THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

TRACE METAL ALTERATIONS DURING ENDRIN INTOXICATION

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

BY

WENDELL L. SOWELL

Oklahoma City, Oklahoma

1967

TRACE METAL ALTERATIONS DURING ENDRIN

INTOXICATION

APPROVED

Carl A Nau

John B. Bruce

Ronald L. Coleman

Clyde W Lawrence

A Bruce Neat

DISSERTATION COMMITTEE

ACKNOWLEDGMENTS

The author expresses expreciation to Doctors Carl A. Nau, John B. Bruce, R. Dale Vliet, Ronald L. Coleman, and Charles H. Lawrence, for serving on his graduate committee.

Special appreciation is extended to Doctors Ronald L. Coleman and Charles H. Lawrence whose kind help and constructive criticism were invaluable in conducting this study and for their guidance in the writing of the dissertation.

Individual consideration is extended to Dr. Robert N. Thompson for technical drawings, Dr. Robert C. Duncan for his assistance in statistical analysis, Dr. Bobby J. Gunter for his assistance in performing the analysis, and Mr. Bill L. Stevenson for his many favors during this study.

Recognition and appreciation is extended to the U. S. Public Health Service whose support under the Public Health Training Program Grant No. EH67-635 made this study possible.

The author expresses his deepest appreciation to his wife for patience and encouragement during the course of this study and for assistance in preparation of the statistical data.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

TRACE METAL ALTERATIONS DURING ENDRIN INTOXICATION

CHAPTER I

INTRODUCTION

Throughout history, there has been a struggle between man and insects that has continued without letup to the present time, and will continue, no doubt, as long as a member of the human race endures.

There are some people who embrace the idea that man is dominant over insects. However, let us recall that the outcome of most of the great wars of history was not decided by man on the battle fields but by insects, as a consequence of insect-borne diseases. The Allies had to defeat the insects in the South Pacific during World War II before they could attack the enemy forces (31). The great plagues of history were caused by insect-borne diseases. On two occasions, insects arrested the construction of the Panama Canal. Efforts taken to eliminate damage caused by insect vectors of human diseases is demonstrated by the rapid growth of chemical pest control which has

grown to a multi-million dollar enterprise (31).

In the agriculture field, much progress has been made with the aid of economic poisons, but still there are animal losses from insects estimated at $4 billion annually (31). Obviously, there is much room for improvement. The wastage of food consumed or defiled by rodents and insects during a period when millions of people throughout the world are hungry is tragic, inexcusable, and could be prevented by the use of pesticides (32).

Many insect vectors of important human diseases have been brought under control to such a degree that the once dreaded diseases malaria, yellow fever, cholera, and bubonic plague are no longer scourges of mankind and there are those who feel that we may be well on our way to the eradication of at least some of them (32). Each year, until the advent of insecticides, some 250 million persons were afflicted with malaria throughout the world and some 25 million died annually.

According to Herms and James (69), Hodge reported that one adult fly can deposit 120 to 150 eggs per lot for a total of at least six lots, each separated by 3 to 4 days. Based on this he calculated that

> "a pair of flies beginning operations in April may be progenitors, if all were to live, of 191,010,000,000,000,000,000 flies by August. Allowing one-eighth of a cubic inch to a fly, this number would cover the earth 47 feet deep."

insecticides have made it possible to enhance the quality and quantity of agricultural products and to protect the health of people. A spectacular example is the use of the insecticide, 1,1,1-trichloro-2, 2-bi(p-chlorophenyl) ethane (DDT), in the campaign against malaria by the World Health Organization which reached all but 300 million of the 1,400 million persons who live in malarious regions (other than China, North Korea, and North Vietnam).

The economic poisons constituted a billion dollar business in the United States in 1965. In that year, more than $590 million were spent for agricultural pesticides (139).

In 1962, the United States exported more than 22 million pounds of herbicides, and in the 1962 to 1963 period, herbicides comprised 20 per cent of the total organic pesticide production in the United States. In the years from 1959 to 1962, the value of herbicides exported increased from $6.7 million to $22.4 million. In the same period, fungicides exported increased from $8 million to $26 million (139).

Household, lawn, and garden use accounted for approximately 20 per cent of all the pesticides purchased in 1965. In 1964, the United States exported pesticides valued at $134 million and in the first six months of 1966, the value increased to $92,860,834 which was a 36 per cent increase for 1966 (139).

It is obvious that every state in the United States, every

country in the world, and every individual person is in some way dependent upon and affected by the use of economic poisons for his livelihood and health.

Pesticides may be defined as "materials which kill unwanted organisms" (71). Economic poison means any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any insects, rodents, nematodes, fungi, or weeds, or any of the other forms of life declared to be a pest, and any substance or mixture of substances intended for use as a plant regulator, defoliant, or desiccant (76). This includes all preparations intended for use as insecticides, rodenticides, nematocides, fungicides, herbicides, amphibian and reptile poisons or repellents, bird poisons or repellents, fish poisons or repellents, mammal poisons or repellents, invertebrate animal poisons or repellents, plant regulators, plant defoliants, and plant desiccants (76). All of these substances are subject to control by the Federal Insecticide, Fungicide and Rodenticide Act of 1947 and the Miller Pesticide Residue Amendment to the Federal Food, Drug and Cosmetic Act (71).

Insecticides are classified as inorganic and organic poisons. Inorganic pesticides include metallic and soluble compounds which are effective as gastrointestinal poisons for pests with chewing mouth parts or as contact, protoplasmic poisons and include antimony,

arsenic, barium, cyanide, fluoride, mercury, phosphorus, and thallium. Organic pesticides include six major broad classes; namely benzene hexachloride derivatives and isomers, chlorinated camphenes, chlorobenzene derivatives, carbamates, organic phosphates, pesticides of botanical origin, miscellaneous compounds and cyclodiene or indane derivatives (20). They are also classified into subclasses according to their physiological action such as systemic (1), contact, or stomach poisons. One of the most important insecticide groups is the cyclodienes, which are commonly known as the chlorinated hydrocarbon or indane insecticides. This group includes aldrin, dieldrin, isodrin, and endrin.

The presence of chlorinated pesticides in human tissues throughout the world attests to the almost universal application of and exposure to these materials. The cyclodienes, especially endrin, are deposited in fatty tissue where they remain until they are metabolized and/or excreted in the milk, urine, and/or feces (158). For that portion of the chlorinated hydrocarbons that are subjected to metabolism or degradation in the body, the products formed may be more toxic than the original compound.

In general, the cyclodienes are highly toxic to man, animals and insects through their action on the central nervous system. The most consistent observations following repeated exposure of experi-

mental animals have been changes in the liver and kidneys (158). Endrin, one of the most toxic of the cyclodienes, has induced symptoms such as abdominal discomfort, vertigo, insomnia and weakness of legs during mild intoxication, and nausea and diarrhea during acute exposure. Moderate doses have been observed to produce, without warning, a marked loss of appetite, loss of weight and convulsions (158). Due to this comparatively high toxicity, the Federal Government permits no endrin residual on crops that are to be consumed by humans or marketable animals.

Even though much is known about the pharmacological action of the cyclodienes relatively little is known concerning their molecular base of action. Recent studies concerning similar disease states have indicated that shifts in certain trace metals at the molecular level result in observed clinical phenomena. Such trace metal shifts may not only result from, but also contribute to, the toxic state and as such could provide a basis for the detection and evaluation of intoxication before these gross physiological responses are manifested. Thus, an evaluation of trace metal mobilization appears to be essential to a basic knowledge of the toxic reaction.

CHAPTER II

REVIEW OF LITERATURE

Chemistry of the Cyclodienes

The cyclodiene insecticides are highly chlorinated cyclic hydrocarbons characterized by an endomethylene-bridge structure. Synthesis of these cyclodienes, except toxaphene, is by a Diels-Alder diene reaction. Structural isomers (endo- and exo-forms) are possible in the bridged-ring compound. The isomers are named from the relative positions occupied by the rings in relation to each other. Attachments can be made in two positions (as shown in Figure 1) using carbon atoms 7 at the top of the drawings as the beginning point of the triangle, and carbon atoms 1 and 2 are used to determine the isomeric positions of the ring structures (51).

Rings attached to carbon atoms 1 and 2 may be in one of two positions. If the adjacent ring lies outside an angle drawn from carbon atom 7 through carbon atom 2, it is identified as an exo derivative, while those rings which lie inside the angle are called endo derivatives (51). The second ring attached to carbon atoms 1 and 2 becomes

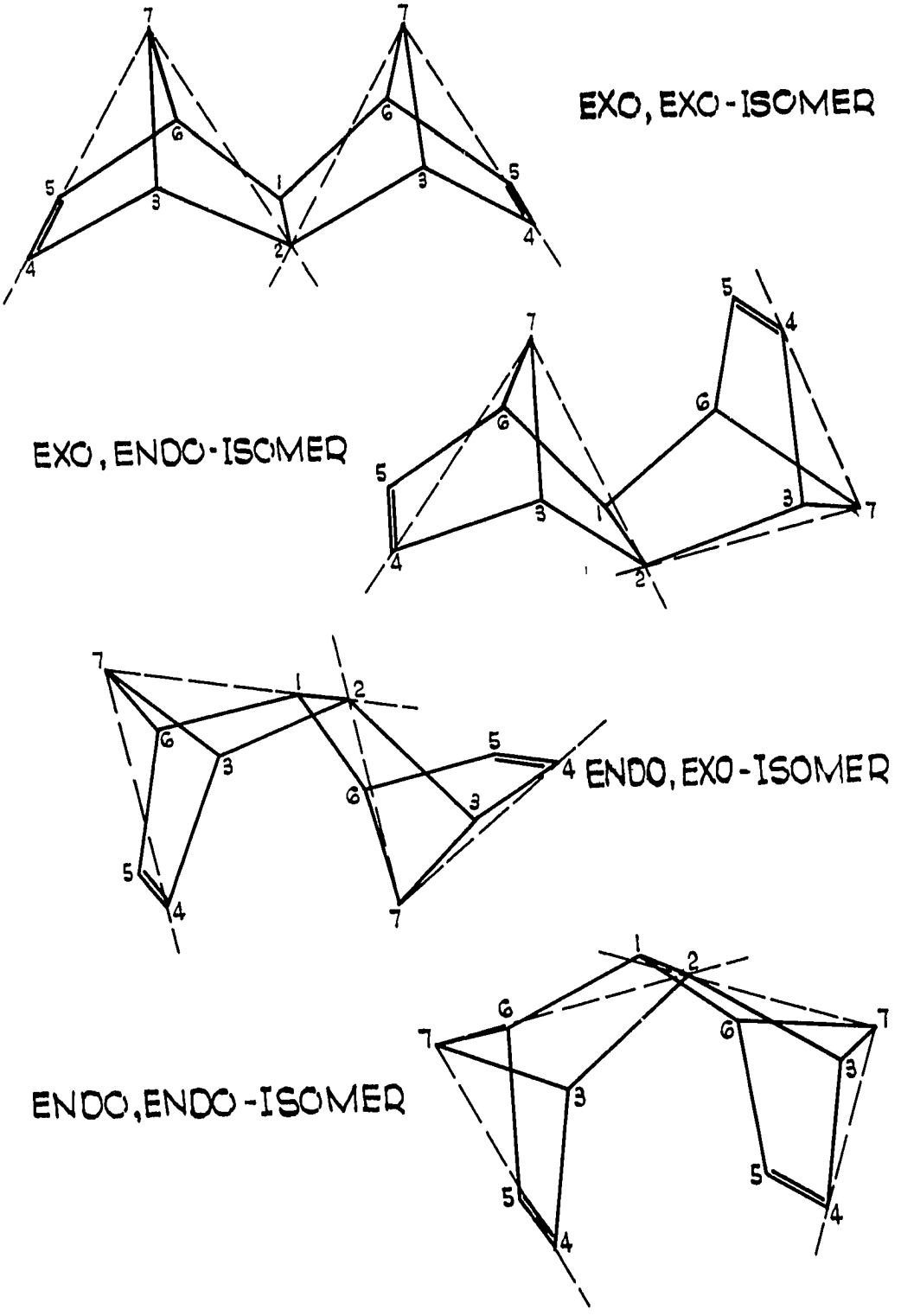

Figure 1. Structual isomers (endo- and exo-forms).

either exo- or endo- with respect to the ring containing carbon atoms 1 through 7. This second ring may also have attachments that can be exo- or endo- so that four theoretical isomers may exist: exo, exo-; exo, endo-; endo, exo-; and endo, endo-. Oxidation of the 6,7-positions with per-acids gives the 6,7-epoxy derivatives (51).

Aldrin

In 1949, Kearns et al. (82) published the first report on a new insecticide that was introduced in 1948 and called "Compound 118." Later this insecticide was assigned the coined name, ALDRIN, because it was synthesized by the Diels-Alder reaction. The endo-exo isomer aldrin is a product having not less than 95 per cent of its principal constituent the chemical 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo, exo-5,8-dimethanonaphthalene (51) (Figure 2). The principal component is a white crystalline solid which has a melting point of 100 to 102°C. This compound has no odor at room temperature, but has a pine-like odor when heated to higher temperatures. Epoxidation of the 6,7 positions of aldrin with per-acids forms the endo-exo epoxide, dieldrin (51).

The initial insecticidal toxicity of aldrin is relatively high and it also has a comparatively high chronic and dermal toxicity to animals. Usually, the residual action is short, and the residues do

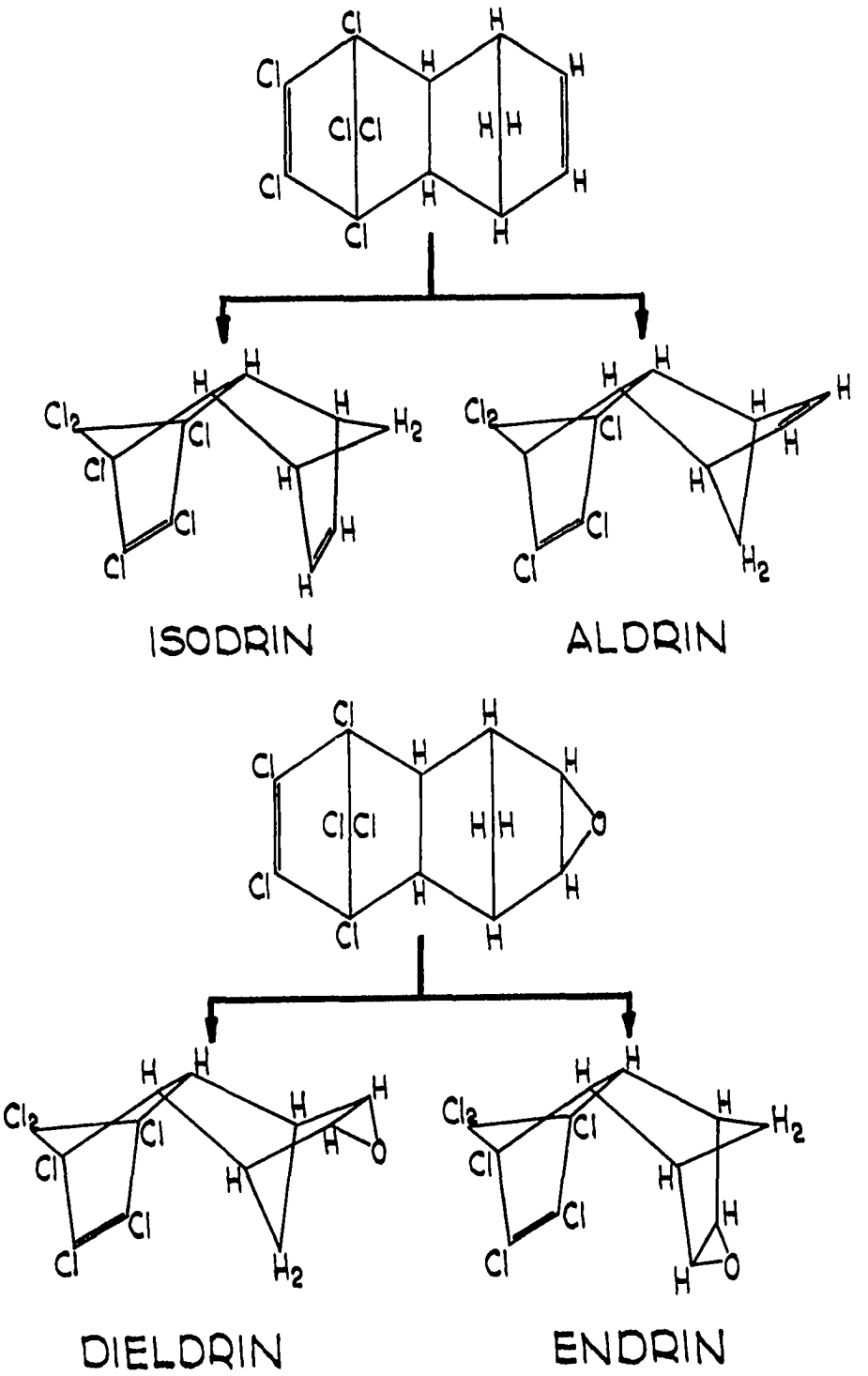

Figure 2. Structural configurations of the cyclodienes.

not persist longer than three weeks, with no phytotoxicity when applied as directed.

Isodrin

The endo-endo isomer of aldrin was isolated in the latter part of 1950 (51), and was given the name "Compound 711." Later the coined name of ISODRIN was given to this isomer. Chemically, isodrin is 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-endo-5,8-dimethanonaphthalene (51) (Figure 2), and is a white crystalline solid which is not decomposed by alkalies but is slowly decomposed when heated above 100°C. It has a high acute toxicity to warm-blooded animals and a low toxicity to flies. Isodrin is synthesized by slowly reacting cyclopentadiene with the condensation product of hexachlorocyclopentadiene and vinyl chloride (51). The 6,7-positions are oxidized with per-acids to give the 6,7-epoxy derivative endrin.

Dieldrin

Kearns et al. in 1948 (82) first described this insecticide under the name "Compound 497," and DIELDRIN was coined in 1949 and registered as a generic name for the "Compound 497." This name was chosen because this compound was synthesized by the Diels-Alder condensation reaction like aldrin. The active insecticidal product

contains not less than 85 per cent of its principle constituent, the chemical 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene (51) (Figure 2). It is a buff-colored crystalline solid with very little odor and a melting point of 150°C in mixtures and 175 to 176°C in the pure state (51). Below 43°C, it is less volatile than DDT or aldrin and is more volatile at higher temperatures. It is relatively stable in spite of the epoxy linkage. Dieldrin has a higher toxicity to some insects than DDT or aldrin and is considered a general poison with a pronounced residual action, and very little phytotoxicity. It is both a stomach and contact poison and readily absorbed through the skin in lethal quantities.

Endrin

Endrin (the stereoisomer of the principle constituent of dieldrin) is a white crystalline solid, having the endo-endo configuration (Figure 1) with respect to the two methano portions of the formula, and is defined as 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-endo-5,8-dimethanonaphthalene (157) (Figure 2). This formulation was formerly called "Compound 269." Later it was given the coined name ENDRIN. This isomer is stable to basic reagents and alkalies, but when treated with acids or heated above 200°C, a rearrangement of the molecule occurs with

the production of two derivatives; one referred to as the delta keto-153 derivative, 1,8,9,10,11-hexachloropentacyclo-(6.2.$1^{3,6}$.$0^{2,7}$.$0^{4,10}$)-dodecane-5-one (127), and the other derivative is an aldehyde, 4,5,6,7,8,8-hexachloro-4,7-methano-3,5,6-methenoindane-1-carboxyaldehyde (119). Endrin is soluble in the usual organic solvents, but insoluble in water. Its greatest solubility is in aromatic hydrocarbons such as benzene and xylene (51). It was introduced in the early 1950's and has found extensive field use because of a striking potency against many household and field insects (51). Endrin is considered a general poison, killing both by contact and stomach action. It is toxic to animals and insects in small quantities and absorbed into the body by one of three methods: inhalation, ingestion, or through the intact skin. Once endrin enters the body, it can be stored in the fatty tissue or secreted via the feces and milk (158).

Endrin dissipates from the fatty tissues more expeditiously than the other cyclodiene insecticides (158). It appears to be transitory in the body and no evidence of metabolites or end products have been reported. The exact cause of death from endrin has not been determined; neither have the deposition, distribution, and quantity of endrin in the individual cell fragments been evaluated.

Endrin can undergo isomerization, rearrangement, and decomposition when exposed to heat, sunlight, and ultraviolet irradi-

ation. This instability of endrin under these special conditions can be both a blessing and a curse. Degradation under ultraviolet irradiation can be used for identification of endrin on thin layer chromatograms. At the temperature required in gas chromatography, pure endrin will give rise to multi-peak chromatograms (57) (119). Two major peaks of almost equal size are the result of thermal isomerization of endrin to a ketone and a new isomeric aldehyde (Figure 3). Intermediate to the formation of the ketone and the aldehyde is an enol-like compound (Figure 4) (146).

Sunlight produces the delta-keto isomer, a mixture of polymers, and a carbonyl type compound (126). Ultraviolet irradiation (2537 Å) produces many of the same products and the mechanism is thought to involve either a hydride shift or a hydrogen abstraction of an epoxy-hydrogen (119). Uniquely caused by 2537 Å is a hydrogen atom replacement of a chloride atom adjacent to the double bond.

In general, the compounds produced by thermal, photochemical, or ultraviolet isomerization appear to be the same.

Toxicology of Endrin

Humans

All methods of manufacture, formulation, and use of endrin are similar to that employed in the handling of other economic poisons of similar toxicity, yet only 214 cases of poisoning have

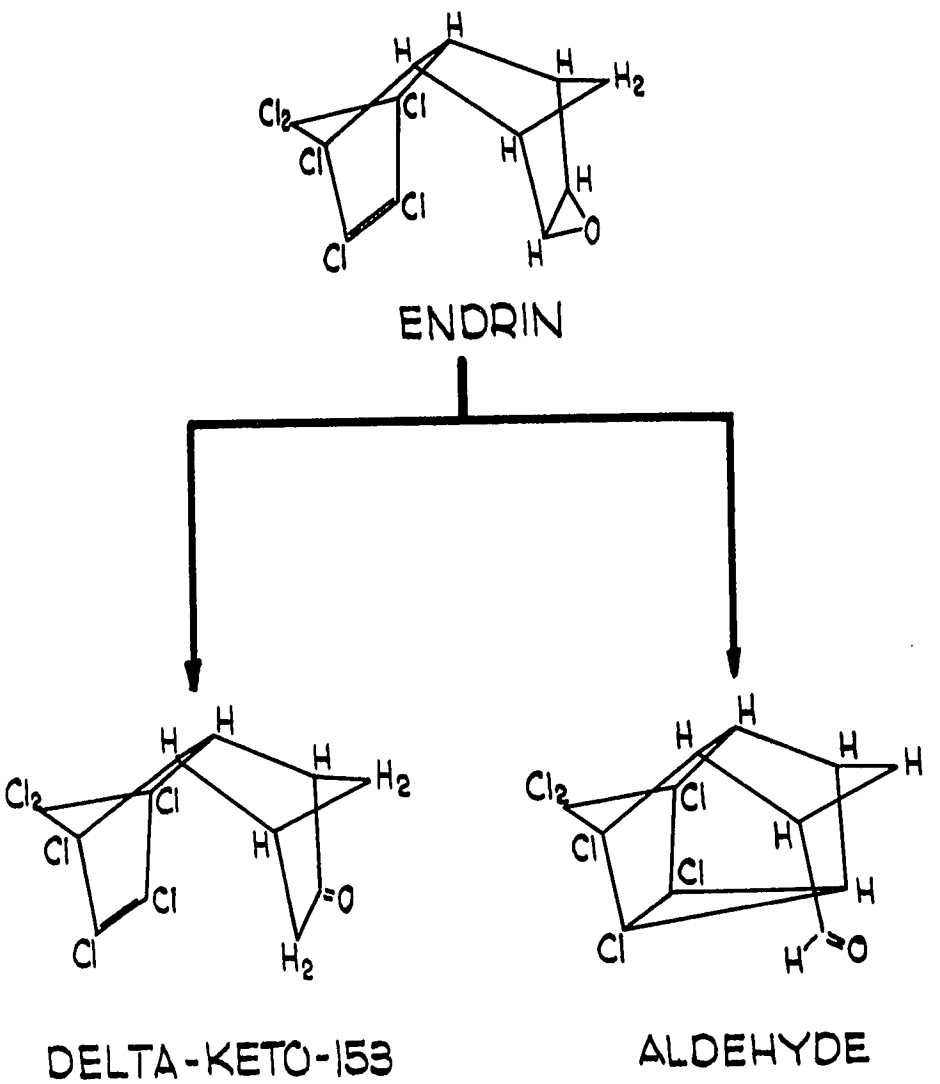

Figure 3. Endrin derivatives

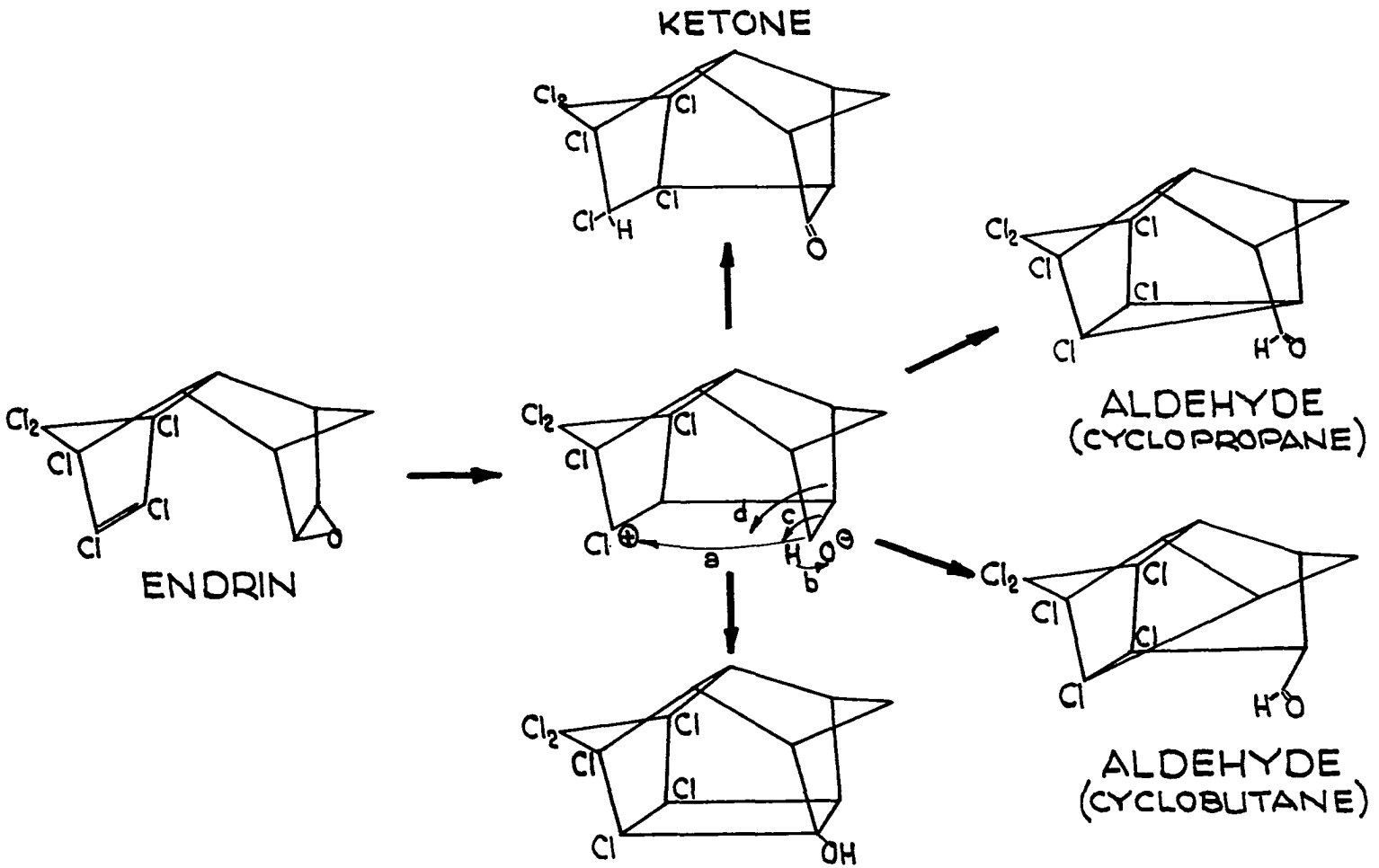

Figure 4. Physical degradation scheme for endrin.

been reported (158). These 214 cases include suicidal attempts, accidental ingestion, occupational exposure, and consumption of food contaminated with endrin. Only a few cases of intoxication have occurred among personnel engaged in the processing of endrin, and no deaths are known to have occurred. The low incidence of casualties might conceivably be explained by the care taken in the safe methods of use, or safe handling in every aspect in the manufacture of endrin, but these do not appear to explain or coincide with the facts.

The best description available of the human reaction to a single oral dose of endrin is that of Davies and Lewis (27), in which a group of 59 persons in Italy were poisoned by eating contaminated bread. The bread was made from flour that had become contaminated in transit.

Symptoms, which developed in about 3 hours in 30 of the 59 people were epileptiform in character (158). Dizziness, weakness in the legs, abdominal discomfort, and nausea occurred in the less severe cases. Several of the individuals were confused and a few complained of temporary deafness. Weakness, insomnia, and anorexia persisted for several days in a few cases, but most of those affected were improved the second day regardless of whether or not they had experienced convulsions (158). It is of significance that from all these illnesses there were no fatalities.

Brief, but effective, exposure by other than oral routes has produced characteristically the same sequence of symptoms, beginning with a sudden attack of convulsions. Very often vomiting is the first indication of the absorption of a toxic amount of endrin (180).

Of immense significance is the fact that after the poisoning occurred in 1956, none of the persons affected suffered anything unfavorable which could in any way be associated with the endrin poisonings.

A careful appraisal of a 10-year period between 1952 and 1962 has disclosed only 43 cases of chronic human intoxication. Twenty-seven of the 43 were so mild that no time was lost from work and no medical attention was necessary, but the other 16 involved loss of consciousness or convulsions and four of the 16 were fatal. In the 16 cases, there was a history of gross over-exposure. After having endured muscle twitching, loss of consciousness, or convulsions, 12 patients made a complete recovery within 24 to 96 hours, and many resumed work in 3 days without further incident (158).

Sixty-four non-occupational intoxications have occurred in the various countries of the world over a period of 10 years. In the Sudan, three villagers drank water from a ditch contaminated with endrin, and each one became ill with nausea, vomiting, headache,

and dizziness, but had no convulsions and recovered within 24 to 48 hours. A man in Spain consumed freshly-sprayed blackberries and became ill with convulsions, but recovered promptly. Jacobziner and Raybin (77) described one case where a child in Venezuela became ill from contamination of the skin.

Over a period of 10 years, a total of 30 cases of accidental ingestion of endrin has taken place due to gross negligence. This is a relatively minor number when compared to all the accidental poisonings caused by the ingestion of such things as kerosene, bleaches, aspirin, and other common household products. There have been a total of 24 fatalities from this group of intoxications and five non-fatal cases which manifested convulsions (158). All these persons consumed endrin in large amounts.

There have been 69 cases of suicide by ingestion of endrin over a period of 10 years in eight different countries. Other than the suicides, there were 145 cases of intoxication, and of these there were 28 fatalities (including four in the USA, and Canada), or an average of less than three fatalities per year (138).

Fifty-one of the 53 patients reported to have had convulsions recovered quickly and completely. The two exceptions were both infants who had absorbed very large amounts of endrin; one by accidental ingestion (158), the other by gross skin exposure

(77). This latter incident was the case of a small child in Maracaibo, Venezuela, who, after playing in a puddle of endrin mistakenly used for house-spraying, had severe convulsions with prolonged coma and cerebral anoxia which resulted in decerebrate rigidity and permanent brain injury.

Fish

The significance of endrin becomes more complicated when it is used near fish since most species are affected by small concentrations of endrin. Several investigators (39) (45) (46) (47) (48) (158) (173) have studied the toxicity of endrin to several different species of fish and fresh water shrimp. Parameters affecting resistance are genetic selectivity, unidentified metabolites formed in the body, structure of red blood cells, and reproductive capacity.

Under laboratory conditions, lethal concentrations of endrin to fish were studied and found to range between 1 ppb and 500 ppb (158). The fact that endrin partitions from water into mud serves as a protective measure; consequently, fish can tolerate a higher concentration of endrin in their natural habitat than in the laboratory (158).

No change was noted in the size of the liver, level of serum chloride, or gamma globulin; however, the concentrations of serum sodium, potassium, calcium, and uric acid were consistently lower

in the treated fish than in the controls (39). Since serum cholesterol levels in endrin-exposed puffers was higher in test fish than controls and cholesterol metabolism is primarily a function of the liver, Eisler and Edmunds (39) suggested that endrin enhances cholesterol production by the liver or inhibits its excretion to the bile duct. This rapid transfer of sodium, potassium, and calcium from the liver into the serum as the concentration of endrin increases supports the theory that endrin impairs the liver function of fish.

Other Animals

Exposure to airborne endrin under conditions found to be non-lethal to rats, mice, guinea pigs, hamsters, and cats was found to be fatal to rabbits (162). The loss of appetite, occasional vomiting and hypersensitivity to physical stimuli or noise are usually the first indications of endrin intoxication in dogs (162). In view of the fact that rats were able to tolerate 5 to 10 times as much endrin in their diet as dogs (109), species variation was obvious in the capability to resist the effects of chronic doses of endrin. Sex difference in the same species was not always marked; however, female rats were found to be more susceptible to endrin than males with the reverse being true for quail (30)(109).

Following prolonged absorption of endrin, animals exhibited

diffused degenerated lesions of the brain, liver, kidney, and adrenal tissues (180). Necrosis of the proximal and distal convoluted tubes of the kidney and fatty vacuolization of the cytoplasm of the hepatic cells were frequent findings even when other organs were normal (180). Cellular degeneration has been noted in rat livers even when there was no cytoplasm vacuolization. Basophilic cells were noted in zones around the central veins in the liver lobules as early as the seventh day after ingestion of a single dose of 3.5 mg/kg of body weight. These lesions advanced in intensity as the ingestion of endrin continued (180).

Treon (159) observed pulmonary hyperemia and edema in animals that died from extremis resulting from terminal dyspnea and hypoxia rather than from endrin. Irregularly distributed foci of swollen hepatic cells with cytoplasmic granules in their periphery were observed in rodents that survived two years of endrin ingestion (159). No increase in incidence of tumors was noted in animals subjected to endrin when compared with a control group not exposed to endrin (159). Increase in the permeability of the so-called blood-brain barrier was observed (147) following the absorption of endrin, and the same author found that intravenous injection of trypan blue blocked the initial acute reaction to the absorption of endrin. Treon (159) observed that, when incorporated into the diet of dogs, endrin was stored in the fat and

liver with smaller quantities in the kidneys, muscle, and brain, and the amount stored did not increase in proportion to the amount administered.

Endrin storage in the body depends on the lipid content of the tissues; however, it was found that female rats had a higher storage rate than male rats (86) (160). Nelson et al. (109) reported that endrin was absorbed on the alkaline phosphatase in the blood of rats; however, Weil and Russel (174) observed that the alkaline phosphatase levels decreased in the blood of rats after eight hours of fasting.

Physiological Mechanism

Intravenous injection of endrin in anesthetized pigeons induced a number of changes in telencephalic neuronal function (125). Dosages of 4 mg/kg or more caused seizure activity throughout the telencephalon; however, at the 2 to 3 mg/kg dosage level the seizure activity was limited primarily to the ectostriatum by stimulation of the nucleous rotundus, a diencephalic visual projection area. The reticular formation functions tested were affected very little by endrin at any dosage tested. It was suggested that relatively low levels of endrin found in the brain may impair visual function in birds and that this visual impairment could be a major factor underlying the well-known sensitivity of birds toward the chlorinated hydrocarbon pesti-

cides (125). Revzin (125) observed that the slope of the dose-response curve was very steep since 1.2 mg/kg was an LD_0 whereas 2.0 mg/kg approximated an LD_{100} with an LD_{50} of 1.5 mg/kg. No evidence of toxicity was observed in the birds 4 hours after injection of the pesticide (125).

Pentobarbital in sufficient doses completely blocks the electrographic effects of endrin. Given in convulsive doses, endrin had a diphasic action upon the effects of ascending reticular activating system stimulation (125).

Emerson et al. (42) observed that dogs treated with lethal amounts of endrin followed the usual pattern of symptoms and that their tolerance for barbiturate increased greatly during intoxication, even though the barbiturate decreased the arterial blood pressure. Decreased venous blood pH, increased rectal temperature, hemoconcentration, and leukocyte concentration also occurred. Succinylcholine completely prevented convulsions in endrin-treated dogs, and in these dogs, arterial pressure always increased initially but fell subsequently.

Endrin administered to dogs produced cardiovascular alterations such as hypertension and severe bradycardia (41). The bradycardia appeared to result from a potentiation of acetylcholine and increased vagal activity. This potentiation of acetylcholine resulting in

bradycardia could be related to an accumulation due to a fall in pH since cholinesterase activity decreases with decreasing pH. These results have been noted in dogs treated with dieldrin and aldrin (58) (59).

Increased glomerular filtration rate and renal blood flow with hypertension and bradycardia was observed by Reins et al. (123) for 1 to 2 hours after acute exposure of dogs to endrin (123). Moribund animals revealed significant changes in renal function which were secondary to alterations in systemic hemodynamics (123). Increased renal resistance suggests that endrin stimulates the sympatho-adrenal system and that the renal vascular resistance was due to circulating catecholamines and to autoregulation within the kidney (123). Adrenal gland discharges do not influence systemic hypertension or bradycardia, but stimulation of central nervous system or carotid and cardiac reflexes are overriding humoral effects, or bradycardia can be produced by release of acetylcholine and/or inhibition of acetylcholine esterase (123).

Catecholamines at high levels are a factor in the lethal cardiovascular effects of DDT (120) (148). Endrin caused an increase in the venous return and a corresponding elevation of disc output, with no change in peripheral resistance (124). Abdominal visceral vasculature was identified as the origin of the increased cardiac inflow

following endrin intoxication (124). Hematocrit value increased even in eviscerated dogs when the splenic stores of red blood cells were removed (124). Hypertension developed much less and slower in adrenalectomized dogs than in intact animals (124). Endrin produced a decreased oxygen uptake and reduced metabolic activity (124).

Noticeable rise in cardiac inflow coincided with a steady drop in resistance (70). Left atrial pressure increased strikingly within 15 minutes after treatment with endrin, but the right atrial pressure held steady (70). In some experiments, the left heart failed. Injection of endrin produced striking increases in oxygen uptake and carbon dioxide production. Acidosis developed within an hour in dogs dosed with endrin and the pH was constantly below 7.0. Endrin appeared to have two actions when it was administered in LD_{75} doses: one on the central nervous system and one on the left ventricle (70). Gowdey et al. (58) reported that the chlorinated hydrocarbin insecticides exerted their effects through stimulation of the central mechanisms, not peripherally. A prominent feature was the striking rise in venous return after endrin treatment (70). Convulsions apparently originate from a direct action of endrin on the central nervous system (70) (Figure 5).

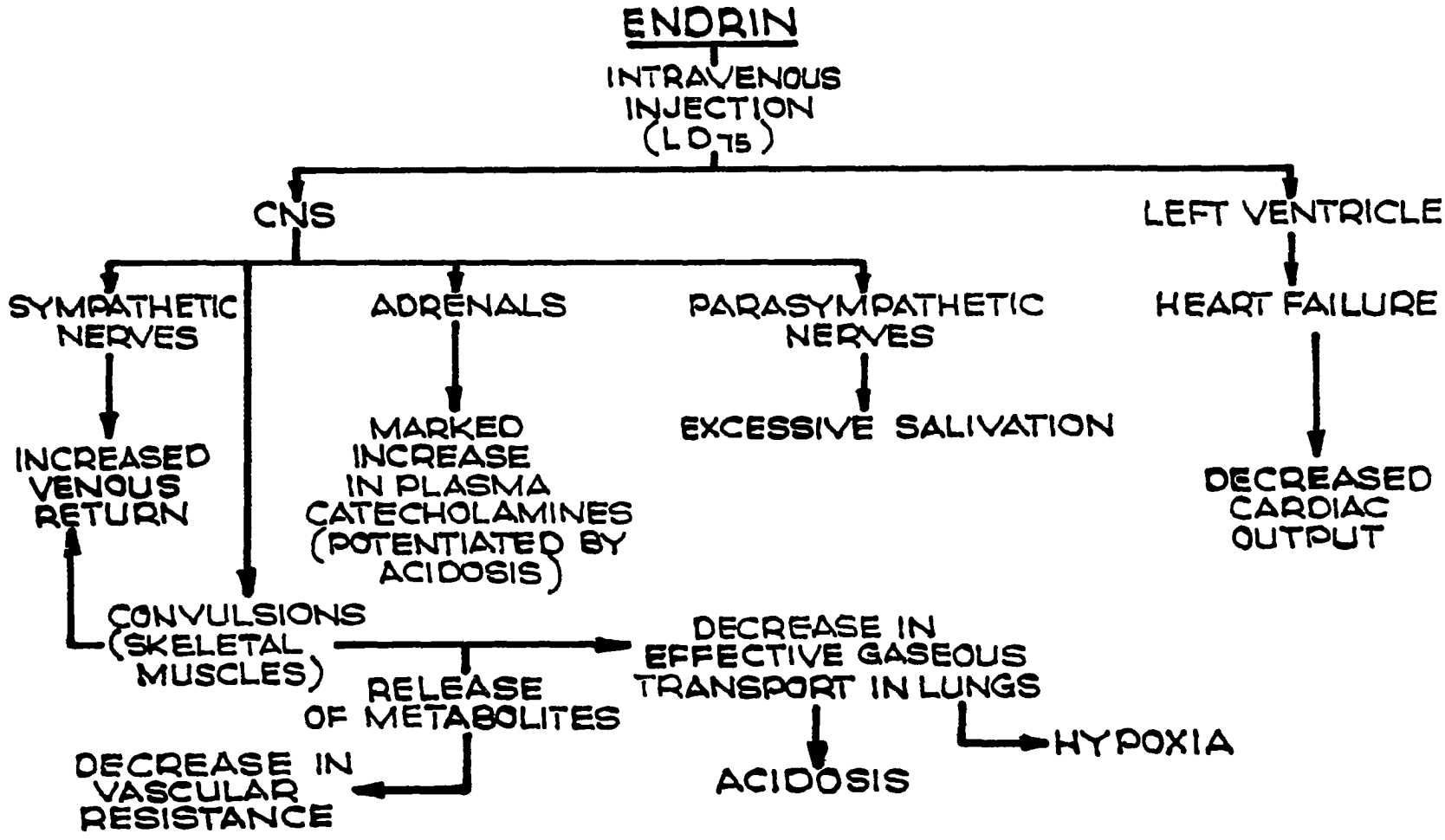

Figure 5. Physiological changes produced by endrin.

Metabolism

In 1959, Perry (114) made the following statement:

> Despite considerable work on this subject, there is no conclusive evidence at the present time that any of the chlorohydrocarbon insecticides exert lethal action by inhibiting a vital enzyme or enzyme system.

According to Perry (115), Sacktor in 1949 showed that DDT partially blocked a vital metabolic pathway and cytochrome oxidase. Sacktor (130) (131) showed that DDT-resistant house flies had 50 per cent more cytochrome oxidase than a susceptible strain of house flies. Chlorinated insecticides completely inhibited cytochrome oxidase in the muscle of the cockroach in small doses (106). Barsa and Ludwig (7) showed that the amount of inhibition by 10^{-3}M DDT was dependent on the concentration of both cytochrome oxidase and succinic dehydrogenase. Brown and Brown (18) observed that neither DDT nor methoxychlor induced a significant decrease in cockroach muscle cytochrome oxidase activity in vivo; therefore, the authors concluded that the in vitro effects observed earlier by several investigators were largely due to the adsorption of hydrophobic colloid particles of DDT on the hydrophilic enzyme surface. Perry in 1959 (114) found that DDT did not involve the carbonic anhydrase system in the American cockroach or house fly as a mechanism of detoxification. Sacklin et al. (129) observed that 5×10^{-3}M DDT completely inhibited

the oxidation of the citric acid cycle intermediates and oxidative phosphorylation of subcell particles from the house fly.

According to Perry (114), Tomizawa and Koike observed that lindane did not effect cholinesterase or alkaline phosphatase, but it had a slight inhibitory effect on fumaric dehydrogenase and moderate in vivo inhibitory effect on citric dehydrogenase of the rice stem borer.

Sternburg et al. (151) isolated the enzyme DDT-dehydrochlorinase which catalyzes the dehydrochlorination of DDT in vitro. He also (150) observed that only DDT-resistant strains of house flies contained this enzyme.

Perry (116) isolated from both resistant and susceptible body lice an enzyme identified as DDT-ase that detoxifies DDT in vitro at the same rate, but only the resistant strain was capable of detoxifying DDT in vivo. Lindquist and Dahm (93) detected small amounts of DDE but large quantities of DDT and three unidentified metabolites in the excreta of DDT-treated Madeira roaches.

Giannotti et al. (53) showed that aldrin was partially converted to dieldrin in the cockroach. Perry (116) observed that 83 per cent of the applied aldrin was metabolized to dieldrin within 24 hours following topical application, and the conversion was complete at the end of 96 hours with 5 to 8 per cent of total radioactivity found in the excreta. After application of Cl^{36} or C^{14} dieldrin, 8 to

12 per cent of the total radioactivity was detected in the excreta. Therefore, it appeared to Perry (116) that dieldrin was not metabolized by resistant insects and was retained unchanged in non-sensitive tissues, probably the body fat.

Chlorinated insecticides were studied by Weikel et al. (173) to determine their effect on the ion movement across the rabbit erythrocyte membrane. They observed that lindane, DDT, dieldrin, methoxychlor and aldrin inhibited the phosphate exchange rate, but DDT produced a selective increase in the permeability of the erythrocytes to sodium. Weikel et al. (173) showed that aldrin produced a loss of phosphate and potassium from the erythrocytes.

The toxic manifestations of the cyclodiene insecticides are controlled by their low solubility in water and their high solubility in fat. Dieldrin toxicity is uniform for a particular solvent and concentration, but the quantity finally absorbed depends upon the contents of the animal's gastrointestinal tract and its rate of peristalsis (68). The short period of time from treatment to onset of symptoms would suggest that dieldrin and other cyclodiene insecticides are absorbed from the upper portion of the gastrointestinal tract (68). Moss and Hathway (107) showed that dieldrin was transported in the blood following absorption on lipo-proteins and certain other proteins, and the rate of excretion was influenced by the nutritional state of the rat.

If the rat was starved the excretion rate increased. When the bile duct was cannulated, fat absorption was stopped completely, Cl^{36} increased in the bile, and the quantity of unchanged dieldrin excreted in the feces was greater than in the normal rat (68). In weight loss, production of polar metabolites, and excretion by the bile duct, there must be an excess capacity to metabolize dieldrin or the metabolizing enzyme must be readily stimulated (68). The principle metabolite which was more polar than dieldrin was excreted in the feces (68) and contained 60 per cent of total chlorine excreted. Studies on dieldrin metabolism in rats have demonstrated that there are many unidentified metabolites (68).

Hathway et al. (66) observed that increases of lactic acid and pyruvic acid in rat brain during acute aldrin poisoning were associated with hyperactivity of the brain, and that an increase of alanine concentration in the cerebrum occurs before the convulsions. During the dieldrin-induced seizure, the concentration of ammonia was out of phase with the actual convulsions. Telodrin produces intermediate metabolites in the brain (66). Alanine and glutamine concentration increases as well as liberation of ammonia before the onset and during the course of the convulsions (66).

Hosein and Proulx (75) preincubated brain tissue with dieldrin and observed a decreased oxygen consumption by the tissue, and this

inhibition was found to be due to action of dieldrin on various dehydrogenases and cytochromes. Other studies by these same authors showed that dieldrin would inhibit anaerobic glycolysis as well as the hydrolysis of acetylcholine by brain acetylcholinesterase and it would also increase the activity of the nonspecific serum esterases.

The material in the acid-precipitate from dieldrin-treated rat brain tissue was identified as a mixture of free betains: gamma-butyrobetaine, crotonbetaine, and carnitine. Other substances from the brain extract were identified as choline, acetylcholine, and propionylcholine. Since it had been reported that alkaline reineckate precipitation selectively removed conjugated betains from solution, the materials found in this fraction were identified as beta-alanine, and two other substances, ribose and phosphoric acid, as the phosphate part of coenzyme A (75).

Ludwig et al. (94) in 1964, fed C^{14} aldrin to male rats daily for three months. At the end of the eighth week the rats excreted daily the amount that had been administered each day, and at the end of the experiment, approximately 90 per cent of the active material had been excreted. Twelve weeks after the end of feeding, approximately 99.5 per cent of the active material had been excreted. The active material in the feces and urine consisted of aldrin, dieldrin, and considerable amounts (up to 75 per cent in the feces and up to 95

per cent in the urine) of a mixture of hydrophilic metabolic products. There were at least two different products in the feces and in the urine, with the principle metabolite of each being different; however, the secondary metabolites had the same R_f-values.

In 1964, Poonawalla and Korte (122) studied the distribution of C^{14} chlordane by intravenously injecting male rats with 27 µg of active material. Hydrophilic metabolism products in the feces accounted for 75 per cent of the radioactivity, and large amounts of hydrophilic metabolites were detected in the entire alimentary tract and in the kidneys, with unchanged chlordane detected only in the subcutaneous fat.

Rabbits were dosed orally twice weekly with C^{14} dieldrin for a period of 22 weeks by Korte and Arent (85). At the end of the feeding period the animals had excreted 42.2 per cent of the administered radioactivity (29.7 per cent in the urine and 12.5 per cent in feces). After 52 weeks, the excretion in the urine had increased to 43.1 per cent, while the radioactivity in the feces had decreased rapidly after termination of dieldrin administration. Six metabolites were isolated from the urine with the principle metabolite, which was present in amounts of about 86 per cent, and had a melting point of 130 to 131°C. From the structure, Korte and Arent (85) concluded that the epoxy ring system of dieldrin had hydrolized in vivo, leading

to a 6.7-trans-dihydroxy-dihydro-aldrin. The structural formula of this metabolite was one of the two enantiomorphic isomers of the synthetic racemic 1.2.3.4.10.10-hexachloro-6.7-trans-dihydro-1.4-endo-5.8-exo-dimethano-1.4.4a.5.6.7.8.8a-octahydro-naphthalene. Acute oral toxicity of the 6.7-trans-dihydroxy-dihydro-aldrin in mice (LD_{50}-1250 mg/kg) was less than dieldrin. The metabolite was injected into rats and within 3 days 82.2 per cent of the radioactivity was found in the excreta, 84 per cent as unchanged trans-dihydroxy-dihydro-aldrin and 16 per cent of a more hydrophilic compound that was identical to one metabolite found in dieldrin metabolism studies (85).

Trace Metals

Many elements occur in living tissue in small quantities and are frequently referred to as trace elements, minor elements, oligo-elements, or micro-nutrient elements. All of these terms will be referred to as trace metals for the purposes of this investigation. Frequently, iron is not included in this group due to its high mammalian requirement, primarily in the hemoglobin molecule. Some investigators include iron because it functions as a component of several oxidative enzymes in a manner similar to copper and other trace metals (163). Some mammals require many times more copper than

cobalt, and the concentrations of zinc in animal tissues are much greater than either manganese or nickel (163). In this study, iron, magnesium, zinc, and copper will be referred to as trace metals.

Several trace metals are essential dietary components paramount to the enzymatic processes of the living organism; additional ones exhibit certain specific metabolic activities (163). The study of the trace metals began approximately 100 years ago (163). According to Underwood (163), Bernard and MacMunn opened the way to the discovery of the metalloenzymes and to metal-enzyme catalysis. Kehoe et al. (83) and Wright and Papish (179) made a series of detailed studies of the distribution of trace metals in biological materials.

Green (60) said that "enzymic catalysis is the only rational explanation of how a trace of some substance can produce profound biological effects." Trace metals may perform various enzymic reactions that range from weak ionic strength effects to the highly specific associations found in metalloenzymes. In the metalloenzymes, the ion is so firmly attached and such an integral part of the enzyme molecule, it cannot be reversibly removed without permanent loss of enzymatic activity.

Enzymes are not noticeably affected by the presence or absence of salts, but the presence of certain ions are absolutely necessary for the activity of some enzymes, while other ions are

highly toxic to nearly all enzymes. One ion may be toxic to certain enzymes and act as an activator for others; the same ion may inhibit an enzyme at one concentration and be an activator of the same enzyme at another concentration (33).

There are some 15 different metal cations with atomic numbers between 11 and 55 that have been found to activate one or more enzymes. Four of the most important cations are Zn^{++}, Cu^{++}, Mg^{++} and Fe^{++} as based on the total number of effected enzymes. Ionic size is one of the most important factors determining which metal activates a particular enzyme. Radii of the metal activators lie within a narrow zone and in the middle range of observed atomic radii (33). In general, activators are not interchangeable. In some enzymes only one metal is an activator and in other cases two or three metals are capable of activating a single enzyme. Mg^{++} is the natural activator of most of the enzymes which act on phosphorylated substrates, notably the kinases, the synthetases, and the enzymes that hydrolyze phosphoric acid anhydrides, but not the phosphorylases. More is known about metalloenzymes than the so-called metal ion-activated enzymes (163). A fixed number of metal atoms are firmly associated with the protein molecule in metalloenzymes (163). A rapidly increasing number of metalloenzymes have been isolated from living tissue in recent years (163); some of the more important ones containing iron

are: the cytochromes, catalase, cytochrome c-reductase, and fumaric dehydrogenase. Those containing copper are ascorbic acid oxidase, uricase, cytochrome oxidase, and δ-aminolevulinic acid dehydrase and those containing zinc are carbonic anhydrase, carboxypeptidase, alkaline phosphatase, and several pyridine nucleotide dehydrogenases (163). Some of the most important enzymes containing magnesium are kinases, synthetases (those which act on phosphorylated substrates), and those that hydrolyze phosphoric acid anhydrides.

Iron

Iron was used by the ancient Greeks in treatment of muscular weakness and wounds of war. Investigations of the cytochromes by Keilin and others in the 1920's clearly established a broader concept of the biological significance of iron (163). New emphasis was placed on the relation of iron to the basic processes within the tissues upon the discovery of iron-containing flavoprotein enzymes. In 1937, McCance and Widdowson (100) found that there was no organ which excreted iron, and that the capacity to regulate the amount of iron in the body must therefore remain in the absorptive mechanism. Notwithstanding the enormous progress made in the last two decades, many physiological mechanisms embracing iron remain nebulous.

The iron concentration in the body varies with the species,

sex, age, nutrition, and state of health. In man, the iron content is between 0.006 and 0.007 per cent of the entire body weight, and in the adult rat, 0.005 per cent (145). Variations in liver iron stores and in hemoglobin levels are noted among species at birth; for example, the pig has relatively little iron stores in its body at birth, but in contrast, the body iron content of the newborn rabbit is exceptionally high. When both male and female rats were fed on the same diet, the females accumulated more iron in their livers (113). Usually the liver and spleen rank highest in iron content, followed by the kidney, heart, skeletal muscles, pancreas, and brain, which normally contain only a half to a tenth of the concentrations in the liver and spleen (163). Gross et al. (62) reported vast increases in the iron content of the liver, up to a total of 10 g in human malignancy and chronic infections. As much as 50 g may accumulate in the human body in the final stages of hemochromatosis (34); conversely, in conditions such as iron deficiency and hemorrhagic anemia, the iron content of the liver, spleen, and bone marrow is reduced below normal (163).

Iron has been known for many years to occur in the plasma in three forms: protein bound, "acid soluble iron," and plasma hemoglobin iron. Improved methods of analysis have shown that in disease, iron may occur in the plasma in at least seven different complex forms (89). Normally, the major part of iron exists in the

plasma bound to the specific iron-binding protein, transferrin. A diminutive amount of iron is always present in the hemoglobin firmly bound to haptoglobin, the normal hemoglobin-binding plasma protein. In severe hepatocellular damage, the plasma sometimes contains ferritin, a normal intracellular iron-apoferritin complex (89). Iron metabolism had been known many years before it was verified that "serum iron" circulates with the plasma firmly chelated to the specific metal-combining β_1-globulin, usually named transferrin (Tr) or siderophilin. Laurell and Ingelman (90) resolved that every Tr molecule can firmly bind two atoms of ferric iron or loosely bind cupric copper or zinc. In the CuTr complex, iron can readily replace copper (72). In the complex, the iron is trivalent and essentially bound with ionic bonds (38). The normal total iron-binding capacity ranges between 250 to 400 μg of iron per 100 ml of serum and normally is a measure of concentration of Tr-bound iron in the plasma (10). The FeTr complex may be regarded as a small, readily available, atoxic, circulating iron depot. Gitlin et al. (55) and Jager and Gubler (78) established that the iron in the FeTr complex has a much shorter biological half-life in the circulation than Tr itself, which proves that the reaction between the iron and Tr is normally reversible in plasma, and that the Tr serves as a true carrier of iron with the protein molecule taking up and giving off iron according to

environmental requirements.

Normally, plasma contains 0.20 to 0.32 g of iron per 100 ml, but during pregnancy, in chronic iron deficiency, and occasionally in the initial phase of acute hepatitis, higher values develop. In acute and chronic active diseases the concentration of plasma Tr decreases (23), and the higher the activity of the disease, the lower the Tr concentration will be. Usually the cells in the bone marrow utilize 70 to 80 per cent of the daily iron turnover, but in active diseases more plasma iron than normal is retained by the reticulo-endothelial cells, and less is utilized by the bone marrow (89). In the presence of inflammation, there appears to be a defect since more iron is released from the tissues to the plasma transferrin pool (52). Both the plasma iron and Tr values fall rapidly in acute diseases (23). Iron ion activity in plasma can be determined by the ratio between the iron-bound and free transferrin.

Normally, hemoglobin in small amounts (1 to 3 mg/100 ml) can be detected in plasma from which the erythrocytes have been removed (89). Most of the time the circulating plasma hemoglobin is bound to haptoglobin, the hemoglobin-binding plasma protein. Haptoglobins link hemoglobin more strongly than any other plasma proteins. In the plasma, haptoglobin has a great affinity for hemoglobin and no measurable quantity of free hemoglobin occurs in the plasma until all

haptoglobin has been saturated. Haptoglobinemia is usually found in individuals with a profound shortened life span of the erythrocytes, and in patients with pernicious anemia (111). Since haptoglobin forms relatively high molecular weight complexes, the hemoglobin is not excreted in the urine. In oxyhemoglobin, the bond between heme and globin is covalent, but in reduced hemoglobin and in methemoglobin, the bond is ionic (163).

Hemoglobin, the pigment of the red blood cells is a complex of basic protein, globin, and four ferroprotoporphyrin or heme moieties. When under the influence of oxidants, the iron atom is oxidized to the ferric state and loses its capacity to transport oxygen. Ferritin has a central nucleus of iron, stored in six micelles arranged at the corners of a regular octahedron, surrounded by a shell of protein, spherical in shape (108), and contains up to 20 per cent iron.

Hemosidern, an amorphous compound containing up to 35 per cent iron, condenses into an essentially protein-free aggregate comprised of ferric hydroxide (141), and is a valuable aid in diagnosing iron deficiency anemia. The stored iron in the form of ferritin and hemosiderin is readily available from the liver and spleen for erythropoiesis and for placental iron transfer to the fetus (140). Normal human adults excrete between 0.1 and 0.3 mg/day in the urine (6), but this amount may be increased up to 10 mg/day under

certain controlled conditions. Iron is also excreted in sweat and other means in small quantities. It has been estimated that from all sources, adult humans lose between 0.5 and 1.0 mg/day of iron (35).

In animals with single stomachs, iron is absorbed mainly in the duodenum (19) and only in the ferrous state (105), but in the rat and dog ferric as well as ferrous salts are utilized (162). It is well known that substances such as ascorbic acid and cysteine in food assist in the reduction of iron from the ferric to ferrous iron in virto (121). High levels of phosphates and phytates reduce the absorption of iron by formation of insoluble salts (170) (175). Bothwell et al. (15) suggest that factors controlling iron absorption other than dietary are the rate of erythropoiesis and the condition of the iron stores. Conditions such as coeliac disease, steatorrhea, and hemochromatosis, produce constitutional defects that break down normal absorption of iron (118). Joseph (80) used radioactive iron in normal patients with iron deficiency anemia and observed that the normal patients absorbed 2 to 20 per cent and the iron deficiency patients absorbed 20 to 60 per cent of an oral dose. An increase in iron absorption occurs in aplastic anemia, hemolytic anemia, pernicious anemia, pyridoxine deficiency, hemochromatosis and transfusional hemosiderosis (118).

In the normal human male, a total of 800 to 900 g of hemoglobin is synthesized and destroyed every 120 to 125 days (163).

Moore (104) administered radioactive iron, and within 4 to 8 hours, hemoglobin was identified in the peripheral blood. Within 7 to 14 days, 70 to 100 per cent of the isotope was detected in the circulating hemoglobin. The transfer of iron from the plasma to the liver cells depends upon the energy-yielding ractions in liver cells for the continued synthesis of adenosine trophosphate, which together with ascorbic acid, reduces the ferric iron of transferrin to the ferrous state, thus releasing it from its bond to protein and making it available for incorporation into ferritin (163). A decreased oxygen supply to the liver cell releases the iron from the ferritin to the plasma, which results in the breakdown of nucleotides, including adenosine triphosphate, and elevates the cellular levels of xanthine and hypoxanthine.

In spite of the advancement in the field of medicine and improvement in methods of analysis, iron deficiency is almost as prevalent today as it was 30 years ago, and is now the most frequently encountered deficiency. In adult males, loss of blood is the main cause of iron deficiency, whereas, in women and children, it is at least in part due to dietary deficiencies. Many clinical symptoms such as listlessness and fatigue, palpitation on exertion, stomatitis, and a sore mouth are manifested in iron deficiency. Children exhibit anorexia, depressed growth, and decreased resistance to infection in

iron deficiency (163). Iron deficiency also results in an anemia of the hypochromic or microcytic type, along with a normoblastic or hyperplastic bone marrow that contains very little or no hemosiderin (163). Beutler (10) demonstrated prominent reduction in cytochrome c in the liver and kidneys of iron-deficiency rats; no decrease in catalytic activity of red cells (13); a minute, but striking, decrease in cytochrome oxidase in the kidneys, but not in the heart (11); and partial exhaustion of succinic dehydrogenase in the kidneys and heart, but not in the liver (14). Beutler (12) examined iron-deficient rats and found that the non-iron-containing enzyme, aconitase, was reduced in the kidneys, but not in the heart, liver, or brain.

Iron deficiency occurs in infants between 6 and 24 months of age and is characterized by abnormalities in erythrocyte morphology and in iron metabolites (163). Blood in a newborn infant usually contains 20 to 22g Hb/100 ml, which represents about 200 to 220 mg of iron (97). Many facets such as chronic loss of blood, peptic ulcers, hemorrhoids, hookworm infestation, or malignant changes in the intestinal or urogenital tracts may be responsible for deficiency states (163).

Magnesium

Magnesium, like calcium, is an ion of remarkable physio-

logical significance since it is indispensable to life of the mammalian organism. Meltzer and Auer (101) recognized that two drops of magnesium sulphate injected intracerebrally into dogs resulted in complete anesthesia and relaxation for several hours. Meltzer and Auer (102) injected laboratory animals subcutaneously with magnesium sulfate and provoked general anesthesia. The most significant findings reported by Meltzer and co-workers were: (a) that a hypodermic injection of magnesium sulfate had a profound effect on the nervous system; (b) that a subcutaneous injection of magnesium sulfate had no purgative action; (c) that magnesium was excreted to a great extent through the kidneys; (d) that the action of calcium in the body was antagonistic to that of magnesium; and (e) that the effects of magnesium given parenterally could be reversed by intravenous injection of calcium chloride. The margin between an anesthetic dose and one that institutes respiratory paralysis is very slight (102). According to Neuwirth and Wallace (110) the first evidence of depressed central nervous system activity appears at a serum magnesium concentration of 5 to 6 mg/100 ml. With a concentration of 14 mg/100 ml, ataxia appears; whereas, a concentration of 20 mg/100 ml results in complete unconsciousness.

Complicated modifications in the magnesium and calcium content of the body and blood picture were noted in magnesium de-

ficient experimental animals (112). First, the deficiency was characterized by vasodilation and hyperemia. The plasma level initially dropped sharply and within 2 weeks rose to a peak at which time hyperexcitability developed. Malnutrition, cachexia and renal damage marked the second stage. The erythrocyte magnesium level dropped to half of the normal value where it remained constant; meanwhile, the calcium concentrations in the heart and muscle were amplified by 50 to 100 per cent, and the concentration in the kidney increased as much as 15-fold (2). Watchorn and McCance (172) made a subacute magnesium deficiency study in rats and found the bones, blood and teeth permanently deficient in magnesium while the liver showed an increase and the kidney was found to be calcified.

The calcification of the kidney occurring in magnesium-deficiency is related directly to bone metabolism (2). Histological studies indicate that magnesium affects bone metabolism and when this element is deficient, bone is not formed, does not grow, or actually atrophies (102). Leukocytosis also develops during the hyperemic aspect of the syndrome (81). Mononuclear leukocytes and neutrophils contribute to the syndrome, but eosinophils increase 1000 per cent above the average level.

Greenberg et al. (61) and MacIntyre and Davidson (95) observed that extenuated magnesium expropriation brings forward

degeneration of the kidneys as manifested by degenerative changes in the tubules and calcification in the corticomedullary zone and later the cortex. A marked decrease of mitochondrial enzyme activity was accompanied by a low plasma magnesium level, and it appeared that the cells of the kidney depend on a constant store of magnesium (2).

Widdowson et al. (176) studied the magnesium content of humans and found the concentration to range between 22.7 and 35.0 mEq/kg of wet tissue (3). It has been estimated that the skeletal content of magnesium is about 60 per cent of the total body concentration of magnesium (2) and the muscle content of magnesium is 16 mEg/kg (142). According to Shohl (142), the tissue concentrations of magnesium in mEq/kg of wet weight of tissue are as follows: blood cells, 4.2; serum, 2.5; brain, 11.0; kidney, 14.4; liver, 15.3; spleen, 10.2; skeleton, 74.6; and muscle, 16.1. The mean value of the magnesium concentration in hospital patients was 1.59 mEq/l; however, lower values were obtained in patients with congestive heart failure, cirrhosis, or renal failure after dialysis (2) (40) (144). Aikawa (2) reported that magnesium was located mostly within the cells and that serum magnesium represents only a small portion of the total body magnesium. This serum fraction is the most accessible for analysis and of the utmost clinical importance (2). A greater value of magnesium was observed in the red cells of patients with reticulocytosis,

with this increased concentration taking place in the cells of the bone marrow (54). Patients with hyperthyroidism, malnutrition, or chronic liver disease have below average concentration of erythrocyte and serum magnesium (171). In some disease such as viral hepatitis and hypoparathyroidism, there is evidence that the magnesium values become altered in the red cells but not in the plasma (171). In diseases like spinal meningitis, the spinal fluid magnesium reaches a level much higher than the normal serum levels; whereas, the concentration in the spinal fluid is normally only slightly above the plasma value (25).

Several investigators used intravenous injection of Mg^{28} to study the tissue uptake of this metal in many species including man (3) (4) (17) (49) (56) (91) (99) (143). The data from these experiments indicate that Mg^{28} in the extracellular fluid very rapidly reaches equilibrium with the established magnesium. Some tissues such as heart, liver, kidney, adrenals, lymph nodes, and appendix take up the Mg^{28} exceedingly fast. Mature animals do not take up magnesium in the bone and muscle as fast as the growing animals and fetuses. Since equilibrium in dogs and human beings extends over a long period of time (60 hours) the method used to quantitate potassium cannot be used to quantitate magnesium in these species (2).

Insulin appears to play the same type role in the metabolism

of magnesium as it plays in the metabolism of glucose and potassium (2). Magnesium needs insulin in its transfer across the cell membrane. Without magnesium the cells cannot function properly and the oxidation of glucose and the oxidative phosphorylation along with the production of adenosine triphosphate would not take place (2). From many studies of different factors concerned with the uptake of magnesium, it appears that magnesium metabolism may be incited by any condition which modifies cellular entirety or capacity, cellular purpose, stored products or the act of synthesis (2). The occurrence of hypermagnesemia usually is associated with renal insufficiency (2). Magnesium appears to play a role in hibernation according to studies made on hedgehogs by Suomalainen (156). This investigator found that the serum magnesium concentration in the fall averaged 3.2 mg/100 ml and 5.43 mg/100 ml in early January.

Various studies on the role of magnesium have been made since Peters and Van Slyke (117) introduced their report on magnesium metabolism with a comment that no clinical significance had, up to that time, been attached to changes in magnesium metabolism. No serum alterations in the concentration of magnesium have been reported for the following diseases: thyroid disease, schizophrenia, basophilic adenoma, Addison's disease, cardiac disease, steatorrhea, progressive muscular dystrophy, familar periodic paralysis, myotonia,

atrophica, and neurocirculatory asthenia (2). A rise above normal serum magnesium values have been reported in tuberculosis, renal insufficiency, toxemia of pregnancy (67), and diabetic acidosis (2).

Between 1904 and 1912, Richard Willstatter (43), a German organic chemist, demonstrated that chlorophyll consisted of the porphin system and a central atom of magnesium with a complex linkage. He also showed that magnesium could be split off from chlorophyll with dilute acids and furthermore that the reaction was reversible.

Zinc

According to Vallee (164) the role of zinc, as an essential nutrient for plants and animals, has been recognized ever since Raulin in 1869 showed it to be necessary for the growth of Aspergillus niger. According to Vallee (164) the presence of zinc was first demonstrated in human liver by Raoult and Breton in 1877. In 1934 evidence was introduced to show that zinc was essential to normal growth and development of animals (164). Stirn et al. (153) in 1935 noted that zinc was essential to rats. Keilin and Mann (84) in 1940 isolated and purified the enzyme carbonic anhydrase, which contained 0.33 per cent zinc as part of its molecule. This was the first evidence offered to explain a mode of action of zinc. This enzyme catalyzes the dehydration of carbonic acid and participates in the elimination of CO_2

in the lung and its incorporation in the tissues (165). A great variety of proteins combines with metal cations in a specific way, depending on the reactivity and location of the protein's combining sites. Complexes formed most often are completely reversible (89). Zn^{++} complexes shift the points of minimum solubility of the plasma proteins toward the neutral region. Metal-protein complexes are complicated by the fact that groups most likely to bind with metal cations are those with demonstrable affinity for protons (89).

Zinc, copper, and cadmium combine with the imidazole groups of human and bovine serum albumins (64) (157). Gurd and Goodman (64) found that Zn^{++} and Cd^{++} compete directly for these binding sites. While the interaction of Cd^{++} and Zn^{++} with albumin appears to be similar, the alkaline phosphatase of plasma is soluble in the presence of Cd^{++} but insoluble in the presence of Zn^{++} (89). The zinc-protein complexes are in part insoluble at an ionic strength of 0.15 and a pH 7 (89).

Gurd and Goodman (64) studied the interaction of zinc with human albumin and determined the role of the imidazole group in the binding of Zn^{++} and found that each albumin molecule binds 16 Zn^{++}. Plasma concentration of the metal is normally 90 to 100 μg/100 ml, which corresponds to about 1 Zn^{++} per every 50 protein molecules in the plasma (89).

It has been shown that zinc exists in the plasma proteins in at least two types of linkage: one corresponds to a firm bond (30 to 40 per cent), and the other to a loose bond (60 to 70 per cent) in the physiological pH range (89). Zinc in the firmly-bound form may represent a chemically and physiologically homogenous or heterogeneous fraction (89). Enzymes, such as carbonic anhydrase and carboxypeptidase along with alcoholic, glutamic, and lactic dehydrogenases, contain firmly-bound zinc (165), but the concentrations of these enzymes in the plasma are not high enough to account for the bulk of the firmly-bound plasma zinc (89). The ratio between the loosely- and firmly-bound zinc in the dog and human plasma is about the same (89). The rapid uptake of Zn^{65} by the γ-globulins suggest that zinc in these complexes is either exchangeable in the plasma in vivo, or that zinc-protein complexes have a very high turnover in vivo (89). Plasma zinc represents less than 1 per cent of the total body zinc (89).

Analysis of vertebrate organs, including man, showed the presence of zinc in quantities varying from 10 to 200 μg/g (166). Most organs, including the pancreas, contain between 20 and 30 μg of zinc/g of wet tissue (164), while bone, liver, and voluntary muscle contain approximately double this amount (164). Newborn rat livers contain two to three times the quantity of zinc as that of an adult rat, and within 2 weeks,, the concentration decreases to approximately

100 ppm, which is the content of adult livers (9). Zinc content of an adult fat-free body weighing 70 kg, varies from 1.36 to 2.32 g. The iron content varies from 4.2 to 6.1 g, and the copper content, 81 to 230 mg (163).

Montgomery et al. (103) and Sheline et al. (136) (137) studied the distribution of Zn^{65} in the mouse and dog and in both species the liver was found to contain the largest fraction of the injected dose. The greatest radioactivity was found in the erythrocytes, brain, skeletal muscle, and skin, with decrease in activity being more rapid in the mouse than in the dog. Feaster et al. (44) observed that Zn^{65} was absorbed at a very low rate from the gastrointestinal tract of the rat; however, once absorbed, the isotope moved freely from the dam across the placenta to the fetus, and more than half the dose retained was transferred to the young through the milk within ninety-six hours.

Relatively high concentrations of zinc have been detected in the male genital organs (164). On a dry weight basis, there is 874 $\pm$ 63 µg zinc/g in the dorsolateral rat prostate; the rabbit prostate contains 1296 $\pm$ 31 µg zinc/g, and the normal human prostate, 859 $\pm$ 96 µg zinc/g (98). Carbonic anhydrase activity increases simultaneously with the zinc content, and the concentration of the enzyme accounts for only 3.5 per cent of the total zinc of the dorsolateral rat prostate (164). Rats fed a diet containing less than 0.5 µg zinc/g resulted in

degeneration of the testes, hyperplasia of the coagulating glands, the seminal vesicles and prostate, and relative or complete decrease in the number of sperm in the epididymes; however, all these changes except testicular atrophy were reversed when zinc was added to the diet (164).

The function of zinc in the crystallization of insulin at pH levels near 6 has been known for many years (65). The final product contains 0.52 per cent zinc, 0.77 per cent cadmium, and 0.44 per cent cobalt. Erythrocyte zinc concentration and carbonic anhydrase activity vary directly with the hematocrit level and hemoglobin concentration in normal people, as well as those afflicted with anemias, polycythemia vera, secondary polycythemia, leukemia, and congestive heart failure (164). Lowered serum zinc levels have been found in patients suffering from acute infections, diabetes, cirrhosis, myocardial infarction, and pernicious anemia. Patients suffering from hyperthyroidism, hypertension, polycythemia vera, and eosinophilia, along with those receiving adrenaline and thyroxine, as well as those receiving x-ray irradiation, have serum zinc levels higher than normal. Vallee et al. (168) observed increased erythrocyte zinc concentration in sickle cell anemia and pernicious anemia patients. However, in patients with untreated pernicious anemia, the erythrocyte zinc concentration and carbonic anhydrase activity were observed to be nearly normal, even

though the hematocrit value, erythrocyte count, and hemoglobin levels were noticeably decreased (164). Leukocyte zinc concentration in patients with various acute and chronic lymphatic and myelogenous leukemia was found to be greatly decreased, but could be returned to normal levels by proper therapy. Administration by injection was not successful in inducing a return of the zinc concentration to normal levels (178).

Intake of daily zinc concentration by humans has been reported to range from 10 to 15 mg/day, with 5 to 10 mg/day excreted in the stool, and 0.4 to 0.5 mg/day in the urine; however, patients with zincuria excreted 2.1 mg of zinc in their urine daily.

Many articles on the necessity of zinc in the human diet for normal growth and development and for medication have been published. In zinc-deficient rats, the pancreatic amylase and protolytic activity were lowered significantly, and were not restored by addition of zinc *in vitro* or *in vivo*. Wachtel *et al.* (169) in 1941 found that the uric acid content of zinc-deficient rats was double its normal value. Day and Skidmore (29) in 1947 found that zinc deficiency in the rat caused a decrease in liver catalase activity which could not be restored by addition of zinc to the diet. Liver esterase activity and riboflavin content of the liver, kidneys, heart, and lungs are not altered. Hyper-

keratinization, thickening of the epidermis, intra- and intercellular edema of the skin and mucous membranes of the esophagus and mouth were found on histological studies of zinc-deficient rats by Day and McCollum (28) and Follis et al. (50). Hogs need a higher zinc content in their diet than mice or rats. When the diet contains a high calcium content, the zinc content has to be increased in proportion or a disease known as parakeratosis will develop (164).

Vallee et al. (167) recently described a condition in humans known as post-alcoholic cirrhosis marked by gross abnormalities in zinc metabolism and lowered serum zinc concentration. In these patients, the magnesium and copper content did not change. When rats were fed a diet containing 1 per cent zinc, a severe anemia developed in 3 to 5 weeks ; however, a diet as high as 10 per cent zinc could be tolerated for only a few weeks. When copper was added to the diet, the hemoglobin was maintained at a higher level than normal; however, a mixture of iron, copper, and cobalt maintained the hemoglobin at normal levels. Sadasivan (132) in 1951 observed an increase in urinary uric acid and creatinine during a high zinc diet.

It has been shown indirectly that there is a demand for zinc in wound healing, for example, severe thermal burns (154). In E. coli endotoxin shock, a drastic decrease has been observed in the

plasma zinc concentration (63).

Copper

It has been well established that copper is necessary for the formation of hemoglobin, absorption of iron from the intestinal tract, and mobilization of iron from the tissue stores of mammals. The exact function of copper is unknown, but the influence of copper on the absorption of iron appears to depend on the concentration in the tissues rather than on the concentration in the diet. Holmberg and Laurell (73) found that more than 90 per cent of the serum copper was firmly bound to an γ_2-globulin and contained 8 atoms of copper per molecule of ceruloplasmin and that the oxidase activity of ceruloplasmin was controlled by the serum copper level. The nature of the copper linkage suggests that not all copper atoms are bound in the same manner. The properties of ceruloplasmin simulate those of the laccases, and catalyzes, in vitro, the oxidation of many substances in the presence of molecular oxygen. Except in Wilson's disease, the level of ceruloplasmin is indicated by the serum copper values (Cu/s). The variation of ceruloplasmin in certain other diseases can be construed from data obtained from the fluctuation of the serum copper in physiological and pathological conditions (74) (96). Lang and Renschler (88), utilizing intravenous injections of Cu^{64}, observed that the liver was the only organ in which

Cu^{64} increased in the ceruloplasmin-containing fraction and since this occurred before ceruloplasmin-containing Cu^{64} appeared in the plasma, they concluded that ceruloplasmin was synthesized in the liver. The normal value of Cu/s at birth is only 50 µg/100 ml, but at 1 year of age, the concentration is the same as found in adults (5). Ceruloplasmin is formed throughout the intrauterine life, and it does not deviate with sex. The stage and advancement of malignant diseases and extent of necrosis, as well as status and continuance of febricity, varies the Cu/s values. In complications (infectious rheumatoid arthritis and liver diseases) during pregnancy, the Cu/s values may increase to 600 µg/100 ml (89). Subnormal Cu/s values have been observed in severe hypoproteinemia (malnutrition and gastrointestinal disorders) probably resulting from an amino acid deficiency (22). It was also observed that the ceruloplasmin value decreased in adults with nephritic syndromes which may have been caused by urinary loss. Many investigators suggest that the low ceruloplasmin level in Wilson's disease was due to impaired synthesis of ceruloplasmin (36) (79) (133). The turnover of the albumin copper is high, indicating a rapid exchange with extravascular copper pools (8) (36) (79) (133).

Numerous copper proteins have been isolated from plant and animal tissues and several of these have been shown to be enzymes with oxidative functions (163). Evidence has been derived to indicate

that tyrosinase, laccase, ascorbic acid oxidase, cytochrome oxidase, uricase, butyryl CoA dehydrogenase, and δ-aminolevulinic acid dehydrase are copper (containing) compounds (152). Copper has been shown to be implicated in the metabolic process of pigmentation, keratinization of wool, bone formation, reproduction, myelination of the spinal cord, and the hematopoiesis (163). According to the literature reviewed, the concentration and distribution of copper in the body varies, but estimates of 100 to 150 mg of total copper or 1.5 to 2.5 ppm have been noted. In the adult rat, muscles contain 21 per cent, bones 23 per cent, liver 13 per cent, and skin 36 per cent of the body copper (92). In order of concentration the tissues are: liver, heart, kidney, hair, brain, spleen, bones, and the endocrine glands (pituitary, thyroid, and thymus) (163). Some tissues such as endocrine glands, muscles, heart, brain, and skin are very resistant to change in copper concentration; whereas, the liver, kidney, spleen, and lungs can vary greatly by high diet intake. The liver concentration of copper has been studied in many species and these studies indicate that the concentration and copper levels depend upon the species, age, nature of diet, and occurrence of certain disease conditions (163). It has been suggested that the high liver concentration of copper at birth functions as storage copper to overcome the low copper content of maternal milk. Intake of molybdenum will limit the copper storage in proportion to the in-

organic sulfate content of the diet (163).

Human diseases such as Mediterranean anemia, hemochromatosis, Wilson's disease (hepto-lenticular degeneration), cirrhosis and yellow atrophy of the liver, tuberculosis, carcinoma, and severe chronic diseases accompanied by anemia are characterized by exceptionally high liver copper levels (21). The concentration of copper in blood of healthy animals varies greatly from organ to organ in the same animal, but the patterns from species to species are very similar; for example, in man and cattle the concentration of copper is evenly divided between plasma and RBC (5) (21) (37) (108) (163). In man, above average concentration of copper prevails in a great number of chronic and severe diseases, including leukemia, Hodgkin's disease, diverse anemias, "collagen" disorders, hemochromatosis, and myocardial infarction (171). In dogs, copper is absorbed from the acid section of the upper jejunum while in pigs it is absorbed from the small intestin and colon, and in man it is believed to be absorbed from the first section of small intestine (26) (128). The blood plasma transports absorbed copper (loosely bound to a plasma protein probably serum albumin) to the parenchymal cells of the liver and kidney (163). The major route of excretion appears to be the biliary system, but when this system is obstructed, increased urinary excretion takes place (163). A great array of clinical irregularities, such as anemia,

depressed growth, bone disorders, neonatal ataxia, heart failure, and gastrointestinal disturbances have been connected with a regimen deficiency of copper or responds to copper therapy (163). The first evidence of copper deficiency is a slow exhaustion of body copper deposits, and then a constant drop in the blood copper concentration below that demanded for ideal hematopoiesis (163). Lahey et al. (87) contributed evidence to support the theory that copper was important in the erythrocyte maturation process when they reported that pigs and dogs had fewer reticulocytes in their blood during copper anemia than they had during iron anemia, and that animals suffering from both iron and copper deficiency anemia had a striking reticulocyte response upon the addition of copper to the diet, but had very little response when iron was added to the diet.

Schultze (134) was the first to show that there was a loss of cytochrome oxidase from the tissues in copper deficiency animals. Copper is specifically connected to the synthesis of the prosthetic group of cytochrome oxidase as one of its basic functions (163). Skeletal changes are unrelated to the anemia in copper deficiency. Bones in copper deficiency are distinguished by below average cortices, deficient trabeculae and wide epiphyses, together with normal ash, calcium, phosphorus and CO_2 contents (163).

Copper demand cannot be based on the copper intake because many dietary constituents may dominate copper assimilation and utilization (163). Copper deficiency associated only with iron deficiency anemia and hypoproteinemia has been demonstrated only in infants (155). Scoular (135) estimated that children could retain about 0.06 to 0.10 mg Cu/kg body weight; therefore, a daily intake of 1.2 to 3.0 mg would be required.

The liver has the ability to retain large amounts of ingested copper without physiological damage, but if the excessive liver copper escaped into the blood stream, extensive hemolysis and jaundice would ensue and death would occur (163). Boyden et al. (16) have maintained rats on diets containing 500 ppm copper (about 100 times the normal intake) without apparent damage to health or growth. In cattle and sheep, copper poisoning associated with chronic or sudden hemolytic icterus and hemoglobinuria has been reported (163). Very little is known about the levels of copper that will result in acute toxicity in man, but it would require approximately ten times the daily dietary intake, an unlikely situation except for certain occupational conditions (163).

CHAPTER III

PURPOSE AND SCOPE

In the field of insecticide toxicity the literature indicates that many investigations have been carried out under a variety of conditions on many species of laboratory animals. A large number of investigations have also been performed with endrin in acute and subacute toxicity studies. Even though several reports have been made on the pharmacological action of this cyclodiene on the central nervous system, the exact mechanism of this action has not been demonstrated in man or animals. Chronic toxicity studies show that repeated doses of the cyclodienes produce microscopic changes in the liver and the kidneys in some experimental animals. Lesions may also be produced in man and animals by a single acute or subacute dose.

The administration of cyclodiene insecticides orally or parenterally to rats in doses above the LD_{50} level will produce terminal convulsions. Analysis of the brain of the convulsing animals revealed the presence of betaine CoA esters, released in the brain tissue through damage to the brain cell mitochondria (75). Dieldrin,

one of the cyclodiene insecticides, inhibits various dehydrogenases and cytochrome b metabolism (75). This insecticide also inhibits anaerobic glycolysis and the hydrolysis of acetylcholine by brain acetylcholinesterases. Several of the enzymes are inhibited by poisons, competitive inhibitors, various "mixed-type" inhibitors, heavy metals, non-competitive inhibitors, and other types of inhibitors that block one or more steps in a particular metabolic pathway.

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

This research was designed in order to amplify and elucidate the presence and variations in the trace metal concentrations influenced by oral doses of the insecticide endrin administered under controlled conditions. The relationship of trace metals and endrin has not been previously investigated nor has the concentrations of various metals in normal rat tissues been adequately reported. The explicit purposes of the present investigation were to dose rats with subacute and acute oral doses of endrin in order to study variations of iron, magnesium, zinc, and copper in (a) plasma, (b) red blood cells, (c) liver, kidney,

spleen, brain, and heart, and (d) urine and feces. Hematocrit values were also determined and compared. The ultimate objective of the long term project for the Institute of Environmental Health, Department of Preventive Medicine and Public Health, University of Oklahoma Medical Center, is to provide a foundation for a diagnostic technique in the course of insecticide intoxication. The evaluation of trace metal involvement during the course of endrin intoxication is one phase of this goal. If the disclosure and employment of the character of trace metals as such a tool appears to be of little usefulness, it must be recalled that only a few years ago the role of trace metals in the formation of protein complexes and the role of trace metals in numerous diseases were unknown, except in some specific nutritional deficiencies.

CHAPTER IV

EQUIPMENT, METHODS AND PROCEDURE

The experimental animals consisted of adult male Holtzman albino rats (300 to 425 g each with an average weight of 403 g) purchased from the Holtzman Company, Houston, Texas. They were acclimated to the laboratory environment for 2 weeks and then examined for symptoms of illness after which time they were randomized into three groups and placed in individual metabolic cages. The control and chronic groups consisted of 10 rats each, while the acute group was composed of 12 rats. Throughout the experiment all animals received water and Rockland Mouse Breeder diet 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, over an 8-day period, a total oral dose of 8 mg of endrin per kg of body weight. This pesticide, which was prepared by dissolving 5 g of endrin in 100 ml of commercial grade peanut oil, was administered via a stomach catheter in 4 doses starting with 3 mg/kg on the first day, 2 mg/kg

on the third and fifth days, and 1 mg/kg on the eighth day. This group along with the control group was sacrificed on the ninth day.

The animals in the acute group were orally exposed to a single dose of 25 mg/kg of body weight and the onset and duration of each convulsion was recorded. Sacrifice was effected at the initiation of the third convulsion. Since the lapsed time between administration of the endrin and the occurrence of the third convulsion was rather constant (2 ± 0.5 hours), all acutely exposed animals were closely grouped at the same degree of intoxication.

The animals were sacrificed by first placing each under anesthesia with diethyl ether then opening the abdominal cavity and exsanguinating the animal by inserting a needle attached to a syringe into the abdominal aorta just above the iliac arch. The blood which ranged in volume from 3 to 11 ml per rat was then transferred to a test tube. The syringes, needle, and test tube, as well as the capillary tubes used in the later analyses of blood, were pretreated with heparin.

After the animals had been bled, the liver, right kidney, spleen, brain, and heart were removed from the body, blotted dry, placed in tared beakers, and the wet weight determined. Then the tissues were dried in an oven at 105°C for 16 hours, placed in a dessicator over night, and the dry weight determined. The moisture

weight was derived from the difference between the organ total wet weight and the subsequent dry weight. Next the tissues were transferred to a furnace and burned at 500° C for 8 hours. The ash weight was determined after dessication over night. The weight lost during the ashing process was termed volatile weight and was derived by the difference between the dry and ash weight. After the weight of the ash was determined, 1 ml of 0.0324 N HCl for each mg of ash was added to the container. The containers were then covered and allowed to stand over night. The acid and ash residue were transferred to a test tube, sealed with parafilm, and stored under refrigeration until analysis. Urine and feces from control and chronic animals were collected on alternate days and pooled. The air-dried feces were ashed and solubilized in the same manner as the tissue samples. The urine was diluted with 5 parts 0.0324 N HCl and analyzed in the same manner as the plasma.

The hematocrit ratio for each animal in each group was determined in capillary tubes centrifuged at 15,000 x g for 5 minutes. The remaining portion of the blood sample was then separated into plasma and red blood cells (RBC) by centrifugation under the same conditions. One ml of plasma was withdrawn and diluted with 5 parts 0.0324 N HCl and 1 ml of packed RBC was withdrawn and diluted with 35 parts of the dilute acid. The concentrated solutions were preserved

by freezing and the diluted samples were stored under refrigeration until analysis. Preparation of tissue and blood fractions in this manner permitted direct aspiration of the samples into the burner of the atomic absorption unit, and resulted in more reproducible results with fewer mechanical difficulties than encountered in analysis by other methods.

A set of standard solutions containing 10,000 ppm of iron, magnesium, zinc and copper, respectively (Fisher Scientific Company, Fairlawn, New Jersey), were serially diluted with 0.0324 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: iron, 1 to 25 μg/ml; magnesium, 0.1 to 15 μg/ml; zinc, 0.25 to 2.0 μg/ml; and copper, 0.25 to 5.0 μg/ml. In order to overcome phosphate bonding, the magnesium standards, as well as all samples prepared for magnesium analysis, were provided with 0.05 ml of concentrated strontium chloride (50.0 g/l) per ml of sample. During analysis, appropriate standards were repeated with every tenth sample.

All the samples from the experimental animals (plasma, RBC, feces, urine, liver, kidney, spleen, brain and heart) were analyzed for iron, zinc, copper, and magnesium in that order.

These analyses were accomplished with a Jarrell-Ash Atomic Absorption Spectrophotometer Model 82-362 utilizing a 0.5 meter Ebert mount monochrometer having a grating of 30,000 lines per inch

in the ultraviolet range (Figures 6 and 7). The hollow cathode tubes, which provided the characteristic photons for each metal, were operated at the following amperages: (a) iron, 11.6; (b) magnesium, 10.0; (c) zinc, 6.0; and (d) copper, 7.0. Energy necessary for placing the atoms in a neutral state was provided by a direct aspiration Hecto burner using hydrogen and air at the following pressures for the individual metals: (a) iron, hydrogen 12.5 psi and air 15 psi; (b) magnesium, hydrogen 5.0 psi and air 15 psi; (c) zinc, hydrogen 15 psi and air 21 psi; and (d) copper, hydrogen 12.5 psi and air 15 psi. An RCA photomultiplier tube Model IR 106 was operated at the following voltages and wavelengths: (a) iron, 760 and 2483 Å; (b) magnesium, 450 and 2851 Å; (c) zinc, 450 and 2138 Å; and (d) copper, 450 and 3247 Å.

The data were subjected to analysis of variance and the various parameters and treatment groups were compared utilizing Duncan's New Multiple Range Analysis (149). The statistical treatment employed is exemplified in Appendix I and all other data are recorded in Appendix II. The term "significant" as used herein implies statistical significance at the 0.05 level.

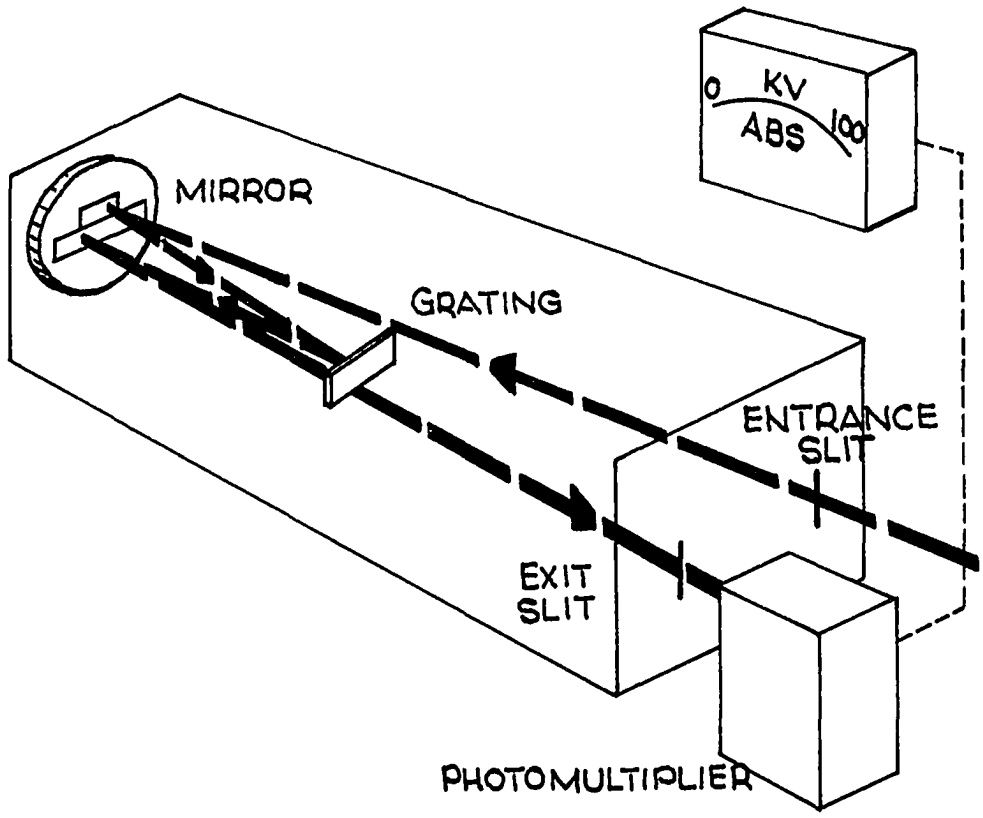

Figure 6. Optical design of the Jarrell-Ash Ebert spectograph, with photomultiplier and recorder attachments.

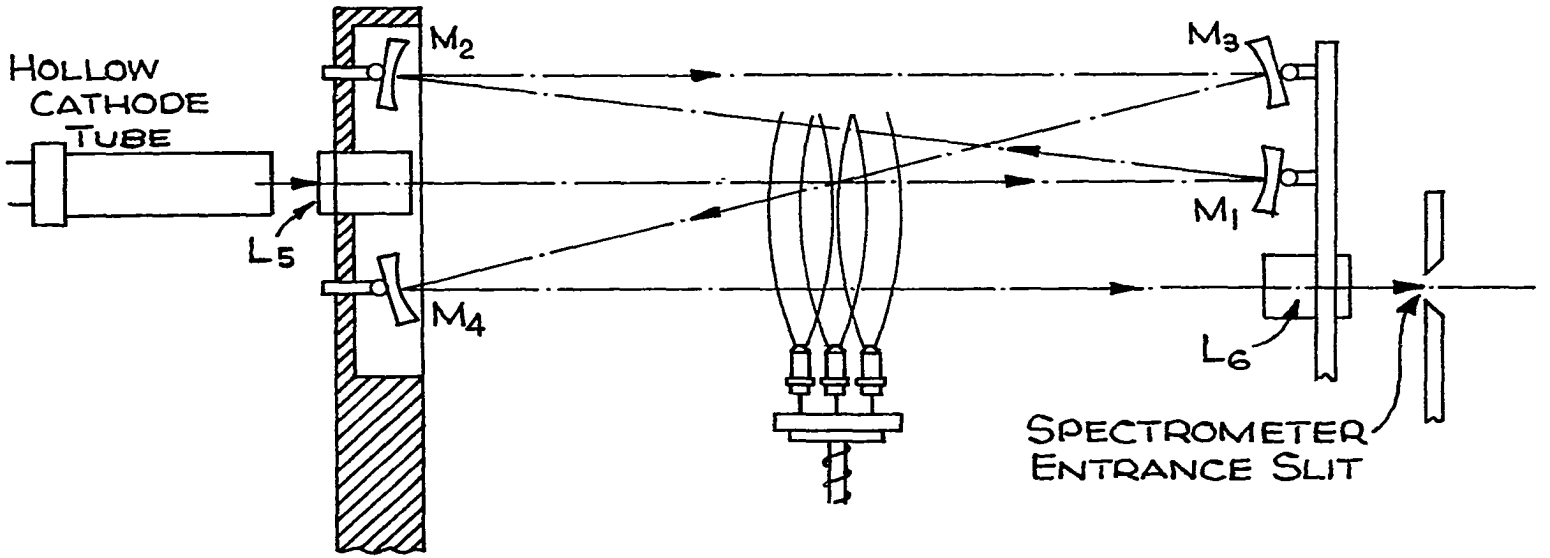

Figure 7. Multi-pass optical system composed of the new Corning optics.

CHAPTER V

OBSERVATIONS AND DISCUSSION

The observations on the acute, chronic, and control experimental groups of rats are presented, initially, as a consideration of the response of the selected metals in the various organs and in the circulatory and excretory systems. Next, the observations are considered from the standpoint of each metal and its response throughout the animal. The comparisons of the treatment and control experimental parameters are shown in Tables 2 through 13 (Appendix II), and these data are summarized in Table 1, which indicates the statistically significant increases and decreases by directional arrows.

Organs and Excretions

Liver

Acute versus Control. All organ size parameters demonstrated significant increases varying from 25 per cent in the wet weight to 200 per cent in the ash content. In spite of these changes, the total organ content of iron and magnesium indicated no appreciable

TABLE 1

SUMMARY OF COMPARISON OF THE TREATMENT PARAMETERS

	Wet Weight	Moisture	Volatile	Ash	Total Metal				Metal Concentration			
					Fe	Mg	Zn	Cu	Fe	Mg	Zn	Cu
Liver												
acute	↑	↑	↑	↑	-	-	↑	↓	-	↓	-	↓
chronic	-	-	-	-	↑	↑	↑	-	-	↑	-	↓
Kidney												
acute	↑	↑	-	-	↑	↑	↑	-	↑	↑	↑	-
chronic	-	-	-	↓	-	-	-	↑	↑	-	↑	↑
Spleen												
acute	-	-	-	-	↑	↑	↑	↓	↑	↑	↑	↓
chronic	↓	↓	↓	↓	-	↓	-	↓	-	-	-	-
Brain												
acute	↑	↑	-	-	-	↓	↑	↓	-	↓	-	↓
chronic	-	↑	-	-	-	-	-	-	-	-	-	-
Heart												
acute	↑	↑	↑	↑	↑	↑	↑	↓	↑	↑	↑	↓
chronic	-	-	-	↓	-	↓	-	-	↑	-	-	-
RBC												
acute									-	↓	↓	-
chronic									-	-	↓	↓
Plasma												
acute									-	↓	-	↑
chronic									↓	-	↓	↓
Feces-chronic[a]					↓	↑	↓	↓	↑	↑	↓	↑
Urine-chronic[a]					↑	↑	↓	↓	↑	↑	↓	-

[a]Arrows indicate directional changes and not statistical significance since the samples were pooled and F-values could not be determined.

changes in their normal values of 393 μg/mg and 1336 μg/mg. On the other hand, the total amount of zinc did increase significantly, while the total copper content decreased.

The decreases in the unit concentrations of magnesium and copper are predictable since their total organ contents remained constant or decreased while the organ size increased. The lack of change in the zinc concentration indicates that, even though the total amount of zinc present increased, the rate of increase was proportional to the increase in size of the organ.

Chronic versus Control. All organ size parameters were essentially unchanged at the end of the chronic exposure study; however, significant increases of 30, 35, and 30 per cent were observed in the total organ content of iron, magnesium, and zinc. Conversely, copper, which appears to be one of the more mobile metals, demonstrated a stationary level of 0.15 μg. In view of the facts that the organ size remained constant and the metal contents increased or remained constant, corresponding behavior of the unit concentrations is to be expected; however, due to the complexity of the biological function of the liver, only an anticipated increase in the concentration of magnesium was observed.

Kidney

Acute versus Control. Since only the wet and moisture weights of the kidney increased and their increases were of the same direction and magnitude, increases in the intracellular and extracellular fluids would account for the increased moisture weight and its accompanying wet weight.

Proportional increases in concentration and total kidney metal content were detected for iron (100 per cent), magnesium (20 per cent), and zinc (50 per cent). Therefore, the end result of treatment was an increased concentration of iron, magnesium, and zinc above and beyond considerations of increased organ size. No vital changes were observed in copper of the kidney as a result of the acute endrin treatment.

Chronic versus Control. In the chronic treatment, the means of wet, moisture, and volatile kidney weights revealed no significant alterations from the control but the mean ash weight showed a significant decrease. Significant increases of 50 and 25 per cent in the concentrations of iron and zinc were reflected by near significant increases in their respective total amounts in the kidney. Kidney magnesium was not altered by the chronic treatment. An increase of 60 per cent in the total and concentrations of copper in the

chronic treatment kidney is the only case of an organ copper increase revealed in this investigation.

Spleen

Acute versus Control. Table 1 indicates that there were no significant changes in the total weight, moisture content, volatile component, or ash content of the spleens from the acute treatment. However, the total amounts of iron, magnesium and zinc present in this organ exhibited significant increases of 70, 45, and 75 per cent, respectively, above their control values of 50, 84, and 10 µg. Since the total amounts of these metals increased while no significant changes in the organ size occurred, the significant increases of 75, 50, and 80 per cent in the unit concentrations of these three metals are understandable. Copper, on the other hand, exhibited a significant 35 per cent decrease in the total metal present (1 µg) and a corresponding decrease in its concentration.

Chronic versus Control. Endrin administration under chronic conditions resulted in significant decreases of 15, 20, 20, and 45 per cent, respectively, in the total organ wet, moisture, volatile, and ash weights. Even with significant decreases in the organ weight parameters, the concentrations of the metals were not altered by the

chronic treatment. Although the unit concentrations of magnesium and copper were not changed by the chronic treatment, a 30 per cent decrease in both the total magnesium and copper contents were accompanied by and correlated with a generalized 30 per cent decrease in the spleen organ weight.

Brain

Acute versus Control. As in the case of the kidney under acute treatment, only the wet and moisture weights increased, again suggesting that both weight increases were the result of a moisture influx. Brain iron was not altered by treatment. A 20 per cent decrease in magnesium total content with an increase in brain moisture yielded an expected 25 per cent decrease in concentration. Since the zinc concentration did not change, the total zinc present increased in proportion to the increase in organ size. A 30 per cent increase in total zinc was found. In a directional shift, copper exhibited a decrease of some 50 per cent in total and concentration quantitities.

Chronic versus Control. Even though there was an increase in the moisture content of the brain, consideration of the data and organ physiology casts doubt on the biological significance of this single statistical observation resulting from chronic endrin treatment.

Heart

Acute versus Control. Statistically significant but comparatively small increases of 10 to 15 per cent were observed in all organ size parameters. Total iron, magnesium, and zinc content increased 50, 30, and 50 per cent above their control values of 2.0, 129, and 0.96 μg. In view of the increase in organ size as well as the increases in the total metallic content, the increases in unit concentration of 55, 15, and 55 per cent in iron, magnesium, and zinc, indicates a marked influx of these metals into the heart. Copper, on the other hand, indicates a significant reduction of 50 per cent in both the total metal as well as concentration.

Chronic versus Control. Of the four size parameters, only ash weight demonstrated a significant change and this was a decrease. An increase in the concentration of iron was observed, but this observation was not validated by a significant increase in the total organ content of this metal. A similar situation exists in the case of magnesium where the total decreased with no significant shift observed in the unit concentration. Zinc and copper demonstrated no alteration as a result of chronic endrin treatment.

Hematocrit

Chronic versus Control. The mean hematocrit ratio for the control animals was 46.9, while that for the chronic treatment showed a non-significant increase to 49.8; however, the increased value of 52.9 exhibited by the acute animals was statistically significant.

Red Blood Cells

Acute versus Control. Iron and copper concentration did not deviate significantly from their control values of 1049 and 0.40 µg/ml of packed cells; however, magnesium did decrease significantly from 23.5 to 17.5 µg/ml. Zinc was also mobilized out of the RBC as evidenced by a decrease from 17.4 to 11.1 µg/ml.

Chronic versus Control. The chronic animals manifested no alterations in iron or magnesium RBC concentrations. Declines of 40 and 30 per cent in the zinc and copper values reveal evidence of a marked transportation of these metals out of the RBC during chronic endrin intoxication.

Plasma

Acute versus Control. By comparison, the acute animals

exhibited no changes in plasma iron and zinc levels in relation to the control values of 7.43 and 127 µg/ml; however, the magnesium control value of 8.3 µg/ml experienced a significant decrease of 10 per cent. The generalized organ decreases in copper content are reflected by a doubling of the control plasma copper concentration value of 0.30 µg/ml.

Chronic versus Control. Depletions of 20, 50, and 30 per cent were observed in base line values of 7.43, 1.27 and 0.20 µg/ml for the iron, zinc, and copper content of the plasma; however, the plasma magnesium content revealed no change from the base line value.

Feces

Chronic versus Control. Fecal content of the control animals averaged 1.5 dry weight (200.2 mg ash/animal/day), while the chronic endrin treatment animals averaged 0.55 g dry weight (5.5 mg ash/animal/day). Chronic endrin treatment animals excreted 0.0062 µg of iron/day, while the control animals excreted 0.0073 µg of iron/day. The chronic group excreted 495.4 µg of magnesium/day and the control animals excreted 111.8 µg of magnesium/day. No zinc was detected in the feces of the endrin treated animals; however 0.0202

µg/day was excreted by the control rats. The control animals excreted 0.0057 µg of copper/day which corresponds to twice as much copper as the chronic treatment value of 0.0024 µg/day; however, the control treatment feces averaged four times as much ash content as the chronic rats; consequently, the concentration of copper in the feces from the chronically exposed animals was higher than the control values.

Urine

Chronic versus Control. The control animals had a urinary output that averaged 15.5 ml/animal/day; whereas, this volume dropped to 9.6 ml/animal/day for the chronic treatment rats. Chronic endrin treatment resulted in an increased urinary excretion of iron from 2.52 µg/ml (daily total 39 µg) to 5.1 µg/ml (daily total 49 µg). Magnesium urinary excretion followed the same pattern of increase, rising from a non-detectable level in the control urine to 2.1 µg/ml which corresponded to a daily excretion of 20 µg for the chronic animals. The deviation in zinc excretion was of the opposite direction: control values of 1.50 µg/ml (total of 23 µg/day) dramatically decreased to 0.102 µg/ml which represented an excretion total of 0.98 µg/day. The concentration of copper remained constant at 0.14 µg/ml, but the reduced urinary volume of the chronic animals re-

sulted in a decrease of total daily copper excretion of 2.17 to 1.34 μg.

Metals

Iron

Of the five organs examined during acute intoxication, three (kidney, spleen and heart) indicated significant changes in the concentration as well as the total iron present. In all cases where alterations were observed, they were increases and except for the spleen, these increases paralleled increases in the organ size. Only two organs (liver and brain) maintained the control levels of iron and this occurred in opposition to increases in the size of the organs. Since the concentration of this metal in the RBC and plasma did not change significantly, the mobilization of iron into various organs apparently did not occur at the expense of the blood.

The results of the chronic study were similar to those of the acute in that three organs indicated changes in the iron present while two organs (brain and spleen) remained essentially unchanged; furthermore, where alterations did occur, they were manifested as increases. While the behavior of iron in the chronic tissues could not be rationalized on the basis of changes in the various organ size parameters, the observed increase was supported, at least in part, by decreases in the plasma and feces and an increase in the urine which correlated

with the increases in the concentration in the kidney.

Magnesium

The acute treatment tissues revealed significant changes in the concentration and/or total magnesium present in all five organs examined. In each case where alterations were observed during this treatment increases were noted except for the brain and liver which demonstrated decreases. In the heart and kidney, the increases in magnesium paralleled increases in organ size while the changes in the spleen, liver, and brain were in opposition to the reactions of the organ size parameters. The mobilization of magnesium into the heart, spleen and kidney was apparently acquired at the expense of the liver, brain, RBC, and plasma.

Of the five organs examined during chronic exposure only the liver demonstrated an increase in the magnesium levels while the heart and spleen indicated net losses and the kidney and brain remained at control levels. The increase in the total as well as concentration of magnesium in both the urine and feces indicates a net loss of this metal from the chronically exposed animal. This loss, as well as that amount transported to the liver, was apparently supplied by the spleen and heart since all other organs along with the RBC and plasma, remained essentially unchanged.

Zinc

The results of the examination of all five acute treatment organs revealed increases in the concentration and/or total zinc present. In all cases except the spleen, the increases in the total metal correlated with increases in organ size. In the case of the kidney and heart, the increases in the total as well as the unit concentration of zinc, in view of the increased organ size, indicate that acute treatment effected a marked influx of this metal into these two organs. The observed increases occurred, at least in part, at the expense of the blood, specifically the RBC.

During chronic intoxication, only two organs (liver and kidney) indicated changes in their zinc levels, and in both cases these were increases which occurred at a consistent organ size. All other organs studied maintained control levels of this metal in spite of changes in the organ size parameters. While the observations of zinc in chronic tissues could not be explained on the basis of changes in various organ size parameters, the increases appeared to be sustained by decreases in the plasma, RBC, feces, and urine.

Copper

Of the organs examined during acute exposure, the liver, spleen, brain, and heart demonstrated significant decreases in both

the unit concentration and total copper present. In all cases, the observed decreases were in opposition to changes in the size of the organs; therefore, extensive organ losses of this metal occurred. The kidney was the only acutely exposed organ that maintained control levels of copper, and again this was accomplished contrary to an increase in organ size. The observed decreases in organ copper were supported by an increase in the concentration of this metal in the plasma. This suggests that the kidney is the target organ for the mobilized copper and that the plasma is the vehicle responsible.

Examination of all the organs of the chronic treatment revealed significant changes in the concentration and/or total copper present in three organs (liver, kidney, and spleen). In the liver, the concentration decreased without any changes in either the total copper content or the weight parameters, while in the spleen the total decreased along with significant decreases in the organ weight parameters. The kidney was the only organ which manifested increases and this was observed even though there were no changes in the organ size. Only two organs (brain and heart) maintained control levels of copper and this occurred while the organ weights remained at control levels. In general, the copper was mobilized out of the

tissues, the RBC and the plasma into the kidney. This supports the observation that the kidney is the target organ for copper mobilized during endrin intoxication. Since copper was transported to and accumulated by the kidney, the decrease of this metal in the urine suggests that its molecular association (possibly ceruloplasmin) prohibited its excretion.

CHAPTER VI

SUMMARY AND CONCLUSIONS

This investigation was concerned with the measurement of endrin effects as evidenced by alterations in the concentration and total quantities of iron, magnesium, zinc, and copper in the liver, kidney, spleen, brain, heart, red blood cells, plasma, urine, and feces of the adult male Holtzman albino rat. Thirty-two animals were randomly divided into a control group of 10; a group of 12 which received a single acute dose of 25 mg/kg of body weight; and a chronic group of 10; which received, over an 8-day period, 4 doses totaling 8 mg/kg of body weight.

Based on a statistical analysis of over 1,500 determinations made throughout this investigation, the following conclusions have been drawn:

1. In general, acute exposure had more effect on organ size than did chronic exposure. Typically this was manifested as significant increases in the principal organ size parameters in the acutely exposed animals while the same observations made during chronic exposure

indicate no appreciable change. The exception occurred in the spleen, which indicated changes in size during chronic rather than acute intoxication and the changes were decreases rather than increases.

2. Acute treatment seemed to have more effect on metal mobilization (concentration as well as total organ content) than did chronic exposure. The general pattern was significant increases in iron, magnesium and zinc and significant decreases in copper. It was observed that whenever both iron and magnesium increased, zinc also increased.

3. As might be expected, each organ presented an individual pattern of trace metal content and changes due to endrin intoxication. Of the organs studied, the brain was the most resistant to change in organ size and metal content. This was especially evident during chronic exposure. The spleen and heart (in that order) had the greatest number of significant alterations, the most significant mobilization of metals above and beyond considerations for organ size parameters, and in general, the greatest magnitude of metal responses. The kidney and liver were next in order of change.

4. In general, the acute and/or chronic endrin treatments resulted in increases in the iron content of all organs but the brain. The organ iron increase was not at the expense of the blood nor was it appreciably reflected by its change in the urine and feces.

5. Acute exposure appeared to effect a magnesium migration into the organs at a greater extent than did the chronic treatment. Chronic treatment did not alter magnesium concentration in the blood while the excretion of magnesium was greatly increased (principally in the urine).

6. In those organs where the zinc content was altered, the direction was always an increase. This organ zinc increase appeared to be at the expense of the red blood cell and plasma (except for plasma zinc of the acute treatment). The increased requirement for zinc in chronic exposure was definitely reflected in the reduced urinary and fecal excretion of zinc; in fact, fecal zinc excretion dropped to a non-detectable value.

7. Acute treatment effected emphatic changes in both the total and the concentration of copper in the liver, spleen, brain, and heart. The kidneys were the only organs in which this metal remained at control levels. The large amount of copper thus mobilized was manifested by a 5-fold increase in its concentration in the plasma. On the other hand, during chronic exposure, the kidney was the only organ which indicated an increase in copper. The suggested role of the kidney as a target organ for this metal is supported by copper decreases in the spleen, RBC, plasma, and feces. The decrease in the total amount of this metal passing in the urine indicates a kidney

retention and implies that the mobilized copper is associated with a molecule (possibly ceruloplasmin) that is not subject to appreciable excretion.

8. The following metal alterations should be further studied as possible additional toxicological tools for chronic endrin exposure: (a) the appearance of magnesium in the urine along with an increase in this metal in the feces; (b) marked decreases in the fecal and urinary zinc levels; and (c) significant decreases in the concentration of copper and zinc in both the RBC and plasma.

BIBLIOGRAPHY

1. Adkins, T. R., Jr.; Sowell, W. L.; Arant, F. S. "Systemic Effect of Selected Chemicals on the Bed Bug and Lone Star Tick When Administered to Rabbits," J. Economic Ent., Vol. 48 (1955), pp. 139-141.

2. Aikawa, J. K. The Role of Magnesium in Biologic Processes. Springfield, Illinois: Charles C. Thomas, 1963.

3. Aikawa, J. E., et al. "Magnesium Metabolism in Rabbits Using Mg^{28} as a Tracer," Amer. J. Physiol., Vol. 197 (1959), pp. 99-101.

4. Aikawa, J. K.; Gordon, G. S.; Rhoades, E. L. "Magnesium Metabolism in Human Beings: Studies With Mg^{28}," J. Appl. Physiol., Vol. 15 (1960), pp. 503-507.

5. Axtrup, S. The Blood Copper in Anaemias of Children. Lund, Sweden: Lindstedts Universitetsbokhandel, 1946.

6. Barer, A. P.; Fowler, W. M. "Urinary Iron Excretion," J. Lab. Clin. Med., Vol. 23 (1937), pp. 148-155.

7. Barsa, M. C.; Ludwig, D. "Effects of DDT on the Respiratory Enzymes of the Mealworm Tenebrio Molitor L. and of the Housefly Musco domestico L.," Ann. Ent. Soc. America, Vol. 52 (1959), pp. 179-182.

8. Bearn, A. G.; Kunkel, H. G. "Localization of Cu^{64} in Serum Fractions Following Oral Administration: An Alteration in Wilson's Disease," Proc. Soc. Exptl. Biol. Med., Vol. 85 (1954), pp. 44-48.

9. Bergel, F., et al. "Cellular Constituents: Major and Minor Metals in Normal and Abnormal Tissues. Part I. Analysis of Wistar Rat Livers for Copper, Iron, Magnesium, Manganese, Molybdenum, and Zinc.," J. Pharm. and Pharm., Vol. 9 (1957), pp. 522-531.

10. Beutler, E. "Iron Enzymes in Iron Deficiency. I. Cytochrome c," Am. J. Med. Sci., Vol. 234 (1957), pp. 517-527.

11. Beutler, E. "Iron Enzymes in Iron Deficiency. IV. Cytochrome Oxidase in Rat Kidney and Heart," Acta Haematol., Vol. 21 (1959), pp. 371-377.

12. Beutler, E. "Iron Enzymes in Iron Deficiency. VI. Aconitase Activity and Citrate Metabolism," J. Clin. Invest., Vol. 38 (1959), pp. 1605-1616.

13. Beutler, E.; Blaisdell, R. K. "Iron Enzymes in Iron Deficiency. III. Catalase in Rat Red Cells and Liver With Some Further Observations on Cytochrome c," J. Lab. Clin. Med., Vol. 52 (1958), pp. 694-699.

14. Beutler, E.; Blaisdell, R. K. "Iron Enzymes in Iron Deficiency. V. Succinic Dehydrogenase in Rat Liver, Kidney and Heart," Blood, Vol. 15 (1960), pp. 30-35.

15. Bothwell, T. H.; Pirzio-Biroli, G.; Finch, C. A. "Iron Absorption. I. Factors Influencing Absorption," J. Lab. Clin. Med., Vol. 51 (1958), pp. 24-36.

16. Boyden, R.; Potter, V. R.; Elvehjam, C. A. "Effect of Feeding High Levels of Copper to Albino Rats," J. Nutr., Vol. 15 (1938), pp. 397-402.

17. Brandt, J. L.; Glaser, W.; Jones, A. "Soft Tissue Distribution and Plasma Disappearance of Intravenously Administered Isotopic Magnesium with Observations on Uptake in Bone," Metabolism, Vol. 7 (1958), pp. 355-363.

18. Brown, B. E.; Brown, A. W. A. "The Effects of Insecticidal Poisoning on the Level of Cytochrome Oxidase in the American Cockroach," J. Econ. Ent., Vol. 49 (1956), pp. 675-679.

19. Brown, E. B.; Justus, B. W. "In vitro Absorption of Radioiron by Everted Pouches of Rat Intestine," Am. J. Physiol., Vol. 194 (1958), pp. 319-326.

20. Brown, R. L. Pesticides in Clinical Practice. Charles C. Thomas, Publisher, 1966.

21. Cartwright, G. E. Symposium on Copper Metabolism. W. D. McElroy and B. Glass, eds. Baltimore, Maryland: Johns Hopkins Press, 1950. p. 274.

22. Cartwright, G. E.; Gubler, C. J.; Wintrobe, M. "Studies on Copper Metabolism. XI. Copper and Iron Metabolism in the Nephrotic Syndrome," J. Clin. Invest., Vol. 33 (1954), pp. 685-698.

23. Cartwright, G. E.; Wintrobe, M. M. "Chemical, Clinical, and Immunological Studies on the Products of Human Plasma Fractionation. XXXIX. The Anemia of Infection. Studies on the Iron-binding Capacity of Serum," J. Clin. Invest., Vol. 28 (1949), pp. 86-98.

24. Claude, B. E.; Ferguson, E. D. "Susceptibility and Resistance of Mosquito Fish to Several Insecticides," J. Econ. Ent., Vol. 57 (1964), pp. 430-431.

25. Cohen, H. "Chemical Studies Bearing on the Formation of Cerebrospinal Fluid," Brain, Vol. 50 (1923), pp. 601-607.

26. Cox, W. J.; Mueller, A. J. "The Composition of Milk From Stock Rats, and an Apparatus for Milking Small Laboratory Animals," J. Nutr., Vol. 13 (1937), pp. 249-261.

27. Davies, G. M.; Lewis, F. "Outbreak of Food Poisoning from Bread Made of Chemically Contaminated Flour," Brit. Med. J., Vol. 2 (1956), pp. 393-398.

28. Day, H. G.; McCollum, E. V. "Effects of Acute Dietary Zinc Deficiency in the Rat," Proc. Soc. Exptl. Biol. Med., Vol. 45 (1940), pp. 282-284.

29. Day, H. G.; Skidmore, B. E. "Some Effects of Dietary Zinc Deficiency in the Mouse," J. Nutr., Vol. 33 (1947), pp. 27-38.

30. DeWitt, J. B. "Chronic Toxicity to Quail and Pheasants of Some Chlorinated Insecticides," J. Agr. Food Chem., Vol. 4 (1956), pp. 863-866.

31. Decker, G. C. "Don't Let the Insects Rule," J. Agri. Food Chem., Vol. 6 (1958), pp. 98-103.

32. Decker, G. C. "Changing Horizons," Presidential Address, Third Annual Meeting, Entomological Society of America, November, 1955, Bulletin of the Entomological Society of America, Vol. 2 (1956), pp. 2-5.

33. Dixon, M.; Webb, E. C. Enzymes. 2d ed.; 3rd impression; New York and London: Academic Press, Inc., 1965.

34. Drabkin, D. L. "Metabolism of the Hemin Chromoproteins," Physiol. Revs., Vol. 31 (1951), pp. 345-431.

35. Dubach, R.; Moore, C. V.; Callender, S. "Studies in Iron Transportation and Metabolism. IX. The Excretion of Iron as Measured by the Isotope Technique," J. Lab. Clin.Med., Vol. 45 (1955), pp. 599-615.

36. Earl, C. J.; Moulton, M. J.; Selverstone, B. "Metabolism of Copper in Wilson's Disease and in Normal Subjects," Am. J. Med., Vol. 17 (1954), pp. 205-213.

37. Eden, A.; Green, H. H. "Micro-determination of Copper in Biological Material," Biochem. J., Vol. 34 (1940), pp. 1202-1208.

38. Ehrenberg, A.; Laurell, C. B. "Magnetic Measurements on Crystallized Fe-Transferrin Isolated from the Blood Plasma of Swine," Acta Chem. Scand., Vol. 9 (1955), pp. 68-72.

39. Eisler, R.; Edmunds, P. H. "Effects of Endrin on Blood and Tissue Chemistry of a Marine Fish," Transactions of The American Fisheries Society, Vol. 95 (April 1966), pp. 153-159.

40. Elkinton, J. R. "The Role of Magnesium in the Body Fluids," Clin. Chem., Vol. 3 (1957), pp. 319-331.

41. Emerson, T. E., Jr. "Mechanisms of Hemoconcentration in the Dog During Acute Endrin Insecticide Poisoning," Can. J. Physiol. Pharm., Vol. 43 (1965), pp. 793-800.

42. Emerson, T. E., Jr.; Brake, C. M.; Hinshaw, L. B. "Cardiovascular Effects of the Insecticide Endrin," Can. J. Phys. Pharm., Vol. 42 (1964), pp. 41-51.

43. Encyclopaedia Britannica, 7:88, 23:634. Chicago: William Benton, Publisher, 1958.

44. Feaster, J. P., et al. "Absorption, Deposition, and Placental Transfer of Zn^{65} in the Rat," Am. J. Physiol. Vol. 181 (1955), pp. 287-290.

45. Ferguson, D. E.; Bingham, C. R. "Endrin Resistance in the Yellow Bullhead, Ictalvrus Natalis," Transactions of The American Fisheries Society, Vol. 95 (1966), pp. 325-326.

46. Ferguson, D. E.; Bingham, C. R. "The Effects of Combinations of Insecticides on Susceptible and Resistant Mosquito Fish," Bulletin of Environmental Contamination & Toxicology, Vol. 1 (1966), pp. 97-103.

47. Ferguson, D. E.; Culley, D. D.; Cotton, W. D. "Tolerances of Two Populations of Fresh Water Shrimp to Five Chlorinated Hydrocarbon Insecticides," J. Mississippi Acad. Sci., Vol. 11, pp. 235-237.

48. Ferguson, D. E., et al. "Resistance to Chlorinated Hydrocarbon Insecticides in Three Species of Fresh Water Fish," Bio. Science, Vol. 14 (1964), pp. 43-44.

49. Field, A. C. "Balance Trials with Magnesium-28 in Sheep," Nature, Vol. 183 (1959), pp. 983-983.

50. Follis, R. H.; Day, H. G.; McCollum, E. V. "Histologic Studies of the Tissues of Rats Fed a Diet Extremely Low in Zinc," J. Nutr., Vol. 22 (1941), pp. 223-237.

51. Frear, D. E. H. Chemistry of the Pesticides. 3rd ed.; Toronto, New York and London: D. Van Nostrand Co., Inc., 1955.

52. Freireich, E. J., et al. "The Effect of Inflammation on the Utilization of Erythrocyte and Transferrin Bound Radioiron for Red Cell Production," Blood, Vol. 12 (1957), pp. 972-983.

53. Giannotti, O.; Metcalf, R. L.; March, R. B. "The Mode of Action of Aldrin and Dieldrin in Periplaneta americana (L.)," Ann. Ent. Soc. America, Vol. 49 (1956), pp. 588-592.

54. Ginsburg, S., et al. "Magnesium Metabolism of Human and Rabbit Erythrocytes," Blood, Vol. 20 (1962), pp. 722-729.

55. Gitlin, D.; Janeway, C. A.; Farr, L. E. "Studies on the Metabolism of Plasma Proteins in the Nephrotic Syndrome. I. Albumin, γ-Globulin and Iron Binding Globulin," J. Clin. Invest., Vol. 35 (1956), pp. 44-56.

56. Gilbert, D. L. "Magnesium Equilibrium in Muscle," J. Gen. Physiol., Vol. 43 (1960), pp. 1103-1118.

57. Goodwin, E. S., et al. "The Analysis of Crop Extracts for Traces of Chlorinated Pesticides by Gas-liquid Partition Chromatography," Chem. and Ind. (London: 1960), pp. 1220-1221.

58. Gowdey, C. W., et al. "The Pharmacological Properties of the Insecticide Dieldrin," Can. J. Biochem. Physiol., Vol. 32 (1954), pp. 498-503.

59. Gowdey, C. W., et al. "A Study of the Pharmacological Properties of the Insecticide Aldrin (hexachlorohexahydrodimethanonaphthalene)," Can. J. Med. Sci., Vol. 30 (1952), pp. 520-533.

60. Green, D. E. "Enzymes and Trace Substances," Advances in Enzymol., Vol. 1 (1941), pp. 177-198.

61. Greenberg, D. M.; Lucia, S. P.; Tufts, E. V. "The Effects of Magnesium Deprivation on Renal Function," Amer. J. Physiol., Vol. 121 (1938), pp. 424-430.

62. Gross, H. M.; Sandberg, M.; Holly, O. M. "Changes in Copper and Iron Retention in Chronic Diseases Accompanied by Secondary Anemia. II. Changes in Liver, Spleen and Stomach," Am. J. Med. Sci., Vol. 204 (1942), pp. 201-205.

63. Gunter, B. J. "The Effect of Endotoxin Shock on Blood Zinc Levels and Plasma Protein Concentrations." Unpublished Ph.D. dissertation, University of Oklahoma, 1967.

64. Gurd, F. R. N.; Goodman, D. S. "Preparation and Properties

of Serum and Plasma Proteins. XXXII. The Interaction of Human Serum Albumin with Zinc Ions," J. Amer. Chem. Soc., Vol. 74 (1952), pp. 670-675.

65. Hallas-Møller, K.; Peterson, K.; Schlichtkrull, T. "Crystalline and Amorphous Insulin-Zinc Compounds with Prolonged Action," Science, Vol. 116 (1952), pp. 394-398.

66. Hathway, D. E.; Mallinson, A.; Akintonwa, D. A. A. "Effects of Dieldrin, Picrotoxin and Telodrin on the Metabolism of Ammonia in Brain," Biochem. J., Vol. 94 (1965), pp. 676-686.

67. Haury, V. G.; Cantarow, A. "Variation of Serum Magnesium in 52 Normal and 440 Pathologic Patients," J. Lab. Clin. Med., Vol. 27 (1942), pp. 616-622.

68. Heath, D. F.; Vandekar, M. "Toxicity and Metabolism of Dieldrin in Rats," Brit. J. Industr. Med., Vol. 21 (1964), pp. 269-279.

69. Herms, W. B.; James, M. T. Medical Entomology. New York: The Macmillan Company, 1961.

70. Hinshaw, L. B., et al. "Effects of the Insecticide Endrin on the Cardiovascular System of the Dog," J. Pharm. Exptl. Therap., Vol. 153 (1966), pp. 225-236.

71. Hodges, R. E. "The Toxicity of Pesticides and Their Residues in Food," Nutr. Rev., Vol. 23 (The Nutrition Foundation, Inc.: 1965), pp. 225-230.

72. Holmberg, C. G.; Laurell, C. B. "Investigations in Serum Copper. I. Nature of Serum Copper and Its Relation to the Iron-binding Protein in Human Serum," Acta Chem. Scand., Vol. 1 (1947), pp. 944-950.

73. Holmberg, C. G.; Laurell, C. B. "Investigations in Serum Copper. II. Isolation of the Copper Containing Protein, and a Description of Some of its Properties," Acta Chem. Scand., Vol. 2 (1948), pp. 550-556.

74. Holmberg, C. G.; Laurell, C. B. "Oxidase Reactions in Human

Plasma Caused by Coeruloplasmin," Scand. J. Clin. Lab. Invest., Vol. 3 (1951), pp. 103-107.

75. Hosein, E. A.; Proulx, P. "Chemical and Biochemical Analyses on Brain Tissue Preparations During the Epileptiform-like Activity of Dieldrin and Other Cerebral Convulsants," J. Agr. Food Chem., Vol. 8 (1960), pp. 428-431.

76. Interpretation Number 3 of the Regulations for the Enforcement of the Federal Insecticide, Fungicide, and Rodenticide Act, USDA, Nov., 1964, Title 7 - Agriculture, Chapter III - Agriculture Research Service, Department of Agriculture. Part 362, Sec. 362.101.

77. Jacobziner, H.; Raybin, H. W. "Poisoning by Insecticide (Endrin)," N. Y. State J. Med., Vol. 102 (1959), pp. 2017-2022.

78. Jager, B. V.; Gubler, C. J. "An Immunologic Study of the Iron-Binding Protein," J. Immunol., Vol. 69 (1952), pp. 311-318.

79. Jensen, W. N.; Kamin, H. "Copper Transport and Excretion in Normal Subjects and in Patients with Laennec's Cirrhosis and Wilson's Disease: A Study with Cu^{64}," J. Lab. Clin. Med., Vol. 49 (1957), pp. 200-210.

80. Josephs, H. W. "Absorption of Iron as a Problem in Human Physiology," Blood, Vol. 13 (1953), pp. 1-54.

81. Kashiwa, H. K.; Hungerford, G. F. "Blood Leucocyte Response in Rats Fed a Magnesium Deficient Diet," Proc. Soc. Exptl. Biol. Med., Vol. 99 (1958), pp. 441-443.

82. Kearns, C. W.; Weinman, C. J.; Decker, G. C. "Insecticidal Properties of Some New Chlorinated Organic Compounds," J. Econ. Ent., Vol. 42 (1949), pp. 127-134.

83. Kehoe, R. A.; Cholak, J.; Story, R. V. "A Spectrochemical Study of the Normal Ranges of Concentration of Certain Trace Metals in Biological Materials," J. Nutr., Vol. 19 (1940), pp. 579-592.

84. Keilin, D.; Mann, T. "Carbonic Anhydrase. Purification and Nature of the Enzyme," Biochem. J., Vol. 34 (1940), pp. 1163-1176.

85. Korte, F.; Arent, H. "Metabolism of Insecticides, IX (1) Isolation and Identification of Dieldrin Metabolites from Urine of Rabbits after Oral Administration of Dieldrin-^{14}C," Life Sciences, Vol. 4 (1965), pp. 2017-2026.

86. Kunze, F. M.; Lang, E. P. "Toxicants in Tissues of Rats on Diets Containing Dieldrin, Aldrin, Endrin, and Isodrin," Fed. Proc., Vol. 12 (1953), p. 339.

87. Lahey, M. E., et al. "Studies on Copper Metabolism. II. Hematologic Manifestations of Copper Deficiency in Swine," Blood, Vol. 7 (1952), pp. 1053-1074.

88. Lang, N.; Renschler, H. E. "Untersuchungen Zum Ort der Coeruloplasminbildung mit Radiokupfer (^{64}Cu)," Z. ges. exptl. Med., Vol. 130 (1958), pp. 203-214.

89. Laurell, C. B. "Metal-binding Plasma Proteins," The Plasma Proteins. F. W. Putnam, ed. New York and London: Academic Press, Inc., 1960.

90. Laurell, C. B.; Ingelman, B. "The Iron-binding Protein of Swine Serum," Acta Chem. Scand., Vol. 1 (1947), pp. 770-776.

91. Lazzara, P., et al. "Tissue Distribution, Kinetics and Turnover of Mg^{28} in the Dog," Clin. Res., Vol. 10 (1962), p. 252.

92. Lindow, C. W.; Peterson, W. H.; Steenbock, H. "The Copper Metabolism of the Rat," J. Biol. Chem., Vol. 84 (1929), pp. 419-436.

93. Lindquist, D. A.; Dahm, P. A. "Metabolism of Radioactive DDT by the Madeira Roach and the European Corn Borer," J. Econ. Ent., Vol. 49 (1956), pp. 579-584.

94. Ludwig, G.; Weis, J.; Korte, F. "Excretion and Distribution of Aldrin ^{14}C and its Metabolites After Oral Administration

for a Long Period of Time," Life Sciences, Vol. 3 (1964), pp. 123-130.

95. MacIntyre, I.; Davidsson, D. "The Production of Secondary Potassium Depletion, Hypercalcaemia and Nephrocalcinosis by Magnesium Deficiency," Biochem. J., Vol. 69 (1958), pp. 6P-7P.

96. Markowitz, H., et al. "Studies on Copper Metabolism. XIV. Copper, Ceruloplasmin and Oxidase Activity in Sera of Normal Human Subjects, Pregnant Women, and Patients with Infection, Hepatolenticular Degeneration and the Nephrotic Syndrome," J. Clin. Invest., Vol. 34 (1955), pp. 1498-1508.

97. DeMarsh, A.B.; Windle, W. F.; Alt, H. L. "Factors Influencing the Blood Picture of the Newborn," Am. J. Diseases of Children, Vol. 75 (1948), pp. 863-871.

98. Mawson, C. A.; Fischer, M. I. "The Occurrence of Zinc in the Human Prostate Gland," Can. J. Med. Sci., Vol. 30 (1952), pp. 336-339.

99. McAleese, D. M.; Bell, M. C.; Forbes, R. M. "Magnesium-28 Studies in Lambs," J. Nutr., Vol. 74 (1961), pp. 505-514.

100. McCance, R. A.; Widdowson, E. M. "The Absorption and Excretion of Iron Following Oral and Intravenous Administration," J. Physiol., Vol. 94 (1938), pp. 148-154.

101. Meltzer, S. J.; Auer, J. "Physiological and Pharmacological Studies of Magnesium Salts. I. General Anesthesia by Subcutaneous Injections," Amer. J. Physiol., Vol. 14 (1905), pp. 366-388.

102. Meltzer, S. J.; Auer, J. "Physiological and Pharmacological Studies of Magnesium Salts. II. The Toxicity of Intravenous Injections: In Particular the Effects upon the Centres of the Medulla Oblongata," Amer. J. Physiol., Vol. 15 (1906), pp. 387-405.

103. Montgomery, M. L., et al. "The Elimination of Administered Zinc in Pancreatic Juice, Duodenal Juice and Bile of the Dog as Measured by its Radioactive Isotope (Zn^{65})," J. Exptl. Med., Vol. 78 (1943), pp. 151-159.

104. Moore, C. V. "Iron Metabolism and Nutrition," Harvey Lectures Series, Vol. 55 (1961), pp. 67-101.

105. Moore, C. V., et al. "Absorption of ferrous and Ferric Radioactive Iron by Human Subjects and Dogs," J. Clin. Invest., Vol. 23 (1944), pp. 755-767.

106. Morrison, P. E.; Brown, A. W. A. "The Effect of Insecticides on the Cytochrome Oxidase Obtained from the American Cockroach," J. Econ. Ent., Vol. 47 (1954), pp. 723-730.

107. Moss, J. A.; Hathway, D. E. "Transport of Organic Compounds in the Mammal," Biochem. J., Vol. 91 (1964), pp. 384-393.

108. Muir, A. R. "The Molecular Structure of Isolated and Intracellular Ferritin," Quart. J. Exptl. Physiol., Vol. 45 (1960), pp. 192-201.

109. Nelson, S. C., et al. "Serum Alkaline Phosphatase Levels, Weight Changes, and Mortality Rates of Rats Fed Endrin," J. Agr. Food Chem., Vol. 4 (1956), pp. 696-700.

110. Neuwirth, I.; Wallace, G. B. "A Note on the Absorption, Serum Concentration, and Narcotic Effects of Magnesium," J. Pharm. Exptl. Therap., Vol. 45 (1932), pp. 109-112.

111. Nosslin, B. F.; Nyman, M. "Haptoglobin Determination in Diagnosis of Haemolytic Diseases," Lancet, Vol. 1 (1958), pp. 1000-1001.

112. Orent, E. R.; McCollum, E. V. "Effects on the Rats of Deprivation of Magnesium," J. Biol. Chem. (Scientific Proc), Vol. 92 (1931), XXX-XXXI.

113. Otis, L.; Smith, C. M. "Further Evidence of Sex Variation in the Utilization of Iron by Anemic Rats," Science, Vol. 91 (1940), pp. 146-147.

114. Perry, A. S. "Metabolism of Insecticides by Various Insect Species," J. Agr. Food Chem., Vol. 8 (1960), pp. 266-272.

115. Perry, A. S. "Biochemical Aspects of Insect Resistance to the Chlorinated Hydrocarbon Insecticides," Miscellaneous Publications of The Entomological Society of America, Vol. 2 (1960), pp. 119-137.

116. Perry, A. S. "Technology Branch Summary of Investigations, No. 10. Atlanta, Georgia: Bureau of State Services, Communicable Disease Center, U. S. Department of Health, Education and Welfare, Public Health Service, July-December, 1956. p. 159.

117. Peters, J. P.; VanSlyke, D. D. Quantitative Clinical Chemistry. I. Interpretations. Baltimore, Maryland: Williams & Wilkins Co., 1931.

118. Peterson, R. E.; Ettinger, R. H. "Radioactive Iron Absorption in Siderosis (Hemochromatosis) of the Liver," Amer. J. Med., Vol. 15 (1953), pp. 518-524.

119. Phillips, D. D.; Pollard, G. E.; Soloway, S. B. "Thermal Isomerization of Endrin and Its Behavior in Gas Chromatography," J. Agri. Food Chem., Vol. 10 (1962), pp. 217-221.

120. Phillips, F. S.; Gilman, A.; Cresceitell, F. N. "Studies on the Pharmacology of DDT (2,2,Bisparachlorophenyl-1,1,1, Trichloroethan). II. The Sensitization of the Myocardium to Sympathetic Stimulation During Acute DDT Intoxication," J. Pharm. Exptl. Therap., Vol. 86 (1946), pp. 222-228.

121. Pirzio-Biroli, G.; Bothwell, T. H.; Finch, C. A. "Iron Absorption. II. The Absorption of Radioiron Administered with a Standard Meal in Man," J. Lab. Clin. Med., Vol. 51 (1958), pp. 37-48.

122. Poonawalla, N. H.; Korte, F. "Metabolism of Insecticides, VIII (1): Excretion, Distribution and Metabolism of α-Chlorodan-^{14}C by Rats," Life Sciences, Vol. 3 (1964), pp. 1497-1500.

123. Reins, D. A.; Holmes, D. D.; Hinshaw, L. B. "Acute and

Chronic Effects of the Insecticide Endrin on Renal Function and Renal Hemodynamies," Can. J. Phys. Pharm. Vol. 42 (1964), pp. 599-608.

124. Reins, D. A., et al. "Effect of Endrin on Venous Return and Catecholamine Release in the Dog," Can. J. Phys. Pharm., Vol. 44 (1966), pp. 59-67.

125. Revzin, A. M. "Effects of Endrin on Telencephalic Function in the Pigeon," Toxi. and Appl. Pharm. Vol. 9 (1966), pp. 75-83.

126. Rosen, J. D.; Letter, January 19, 1967. College of Agriculture and Environmental Science, Department of Agricultural Chemistry, Rutgers, The State University, New Brunswick, New Jersey, 08903.

127. Rosen, J. D.; Sutherland, D. J.; Lipton, G. R. "The Photochemical Isomerization of Dieldrin and Endrin and Effects on Toxicity," Bulletin of Environmental Contamination & Toxicology, Vol. 1 (1966), pp. 133-140.

128. Sachs, A., et al. "Copper and Iron in Human Blood," Arch. Intern. Med., Vol. 71 (1943), pp. 489-501.

129. Sacklin, J. A.; Terriere, L. C.; Remmert, L. F. "Effect of DDT on Enzymatic Oxidation and Phosphorylation," Science, Vol. 122 (1955), pp. 377-378.

130. Sacktor, B. "A Comparison of the Cytochrome Oxidase Activity of Two Strains of Adult Houseflies," J. Econ. Ent., Vol. 43 (1950), pp. 832-838.

131. Sacktor, B. "Some Aspects of Respiratory Metabolism During Metamorphosis of Normal and DDT-resistant Houseflies," Biol. Bull., Vol. 100 (1951), pp. 229-243.

132. Sadasivan, V. "Studies on the Biochemistry of Zinc. I. Effect of Feeding Zinc on the Liver and Bones of Rats," Biochem. J., Vol. 48 (1951), pp. 527-530.

133. Scheinberg, I. H.; Morell, A. G. "Exchange of Ceruloplasmin Copper with Ionic Cu^{64} with Reference to Wilson's Disease," J. Clin. Invest., Vol. 36 (1957), pp. 1193-1201.

134. Schultze, M. O. "The Effect of Deficiencies in Copper and Iron on the Cytochrome Oxidase of Rat Tissues," J. Biol. Chem., Vol. 129 (1939), pp. 729-737; "The Relation of Copper to Cytochrome Oxidase and Hematopoietic Activity of the Bone Marrow of Rats," J. Biol. Chem., Vol. 138 (1941), pp. 219-224.

135. Scoular, F. I. "A Quantitative Study, by Means of Spectrographic Analysis, of Copper in Nutrition," J. Nutr., Vol. 16 (1938), pp. 437-450.

136. Sheline, G. E., et al. "Studies on the Metabolism of Zinc with the Aid of its Radioactive Isotope. I. The Excretion of Administered Zinc in Urine and Feces," J. Biol. Chem., Vol. 147 (1943), pp. 409-414.

137. Sheline, G. E., et al. "Studies on the Metabolism of Zinc with the Aid of its Radioactive Isotopes. II. Distribution of Administered Radioactive Zinc in Tissues of Mice and Dogs," J. Biol. Chem., Vol. 149 (1943), pp. 139-151.

138. Shell International Chemical Co. "The Safe Handling and Toxicology of Endrin." Undated bulletin.

139. Shepard, H. H.; Mahan, J. N. "Use of Agr Chemical Continues to Rise," Chem. Eng. News, Vol. 44 (1966), pp. 82A-85A.

140. Shoden, A.; Gabrio, B. W.; Finch, C. A. "The Relationship Between Ferritin and Hemosiderin in Rabbits and Man," J. Biol. Chem., Vol. 204 (1953), pp. 823-830.

141. Shoden, A.; Sturgeon, P. "Formation of Hemosiderin and its Relation to Ferritin," Nature, Vol. 189 (1961), pp. 846-847.

142. Shohl, A. T. "Mineral Metabolism," American Chemical Society Monograph, Series No. 82. New York: Reinhold Publishing Corp., 1949.

143. Silver, L., et al. "Magnesium Turnover in the Human Studied With Mg^{28}," J. Clin. Invest., Vol. 39 (1960), pp. 420-425.

144. Simonsen, D. G.; Westover, L. M.; Wertman, M. "The De-

termination of Serum Magnesium by the Molybdivanadate Method for Phosphate," J. Biol. Chem., Vol. 169 (1947), pp. 39-47.

145. Smthy, C. V.; Miller, R. C. "The Iron Content of the Albino Rat at Different Stages of the Life Cycle," J. Nutr., Vol. 1 (1929), pp. 209-216.

146. Soloway, S. B., et al. "Skeletal Rearrangements in Reactions of Isodrin and Endrin," J. Am. Chem. Soc., Vol. 82 (1960), pp. 5377-5385.

147. Speck, L. B.; Maaske, C. A. "The Effects of Chronic and Acute Exposure of Rats to Endrin," AMA Arch. Ind. Health, Vol. 18 (1958), pp. 268-272.

148. Stavinoha, W. B.; Rieger, J. A., Jr. "The Effects of DDT on the Urinary Excretion of Epinephrine and Norepinephrine," Tox. and Appl. Pharm., Vol. 8 (1966), pp. 365-368.

149. Steel, G. D.; Torrie, J. H. Principles and Procedures of Statistics. New York, Toronto, London: McGraw-Hill Book Co., Inc., 1960.

150. Sternburg, J.; Kearns, C. W.; Moorefield, H. "DDT-Dehydrochlorinase, an Enzyme Found in DDT-resistant Flies," J. Agri. Food Chem., Vol. 2 (1954), pp. 1125-1130.

151. Sternburg, J.; Vinson, E. B.; Kearns, C. W. "Enzymatic Dehydrochlorination of DDT by Resistant Flies," J. Econ. Ent., Vol. 46 (1953), pp. 513-514.

152. Steyn-Parve, E. P.; Beinert, H. "On the Mechanism of Dehydrogenation of Fatty Acyl Derivatives of Coenzyme A. VII. The Nature of the Green Color of Butyryl Dehydrogenase," J. Biol. Chem., Vol. 233 (1958), pp. 853-861.

153. Stirn, F. E.; Elvehjem, C. A.; Hart, E. B. "The Indispensibility of Zinc in the Nutrition of the Rat," J. Biol. Chem., Vol. 109 (1935), pp. 347-359.

154. Strain, W. H.; Fories, W. J.; Hinshaw, J. R. "Zinc Studies in Skin Repair," Surg. Forum, Vol. 11 (1960), p. 11.

155. Sturgeon, P.; Brubaker, C. "Copper Deficiency in Infants," AMA J. Diseases of Children, Vol. 92 (1956), pp. 254-265.

156. Suomalainen, P. "Artificial Hibernation," Nature, Vol. 144 (1939), p. 443.

157. Tanford, C. "The Effect of pH on the Combination of Serum Albumin with Metals," J. Amer. Chem. Soc., Vol. 74 (1952), pp. 211-215.

158. The Toxicology Pharmacology and Use Experience of Endrin. Shell Chemical Company, New York 20, N. Y., June 1,. 1964.

159. Treon, J. F. "The Toxicity of Endrin for Laboratory Animals," The Kettering Laboratory, Department of Preventive Medicine and Industrial Health, College of Medicine, University of Cincinnati, Cincinnati, Ohio, 1955.

160. Treon, J. R. "The Toxicology and Pharmacology of Endrin (A Review of the Literature and of Work in Progress)," The Kettering Laboratory, University of Cincinnati, August 9, 1956.

161. Treon, J. R.; Cleveland, F. P.; Cappel, J. "Toxicity of Endrin for Laboratory Animals," J. Agr. Food Chem., Vol. 3 (1955), pp. 842-848.

162. Underwood, E. J. "A Comparison of Ferrous and Ferric Iron in the Nutrition of the Rat," J. Nutr., Vol. 16 (1938), pp. 299-308.

163. Underwood, E. J. Trace Elements in Human and Animal Nutrition. New York and London: Academic Press, Inc., 1962.

164. Vallee, B. L. "Zinc," Mineral Metabolism, Vol. II, The Elements, Part B. E. L. Comar, Felix Bronner, Eds. New York and London: Academic Press, Inc., 1962.

165. Vallee, B. L. "Zinc and Metalloenzymes," Advances in Protein Chem., Vol. 10 (1955), pp. 317-384.

166. Vallee, B. L., et al. "The Relationship Between Carbonic Anhydrase Activity and Zinc Content of Erythrocytes in Normal, in Anemic and Other Pathological Conditions," Blood, Vol. 4 (1949), pp. 467-478.

167. Vallee, B. L., et al. "Zinc Metabolism in Hepatic Dysfunction. I. Serum Concentration in Laennec's Cirrhosis and Their Validation by Sequential Analysis," New Eng. J. Med., Vol. 255 (1956), pp. 403-408.

168. Vallee, B. L.; Fluharty, R. G.; Gibson, J. G. "II, Distribution of Zinc in Normal Blood and Organs Studied by Means of Zn^{65}," Acta Unio. Intern. contra Cancrum., Vol. 6 (1949), pp. 869-873.

169. Wachtel, L. W., et al. "Blood Uric Acid and Liver Uricase of Zinc Deficient Rats on Various Diets," J. Biol. Chem., Vol. 138 (1941), pp. 361-368.

170. Walker, A. R. P.; Fox, F. W.; Irving, J. T. "Studies in Human Mineral Metabolism. I. The Effect of Bread Rich in Phytate Phosphorus on the Metabolism of Certain Mineral Salts with Special Reference to Calcium," Biochem. J., Vol. 42 (1948), pp. 452-462.

171. Wallach, S., et al. "Plasma and Erythrocyte Magnesium in Health and Disease," J. Lab. Clin. Med., Vol. 59 (1962), pp. 195-210.

172. Watchorn, E.; McCance, R. A. "Subacute Magnesium Deficiency in Rats," Biochem. J., Vol. 31 (1937), pp. 1379-1390.

173. Weikel, J. H., Jr.; Lang, E. P.; Tomchick, R. "Ion Movement Across the Rabbit Erythrocyte Membrane as Affected by Chlorinated Insecticides," Division of Pharmacology, Bureau of Biological and Physical Sciences, Food and Drug Administration, presented to the American Society for Pharmacology and Experimental Therapeutics, Nov. 9, 1956, pp. 262-272, at French Lick, Indiana.

174. Weil, L.; Russell, M. A. "Studies on Plasma Phosphatase Activity in Relation to Fat Metabolism in Rats," J. Biol. Chem., Vol. 136 (1940), pp. 9-23.

175. Widdowson, E. M.; McCance, R. A. "Sexual Difference in the Storage and Metabolism of Iron," Biochem. J., Vol. 42 (1948), pp. 577-581.

176. Widdowson, E. M.; McCance, R. A.; Spray, C. M. "The Chemical Composition of the Human Body," Clin. Sci., Vol. 10 (1951), pp. 113-125.

177. Wintrobe, M. M.; Cartwright, G. E.; Gubler, C. J. "Studies on the Function and Metabolism of Copper," J. Nutr., Vol. 50 (1953), pp. 395-419.

178. Wohl, M. G.; Goodhart, R. S. "Zinc," Modern Nutrition in Health and Disease. New York and London: Academic Press, 1956. pp. 45-53.

179. Wright, N. C.; Papish, J. "The Inorganic Constituents of Milk," Science, Vol. 69 (1928), p. 78.

180. Zavon, M. R. "The Toxicology and Pharmacology of Endrin," The Kettering Laboratory, Department of Preventive Medicine and Industrial Health, College of Medicine, University of Cincinnati, Cincinnati, Ohio.

APPENDIX I

AN EXAMPLE OF THE STATISTICAL TREATMENT

APPLIED TO ALL DATA

CONCENTRATION OF COPPER IN THE LIVER

	Concentration in ug/mg of ash		
Replications	Control	Chronic	Acute
1	0.20	0.14	0.032
2	0.14	0.12	0.025
3	0.14	0.19	0.031
4	0.16	0.08	0.030
5	0.18	0.13	0.029
6	0.14	0.07	0.027
7	0.20	0.08	0.021
8	0.13	0.05	0.031
9	0.13	0.18	0.034
10	0.11	0.12	0.039
11	-	-	0.024
ΣX	1.53	1.16	0.323
$\bar{X}$	0.15	0.12	0.029
ΣX^2	0.2427	0.1540	0.0097
$(\Sigma X)^2/N$	0.2341	0.1346	0.0095

Σx^2	0.0086	0.0194	0.0002
s^2	0.0010	0.0022	0.00002

$$\Sigma X^2 = \Sigma X^2 - \frac{(\Sigma X)^2}{n}$$

$$s^2 = \frac{\Sigma x^2}{n-1}$$

$$\Sigma x_1^2 = \Sigma X^2 - \frac{(\Sigma X)^2}{N} = 0.2424 - 0.2341 = 0.0086$$

$$s_1^2 = \frac{\Sigma x_1^2}{n_1 - 1} \quad \frac{0.0086}{9} = 0.0010$$

THE ANALYSIS OF VARIANCE FOR ANY NUMBER OF GROUPS WITH UNEQUAL APPLICATION

Location	r_1	$\Sigma_j X_{ij}$	$\bar{X}_i$	$\Sigma_j X_{ij}^2$	X_i^2/r_i	$\Sigma_j X_{ij}^2 = \Sigma_j (X_{ij} - \bar{X}_2)^2$
Control	10	1.53	0.15	0.2427	0.2341	0.0086
Chronic	10	1.16	0.12	0.1540	0.1346	0.0194
Acute	11	0.323	0.029	0.0097	0.0095	0.0002
Total	31	3.013	0.299	0.4064	0.3782	0.0282

$$\text{Correction factor} = C = \frac{X^2}{\Sigma r_i} = \frac{\Sigma_{ij} X}{\Sigma r_{ij}} = \frac{(3.013)^2}{31} = \frac{9.078}{31} = 0.2928$$

$$\text{Total Sum Squares} = \Sigma_{ij} X_{ij}^2 - C$$

Total SS $= 0.4064 - 0.2928 = 0.1136$

Treatment Sum Squares $\sum \frac{X_{i.}^2}{r_i} - C = \frac{X_1^2}{r_1} + \ldots + \frac{X_{t.}^2}{r_t} - C$

Treatment SS = 0.3782 - 0.2989 = 0.0854

Error SS = total SS - treatment SS

= 0.1136 - 0.0854 = 0.282

ANALYSIS OF VARIANCE SUMMARIZED

Source of Variation	df	SS	MS	F
Among treatments	2	0.1136	0.0568	56.80*
Within treatments	27	0.282	0.00101	-
Total	29	-	-	

$S_{\bar{d}} = \sqrt{\text{Error MS } \frac{1}{2}(\frac{1}{r_i} + \frac{1}{r_j})}$

$\frac{1}{2}(\frac{1}{r_i} + \frac{1}{r_j})$ for 10 and 11 replications $= \frac{1}{10} + \frac{1}{11} = 0.095$

$\frac{1}{2}(\frac{1}{r_i} + \frac{1}{r_j})$ for 10 and 10 replications $= \frac{1}{2}(\frac{1}{10} + \frac{1}{10} = 0.1$

$\sqrt{\text{Error MS } \frac{1}{2}(\frac{1}{r_i} + \frac{1}{r_j})} = \sqrt{\text{Error MS } \frac{1}{2}(0.1 + 0.1)} =$

$\sqrt{\text{Error MS } (0.1)}$

$\sqrt{0.001\ (0.095)} = \sqrt{0.000095} = 0.00948$

$0.00948 \times 3.04 = 0.02888$

$\sqrt{0.001\ (0.1)} = \sqrt{0.0001} = 0.01$ $\qquad 0.01 \times 2.90 = 0.0290$

3.04 from table A.7 Steel & Torrie for 0.05 level 3 + 28° df.

2.90 from table A.7 Steel & Torrie for 0.05 level 2 + 28° df.

The means of treatment are ranked from highest to lowest.

A - 0.15, B - 0.12, C - 0.029

A - C = 0.15 - 0.029 = 0.121
0.121>0.02888 ∴ Sig. difference

A - B = 0.15 - 0.12 = 0.03
0.03>0.29 ∴ Sig. difference

B - C = 0.12 - 0.029 = .091
0.091>0.0290 ∴ Sig. difference

APPENDIX II

TABLE 2

TISSUE WEIGHTS FOR CONTROL RATS

Tissue		Grams			
		Wet	Ash	Moisture	Volatile
Liver	$\bar{x}$	10.26456	0.19005	6.929	3.15
	s^2	1.26126	1.90180	0.593	0.13
	n	10	10	10	10
Kidney	$\bar{x}$	1.11244	0.01600	0.786	0.340
	s^2	0.00588	0.00213	0.0027	0.01122
	n	10	10	10	10
Spleen	$\bar{x}$	0.64758	0.01290	0.477	0.157
	s^2	0.00896	0.00190	0.0061	0.00033
	n	10	10	10	10
Brain	$\bar{x}$	1.55128	0.02549	1.174	0.341
	s^2	0.01468	0.01617	0.0093	0.00111
	n	10	10	10	10
Heart	$\bar{x}$	1.12381	0.01412	0.842	0.267
	s^2	0.00777	0.00117	0.0044	0.00056
	n	10	10	10	10
Feces	$\bar{x}$	1.23421	0.16808		

TABLE 3

TISSUE WEIGHTS FOR CHRONIC RATS

Tissue		Grams			
		Wet	Ash	Moisture	Volatile
Liver	$\bar{x}$	9.82125	0.21491	6.647	2.96
	s^2	2.01366	1.96285	0.996	0.16
	n	10	10	10	10
Kidney	$\bar{x}$	1.11445	0.01416	0.815	0.286
	s^2	0.01249	0.00111	0.0079	0.00056
	n	10	10	10	10
Spleen	$\bar{x}$	0.53951	0.00935	0.397	0.133
	s^2	0.01607	0.00513	0.0102	0.00056
	n	10	10	10	10
Brain	$\bar{x}$	1.63062	0.02471	1.251	0.352
	s^2	0.00843	0.00335	0.0058	0.00033
	n	10	10	10	10
Heart	$\bar{x}$	1.09027	0.01202	0.811	0.262
	s^2	0.01290	0.00194	0.0082	0.00067
	n	10	10	10	10
Feces	$\bar{x}$	1.04760	0.10657		

TABLE 4

TISSUE WEIGHTS FOR ACUTE RATS

Tissue		Grams			
		Wet	Ash	Moisture	Volatile
Liver	$\bar{x}$	13.07622	0.34942	8.871	3.86
	s^2	2.44518	11.30552	1.144	1.01
	n	12	12	12	12
Kidney	$\bar{x}$	1.24330	0.01698	0.917	0.309
	s^2	0.01156	0.00376	0.0082	0.00045
	n	12	12	12	12
Spleen	$\bar{x}$	0.69270	0.01304	0.525	0.155
	s^2	0.01114	0.00294	0.0061	0.00073
	n	12	12	12	12
Brain	$\bar{x}$	1.65609	0.02697	1.280	0.349
	s^2	0.01046	0.00632	0.0063	0.00036
	n	12	12	12	12
Heart	$\bar{x}$	1.29144	0.01574	0.976	0.299
	s^2	0.00879	0.00277	0.0050	0.00045
	n	12	12	12	12

TABLE 5

TOTAL, MEAN CONCENTRATION AND VARIATION OF IRON IN ASH OF INDICATED TISSUE

Tissues		Control		Chronic		Acute	
		Total µg	µg/mg	Total µg	µg/mg	Total µg	µg/mg
Liver	$\bar{x}$	392.65	2.12	533.31	2.63	527.75	1.64
	s^2	6743.18	0.23	11283.30	0.82	11512.40	0.25
	n	10	10	10	10	12	12
Kidney	$\bar{x}$	19.33	1.19	26.16	1.86	40.40	2.38
	s^2	80.08	0.25	30.55	0.15	115.16	0.45
	n	10	10	10	10	12	12
Spleen	$\bar{x}$	50.16	3.97	53.14	5.66	86.6	6.75
	s^2	266.6	2.18	355.40	1.71	1187.42	8.28
	n	10	10	10	10	12	12
Brain	$\bar{x}$	11.66	0.46	10.06	0.41	10.38	0.39
	s^2	6.36	0.0022	1.68	0.0033	16.10	0.023
	n	10	10	10	10	12	12
Heart	$\bar{x}$	28.08	1.99	33.63	2.80	48.25	3.14
	s^2	10.35	0.047	37.35	0.16	133.56	0.61
	n	10	10	10	10	12	12
RBC	$\bar{x}$		1049.4*		1078.0*		1052.0*
	s^2		259.6		68.2		540.2
	n		10		10		11
Plasma	$\bar{x}$		7.43*		5.82*		6.99*
	s^2		2.70		2.44		2.40
	n		9		10		12
Urine	$\bar{x}$		2.52*		5.1*		
Feces	$\bar{x}$		0.00036		0.00113		

*Expressed in µg/ml.

TABLE 6

TOTAL, MEAN CONCENTRATION AND VARIATION OF MAGNESIUM IN ASH OF INDICATED TISSUE

Tissues		Control		Chronic		Acute	
		Total µg	µg/mg	Total µg	µg/mg	Total µg	µg/mg
Liver	$\bar{x}$	1335.63	7.1	1853.50	8.7	1662.07	5.0
	s^2	89739.83	0.4178	106244.69	0.4122	193972.01	1.6645
	n	10	10	10	10	12	12
Kidney	$\bar{x}$	121.12	7.6	115.68	8.2	156.80	9.3
	s^2	209.63	0.2378	160.79	0.1400	227.61	0.9527
	n	10	10	10	10	12	12
Spleen	$\bar{x}$	83.54	6.4	64.11	6.8	121.4	9.3
	s^2	416.03	1.4489	283.44	0.2578	120.92	0.8154
	n	10	10	10	10	12	12
Brain	$\bar{x}$	151.2	6.0	165.7	6.7	119.4	4.5
	s^2	824.70	0.9222	221.80	0.1889	266.14	1.0327
	n	10	10	10	10	12	12
Heart	$\bar{x}$	129.1	8.8	114.5	9.5	169.6	10.9
	s^2	384.27	0.3244	142.81	0.1222	51.67	2.1636
	n	10	10	10	10	12	12
RBC	$\bar{x}$		23.5*		21.9*		17.5*
	s^2		22.50		19.62		12.59
	n		10		10		12
Plasma	$\bar{x}$		8.3*		7.8*		7.4*
	s^2		0.32		0.16		0.61
	n		10		10		12
Urine	$\bar{x}$		0.0*		2.1*		
Feces	$\bar{x}$		55.34		90.08		

*Expressed in µg/ml.

TABLE 7

TOTAL, MEAN CONCENTRATION AND VARIATION OF ZINC IN ASH OF INDICATED TISSUE

Tissues		Control		Chronic		Acute	
		Total µg	µg/mg	Total µg	µg/mg	Total µg	µg/mg
Liver	$\bar{x}$	182.77	0.99	225.30	1.07	259.26	0.83
	s^2	494.52	0.022	2045.47	0.051	2488.07	0.12
	n	10	10	10	10	12	12
Kidney	$\bar{x}$	16.31	1.02	17.76	1.26	28.22	1.69
	s^2	7.4	0.0156	6.9	0.043	17.30	0.127
	n	10	10	10	10	12	12
Spleen	$\bar{x}$	9.94	0.77	8.60	0.89	17.52	1.39
	s^2	1.91	0.0022	11.42	0.109	4.88	0.44
	n	10	10	10	10	12	12
Brain	$\bar{x}$	14.14	0.56	13.63	0.55	18.84	0.62
	s^2	6.35	0.008	4.15	0.005	2.35	0.008
	n	10	10	10	10	12	12
Heart	$\bar{x}$	13.48	0.96	11.72	0.97	22.00	1.41
	s^2	1.57	0.0033	3.46	0.0075	4.53	0.044
	n	10	10	9	9	12	12
RBC	$\bar{x}$		17.4*		10.3*		11.1*
	s^2		61.75		8.64		6.81
	n		10		10		12
Plasma	$\bar{x}$		1.27*		0.63*		1.21*
	s^2		0.0478		0.0133		0.2518
	n		10		10		12
Urine	$\bar{x}$		1.50*		0.102*		
Feces	$\bar{x}$		0.001		0.0		

*Expressed in µg/ml.

TABLE 8

TOTAL, MEAN CONCENTRATION AND VARIATION OF COPPER IN ASH OF INDICATED TISSUE

Tissues		Control		Chronic		Acute	
		Total µg	µg/ml	Total µg	µg/ml	Total µg	µg/ml
Liver	$\bar{x}$	26.77	0.15	24.66	0.12	10.58	0.029
	s^2	19.56	0.0010	24.24	0.0022	11.04	0.00002
	n	10	10	9	10	11	11
Kidney	$\bar{x}$	6.23	0.39	9.09	0.64	7.4	0.44
	s^2	5.21	0.0194	4.76	0.0133	5.37	0.0222
	n	10	10	10	10	12	12
Spleen	$\bar{x}$	0.92	0.07	0.59	0.07	0.62	0.048
	s^2	0.04	0.00034	0.0188	0.00062	0.0036	0.00064
	n	10	10	9	10	12	12
Brain	$\bar{x}$	3.51	0.14	3.57	0.14	1.74	0.065
	s^2	0.49	0.00027	0.23	0.00074	0.09	0.00014
	n	10	10	10	10	12	12
Heart	$\bar{x}$	4.27	0.30	4.03	0.33	2.33	0.150
	s^2	0.92	0.0038	0.68	0.0058	0.38	0.0019
	n	10	10	10	10	11	11
RBC	$\bar{x}$		0.40*		0.27*		0.30*
	s^2		0.0042		0.0029		0.0332
	n		10		10		12
Plasma	$\bar{x}$		0.20*		0.63*		0.49*
	s^2		0.00049		0.0120		0.0168
	n		10		10		12
Urine	$\bar{x}$		0.14*		0.14*		
Feces	$\bar{x}$		0.00028		0.00044		

*Expressed in µg/ml.

TABLE 9

SUMMARY OF F-VALUES FOR INDICATED COMPARISON OF THE THREE TREATMENT GROUPS

Analysis	Liver	Kidney	Spleen	Brain	Heart
Fe, total µgs	6.54*	16.56*	7.13*	.836	18.51*
Fe, µg/mg ash	6.36*	13.01*	4.89*	1.36	23.00*
Mg, total µgs	5.12*	27.83*	35.87*	6.92*	49.70*
Mg, µg/mg ash	43.15*	18.12*	33.58*	19.19*	13.69*
Zn, total µgs	9.22*	43.12*	43.36*	22.36*	77.84*
Zn, µg/mg ash	2.50	19.55*	21.37*	2.76	81.50*
Cu, total µgs	67.25*	4.03*	64.44*	47.18*	18.32*
Cu, µg/mg ash	56.80*	9.28*	6.25*	60.00*	34.64*
Wet weight	18.09*	7.00*	5.83*	3.15	9.23*
Ash weight	15.04*	9.08*	13.73*	1.73	18.75*
Moisture	5.27*	8.31*	5.96*	4.71*	14.92*
Volatile	17.60*	1.83	3.62*	2.56	9.06*

*Significant - at the 0.05 level.

TABLE 10

COMPARISON OF MEANS BY DUNCAN'S NEW MULTIPLE-RANGE TEST

Analysis	Liver	Kidney	Spleen	Brain	Heart
Fe, total µgs	B C A	C B A	C B A	A C B	C B A
Fe, µg/mg ash	B A C	C B A	C B A	A B C	C B A
Mg, total µgs	B C A	C A B	C A B	B A C	C A B
Mg, µg/mg ash	B A C	C B A	C B A	B A C	C B A
Zn, total µgs	C B A	C B A	C A B	C A B	C A B
Zn, µg/mg ash	B A C	C B A	C B A	C A B	C B A
Cu, total µgs	A B C	B C A	A C B	B A C	A B C
Cu, µg/mg ash	A B C	B C A	A B C	A B C	B A C
Wet weight	C A B	C B A	C A B	C B A	C A B
Ash weight	C B A	C A B	C A B	C A B	C A B
Moisture	C A B	C B A	C A B	C B A	C A B
Volatile	C A B	A C B	A C B	B C A	C A B

A--Control; B--Chronic; C--Acute.

Non-significant differences indicated by underscoring.

TABLE 11

ANALYSIS OF DIFFERENCES IN THE RED BLOOD CELLS BY ANALYSIS OF VARIANCE AND DUNCAN'S NEW MULTIPLE-RANGE TEST

	Red Blood Cells				
Metals	Control	Chronic	Acute	F-Values	DNMRTV
Total µgs Fe	1049.4	1078.0	1052.0	0.868	B C A
Total µgs Mg	23.5	21.9	17.5	6.17*	A B C
Total µgs Zn	17.4	10.3	11.1	6.32*	A C B
Total µgs Cu	0.40	0.27	0.30	2.03	A C B

A--Control; B--Chronic; C--Acute.

Non-significant differences indicated by underscoring.

TALBE 12

ANALYSIS OF DIFFERENCES IN THE PLASMA BY ANALYSIS OF VARIANCE AND DUNCAN'S NEW MULTIPLE-RANGE TEST

	Plasma				
Metals	Control	Chronic	Acute	F-Values	DNMRTV
Total µgs Fe	7.43	5.82	6.99	2.68	A C B
Total µgs Mg	8.3	7.8	7.4	5.37*	A B C
Total µgs Zn	1.27	0.63	1.21	11.05*	A C B
Total µgs Cu	0.20	0.63	0.43	45.73*	B C A

A--Control; B--Chronic; C--Acute.

Non-significant differences indicated by underscoring.

TABLE 13

ANALYSIS OF DIFFERENCES IN THE HEMATOCRIT RATIOS BY THE ANALYSIS OF VARIANCE AND DUNCAN'S NEW MULTIPLE-RANGE TEST

Treatments	Control	Chronic	Acute	F-Value	DNMRTV
$\bar{x}$	46.90	49.75	52.88	5.69*	C B A
s^2	2.27	7.74	37.18		
n	10	10	12		

A--Control; B--Chronic; C--Acute.

Non-significant differences indicated by underscoring.