-aminolevulinic acid dehydrase (357). Red blood cells contain a copper protein called erythrocuprein (235). Cartwright (52) believes that copper is involved in myelination of nervous tissue and the maintenance of normal skin pigmentation. Carnes et al. (49) and O'Dell et al. (267) postulate that copper-deficient animals have defects of vascular integrity

TABLE 2

SUMMARY OF SELECTED ENZYMES AND METAL RELATIONSHIPS

Enzyme	Metal Contained	Metals Added (Activator)	Chlorpromazine Effects	Substrate	Reference
Adenosinetriphosphatase		Mg^{++}	In brain: Inhibits	Transfer PO_4 groups	85 228
Alkaline Phosphatase	Zn		In brain: Increased Decreased In serum: Increased Decreased No change	Monoesters of orthophosphoric acid. Hydrolyze the amidophosphate link of creatine phosphate	63 75 165 54 379 46 177
Carbonic Anhydrase	Zn		Increased in CNS and kidneys	Acts on acids from C_4 to C_{11}	11
Cytochrome Oxidase	Cu		In brain: Inhibitory No change	Reduces cytochrome C	126 376 251
Hexokinase		Mg^{++}	Biphasic	Converts D-hexose into D-hexose-6-Phosphate	239 127
Lactic Dehydrogenase	Zn		In liver: No change	D-Lactate	63
Liver Arginase	Mn		In liver: No change *in vivo*	Arginine	206

ascribable to abnormalities in the biogenesis of elastin. Its action in erythropoiesis is probably due to the increased absorption of iron and its incorporation into protoporphyrin in the formation of heme (357).

The endocrine glands are examples of low copper tissues and the liver, heart, kidneys, hair, and brain, usually in that order, are examples of high copper concentration tissues (357). The copper concentrations in the liver, kidney, spleen, and lungs can be greatly increased by high copper intakes and those of the liver, kidney, spleen, and blood readily reduced under conditions of copper deficiency (357).

Hypercupremia occurs in most chronic and acute infections in man and in leukemia, Hodgkin's disease, various anemias, collagen disorders, hemochromatosis, and myocardial infarction (361). According to Underwood (357) Hill reported elevated serum copper values in some cases of schizophrenia.

The average amount of copper ingested daily by an adult is about 2.5-5.0 mg, 99 per cent of which appears in the stool, and the majority of that portion absorbed is excreted via the bile. About 70 mg appear in the urine per day (357).

Magnesium

Wacker (365) has stated that shifts in body magnesium result in deleterious effects and can produce significant clinical manifestations. The entire human adult body contains about 25 g. It is the fourth most abundant cation being exceeded only by calcium, sodium, and potassium. Hence, the term "trace" can hardly be applied in this situation. Magnesium is mainly intracellular with a concentration of 26 meq/d. Approximately half of the magnesium resides in the skeleton and exchange very

slowly with the extracellular ions. The highest concentrations of magnesium are in the liver and muscle followed by the brain, kidney and red blood cells (365).

About one-third of the serum magnesium is bound to albumin and globulin and the remainder is in ionic form (365).

Magnesium is especially important in activating enzymes which transfer or hydrolyze phosphate groups, or in some cases split peptides as peptidases. Ribonuclease is also a magnesium-dependent enzyme (125).

The average intake of magnesium for an adult is approximately 300 mg although requirements may vary between infants and under certain conditions such as in pregnancy. Seventy-five per cent of the magnesium intake is absorbed (357).

Several diseases have been associated with altered magnesium metabolism. Miller (244) reported a hypomagnesemic tetany. Smith and Hammarsten (339) reported low plasma magnesium in delirium tremens which was followed by improvement upon administration of magnesium.

Vachon and Marchand (358) reported that in all tissues studied, with the exception of bone, magnesium concentration was found to be decreased one hour after morphine administration. Hypermagnesemia was elevated within the first two hours of treatment, slowly returned to normal and became significantly lower 24 hours after morphine injection. Hills et al. (163) described adrenal insufficiency in which there was high level of plasma magnesium.

Manganese

The body of a normal 70 kg man is calculated to contain a total of some 12-20 mg manganese (357). According to Underwood (357) Tauber

and Krause reported that manganese is not specially concentrated in any organ or tissue so far examined. Even the pigmented portions of the eye, which are so rich in zinc and copper, are not exceptionally high in manganese, although the retina appears to be somewhat richer in this metal than are most body tissues. The bones, liver, kidney, pancreas, and pituitary gland normally carry higher concentrations (1-3 p.p.m. Mn on the fresh basis) than do the other organs and the skeletal muscles (0.1 - 0.2 p.p.m.) are among the lowest in manganese of the tissues of the body. In the liver and bones the levels can be raised or lowered by varying the manganese intake of the animal.

Cotzias (67) reported that ingested divalent manganese remains unchanged in the acid stomach, but as soon as it gets to the small intestine it becomes oxidized since alkaline conditions are more favorable for oxidation. Absorbed manganese is transported via β-globulin plasma transmanganin, the trivalent form (67).

Manganese is poorly absorbed from the gut and is excreted very largely in the feces. Underwood (357) reports that the early studies of Greenberg and associates with Mn^{54} indicated that only about 3-4 per cent of the orally administered dose was absorbed in the rats; it quickly appeared in the bile and a very high proportion was excreted in the feces.

Britton and Cotzias (38) and Borg and Cotzias (34) suggested that disorders due to manganese deficiency or excess may result from slow or rapid rates of turnover.

Quite a range of disturbances has been related to lack of dietary manganese in different species under varying conditions, but the cardinal manifestations, namely impaired growth, skeletal abnormalities,

depressed or disturbed reproductive function, and ataxia of the newborn are similar in all species (357).

Everson and Shrader (106) reported an impairment of glucose tolerance and utilization in young adult guinea pigs deficient in manganese. Hurley (184) and Hurley et al. (185) studied maternal dietary deficiency of manganese during pregnancy. They found irreversible congenital ataxia in the offspring of rats, mice and guinea pigs. Supplementing the diet with manganese during gestation completely prevented the development of these abnormalities.

Liver arginase is the only enzyme other than alkaline phosphatase, that has been shown to be significantly reduced in activity in the tissues of manganese-deficient rats and rabbits (357).

Zinc

It is well established that zinc is an essential element for normal growth of most domestic animals (35). The metal is an integral part of the molecular structure of a variety of enzymes including alcohol dehydrogenase, glutamic dehydrogenase, lactic dehydrogenase, carbonic anhydrase, and alkaline phosphatase (357).

An average adult male contains about 2.2 g of zinc. Zinc occurs in all living cells in varying concentrations, with certain portions of the eye, the male sex glands and secretions, the hair, and the bones normally carrying significantly higher levels than other tissues and fluids. Most other organs contain on the average 20-30 mg/g wet weight.

Zinc is a constituent of the plasma or serum, of the erythrocytes and of the leucocytes. In the plasma it exists in both firmly bound and loosely bound fractions, associated with the serum albumin and

globulins and most strongly bound to the latter (357).

The average daily intake of zinc is about 10-15 mg, 4 per cent of which is excreted in the urine and the remainder is excreted in the feces. Very little of absorbed zinc is excreted in the bile (357).

A dietary deficiency of zinc has been demonstrated in mice, rats, pigs, poultry and cattle, but not in sheep or man (357).

Deficiency of zinc produces subnormal growth, poor feeding efficiency, and testicular atrophy in dogs (300), rats (118), pigs (356), and calves (243). Zinc deficient pigs develop parakeratotic skin lesions which are reversed by zinc feeding (356, 167). Zinc deficient chicks exhibit hyperkerotosis and poor feathering along with multiple skeletal defects (254, 267). Chick embryos hatched from zinc deficient eggs show a variety of bizarre congenital malformations (205).

Prasad and associates (290, 291, 292) demonstrated a convincing etiologic role for zinc deficiency in certain Iranian and Egyptian males with iron deficiency anemia, hepatosplenomegaly, short stature and marked hypogonadism. Zinc feeding has been reported to have a salutary effect on alcoholic cirrhotics (361, 166).

Although it seems clear that zinc deficiency *per se* is followed by characteristic sequelae in animals and man, it is not clear that low tissue zinc concentration can be equated with zinc deficiency. In symptomatic zinc deficient swine, skin, muscle, pancreas, intestine and blood may contain normal zinc content (357). However, Prasad *et al.* (293) have suggested that the tissue content of zinc controls physiologic processes through the formation of zinc-dependent enzymes.

Davies *et al.* (79) have demonstrated that plasma zinc concen-

tration is remarkably constant despite a continuous and relatively massive zinc turnover within the body. They demonstrated that glucose administration is followed by prompt 5-10 per cent fall in plasma zinc concentration with recovery within 1-2 hours. Within this narrow limit, zinc concentration remained constant, with day-to-day variations being much less than for Cu, Ca or Mg.

CHAPTER III

PURPOSE AND SCOPE

It is increasingly evident that toxic or adverse effects of drugs will continue to be a health problem, with increasing emphasis on long-term effects. Experience over the past decade has shown us that we can live with those reactions which are associated with valuable drugs and live without drugs of lesser value which may exact too great a price in side reactions. A number of drugs have fallen by the wayside for the latter reason, but overall, the list of safe and effective psychotherapeutic agents has increased considerably in the period, with every expectation that it will continue to do so.

Chronic toxicity studies have shown that repeated doses of chlorpromazine produce several adverse effects, in which trace metal involvement has been implicated, such as copper in the maintenance of normal pigmentation (52), zinc in hepatic dysfunction (166) and high manganese in parkinsonism.

Due to the importance of copper, magnesium, manganese and zinc in the function of certain metalloenzymes and other metabolic activities, as enumerated heretofore, it may be assumed that a concentration shift exhibited by any one of these metals provoke significant biochemical changes in the metabolism of those substrates effected by the enzymes

involved.

A survey of the literature concerning the action of chlorpromazine on the metalloenzymes reveals conflicting points of view. For instance, various authors have reported that chlorpromazine increases, decreases, or has no effect on the alkaline phosphatase level of the various tissues (379, 46, 177, 241, 211, 75).

These conflicting observations and others might be ascribed to different doses, timing, species, and above all in the methodology for the assessment of enzyme activity. In view of the lack of comparability among existing studies, a need for further research in several of the areas covered in this review is evident.

Therefore, it was felt necessary to study systematically the influence of chlorpromazine on trace metals and try to determine relationships of observed trace metal effects to the changes in corresponding metalloenzymes reported in the literature.

This research project will determine the following: (a) which of the organs, brain, heart, kidneys and liver exhibit the most prominent changes in trace metal after chlorpromazine exposure, (b) the effect of varying periods of exposure to therapeutic levels of chlorpromazine on the trace metal status of the rat, (c) the practicality of trace metal methodology in monitoring the living system for adverse effects.

CHAPTER IV

EQUIPMENT AND PROCEDURE

The ninety-six animals used in this study consisted of adult male King-Holtzman descent rats (250 to 350 g each with an average weight of 300 g) purchased from the Stanley-Gumbreck Company, Oklahoma City, Oklahoma. They were acclimated to the laboratory environment for 2 weeks and then examined for symptoms of illness after which time they were randomized into six groups consisting of eight control and eight experimental rats per group. The first five groups of rats were placed in wire cages, eight per cage. The sixth group of rats were placed in individual metabolic cages. Throughout the experiment all animals received water and purina rat pellets ad libitum. Each rat was weighed at the initiation of the study, immediately prior to dosing, and again at sacrifice. The chronically exposed animals received a subcutaneous dose of 10 mg of chlorpromazine per kg of body weight, biweekly. The chlorpromazine was obtained from Smith Kline and French No. 2601A (Lot No. 116-771). The chlorpromazine injection solution was prepared by dissolving 10 mg of chlorpromazine per ml of 0.85 per cent saline, which was made up in deionized water.

The control animals in each group received an equivalent volume of saline.

One group of animals, consisting of eight controls and eight experimentals, was sacrificed at five day intervals during the 25 day study. The animals in the metabolic cages representing the last group.

The animals in the acute group received a single subcutaneous dose of chlorpromazine, 50 mg/kg of body weight. Sacrifice was effected at the third hour post injection.

The animals were sacrificed by decapitation utilizing a commercial decapitator. Mixed trunk blood was collected in tubes. The serum was collected by centrifugation, then decanted into plastic screw top vials and frozen until the metal analyses could be performed.

Organs were obtained consistently in a similar fashion in order to avoid any changes due to surgical manipulation. The brain, heart, liver and kidneys were removed, blotted dry, cleaned of fat and connective tissue where necessary, and placed into preweighed vial and wet weights were determined. All containers were preacid washed and rinsed in deionized water. The samples were frozen until further sample preparation could be performed.

Urine from control and chronic animals in metabolic cages was collected daily and pooled, to which was added 0.5 ml acetic acid as preservative.

Sample Preparations

The urine was aspirated directly into the flame using a premix burner for manganese. For copper, and zinc it was diluted 1 to 5. For magnesium a ten-fold dilution was needed.

One ml of serum was diluted to 5 ml with 4 ml of 10 per cent TCA and centrifuged, then decanted into clean tubes, which permitted

direct aspiration of the samples into the burner of the atomic absorption unit.

The tissues (brain, heart, kidneys and liver) were subsequently thawed, placed into tared beakers and dried in an oven at 105°C for 16 hours. Subsequently the tissues were placed in the electric furnace at 500°C for 16 hours. The ash weight was determined after dessication overnight. After the weight of the ash was determined, 0.6 N HCl was added to each container in the following ratios of mg/ash to ml/acid: brain, 5:1, heart, 2:1, liver, 10:1 and kidneys, 5:1. The containers were then covered with parafilm and allowed to stand overnight. The acid and ash residue were transferred to a plastic screw top vial and stored until analysis.

All samples from the control and experimental animals were analyzed for manganese without further dilution. For copper, magnesium and zinc of brain, heart and kidneys, a ten-fold dilution was necessary. In the liver a fifty-fold dilution was needed for these metals.

A set of standard solutions containing 10,000 ppm of copper, magnesium, manganese, and zinc, respectively (Fisher Scientific Company, Fairlawn, New Jersey) were serially diluted with 0.6 N HCl in order to obtain standards in the concentration ranges anticipated. These dilutions were then used to prepare standard curves covering the indicated ranges: copper, 0.25 to 5.0 mg/ml; magnesium, 2.5 to 20 mg/ml; manganese, 0.05 to 3.0 mg/ml; and zinc, 0.25 to 5.0 mg/ml. During analysis, appropriate standards were repeated with every eighth sample.

These analyses were accomplished with a Jarrell-Ash Atomic absorption Spectrophotometer Model 82-362 utilizing a 0.5 meter ebent mount

monochrometer. Instrumental settings for each particular metal were optimized and a warm-up period was allowed for the whole system to stabilize. The hollow cathode tube current for each metal was set according to the manufacturers recommendations as follows: copper 5 ma, magnesium 6 ma, manganese 5 ma, and zinc 6 ma. The corresponding resonance line for each metal was 3247A°, 2852A°, 2794A° and 2139A°, respectively.

Data were collected and analyzed statistically using the IBM 1800 Computer of the Hospital Systems at University Hospital of the Oklahoma University Medical Center. For statistical purposes the data for the chronic study was treated as a 2 x 4 factorial arrangement (group factor at two levels - treated and control and time at four levels) with eight observations per cell. Primary emphasis in testing hypotheses was centered on the test for simple effects of time within group and group within time. For certain metals and organs, the heterogeneity of variance precluded the use of the usual analysis of variance technique. These are indicated in the tables in the Appendix. Individual test for these were made using the Modified-t.

The data for the acute and metabolic studies were tested for difference between treated and control using the Student's t statistic. For all test of hypothesis the 5 per cent level of significance was used. Data are tabulated in the Appendix.

CHAPTER V

OBSERVATIONS AND DISCUSSION

The observations on the acute, chronic, and control groups of rats are presented as a consideration of the response of copper, magnesium, manganese and zinc in the brain, heart, kidneys, liver, serum and urine. Secondarily, an attempt is made to correlate trace metal changes with selected metalloenzymes and metal activated enzymes. The comparisons of the treatment and control experimental parameters are shown in Tables 4 through 16 (Appendix), and these data are summarized in Table 3, which indicates the statistically significant increases and decreases by algebraic signs.

Trace metal values have been expressed in terms of concentration per ash weight.

Brain

Chlorpromazine appears to have caused only one significant difference between groups in rat brain weight parameters during the 25 days of this study. The ash weight was decreased at the end of 15 days, however in both groups, chronic and control there were significant decreases in wet weight with time. Only two significant alterations in metal concentration between groups occurred; magnesium was decreased at the end of 15 days, and zinc was elevated at the end of 20 days. Brain zinc

TABLE 3

SUMMARY OF COMPARISON OF THE TREATMENT PARAMETERS

Organ Group	Wet Weight	Ash Weight	Cu	Mg	Mn	Zn
Brain						
Acute						
5						
10						
15				-		
20		+				+
25						
Heart						
Acute			-	-		-
5		+		+		+
10		-	+	+		+
15						
20	-			-		+
25				+		
Kidneys						
Acute				+	+	
5						+
10						
15						
20	-		+	-		
25				+		
Liver						
Acute		+	-	-		
5					-	
10						-
15						
20						+
25			+			
Serum						
Acute						
5				+		
10			+			
15						
20						
25						
Urine						
5				-		
10						
15						
20						
25						

Algebraic signs indicate significance and direction of change at 0.05 level.

exhibited the most prominent change in concentration of all tissue metal shifts observed. There was approximately an eleven-fold increase in zinc at this interval. The intra group variability was also significant.

The absence of appreciable copper change in the brain appears to be in disagreement with the observations of Holbrook (168) which showed cerebellar cortex and caudate nuclei copper increases in the guinea pigs. This difference in observed copper alterations might be the result of species variation. On the other hand, our investigation appears to support observations by other investigators on cytochrome C oxidase of rat brain in which it was reported that no inhibitory effect was found (126, 251).

The migration of magnesium out of the liver appears to be in accord with the observations that chlorpromazine inhibits adenosine triphosphatase in liver and brain preparations (27, 81), and with those of Masurat et al. (237) which indicated that chlorpromazine either greatly activated or inhibited hexokinase activity. The biphasic action of chlorpromazine was said to be a function of the relative concentration of drug and Mg-ATP substrate.

The elevation of brain zinc in our investigation would tend to uphold the significant increase in brain alkaline phosphatase reported by Clark (63), however, it is not in accord with the findings by Chagovets et al. (55) which showed a 70-75 per cent decrease in lactic dehydrogenase activity in brain tissue. Nevertheless, the observations discussed above appear to bolster the involvement of alkaline phosphatase in the rats response to chlorpromazine.

Heart

The heart was the most sensitive to weight change parameters. The heart wet weight was significantly decreased at the end of the 20 day interval, the ash weight was increased at the 5 day interval and decreased at the end of 20 days. There were significant changes in ash weight among control and chronic groups with time.

Copper appeared to have been mobilized into the heart at the 10 day interval, however, there was a decreasing copper concentration with time in control and chronic rats. The acute rats exhibited a significant decrease in heart copper concentration at 3 hour post injection.

Magnesium demonstrated a migration into the heart at the 5, 10, and 25 day (metabolic group) intervals. However, there was a decrease in magnesium at the 20 day interval and in the acute rats.

The specimen was not sufficient to determine manganese.

Zinc appeared to migrate into the heart during the 5, 10, and 20 day intervals, and migrate outward in the acute rats.

Kidneys

The only kidney weight parameters observed to be altered was the wet weight at the end of the 20 day interval. Most of the metal changes were inward migrations. Copper was significantly increased at the end of the 20 day interval. There was an elevation in magnesium at the above interval, and in both the acute and the metabolic group (25 day interval).

Manganese demonstrated a consistently decreasing concentration in the kidneys with time in control and chronic groups. There was an increase in manganese concentration of the kidneys of the acute rats.

Liver

The liver of the acute rats demonstrated the only change in liver weight parameters. The ash weight showed a significant increase in this group. Copper was elevated in the metabolic group (25 day interval), and depressed in the acute group.

Magnesium was significantly depressed in the acute group. The chronic rats showed a significant decrease in manganese at the end of the 5 day interval. There was significant intra variation in manganese concentration in the liver of chronic rats.

Several different patterns of changes appear to have occurred in the zinc concentration of the liver. There was an intra as well as an inter group variability, which was very significant at the 10 and 20 day intervals.

The shift in manganese concentration of the liver of acute rats and the absence of alterations in manganese concentration in the liver of the chronic rats appear to be in accord with the reported observations by Klinger *et al.* (206) which did not show any decrease in the activity of liver arginase in the *in vivo* experiment after 89 to 93 days of chlorpromazine treatment.

Serum

There was an intra as well as inter group variability in metal concentration in the serum of the chronic rats which was highly significant. Copper appears to have been mobilized into the serum at the end of the 10 day interval. Our findings are not in complete accord with those of Azima (16) in patients which showed a gradual rise in plasma copper concentration, however, the alterations were in the same direc-

tion. On the other hand, Nunez (265) reported a decrease in plasma copper of nearly all of the 50 patients examined, but an increase in plasma copper of manic depressive patients.

Both magnesium and zinc serum concentrations have shown a consistently decreasing concentration with time in the chronic rats over the 20 days examined.

Urine

Significant metal alterations in the urinary excretion did not occur except for a decrease in magnesium at the end of the 5 day interval. However, no consistent pattern of urinary magnesium concentration was observed due to its erratic behavior in both controls and experimental rats. This inconsistency may possibly be explained by the changing of the food of the metabolic rats on three different occasions.

Inasmuch as all of these metal-enzyme correlations involve different strains of animals, species, doses, routes of administration, time intervals of sampling, and other variables, no definitive statements can be formulated, only suggestions that need to be subjected to further studies. Due to the lack of a consistent pattern of metal alterations between control and chronic treated rats, except for an almost consistent trend of zinc increase in the heart, the author believes that this study should be duplicated and the exposure period extended.

CHAPTER VI

SUMMARY AND CONCLUSIONS

In this series of experiments ninety-six male Holtzman-King descent rats were used to assess the effects of chlorpromazine upon trace metal shifts quantitatively and the time course of the various elements of response was defined. The animals were randomly divided into six control groups of eight; a group of eight which received a single subcutaneous (s.c.) acute dose of 50 mg of chlorpromazine per kg of body weight; and five chronic groups of eight; which received 10 mg/kg of body weight of chlorpromazine (s.c.) biweekly. One group of control and chronic rats was sacrificed at every five-day interval for a period of twenty-five days. The sixth group of controls and chronic treated animals were placed in metabolic cages.

Chlorpromazine effects were determined by alterations in the concentration of copper, magnesium, manganese and zinc in the brain, heart, kidneys, liver, and serum of all groups and the urine of the last (25 days) group.

In the 2,300 determinations made by atomic absorption spectroscopy on the various samples, differences were sought between control and chlorpromazine treated rats. These studies lead to the following conclusions:

1. Analysis of data from this study showed that generally chlorpromazine had inconsistent effects on organ weight parameters. The brain appears to increase in wet weight with time, while the heart increased in ash weight over the 20-day period, and also between chronic and control groups at the 5-day interval. Other organs showed similar inconsistencies.
2. As might be expected, each organ presented an individual pattern of trace metal changes due to chlorpromazine administration. The observed metal alterations were inconsistent. No definite trend was detected.
3. Zinc demonstrated the most significant change of the metals examined. The brain zinc concentration was elevated threefold at the 20-day interval.
4. Manganese was the least responsive to chlorpromazine administration. Its only significant shifts were a mobilization into the kidneys of the acute group, and a decrease in liver manganese concentration at the 5-day interval, and an intra variation in the chronic rats.
5. Copper exhibited significant inter and intra variation in the heart, and significant differences in the serum after 10 days, and in the liver of both acute and 25-day chronic rats.
6. Magnesium demonstrated the most variable response observed. There were either exceedingly large intra or inter variation or both in most of the tissues examined.

7. The significant increase in serum copper after 10 days, and the decrease in serum zinc concentration with time during the 20 days examined, along with the studies by Azima (16) and Nunez (265), tend to indicate that trace metal methodology, in serum, might be useful for monitoring adverse effects due to chlorpromazine administration.
8. In general, evidence from other studies, in conjunction with the present investigation, suggests that chlorpromazine administration induces alterations in various enzymes and associated metals, including alkaline phosphatase, cytochrome oxidase, adenosine triphosphatase and liver arginase.

   These results do not rule out other factors, but it is felt that there is sufficient evidence to indicate that the enzymes previously listed and their related metals are involved in the biochemical response of the rat and other organisms to chlorpromazine administration. But because of the many variables, species of animals, dosages and route of chlorpromazine administration, only suggestions can be made at this time. Further studies, utilizing more frequent sampling and additional animal species, in conjunction with human studies, need to be done before any definitive conclusions can be formulated.
9. The following metal alteration with associated metalloenzyme or metal activated enzyme should be studied further on an hourly basis, followed by daily sampling, then weekly sampling for chronic response to chlorpromazine administra-

tion in order to provide a better understanding of their involvement:

(a) significant increases of zinc in brain tissue, and alkaline phosphatase activity.

(b) significant increases of copper in serum, kidney and liver tissue along with cytochrome oxidase.

(c) the elevation and/or depression of magnesium in heart, brain, kidney, liver and serum in conjuction with adenosine triphosphate and hexokinase activity.

These studies should be done to provide information for evaluating the response of trace metals and their relationship to enzyme changes as an additional toxicological tool for assessing adverse effects.

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APPENDIX

TABLE 4

MEAN WET WEIGHT (grams) AND VARIATION OF INDICATED TISSUE FOR ACUTE AND METABOLIC RATS

Tissues		Control	Acute		Control	25-Day	
		grams	grams	t-value	grams	grams	t-value
Brain	$\bar{X}$	1.685	1.731	0.724	2.094	1.601	2.122
	S^2	0.0132	0.0151		0.3204	0.0038	
	n	7	7		6	6	
Heart	$\bar{X}$	1.048	0.963	1.211	0.952	0.927	0.366
	S^2	0.0190	0.0154		0.0159	0.0142	
	n	7	7		6	6	
Kidneys	$\bar{X}$	2.353	2.223	0.903	2.502	2.415	0.483
	S^2	0.0049	0.0543		0.0986	0.0949	
	n	7	7		6	6	
Liver	$\bar{X}$	9.398	9.188	1.059	11.833	11.593	0.252
	S^2	1.8660	1.1215		1.7956	3.6443	
	n	7	7		6	6	

TABLE 5

MEAN ASH WEIGHT (milligram) AND VARIATION OF INDICATED TISSUE FOR ACUTE AND METABOLIC RATS

Tissues		Control	Acute		Control	25-Day	
		mg	mg	t-value	mg	mg	t-value
Brain	$\bar{X}$	26.543	28.700	1.541	26.500	23.933	1.769
	S^2	6.584	7.1396		4.7524	7.8736	
	n	7	7		6	6	
Heart	$\bar{X}$	11.643	12.543	1.230	9.717	9.433	0.419
	S^2	2.8123	0.937		1.3619	1.3877	
	n	7	7		6	6	
Kidneys	$\bar{X}$	28.916	29.483	0.33	31.633	30.050	0.702
	S^2	9.7406	7.5790		14.9846	15.5078	
	n	7	7		6	6	
Liver	$\bar{X}$	107.857	147.142	2.840[a]	201.550	185.866	0.797
	S^2	308.8455	1029.8323		1306.3887	1011.5582	
	n	7	7		6	6	

[a]Significant at the 0.05 level.

TABLE 6

MEAN CONCENTRATION (μg/mg) AND VARIATION OF COPPER IN ASH OF INDICATED TISSUE FOR ACUTE AND METABOLIC RATS

Tissues		Control	Acute		Control	25-Day	
		μg/mg	μg/mg	t-value	μg/mg	μg/mg	t-value
Brain	$\bar{X}$	0.181	0.178	0.261	0.212	0.210	0.072
	S^2	0.0096	0.0003		0.0038	0.090	
	n	7	7		6	6	
Heart	$\bar{X}$	0.378	0.276	5.754[a]	0.450	0.491	1.335
	S^2	0.0067	0.0015		0.0053	0.0003	
	n	7	7		6	6	
Kidneys	$\bar{X}$	0.628	0.591	0.685	1.370	1.792	1.017
	S^2	0.0049	0.0132		0.7090	0.3204	
	n	7	7		6	6	
Liver	$\bar{X}$	0.241	0.200	2.419[a]	0.121	0.168	2.810
	S^2	0.0014	0.0015		0.0004	0.0003	
	n	7	7		6	6	
Serum	$\bar{X}$	1.490	1.334	1.645	1.995	1.507	1.512
	S^2	0.0520	0.0100		0.5700	0.0529	
	n	7	7		6	6	
Pooled Urine	$\bar{X}$				0.391	0.330	1.721
	S^2				0.00313	0.00302	
	n				5	5	

[a]Significant at the 0.05 level.

TABLE 7

MEAN CONCENTRATION (μg/mg) AND VARIATION OF MAGNESIUM IN ASH OF INDICATED TISSUE FOR ACUTE AND METABOLIC RATS

Tissues		Control	Acute		Control	25-Day	
		μg/mg	μg/mg	t-value	μg/mg	μg/mg	t-value
Brain	$\bar{X}$	11.214	10.918	0.780	11.983	12.048	0.130
	S^2	0.5625	0.4529		0.7191	0.7850	
	n	7	7		6	6	
Heart	$\bar{X}$	18.683	16.582	2.580[a]	23.610	26.532	2.520[a]
	S^2	1.7240	2.9173		3.2616	4.7917	
	n	7	7		6	6	
Kidneys	$\bar{X}$	14.969	16.303	2.883[a]	14.412	17.125	2.948[a]
	S^2	0.7396	0.5417		3.7095	1.3689	
	n	7	7		6	6	
Liver	$\bar{X}$	13.131	11.120	2.580[a]	11.301	13.191	2.120
	S^2	0.3721	3.8416		1.1664	3.5721	
	n	7	7		6	6	
Serum	$\bar{X}$	21.695	22.244	0.567	23.736	24.807	0.848
	S^2	2.3562	4.1943		3.5797	5.9585	
	n	7	7		6	6	
Pooled Urine	$\bar{X}$				61.915	45.301	
	S^2				1444.9197	731.2914	2.680[a]
	n				5	5	

[a]Significant at the 0.05 level.

TABLE 8

MEAN CONCENTRATION (μg/mg) AND VARIATION OF MANGANESE IN ASH OF INDICATED TISSUE FOR ACUTE AND METABOLIC RATS

Tissues		Control	Acute		Control	25-Day	
		μg/mg	μg/mg	t-value	μg/mg	μg/mg	t-value
Brain	$\bar{X}$	0.026	0.027	0.000	0.027	0.027	0.162
	S^2	0.00001	0.0000		0.0000	0.0000	
	n	7	7		6	6	
Heart	$\bar{X}$						
	S^2		SNS[a]			SNS[a]	
	n						
Kidneys	$\bar{X}$	0.052	0.057	5.009[b]	0.066	0.063	0.646
	S^2	0.0000	0.0000		0.00008	0.00003	
	n	7	7		6	6	
Liver	$\bar{X}$	0.132	0.128	0.419	0.137	0.156	1.907
	S^2	0.00012	0.00036		0.00028	0.00032	
	n	7	7		6	6	
Serum	$\bar{X}$						
	S^2		BLDL[c]			BLDL[c]	
	n						
Urine	$\bar{X}$						
	S^2		BLDL[c]			BLDL[c]	
	n						

[a]Sample not sufficient.

[b]Significant at the 0.05 level.

[c]Below detection limit.

TABLE 9

MEAN CONCENTRATION (μg/mg) AND VARIATION OF ZINC IN ASH OF INDICATED TISSUE FOR ACUTE AND METABOLIC RATS

Tissues		Control μg/mg	Acute μg/mg	t-value	Control μg/mg	25-Day μg/mg	t-value
Brain	$\bar{X}$	0.745	0.730	0.503	0.810	0.814	0.087
	S^2	0.0026	0.0036		0.00028	0.00774	
	n	7	7		6	6	
Heart	$\bar{X}$	4.259	3.919	2.548[a]	4.882	5.151	1.005
	S^2	0.0930	0.0306		0.3795	0.0467	
	n	7	7		6	6	
Kidneys	$\bar{X}$	1.641	1.631	0.534	1.830	2.150	2.110
	S^2	0.0112	0.0058		0.0515	0.0864	
	n	7	7		6	6	
Liver	$\bar{X}$	2.145	1.865	2.038	1.644	1.861	1.552
	S^2	0.0132	0.1183		0.0918	0.0250	
	n	7	7		6	6	
Serum	$\bar{X}$	1.219	1.317	0.908	1.464	1.426	0.338
	S^2	0.0520	0.0282		0.0225	0.0548	
	n	7	7		6	6	
Urine	$\bar{X}$				1.8696	1.9082	0.11056
	S^2				0.27961	0.3297	
	n				5	5	

[a]Significant at the 0.05 level.

TABLE 10

A TYPICAL EXAMPLE OF THE STATISTICAL ANALYSIS FOR CHRONIC RATS

Copper Content of Heart Tissue in μg/mg of Tissue Ash

Analysis of Variance

	SS	DF	MS	F
Groups	0.357005745	1	0.357005745	1.577
Time	0.200593687	3	0.668645625	29.548[a]
Groups X Time	0.108031854	3	0.36010618	1.591
Within Cell	0.126722503	56	0.226290184	

Simple Effect

Time within Groups

Group	SS	DF	MS	F
Control	0.844976	3	0.281658	12.44[a]
Chronic	0.126899	3	0.422997	18.69[a]

Group within Time

Days	SS	DF	MS	F
5	0.257556	1	0.257556	1.13
10	0.975156	1	0.975156	4.30[a]
15	0.187056	1	0.187056	0.82
20	0.175564	1	0.175564	0.07

[a]Significant at the 0.05 level.

TABLE 11

MEAN WET WEIGHT (grams) IN INDICATED TISSUES AND TREATMENT GROUPS FOR CHRONIC RATS

Interval (Days)	5	10	15	20	
Brain					
Control	1.5989	1.7227	1.5764	1.6352	[a]
Chronic	1.5784	1.7384	1.6062	1.6194	[a]
Heart					
Control	0.9519	1.0252	0.9968	1.0927	[b]
Chronic	1.0119	0.9301	1.0047	0.9268	[b]
Kidneys					
Control	2.0326	2.1258	2.0802	2.2076	[b]
Chronic	2.0502	2.1364	1.9641	1.8724	[b]
Liver					
Control	9.8977	9.3898	9.4577	9.9501	
Chronic	9.7371	8.9514	9.5452	8.7093	

[a]Significant difference with time.

[b]Significant difference between groups.

TABLE 12

MEAN ASH WEIGHT (milligrams) IN INDICATED TISSUES AND TREATMENT GROUPS FOR CHRONIC RATS

Interval (Days)	5	10	15	20	
Brain					
Control	22.9499	24.9124	22.4499	23.5374	
Chronic	23.8374	24.0624	24.5499[a]	23.9999	
Heart					
Control	8.9499	10.8874	9.4624	11.0499	b
Chronic	11.0499[a]	9.3124[a]	10.0374	12.0499	b
Kidneys					
Control	23.2874	25.2749	23.0124	24.7624	
Chronic	25.2124	24.8999	22.5249	23.2374	
Liver					
Control	139.4249	121.7624	122.7624	137.0124	
Chronic	139.4125	135.2374	125.0499	121.6374	

[a]Significant difference between groups.

[b]Significant difference with time.

TABLE 13

MEAN COPPER CONCENTRATION (μg/mg ASH) IN INDICATED TISSUES AND TREATMENT GROUPS FOR CHRONIC RATS

Interval (Days)	5	10	15	20	
Brain					
Control	0.1469	0.1706	0.1788	0.1753	
Chronic	0.1673	0.1812	0.1742	0.1859	
Heart					
Control	0.4398	0.3836	0.3839	0.2962	a
Chronic	0.4652	0.4329[b]	0.3623	0.3028	a
Kidneys					
Control	0.7096	0.6748	0.6441	0.6319	
Chronic	0.7234	0.7984	0.6861	0.9301[b]	
Liver					
Control	0.2037	0.2004	0.2131	0.1838	
Chronic	0.1988	0.1802	0.2236	0.1874	
Serum					
Control	1.5922	1.4031	1.3306	1.2227	
Chronic	1.5206	1.9562[b]	1.3673	1.5218	

[a]Significant difference with time.

[b]Significant difference between groups.

TABLE 14

MEAN MAGNESIUM CONCENTRATION (μg/mg ASH) IN INDICATED TISSUES AND TREATMENT GROUPS FOR CHRONIC RATS

Interval (Days)	5	10	15	20	
Brain					
Control	12.3374	11.8462	11.4024	11.1549	
Chronic	11.8274	11.6562	10.4149[a]	10.6662	b
Heart					
Control	24.2007	20.9511	21.8692	18.3938	b
Chronic	27.6313[a]	24.3217[a]	19.4169	15.0611	b
Kidneys					
Control[c]	17.9946	16.6934	15.5687	15.9298	
Chronic	19.2622	17.2484	15.3301	14.0957[a]	
Liver					
Control	14.6904	13.3148	13.1948	12.8216	
Chronic	14.1421	15.3736	13.2824	14.5028	
Serum					
Control	22.9817	23.1007	21.8191	19.9902	
Chronic	26.8347[a]	22.4932	23.2269	20.4968	b

[a]Significant difference between groups.

[b]Significant difference with time.

[c]Heterogeneity prevented the use of analysis of variance in this analysis. Individual differences by modified t-test.

TABLE 15

MEAN MANGANESE CONCENTRATION (μg/mg ASH) IN INDICATED TISSUES AND TREATMENT GROUPS FOR CHRONIC RATS

Interval (Days)	5	10	15	20	
Brain					
Control	0.0266	0.0298	0.0224	0.0276	
Chronic	0.0251	0.0288	0.0254	0.0244	
Heart					
Control	SNS[a]				
Chronic	SNS[a]				
Kidneys					
Control	0.0754	0.0776	0.0678	0.0607	[b]
Chronic	0.0853	0.0702	0.0619	0.0527	[b]
Liver					
Control	0.1389	0.1502	0.1329	0.1406	
Chronic	0.1246[c]	0.1464	0.1243	0.1444	
Serum					
Control	BLDL[d]				
Chronic	BLDL[d]				

[a]Sample not sufficient.

[b]Significant difference with time.

[c]Significant difference between groups.

[d]Below detection limit.

TABLE 16

MEAN ZINC CONCENTRATION (μg/mg ASH) IN INDICATED TISSUES AND TREATMENT GROUPS FOR CHRONIC RATS

Interval (Days)	5	10	15	20	
Brain					
Control[a]	0.7506	0.7578	0.7248	0.7993	
Chronic	0.7987	0.7931	0.6679	2.9366[b]	
Heart					
Control[a]	4.7652	4.0492	4.0424	4.1761	
Chronic	5.6551[b]	4.5482[b]	3.8097	5.3579[b]	
Kidneys					
Control	2.0898	1.9032	1.8346	1.8101	
Chronic	2.3776[b]	1.9867	1.7903	1.8179	c
Liver					
Control[a]	1.9666	2.0332	1.8774	1.7874	
Chronic	1.9132	1.6636[b]	1.8901	2.4628[b]	c
Serum					
Control	1.5484	1.6848	1.5488	1.3942	
Chronic	1.7469	1.5882	1.6259	1.2752	c

[a]Heterogeneity prevented the use of analysis of variance in this analysis. Individual differences by modified t-test.

[b]Indicates significant difference between groups.

[c]Indicates significant difference with time.