Effects of Carbondisulfide on Certain Zinc and Copper Containing Enzymes in Rats

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DEEPAK GOPAL SHEWADE

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BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE

PILANI (RAJASTHAN)

CERTIFICATE

This is to certify that the thesis entitled "EFFECTS OF CARBONDISULFIDE ON CERTAIN ZINC AND COPPER CONTAINING ENZYMES IN RATS" submitted by Mr. Deepak Gopal Shewade ID No. 79RH24009 for the award of Ph.D. degree of the Institute, embodies original work done by him under my supervision.

V.N. Sharma

(DR. V.N. SHARMA)
Assistant Professor
Biological Sciences Discipline.

Date: 16-12-1985

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LIST OF ABBREVIATIONS USED

A	Absorbance
ADH	Alcohol Dehydrogenase
AP	Alkaline Phosphatase
DTC	Dithiocarbamate
E	Extinction
i	Concentration of Inhibitor
k_i	Dissociation constant of enzyme-inhibitor (the inhibitor constant)
k_i'	Equilibrium constant for dissociation of inhibitor from enzyme - inhibitor - substrate complex.
k_m	Concentration of substrate giving half maximal velocity (the Michaelis constant)
k_m'	Value of k_m in presence of excess of inhibitor
k_p	Apparant value of k_m
ND	Not detectable
NS	Not significant
p	Significance level
SDDTC	Sodium diethyl dithiocarbamate
V_m	Velocity of an enzyme reaction when saturated with substrate.
V_p	Apparant value of V_m

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C H A P T E R - I

INTRODUCTION

Man has sought continually to improve his material environment and this endeavour has resulted in an extensive increase in the use of chemical compounds in modern industrial, agricultural and therapeutic practices. Some of these compounds are harmless while many others are toxic and endanger human life. Therefore, the study of the mechanism of toxicity caused by hazardous chemicals and its prevention has become a major responsibility of the modern toxicologist.

One of the chemical compounds used today in many industries is carbondisulphide (CS_2) which is known to be toxic (Davidson et al, 1972). Originally CS_2 was not found in nature; it is a man-made product. It was accidently discovered by the German Chemist W.A.Lampodius, in 1797 (see Hamilton, 1937). Since 1851 it has been used extensively in industries because of its exceptional fat-solvent property. Today CS_2 is utilised not only in the manufacture of viscose rayon but also in the cold vulcanization of rubber and in the manufacturing of rubber accelerators, resins, xanthates, thiocyanates, plywood adhesives, pesticides, tires and floatation agents.

The history of CS_2 toxicity begins with its early uses in the cold vulcanization process of rubber and continues with its subsequent use in the manufacture of viscose rayon. The literature dealing with industrial CS_2 toxicology describes polymorphus hazards to health. Studies on the CS_2 toxicity among workers of viscose rayon industries in Finland, Norway, USA, Japan, etc. reveal that CS_2 toxicity lowers their vigilance, intellectual ability, motor coordination, speed of work and glucose tolerance level (Davidson et al, 1972). It increases the suicidal tendency among the people exposed to it and also raises blood plasma creatin and glucose level, frequency/ chances of coronary heart diseases, retinal microaneurisms and diabetogenic action. Besides these effects, CS_2 exposure has been found to decrease Zn, Cu and other metals, ions in the blood (Hernberg et al, 1969; El-Ghazzar et al, 1973).

The precise manner in which CS_2 is handled by the organism has yet to be discovered. The CS_2 is moderately reactive and relatively insoluble in water. Upon entering into the body 90% of it becomes metabolized. The screening of persons exposed to CS_2 for a

long time has revealed the presence of high concentrations of inorganic sulphur, thiocarbamates and 2-mercapto-2-thiazolidones (Pergal et al, 1972). It has been reported that when CS_2 enters the blood stream and other body tissues it combines with the amino group of bioamines and proteins to produce (di) thiocarbamates type compounds which undergo cyclization to thiazolidines. These thiazolidines due to their inherent chelating ability, sequester essential diavalent metal ions, thus depleting the concentration of important metal ions in the body (Pergal et al, 1972). Besides cyclization CS_2 is also oxidised by the liver microsomal (NADH-dependent) monooxidase system into carboxyl sulphide (COS) and finally to CO_2 (Chengelis and Neal, 1980). Freundt et al (1974) reported a hepatogenic action of CS_2 in the subject with a pre-dosing of phenobarbital.

It has already been stated above that CS_2 exposure decreases the body level of metals in general. A large number of enzymes are either metalloenzymes or dependant on the metal cofactors for their biological activities (McKenna and Distefano, 1977; Melson and Weight, 1967). Further, Pergal et al (1972) has reported a decrease in the body zinc level as a consequence of CS_2 exposure. The zinc is known to be an

essential trace element in the body and its essential nature for the living system is fundamentally based on its role as an integral part of a number of metallo-enzymes and as a cofactor for regulating the activity of specific zinc dependent enzymes. Kirchgessner and Roth (1980) observed that level of Zn in cells govern many metabolic processes, specifically carbohydrate, fat and protein metabolism and nucleic acid synthesis or degradation, through the initiation and/or regulation of the activity of Zinc - dependant enzymes. The decrease in an enzyme activity in response to deficient zinc level depends on how tightly the zinc cations are bound to the protein (thermodynamic stability) or how fast the rate of exchange of the ligands is (Kinetic stability). These are also the reasons why only a few of the known zinc metallo-enzymes respond sensitively, and rapidly to a deficient zinc supply, e.g. alcoholic dehydrogenase, glutamate, malate and lactate dehydrogenase, RNA and DNA polymerase, alkaline phosphatase, carbonic anhydrase, carboxy and di-peptidase.

The uptake of Zn by the body is significantly related to that of copper (Underwood, 1971; Hill, 1976; Raghupathy and Sharma, 1985). Hill (1976) has described

an antagonism between Zn and Cu. Thus a study of Zn and its associated biological activities should be accompanied with those of Cu (Raghupathy, 1983).

Though copper is toxic at higher concentrations, it is an essential metal in the body (Grahm & Cordano, 1976). It is not only involved in iron metabolism and haeme biosynthesis (Lee et al, 1976) but also regulates the activity of many important enzymes either being an integral part or a cofactor for regulating the activity of specific enzymes, viz. cytochrome oxidase, tyrosinase, uricase, diamine oxidase (histaminase), etc. (O'Dell, 1976).

The complex nature of the activities of CS_2 makes different approaches to its understanding necessary. In the present investigation, five representative metalloenzymes were selected to study the effects of CS_2 on their activity in Albino rats alongwith aldolase (an mammalian aldolase do not contain Zn). Of the six enzymes two enzymes are Zn-dependent, (viz. alcoholic dehydrogenase, alkaline phosphatase), three are Cu-dependent (viz. histaminase, tyrosinase and uricase) and aldolase does not contain any metal. The above enzymes were selected due to

their availability and accessible methods of bioassay. Since CS_2 is known to get metabolised into dithiocarbamate compounds, the effect of metabolic analogue, sodium diethyldithiocarbamate on these enzymes was also studied to find out whether the change in activity of an enzyme is due to the direct effect of CS_2 or its metabolite thiocarbamates. Since the five enzymes are metal dependant, in vitro experiments were also taken upto find out whether their activity could be reversed when extra Zn^{++} or Cu^{++} is made available.

Thus, the aims of the present investigation were:

(i) to evaluate the effects of acute and chronic doses of CS_2 on two Zn, three Cu dependant, enzymes and aldolase in brain, liver, kidney and testes of albino rats, as these tissues are known to accumulate these metals.

(ii) to study the effect of sodium diethyl dithiocarbamate on the same enzymes to find out if the change in activity of an enzyme is a direct consequence of CS_2 or due to its metabolic analogue sodium diethyl dithiocarbamate,

(iii) to study the level of Zn and Cu in brain, liver, kidney and testes after chronic CS_2 and sodium diethyldithiocarbamate treatment, and

(iv) to find out whether or not the altered activity of these enzymes could be restored if excess of Zn^{++} and Cu^{++} is made available to their respective enzymes in vitro.

C H A P T E R - II

REVIEW OF LITERATURE

The present review of literature is not to give a bibliographic account of the research on CS_2 and its influence in biological system, but an attempt has been made to develop conceptual review of carbondisulphide toxicity.

Epidemiological Studies:

The first report mentioning CS_2 as a potential health hazard came from France in 1950s' and was referred to the Indiana Rubber Industry, in which CS_2 was extensively used (Hamilton, 1925). In 1856, Dr. Auguste Delpech described a case of the sone of a rubber worker who, after three days of CS_2 vapour exposure in his father's workshop was, "striken with a type of raging delirium" during which he "hurled himself at his father to bite him" (cited from Delpech, 1856). In 1880s' rest of the Europe also became aware of CS_2 toxicity. In 1881 Tamassia published his experiments "peracute poisoning by CS_2 of dogs, guinea pigs, and frogs" (see Bashore et al, 1938). By the turn of the century the rubber industry had entered into large scale production, and carbondisulphide exposure was wide spread (Bashore et al, 1938). According to Koester (as described by

Hamilton, 1940) the manifestation of carbondisulphide poisoning varies as those of lead. Laudenheimer (cited from Bryce, 1886) described 50 cases of carbondisulphide insanity. The lasting merit of Laudenheimer's investigation is that it stirred up public opinion over the risks of carbondisulphide in the rubber industry. Severe occupational carbondisulphide intoxication continued to occur (Piorry, 1956; Ross, 1886), despite the warnings of the early investigators. Peterson (1892) a New York physician, was the first to report on carbondisulphide intoxication in USA. Scattered reports of intoxication resulting from exposure to high concentrations of carbondisulphide continued to appear through out the first two decades of this century (Jump and Cruice, 1904; Francine, 1905; Hamilton, 1915).

In 1938, an extensive survey of the viscose industry was published by the Pennsylvania Department of Labour and Industry. Following this, several reports appeared (Rubin and Arieff, 1945; Barthelemy, 1939) and in 1939, the first exposure standard (120 ppm for 8 hours and 30 ppm for 12 hours) was adopted by the American Standards Association.

Vigliani, in 1954, reported that in the 100 workers which he observed from rayon industries in Italy, 88% had polyneuritic symptoms. Gastric disturbances, headaches and vertigo followed in prevalence with 28%, 18% and 18% respectively. "Sexual weakness" and tremors both occured in 16% of the cases and myopathy in 15%. Psychosis were diagnosed in 5% of the 100 workers. The author also reported on 43 viscose rayon workers with CS_2 poisoning, 39 of them from 2 viscose plants, had been diagnosed as having encephalopathy between 1944 and 1953. The typical symptoms observed were asthenia, paresthesia, difficulty in walking, speech alterations, and mental deterioration were common early symptoms. Most workers had experienced a stroke followed by spastic hemiparesis. Extrapyramidal involvement occured in all patients. Cerebral arteriography, EEG, and examination of the fundus oculi indicated that the encephalopathy was vascular in origin. Necropsy of three patients revealed diffuse vascular sclerosis, cerebral atherosclerosis, hyaline fibrosis of the media and thickening of the intima of blood vessels. Most cases (84.6%) of vascular encephalopathy were found in the two monitored plants, occured

in workers from the spinning departments. Lilis et al (1967) found that, at the time of examination, 7 workers were hypertensive, as diagnosed on the basis of blood pressure tests, 9 had a history of high blood pressure but were not hypertensive at the time of testing, and 10 never had high blood pressure. Renal function tests were also performed on these workers. The authors concluded that long term exposure of workers to carbon-disulphide causes vascular disease manifested in the alterations of renal function. These changes were suggested to be the results of activation of the sympathetic division of the autonomic nervous system, producing effects similar to those of epinephrine. Baranowska (1965) concluded that the dermal absorption rate of carbondisulphide from aqueous solutions was high enough to be of significant concern in the workers of viscose rayon industry.

Mack et al (1974) found that in man carbondi-sulphide blocks microsomal mixed-function-oxidases, thereby inhibiting N-demethylation of amidopyrine, with simultenous prolonged excretion of the metabolities 4-aminoantipyrine and acetyl-4-aminoantipyrine. These authors also pointed out the possible serious

implications of even very low concentration of CS_2 exposure (10 - 20 ppm) for 6 hours, which could retard normal metabolism of such frequently taken drugs as analgesics, hypnotics, antidiabetics and anticonvulsants.

Hanninen (1971) administered a battery of psychological tests to 50 viscose rayon workers with carbondisulphide poisoning, 50 workers exposed to carbondisulphide without known poisoning, and 50 unexposed workers. The exposed group showed significant decrease in the speed, vigilance, manual dexterity, and intelligence. In the exposed the changes were less severe. The poisoned group showed latent poisoning characterised by traits indicative of depressive mood, slight motor disturbances and intellectual impairment, whereas clinically manifested poisoning resulted in lowered vigilance, diminished intellectual activity, diminished rational control, retarded speed, and motor disturbances. Exposure in the plant had averaged from 20 to 40 ppm during the years 1950-1959 and from 10 to 30 ppm from 1960 onwards.

Plasma and erythrocyte changes in zinc and magnesium levels such as those found in animals were investigated in Finnish and Norwegian workers exposed to carbon

disulphide by Hernberg et al (1969). The magnesium levels were significantly reduced in erythrocytes of exposed Finnish workers and zinc concentrations were lowered slightly. No such difference was found in Norwegian men. Plasma magnesium was higher for the exposed Finns, but not for the Norwegians; plasma zinc differences were not seen in either group. The geometric mean carbondisulphide concentrations were 4 - 15 ppm in the Finnish rayon factory, and 18 ppm in the Norwegian factory.

Mancuso and Locke (1972), in a mortality study using social security records, found an excess of suicides in workers first employed at one viscose factory between the years 1938 and 1948. In addition to an excess of recorded suicides, there were a number of deaths recorded as other causes which strongly resembled suicide. Certain departments of the plant were primarily responsible for the excess. Unfortunately, carbondisulphide concentrations were not available for the plant as a whole or for the departments with high suicidal rates.

Hernberg et al (1971) examined blood lipids, glucose tolerance, and plasma creatinine in 343 viscose rayon workers who had been exposed to an average of

20 - 40 ppm CS_2 in the 1950s' and 10 - 30 ppm in the 1960s'. This study, part of a study on coronary risk factors and coronary diseases, found that level of fasting glucose, correlated with the exposure time and exposure index in the exposed group with high plasma creatinine concentration. In the same group of workers, Hernberg et al (1973) found that in a 55 year period, 16 exposed men had died from coronary heart disease as compared with three controls and found that the risk of death rose with increasing exposure. Sakurai (1972) studied more than 10 years of blood pressure records in a Japanese viscose rayon plant. Blood pressures were reduced when workers were removed from a carbondisulphide environment and when the environment was improved from a level of 20-50 ppm to a level of 10-25 ppm.

Goto et al (1971), in their study of 214 carbondisulphide workers from 11 Japanese viscose rayon plants and 45 controls, found a trend toward higher blood sugar levels with advancing age in carbondisulphide exposed workers, and suggested a mild diabetogenic action. They also observed a prevalance of retinal microaneurism growing with increasing duration of exposure to carbondisulphide. Candura et al (1979) were able to establish a correlation between blood glucose levels and the

exposure index which was derived from both the intensity and the length of exposure. El-Ghazzar et al (1973) examined 82 workers in the Egyptian viscose rayon industry and 33 controls; and found that carbondisulphide caused depletion of serum zinc with an increase in the rate of zinc excretion, and also an increase in all serum protein fractions. The effects were temporary and improved on cessation of exposure. Cai and Bao (1981) noted that the exposure to carbondisulphide can affect the maternal function of female workers, if carbondisulphide can reach the foetuses through the placenta or babies via mother's milk.

Methodology of Carbondisulphide Exposure Studies: Cohen et al (1959) has reported that carbondisulphide combines with amino groups of aminoacids, proteins and peptides *in vivo* to yield excretable products containing C-SH and C=S groups. The two metabolities, i.e. 2-mercapto-thiazolidone and thiocarbamide, have actually been isolated from the urine samples of carbondisulphide exposed workers (Pergal et al, 1972). These two excretory carbondisulphide products catalyses the oxidation of sodium azide with iodine.

$$2NaN_3 + I_2 \longrightarrow 3N_2 + 2NaI$$

Based upon this observation, a practical test for carbondisulphide exposure has been developed to determine the degree of carbondisulphide exposure by measuring the time of iodine decoloration (Roubal et al, 1963; Vasak et al, 1963). The iodine decoloration correlates linearly with carbondisulphide exposure coefficient (E), expressed as

$$E = C \log t$$

Where t represents time interval required for decoloration of iodine added to urine and C is the concentration of creatinine in µg/lit. The daily integral exposure to CS_2 is estimated approximately from the L obtained at the end of work shift. The validity of iodineazide test has been repeatidely confirmed by subsequent workers (Djuric, 1963; Djuric et al, 1965; Salvadeo et al, 1967; Locati, 1967; Pergal et al, 1972). Though the test has proved unreliable below the permisible limit of 50 mg/m^3 CS_2 exposure, but its sensitivity may be improved by the introduction of iodometric titration (Jakubowski and Piotrowski, 1965). According to many toxicologists (Graevac-Leposavic et al, 1967; Fourier et al, 1971; Magos, 1972; Pergal et al, 1972), iodine test is important for determining carbondisulphide incipient toxicity.

CS_2 Metabolism and Biochemical Toxicity:

CS_2 being lipid soluble and volatile solvent, rapidly enters in the body mainly as vapours through lungs and some gets absorbed through the skin. Its absorption amounts to 80% in rat (Soucek, 1957) and 85% in man (Petrovic and Djuric, 1966) of the inhaled vapours, and CS_2 blood equilibrium is reached with in 1.5 hr. during prolonged exposures. The uptake of CS_2 by tissues other than blood is a slow process and requires several days (Teisinger and Soucek, 1949; Busing et al, 1953; Madlo and Soucek, 1953; Freundt and Schnapp, 1970). Experimental evidences in rats suggest marked accumulation of free as well as bound CS_2 in peripheral nerves, brain and liver etc. The blood to liver ratio of CS_2 concentration becomes 3:2, during prolonged exposure (Soucek 1957; Demus, 1967).

Considerable amount of free blood CS_2 is expired back to the environment amounting to 30 - 50% in rats (Demus, 1967), 10 - 15% in men (Jakubowsky, 1971) and less than 1% is excreted in urine. In human subjects, the level of CS_2 falls from 85% absorbed to 40 - 45% retained when equilibrium is established in 2 hrs, due to its short half-life (Petrovic and Djuric, 1966).

The retained CS_2 undergoes a biotransformation (Brieger, 1961) and displays marked cumulative effects (Magos et al, 1974).

Studies with labled CS_2 on mice and guinea pigs have revealed that about 30% S^{35} is excreted in inorganic form (Strittmatter et al, 1950; Giovine, 1957). Later Magos et al (1974) found excretion of significant amount of organically bound S^{35} in labled CS_2 exposed rats. Savolainen et al (1977) described considerable accumulation of bound as well free CS_2 in the nervous system and alteration in CS_2 metabolism in brain of rats after pretreatment with phenobarbitone.

Cohen and coworkers (1959) in their studies on the biotransformation of CS_2 in the body, found that CS_2 combines with amino groups of bioamines and proteins to produce thiocarbamates type compounds and further cyclize to thiazolidones. Pergal et al (1972) identified products three metabolic products of CS_2 viz. thiocarbamide, 2-mercapto-2-thiazolidine and an unidentified compound in human urine. As a consequence of CS_2 exposure Dematteis (1974) suggested, oxidation of CS_2 liberates highly reactive sulphur radicals and the later becoming attached to microsomal constituents; this binding

appears to initiate the toxic liver changes (Dalvi et al, 1975; Freundt et al, 1974). Recently, Chengelis and Neal (1980) described the oxidation of carbondisulphide by liver microsomal (NADH-dependent) monooxygenase into carbonyl sulphide (COS) which subsequently metabolises to CO_2 in a separate reaction librating sulphur.

Cavelleri and associates (1967) found a linear decrease in urinary excretion of 17-ketosteroids and 17-hydroxy corticosteroids in relation to the CS_2 exposure period, with a simultaneous decrease in ICSH secretion and depressed testicular activity. According to Maugeri et al (1971) the gonadal disfunction is provoked either due to toxic lesion caused directly by CS_2 or by inhibition of ICSH releasing factor from hypothalamus. Lancranjan (1972) found a significant increase in the frequency of hypospermia, teratospermia and asthenospermia in CS_2 affected workers as compared to the normal. Petrov (1969) observed that elevated CS_2 concentration lowered the probability of full term pregnancy. Earlier Bezvershenko (1965) reported elevated quantities of estrogens in urine of CS_2 exposed pregnant women than the normal.

Since the late 1940s' the dominance of reports on generalized vascular changes in cases of CS_2 poisioning caused Attinger to postulate that CS_2 is a primary 'vasotropic' rather than 'neurotropic' poision (see Browning, 1965). Von Rochenberg (1957) found pain in leg calf or difficulty in walking and called CS_2 toxicity 'a primary vascular syndrome' (Browning 1965).

CS_2 appears to affect the coagulation of blood in a complex manner either due to hypoprothrombinemia and lack of factor VII (proconvertin) or reduced blood plasmin activity (see Visconti et al, 1966; Djuric et al, 1971). Numerous old publications have reported the occurance of hypochromic anaemia and morphological changes in erythrocytes. Sidorowicz et al (1980), their analysis of CS_2 poisoning, found retarded maturation and structural disturbances in erythrocytes.

Mihail et al (1968) have reported erythrocyte membrane injuries due to inhibition of membranal acetylcholinesterase system by carbondisulphide. It was found that multiple intravenous doses of CS_2 caused diminition of phagocytotic index without affecting immunological reactivity of the organisms (Bagunowicz, 1970). Biochemical function tests have revealed impaired hepatic

BSP clearance and biliary secretion as well as reduced bilirubin uptake among CS_2 intoxicated workers (Gibson and Roberts, 1972).

Lysina (1967) reported a slight fall in total protein level and albumin to globulin ratio with a simultaneous fatty degeneration and hemorrhage in liver due to CS_2 intoxication. Wieckowski et al(1969) in their CS_2 exposure study, found a slight increase in several globulin fractions in conjuction with hypo-albuminemia. Earlier Visconti (1965) reported the similar types of CS_2 effects along with hepatic dis-function and behavioural changes among the viscose rayon plant workers.

Vascular disturbances now a days figure as a prominent effect of CS_2 poisioning. It is assumed that CS_2 leads to atherosclerosis due to a variety of lipid aberrations mentioned below.

i) Hypercholesterolemia both in animals and human (Wronska-Nofer and Sokal, 1972),

ii) Hyperlipemia, which may be prevented by pretreat-ment with nicotinic acid (Wronska-Nofer, 1970),

iii) Hyperlipoproteinemia exhibiting high frequency of hyper -lipoproteinemia (Manu et al, 1971),

iv) Lipoprotein lipase inhibition by CS_2 leading to low plasma fatty acids level (Wronska - Nofer and Gluszek, 1965),

v) Inhibition of plasma elastase activity, which probably is the atherogenic potency of CS_2 (Vertin, 1966) and

vi) Inhibition of antiheparinic activity of blood (Visconti et al, 1966).

A host of workers have studied the cardiological changes in the CS_2 exposed animal and human subjects (Lewey, 1941; Brieger, 1949; Tiller et al, 1968). The CS_2 exposed individuals show P-wave changes, depression of S-T segment and inversion of T-wave along with double the mortality rate among the CS_2 exposed workers as compared to non-exposed workers.

McDonald (1938) reported that enlargment of blind spots and diminution of pappillary reflex due to CS_2 poisoning. Hotta et al (1972) found retinopathy among the viscose rayon workers in Japan. Recently Raitta et al (1975) described impaired colour discrimination due to impared retinal ganglions or demyelination of optic nerve among the CS_2 exposed subjects from

viscose rayon industries.

Many researchers have reported about 64% choronic gastritis (Brieger, 1967) as revealed by increased uropepsin excretion (Szott and Kuczynska-Sekieta, 1969) and renal disorder both in animal and man (Cohen et al, 1959; Browning, 1965).

The studies of glycogen metabolism in animals and man have revealed depletion in liver glycogen and marked changes in the energy yielding metabolic processes in liver (Minden et al, 1967; Kurzinger and Freundt, 1969; Glinska et al, 1969).

Treatment of animals with CS_2 have been found to decrease levels of transaminase, glucose 6-phosphatase, fructose 1,6-diphosphatase and cytochrome oxidase in rats (Minden et al, 1967), desulfuration of CS_2 leading to sulphur binding to cytochromes and finally the later's destruction (Obrebska et al, 1980), diminution of several serum amino acids, deficiency of pyridoxin and impaired tryptophan metabolism (Afonova, 1965) and decline in cerebral GABA and glutamate with a simultaneous increase in brain glutamine due to decreased glutamate decrabonylase activity (Tarkowski, 1974) in CS_2 exposed workers.

A large number of workers reported that the many CS_2 induced lipid metabolic changes are reversed by exogenous nicotinic acid administration (Wronska-Nofer, 1970; Liniecki, 1960; Wronska-Nofer and Sokal, 1972). Similarly administration of pyridoxine in cases of CS_2 poisioning relieves many neurological disorders (Lazarev et al, 1965; Tarkowski and Cremer, 1972; Gorny, 1971; Kujalova, 1974).

Zinc and Copper:

Zn and Cu are essential trace metals for overall body growth, reproduction and other physiological functions (see Underwood, 1971; Prasad, 1976 , 1980; Sharma et al, 1981). Zn is a principal limiting nutrient in children and adolescents (Prasad, 1980; Casey and Hambidge, 1980). Its deficiency due to marginal or inadequate intake has been reported in USA (Sandstead et al, 1976; Hambidge, 1977), Egypt and Iran (Ronghey et al, 1974), Turkey and Portugal (Halsted et al, 1974) causing acrodermatitis, behaviuoral changes, retarded body growth, reproductive dysfunction etc. The involvement of Cu as an essential nutrient and biochemical, and its physiological role has been reviewed in detail by Underwood (1971) and O'Dell(1976).

The deficiency of Cu leads to neonatal ataxia, hypomyelination, Parkinsonism, Wilson's desease, Menke's syndrome, retarted growth and gonadal dysfunction (Underwood, 1971; Henkin, 1974; Graham and Cordano, 1976).

The uptake of trace elements by the body depends on other trace metals in the diet or environment. The metabolic interrelationship among various metals is of profound biological significance viz. Cu-Zn-Fe, Fe-Zn-Cd, etc (Underwood, 1971; Hill, 1976). Various studies have established an antagonism between Zn and Cu, i.e. an increased Zn uptake by body leads to decrease in body Cu and vice-versa. (Magee and Matrone, 1960; Hill, 1976; Hill et al, 1976; Van Campen, 1969; McClain et al, 1973; Mills, 1974).

Most of the trace metals act either as an integral part of enzymes and harmones or as co-factors for the biological activity of enzymes. More than 20 metalloenzymes of Zn have been identified and many other known to be activated by Zn (Riordon and Vallee, 1976). Similarly, O'Dell (1976) had listed about 12 important Cu containkng enzymes.

The function of Zn in metalloenzymes has been divided into four categories viz. catalytic, structural, regulatory (or modulatory) and non-catalytic (Galdes and Vallee, 1983). Zn in aldolase is directly involved in catalysis by enzyme and its removal from the enzyme results in an inactive apoenzyme. Equine alcohol dehydrogenase contains both catalytic and noncatalytic Zn atoms per subunits, where the coordinate geometry of catalytic zinc fluctuates between tetracoordinate and pentacoordinate properties, reflecting it to be entatic (Vallee and Williams, 1968). According to Dunn (1975), the Zn polarise the substrate molecules on one hand and activate water molecule to act as nucleophile.

The alcoholic dehydrogenase contains either 2 or 4 g atoms of zinc per mole of enzyme (Wartburg et al, 1964; Akeson, 1967; Oppenheimer et al, 1967; Dunn et al, 1979 , Kordaland and Parsons, 1979; Galdes and Vallee, 1983).

Alkaline phosphatase from E. Coli. contains 4 g Zn atoms/mol, of which 2 g atoms are essential for enzyme activity (Plocke et al, 1962), while additional 2 g atoms stabilize, its structure (Simpson and Vallee, 1968, Sowadski et al, 1981). This enzyme shows

quick loss of activity in conditions of Zn deficiency (Kirchgessner et al, 1975).

Horecker et al (1972) described aldolase as a dimeric enzyme containing Zn ion per sub-unit and is known to become inhibited in presence of a chelating agent (Imsande and Handler, 1961).

Malmstrom (1978) has described three forms of copper in Oxidases, viz. (1) Cu^{2+} ions producing intense blue colour with certain proteins, (2) Cu^{2+} ion having low visible extinction relative to 'blue' Cu^{2+}, and (3) Cu^{2+} in an electron paramagnetic resonance nondetectable form. According to Malmstrom (1978) cooperative pairing of the above three types of Cu^{2+} ions allow two electrons reduction of dioxygen to peroxide, e.g. tyrosinase (Mason et al, 1961), uricase, diamine oxidase (Mondovi et al, 1967).

Effect of CS_2 on Zn, Cu and Enzymes:

Trace element shifts are generally considered as biochemical indices of toxic response and clinical signs of poisoning. According to earlier reports (Wronska-Szpasowa et al, 1961; Scheel et al, 1960)

states that CS_2 form reaction products (dithiocarbamates) with amino groups and further cyclize to thiazolidones, which produces metal chelates. Scheel (1965) states that the chelation of metal ions with CS_2 metabolites results in displacement, thereby, depletion of essential metals leading to the degenerative changes in the body tissues. For example depletion of Zn in spinal cord due to CS_2 intoxication will indicate cause of paresis (Scheel et al, 1960). These findings that metal chelate formation is one of the basic mechanism of CS_2 poisoning was further supported by the fact that mineral rich (Zn and Cu) diets prevent derangement of mineral metabolism (Scheel, 1967). The effects of CS_2 on metal balance has been studied by many workers (Rich and Horsfall, 1963; Hernberg and Nordman, 1969; El-Ghazzer, 1973). According to these workers, the CS_2 toxicity lies in its binding directly or through its metabolic products to the bivalent ions.

Mckee et al (1943) in <u>in vitro</u> studies, demonstrated that large quantities of CS_2 slightly inhibit the succinic oxidase system. Similarly Cohen et al (1959) concluded that CS_2 inhibits alkaline phosphatase

activity. Subsequent studies on theeffect of CS_2 on enzymes also concluded that CS_2 inhibits the activities of cytochrome oxidase in the brain tissues (Brieger, 1961), monoamine oxidase system (Magistretti and Peirone, 1961; Vigliani, 1962), several transaminases (Del Faver et al, 1964; Tarkowski and Wronska-Nofer, 1966; Vasak and Kopecky, 1967), lipoprotein lipase (Andri and Cavelleri, 1962), aldolase, glucose 6-phosphate and lactate dehydrogenase, fructose 1,6-diphosphatase and specific esterases (Tarkowski and Wronska-Nofer, 1966).

Nelson and Weight (1967) found that small dose of CS_2 do not influence the monoamine oxidase (MAO) system unless given for a long period, however the high dose of CS_2 does affect the MAO system. Partial inhibition of acetylcholinestrase activity has also been observed in cases of occupational CS_2 intoxication (Mihail et al, 1968). Catechol depleting effects of CS_2 was demonstrated due to dopamine β -hydroxylase inhibition by Truhaut et al (1971). Later McKenna and Distefano (1977) proposed that dithiocarbamate (a metabolic product of carbondisulphide) binds with Cu^{2+} of

dopamine β-hydroxylase, thus making it inactive. The inactive enzyme could be reactivated with the help of supplementary exogenous Cu^{2+}. In a neurotoxicological study, Tarkowski (1974) observed that inhibition of glutamate decarboxylase is a key factor in the neurotoxicity by CS_2. Recently Karakasevic et al (1979) have discovered a decrease in lactate dehydrogenase activity with a simultaneous increase in activities of sorbitol and glutamate dehydrogenases in serum of Yugoslav workers.

C H A P T E R - III

MATERIALS AND METHODS

MATERIALS:

Adult male rats of Porton strain weighing 180-200 g, were used throughout the present investigation. The animals were fed on pellet diet manufactured by Hindustan Lever Ltd., Bombay. The rats were subjected to carbondisulfide (CS_2) as well as to sodium diethyldiothiocarbamate (SDDTC) treatments. The effects of CS_2 and its metabolic analogue (SDDTC) were studied on the following five metalloenzymes and one non-metalloenzyme.

I. Non-metal enzyme:

1. Aldolase (Fructose 1,6-diphosphate:D-glyceraldehyde-3-phosphate-lyase)

II. Zinc containing enzymes:

2. Alcohol dehydrogenase (Alcohol:NAD oxidoreductase)
3. Alkaline phosphatase (Orthophosphoric monoester phosphohydrolase)

III. Copper containing enzymes:

4. Histaminase (Diamine:O_2 oxidoreductase)
5. Tyrosinase (o-diphenol:O_2 oxidoreductase)
6. Uricase (Urate:O_2 oxidoreductase)

The above six enzymes were selected on the basis of facilities available for their assay. For <u>in vitro</u>

studies pure enzymes were procured from Sigma, U.S.A.

EXPERIMENTAL:

In Vivo Experiments:

Two types of treatment were given to the rats viz. Acute and Chronic.

Acute Treatment: For the acute treatment of CS_2, the rats were divided into 9 groups of 10 rats each. Of these 4 groups were intraperitoneally given 400 mg CS_2/kg body weight. The CS_2 injected was dissolved in groundnut oil (Sharma, 1981). Another 4 groups were administered intraperitoneally SDDTC (1.2 g/kg body weight) in aqueous solution. The SDDTC administered was equivalent to 400 mg of CS_2 on molar weight basis. The remaining one group was administered 0.5 ml pure groundnut oil intraperitoneally and was kept as control. The animals after treatment with CS_2 and SDDTC were sacrificed at intervals of 1, 2, 4 and 7 hours. For observing the effects on enzyme activities, brain, liver, kidney and testes were dissected out and stored at 1 - 3°C.

Chronic Treatment: The rats were divided into three groups consisting of 10 rats in each. One group was intraperitoneally given groundnut oil dissolved CS_2 49 mg/kg body

weight and other group was intraperitoneally administered aqueous solution of SDDTC 120 mg/kg body weight. The treatment of CS_2 and SDDTC was continued for 90 days. The third group was administered 0.5 ml groundnut oil and served as control.

During treatment period, the daily food intake and average weight of rats at 15 days interval were also recorded for all the groups. At the end of 90 days, all the rats were sacrificed and brain, liver, kidney and testes were taken out and stored at 1 - 3°C for enzyme assays and metal (Zn and Cu) estimation.

For enzyme assays 10% (w/v) tissue homogenates were prepared in 0.15 M KCl.

PROTEIN ESTIMATION:

The method used was that of Lowry et al (1951).

Reagents:

A - 2% Na_2CO_3 in 0.1N NaOH

B - 0.5% $CuSO_4.5H_2O$ in 1% sodium tartrate.

C - alkaline copper solution: Mixed 50 ml of reagent A with 1 ml of reagent B, used on the same day.

D - Diluted folin reagent: Diluted the folin - Ciocalteu reagent (1:3) with water.

Protein Standard: Diluted solutions of bovine albumin in concentration range of 70-700 µg/ml were used.

Procedure: Taken 0.2 ml of 1000 times diluted tissue homogenate and 1 ml of reagent C in a test tube. Mixed well and allowed to stand for 10 minutes at room temperature. Added 0.10 ml of reagent D with immediate mixing. After 30 minutes absorbance was measured at 750 mµ on Spectronic-20. Calculated the protein concentration by comparison with a standard curve.

ALDOLASE ASSAY:

Aldolase activity was assayed by the modified procedure of Peanasky and Larday (1958), which involved the conversion of fructose 1,6 diphosphate, into dihydroxy acetone phosphate and glyceraldehyde 3-phosphate.

$$\text{Fructose 1,6-diphosphate} \xrightarrow{\text{Aldolase}} \text{Dihydroxy acetone phosphate + glyceraldehyde 3-phosphate}$$

Reagents Used:

(a) 0.01 M fructose 1,6-diphosphate solution

(b) 1 M NaCN prepared fresh before use

(c) 1.0 M NaOH prepared fresh daily

(d) 0.5 M glycylglycine buffer adjusted to pH 7.45

(e) 1.0 M disodium ethylene diamine tetraacetate (EDTA) adjusted to pH 7.9

(f) 60% perchloric acid

(g) 5% ammonium molybdate

(h) Reducing agent : 0.2% 1-amino 2-naphtholsulfonic acid, prepared in solution containing 12% sodium bisulphite and 2.4% sodium sulphite.

Procedure:

The assay mixture consisted of 0.2 ml of fructose 1,6-diphosphate solution, 0.02 ml of cyanide solution, 0.08 ml of glycylglycine buffer, enzyme and water to make up to 0.8 ml.

To inhibit the fructose 1,6-diphosphatase activity in the enzyme solution (tissue homogenate) was treated with 0.1 ml of disodium EDTA solution and held at 0°C for 10 minutes. The reaction was initiated by the addition of enzyme to the reaction mixture at 30°C.

After exactly 5 minutes the reaction was stopped with 1.0 ml of 1M NaOH, and the tubes were allowed to stand at room temperature for 20 minutes. Then 1.4 ml of ice-cold perchloric acid was blown into each tube and tubes were nestled in an ice bath. In case of crude homogenates turbidity was removed by centrifugation and inorganic phosphorus was determined using 2.0 ml aliquots. Water was added to 8.0 ml followed by 1.0 ml of molybdate solution. Then 0.4 ml of reducing agent was added, and water to the 10.0 ml mark. The colour was determined on a spectrophotometer (Cary 17 D) at 660 nm exactly 10 minutes after the addition of reducing agent. Blanks and standards were included in each test.

One unit of enzyme activity is that amount of enzyme which in 5 minutes at 30°C produces 1 µg of alkali labile phosphorus.

ALCOHOL DEHYDROGENASE ASSAY:

Alcohol dehydrogenase activity (ADH) in tissues was determined spectrophotometrically by the method of Theorell and Bonnichsen (1951). Reduction of diphosphopyridine nucleotide (DPN), in the presence of excess of ethanol was recorded at pH 9.6 in 3 minutes, with the

formation of acetaldehyde.

$$\text{Alcohol} + \text{DPN} \xrightarrow{\text{ADH}} \text{Acetaldehyde} + \text{DPNH} + H^+$$

Reagents Used:

(a) 0.1 M glycine-sodium hydroxide buffer, having 9.6 pH

(b) DPN stock solution containing about 10 mg/ml of pure DPN

(c) Ethanol: Absolute alcohol.

Procedure:

Mixed, 3 ml of the buffer, 0.1 ml of the DPN solution, and 0.1 ml of ethanol in a glass cell. To this added 0.1 ml of the tissue homogenate and optical density was read promptly at 340 nm (t = 0). After 3 minutes the change in optical density was recorded. A blank determination was also carried out.

One unit of ADH gives an increase in optical density of 0.045 in 3 minutes.

ALKALINE PHOSPHATASE ASSAY:

p-Nitrophenylphosphate was used as the substrate for the determination (Andersch and Szcypinski, 1947), where paranitrophenol and phosphoric acid (H_3PO_4) were

obtained as products.

$$R\text{-}O\text{-}PO_3H_2 + H_2O \xrightarrow{\text{Alkaline Phosphatase}} H_3PO_4 + R - OH$$

Reagents Used:

(a) Alkaline buffer - substrate solution: consisting of 0.05 M glycine buffer, 5.5×10^{-3}M p-nitrophenylphosphate and having a pH of 10.5

(b) p-Nitrophenol standard solution (5×10^{-5}M) was prepared before use

(c) 0.02N sodium hydroxide solution (NaOH)

Procedure:

Pipetted 1.0 ml of buffer-substrate into test tube and equilibrated for 5 minutes at 37°C. Added 0.1 ml of tissue homogenate to it and mixed. Incubated this mixture at 37°C for 30 minutes. Then added 10.0 ml of 0.02 N NaOH to it. Optical density of the sample was measured at 400 nm on Cary 17 D against apropriate blank.

A unit activity of alkaline phosphatase corresponds to an optical density of 0.084 at 400 nm.

HISTAMINASE ASSAY:

Diamine oxidase (Histaminase) catalyses the oxidation of histamine and various diamines with the consumption of 1 mole of oxygen and the production of 1 mole of aldehyde, 1 mole of NH_3, and 1 mole of hydrogen peroxide (H_2O_2). In the presence of catalase the H_2O_2 is converted to H_2O and 0.5 mole of O_2. The assay was based on the oxygen consumption obtained with histamine as the substrate in the presence of added catalase (Tabor, 1951).

$$RCH_2NH_2 + H_2O + O_2 \xrightarrow{\text{Histaminase}} RCHO + H_2O_2 + NH_3$$

Reagents Used:

(a) 0.02 M Histamine dihydrochloride neutralised with 1N NaOH.

(b) 0.20 M Potassium phosphate buffer pH 7.2

(c) 1 unit/ml dilute crystalline catalase.

Procedure:

1.5 ml of phosphate buffer, 0.25 ml of catalase, and 0.1 ml diamine oxidase solution (Tissue homogenate) was placed in the side arm. After temperature equilibration, the histamine solution was added, and the initial oxygen consumption was measured. A blank

flask was also run without any substrate. The measurements were carried out at 37.5° with air as the gas phase using Warburg apparatus.

One unit of activity is defined as the amount of enzyme giving an oxygen consumption of 1 cmm per hour.

TYROSINASE ASSAY:

Tyrosinase activity in tissue homogenates was assaysed by using method prescribed by SIGMA chemical Company, Missouri, U.S.A. (1983), tyrosinase oxidises catechol to quinone.

$$2\ \text{o-Diphenol} + O_2 \xrightarrow{\text{Tyrosinase}} 2\ \text{o-Quinone} + 2H_2O$$

Reagents Used:

(a) 0.05 M sodium phosphate buffer, pH 6.5 at 25°C

(b) 0.001 M L-Tyrosine substrate solution.

Procedure:

Mixed 10 ml phosphate buffer, 10 ml Tyrosine substrate solution and 9 ml of distilled water in a conical flask. Then oxygenated the mixture for 3-5 minutes. Immediately pipetted 2.9 ml of this mixture

into a quartz cuvette (1 cm lightpath). Equilibrated the mixture at 25°C and monitered A_{280} until constant using Cary 17 D spectrophotometer. To the cuvette added 0.10 ml of the homogenate and mixed by inversion and measured the increase in A_{280} and obtained the ΔA_{280}/minute.

One unit will cause an increase A_{280} of 0.001 per minute at pH 6.5 at 25°C in a 3 ml reaction mixture containing L-tyrosine.

URICASE ASSAY:

The assay used was studied extensively by Practorius (1948). Uricase catalyses the oxidation of uric acid to allantoin.

$$\text{Uric Acid} + O_2 + 2H_2O \xrightarrow{\text{uricase}} \text{Allantoin} + H_2O_2 + CO_2$$

Reagents Used:

(a) Potassium urate solution : 2.0 mg/ml neutralized to pH 8.0 with potassium hydroxide

(b) 0.02 M borate buffer, pH 8.0

Procedure:

A suitable amount of freshly prepared solution of potassium urate was added to a micro cuvette containing

borate buffer to give an absorbance, at 293 nm of about 0.500 in a final volume of 0.98 ml when read against a blank consisting of the same volume of borate buffer alone. Enzyme (Homogenate) was added in equal volume (0.02 ml) to the experimental and blank cuvettes. The decrease in absorbance was determined at 293 nm after 30 seconds.

If the ΔE_{293} per minute is less than 0.100 the rate is zero order for about 3 minutes or more after the first 30 seconds, and it is proportional to enzyme concentration.

One enzyme unit is defined as that amount which will give rise to a ΔE_{293} equal to 1.00 per minute the above conditions.

Estimation of Zn and Cu Levels in Tissues:

Accurately weighed (~ 0.5 gm) wet tissue samples were placed in pre-acid washed beaker. Two ml of fuming nitiric acid (analytical grades) was added to each sample and heated on a hot plate to near dryness and the process was repeated to the above residue. This was followed by the addition of one ml of 70% perchloric acid (analytical grade) and heated till white

fumes of perchloric acid appeared and a clear solution was left. This solution was further concentrated to a negligible quantity. The digested samples were cooled and diluted to 10 ml with glass-distilled deionised water. The diluted samples were fed to Atomic Absorption Spectrophotometer (Perkin-Elmer Model-303) for determination of zinc and copper.

The various parameters used in the instrumental setting for zinc and copper analysis as recommended by Perkin-Elmer Atomic Absorption Spectrophotometer. Instruction Mannual (1976) are as given below:

Parameter	Zinc	Copper
(a) Working standard solution.	2 ppm	1 ppm
(b) Radiation source	Single element Zn-Hollow-Cathode lamp with operating current of 15mA	Multielemental Co-G-Cu-Mn-Ni Hollow-Cathode lamp with operating current of 30mA.
(c) Wave length	214 nm	325 nm
(d) Slit size	4(0.7 nm)	4(0.7 nm)
(e) Function	Absorption	Absorption
(f) Fuel-oxidant system		
i) Fuel oxidant	acetylene-air.	acetylene-air.
ii) Fuel oxidant pressure	8 lb/sq in & 30 lb/sq in respectively	8 lb/sq in & 30 lb/sq in respectively
iii) Flow rate	9:9	9:9
(g) Flame	Lean blue reducing	Lean blue reducing
(h) Maximum linear working range.	2 µg/ml	1 µg/ml

Standard Solutions:

Standard solutions were prepared by dissolving 1.0 gm of pure Zn and Cu metals in 10 ml of fuming nitric acid. Volume was made up to get desired concentrations.

In Vitro Experiments:

In vitro studies were divided in two sets, viz. (1) tissue homogenate experiments and (2) pure enzyme obtained from Sigma Chemicals, U.S.A.

Pure Enzyme Study:

Pure enzymes were obtained from Sigma Chemicals, U.S.A. as given below:

Product No.	Enzyme	E.C.No.	Source
340-L2	Alcoholdehydrogenase	1.1.1.1	Equine liver
A 6253	Aldolase	4.1.2.13	Rabbit muscle
P 3877	Alkaline phosphatase	3.1.31	Bovine (calf) intestine
U 3250	Uricase	1.7.3.3	Porcine liver
T 7755	Tyrosinase	1.14.18.1	Mushroom
D 7876	Histaminase	1.4.3.6	Porcine kidney

Solutions or suspensions of all six pure enzymes selected for the present study, were prepared in 0.15 M KCl separately. Concentrations of enzymes were adjusted to obtain optimum activity. Each of the enzyme solution was put into two sets and the enzyme activity was determined. To these sets 0.0 - 0.5 M of CS_2 and 0.0 - 0.5 M SDDTC were added separately and the enzyme activity was again determined. In cases where inhibition was observed corresponding Zn/Cu (0.5 mM - 6.00 M) metal ions ($ZnSO_4/CuSO_4$) were added in the reaction mixture. The activity of enzymes with so added metal was again determined.

Tissue Homogenate Study:

The CS_2 and SDDTC affected liver tissues were homogenized separately to prepare 10% (w/v) tissue homogenates in 0.15 M KCl along with that of control tissues. The enzyme activity of each of the enzyme were determined, followed by addition of 2.0 mM zinc sulphate and copper sulphate solution into the corresponding metalloenzyme reaction mixture. Again the enzyme activities of all the six enzymes were determined.

C H A P T E R - IV

OBSERVATIONS

The observations on the acute and chronic treatment of CS_2 and its metabolic analogue SDDTC on the activities of three Zn dependant and three Cu dependant enzymes in male albino rats, have been recorded in the following description supported with Tables I - XXI and Figures 1 - 26. The activity of aldolase is Zn dependant in microorganisms but is reported to be independant of Zn in mammalian tissues. This enzyme has been selected to compare the results of metal dependant and metal independant enzymes. In addition to the above behavioural changes, loss in body weight and changes in food intake in the rats under present investigation, have also been recorded.

I. BEHAVIOURAL OBSERVATIONS:

The rats under present investigations, exhibited an intense stimulation after the administration of an acute dose of CS_2 whereas such behaviour was not observed among rats treated with acute dose of SDDTC. Further the CS_2 treated rats acquired mild to intense muscular rigidity, became placid and akinesic, and finally,

passed into coma followed by death within seven hours. The SDDTC treated rats also went into coma but did not die before seven hours.

The chronic treatment of rats with CS_2 showed a slight stimulatory effect immediately after CS_2 injection but became normal, after two hours of the treatment. No behavioural change was observed in the first two months among rats subjected to chronic SDDTC treatment but in third month they showed nasal bleeding.

II. BODY WEIGHT AND FOOD INTAKE:

The changes in the loss of body weight and food intake of rats under chronic CS_2 and SDDTC treatment are given in Tables I and II. The Table I clearly show a progressive loss in body weight of experimental rats while the control one shows gain in their body weight. It can be seen that the chronic CS_2 treatment caused about 2.8% loss in weight whereas the loss in body weight is about 14.2% in case of chronic SDDTC treatment.

III. TISSUE PROTEIN LEVEL:

The changes in the tissue protein level in rats treated with carbondisulphide and SDDTC are tabulated in

TABLE-I : Effect of chronic treatment of CS_2 (40 mg/kg body wt.) and SDDTC (120 mg/kg body wt.) on average body weight of rats (N = 10).

Days	Control (g)	CS_2 (g)	SDDTC (g)
Initial	189.5	188.7	187.3
15	192.0	191.5	188.1
30	197.0	189.9	181.3
45	201.6	188.7	174.1
60	208.1	186.9	168.4
75	209.6	185.8	165.5
90	212.8	183.4	160.7
Change in average body wt.	+12.3%	- 2.8%	-14.2%

TABLE-II : Average food intake (g/day) by rats during CS_2 (40 mg/kg body wt.) and SDDTC (120 mg/kg body wt.) chronic treatment (N = 10).

Days	Control (g)	CS_2 (g)	SDDTC (g)
Initial	6.00	6.10	6.02
15	6.03	6.11	6.00
30	6.00	5.84	5.96
45	6.83	5.64	5.14
60	6.79	5.65	5.85
75	6.73	5.41	5.41
90	6.64	5.34	5.01

Tables III and IV. Table III shows that the acute treatment with CS_2 did not cause significant changes in the tissue protein levels of four tissues, selected for present investigation, viz. brain, kidney, liver and testes, upto 7 hours after which the rats die. The same is trure for acutely treated rats with SDDTC (Table IV). However, in case of chronically treated rats, the liver proteins show significant decrease at $p < 0.01$ level, with CS_2 and SDDTC (Table V). The protein levels of other three tissues does not reveal any significant changes.

IV. ZINC DEPENDANT ENZYMES:

The effects of CS_2 and its metabolic analogue SDDTC have been observed in both acutely and chronically treated rats. The results are recorded under two sub-heads for each enzyme separately.

1. Aldolase:

(a) Acute Treatment:

The CS_2 treatment decreased brain aldolase activity immediately after the injection but the activity was restored within 7 hours. The aldolase activity in testes showed an initial increase in the first hour but decreased by seventh hour. In

TABLE - III : Effect of acute treatment of rats with CS_2 (400 mg/kg body wt.) on tissue protein levels (mg/g tissue ± SE).

Observation Time (hrs)	Brain	Liver	Kidney	Testes
0 (Control)	81.65 ± 6.176	230.6 ± 19.64	138.4 ± 15.36	92.32 ± 11.69
1	80.34 ± 5.80	241.4 ± 20.24	128.0 ± 10.32	84.69 ± 9.08
2	83.04 ± 7.34	221.4 ± 16.68	131.0 ± 14.34	93.31 ± 12.35
4	82.62 ± 6.785	242.6 ± 22.67	140.0 ± 13.45	85.45 ± 8.79
7	78.43 ± 9.027	228.3 ± 34.25	139.0 ± 16.02	88.26 ± 10.64

TABLE - IV : Effect of acute treatment of rats with SDDTC (1.2 g/kg body wt.) on tissue Protein levels (mg/g tissue ± SE).

Observation Time (hrs)	Brain	Liver	Kidney	Testes
0 (Control)	81.65 ± 6.176	230.6 ± 19.64	138.4 ± 15.36	92.32 ± 11.69
1	84.46 ± 7.167	224.3 ± 23.07	142.3 ± 12.54	90.79 ± 9.43
2	87.69 ± 9.181	231.4 ± 16.05	141.8 ± 14.73	87.34 ± 11.84
4	86.31 ± 7.491	233.9 ± 39.80	145.2 ± 16.74	88.78 ± 10.03
7	88.85 ± 10.73	228.7 ± 14.35	144.7 ± 12.11	91.64 ± 7.39

TABLE - V : Effect of chronic treatment of rats with CS_2 (40 mg/kg body wt.) and SDDTC (120 mg/kg body wt.) on tissue Protein levels (mg/g tissue ± SE).

Tissue	Control	CS_2 (a)	SDDTC (b)	p between a & b
No. of Rats	10	10	10	
Brain	77.94 ± 6.948	65.10 ± 5.434 (NS)	71.36 ± 5.403 (NS)	NS
Liver	196.40 ± 14.36	142.8 ± 8.448 ($p < 0.01$)	136.7 ± 7.280 ($p < 0.01$)	NS
Kidney	126.3 ± 9.195	111.6 ± 12.52 (NS)	104.1 ± 8.915 (NS)	NS
Testes	85.59 ± 7.533	84.12 ± 7.281 (NS)	80.14 ± 9.359 (NS)	NS

p - Significance level

NS- Not significant

case of liver, the aldolase activity increased in the 4th hour after initial decrease and then lost its activity by the 7th hour. The kidney tissue did not reveal any significant change in the aldolase activity. A comparative behaviour of aldolase activity in four tissues selected for present investigation, in response to acute carbondisulphide treatment is shown in Figure 1 and significance level is shown in Table VI. As revealed by Table VI, the acute treatment with SDDTC resulted in overall decrease of aldolase activity in all the four tissues viz. brain, liver, kidney and testes without showing any recovery in 7 hours of observation. Further SDDTC treatment showed a severe decrease in aldolase activity as compared to the CS_2 treatment (Table VI; Figure 2).

(b) <u>Chronic Treatment:</u>

Figure 3 and Table VII show the effect of chronic CS_2 and SDDTC treatment on the brain, liver, kidney and testes aldolase activity. It can be seen from Table VIII that aldolase activity decreases in all the four tissues. However, the decrease was highly significant ($p < 0.001$) in case of brain and liver as compared to kidney and testes. In contrast to CS_2, chronic treatment with SDDTC leads to the decrease in aldolase activity in

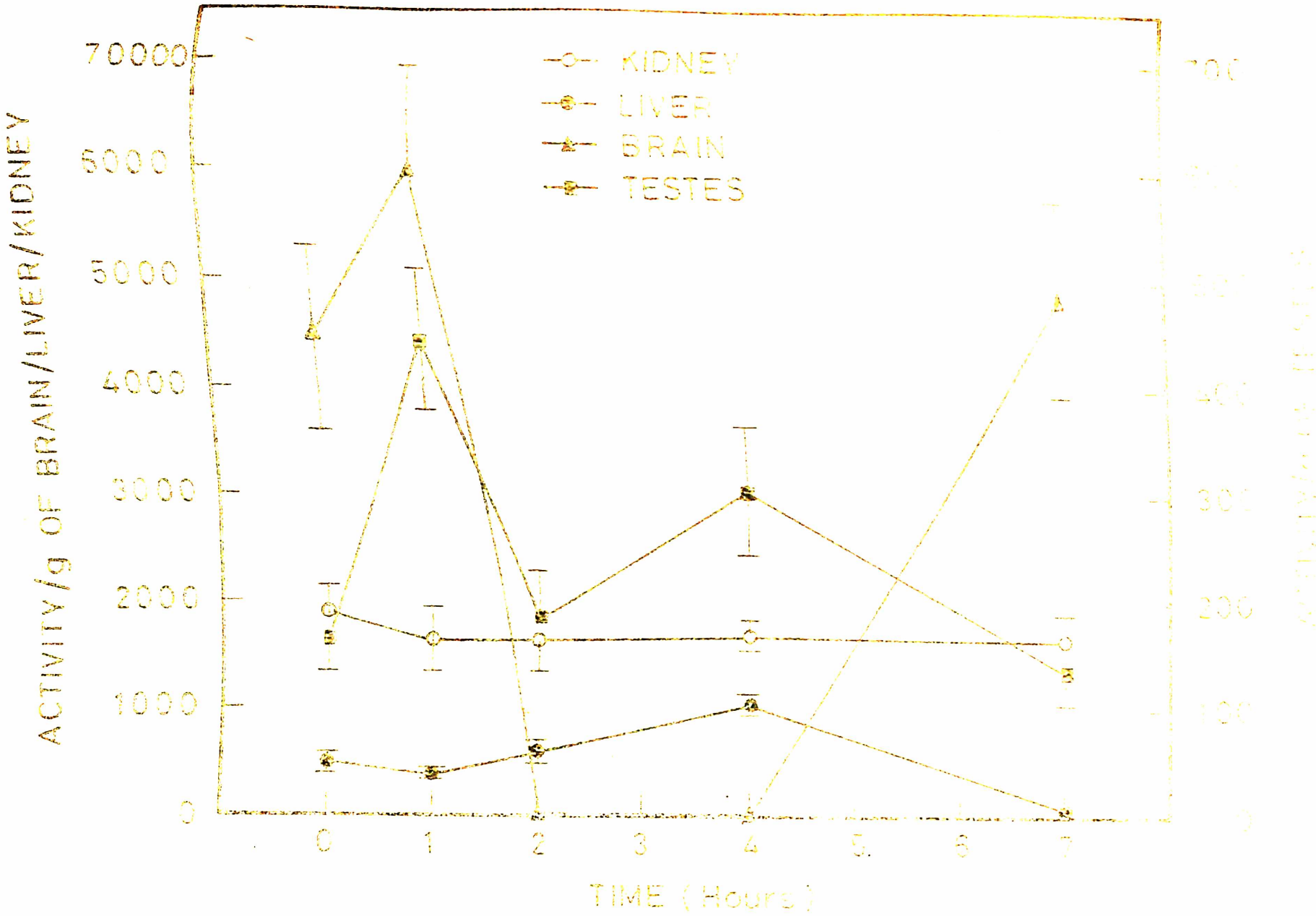


Fig.2 EFFECT OF ACUTE CS_2 (400 mg/kg body wt) TREATMENT ON ALDOLASE ACTIVITY IN RAT TISSUES

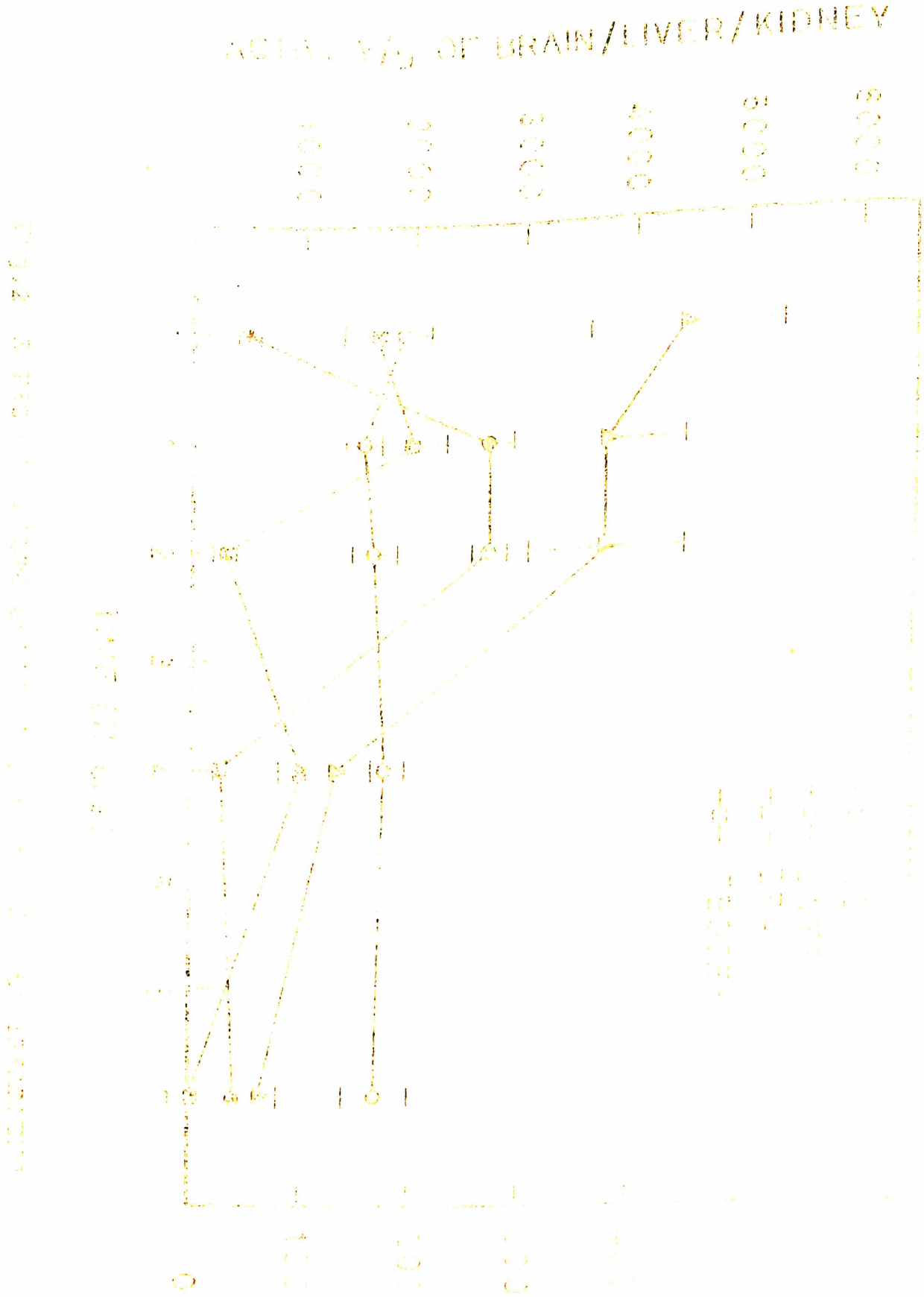


TABLE - VI : Peak effects of acute CS_2 (400 mg/kg body wt.) and SDDTC (1.2 g/kg body wt.) on Aldolase activity (u/g tissue ± SE) in rats in seven hour observations. (Figures in bracket with enzyme activity indicate hour of observation).

Tissue	Control	CS_2 (a)	SDDTC (b)	p between a & b
No. of Rats	10	10	10	
Brain	4470 ± 817	ND (2,4)	650 ± 130 (7) p < 0.001	-
Liver	483.9 ± 77.4	1054 ± 118 (4) p < 0.001	2708 ± 197.8 (1,2) p < 0.001	< 0.001
		64.8 ± 10.2 (7) p < 0.001	267.5 ± 48.1 (4) p < 0.05	< 0.001
Kidney	1920 ± 260	1630 ± 310 (2) NS	1550 ± 170 (1) NS	NS
Testes	164.0 ± 29.5	443.0 ± 66.4 (1) p < 0.01	ND (7)	-

p - Significance level

ND - Not detectable

NS - Not significant.

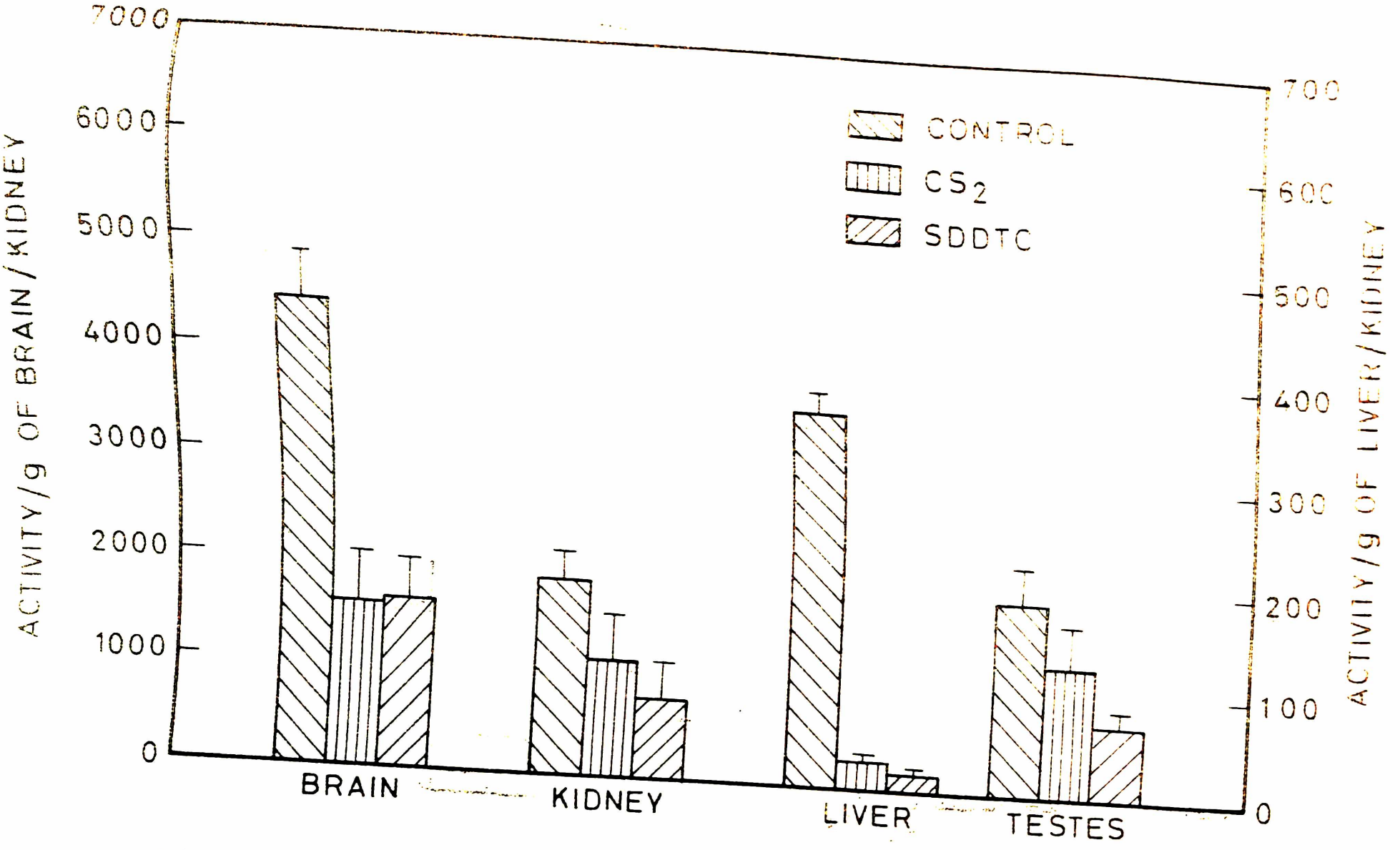


Fig.3 EFFECT OF CHRONIC CS_2 (40 mg/Kg body wt) AND SDDTC (120 mg/Kg body wt) TREATMENT ON ALDOLASE ACTIVITY IN RAT TISSUES

TABLE - VII : Effect of CS_2 (40 mg/kg body wt.) and SDDTC (120 mg/kg body wt.) chronic treatment on tissue Aldolase activity (u/g tissue ± SD) in rats.

Tissue	Control	CS_2 (a)	SDDTC (b)	p between a & b
No. of Rats	10	10	10	
Brain	4432 ± 432.0	1588 ± 438.6 $p < 0.001$	1611 ± 409.4 $p < 0.001$	NS
Liver	369.4 ± 18.31	24.3 ± 1.37 $p < 0.001$	14.0 ± 0.90 $p < 0.001$	< 0.001
Kidney	1847 ± 278.9	1108 ± 434.0 NS	757 ± 337.9 $p < 0.05$	NS
Testes	184.7 ± 34.5	123 ± 42.7 NS	73.9 ± 12.3 $p < 0.01$	NS

p - Significance level

NS - Not significant.

all the four tissues at significance level of $p < 0.001$ in brain, and liver, $p < 0.05$ in kidney and $p < 0.01$ in testes (Table VII). Figure 3 also depicts the similar behaviour of aldolase activity.

2. Alcohol Dehydrogenase:

(a) Acute Treatment:

The effects of acute treatment on rats with CS_2 and SDDTC are shown in figures 4 & 5, and are tabulated in Table VIII. Figure 4 shows that in case of CS_2 treatment, there was an overall decrease in alcohol dehydrogenase activity in all the four tissues i.e. brain, liver, kidney and testes. The maximum decrease in activity occurred at 4th hour. Thereafter the activity increased again partially by 7th hour in case of kidney and brain but continued to decrease in case of liver and testes. However the testes showed a bi-phasic response towards CS_2 treatment i.e. the enzyme activity showed a slight increase in the first two hours after treatment, followed by a constant decrease thereafter.

The acute treatment with SDDTC showed variable behaviour of its activity in the different tissues studied (Figure 5, Table VIII). It can be seen from

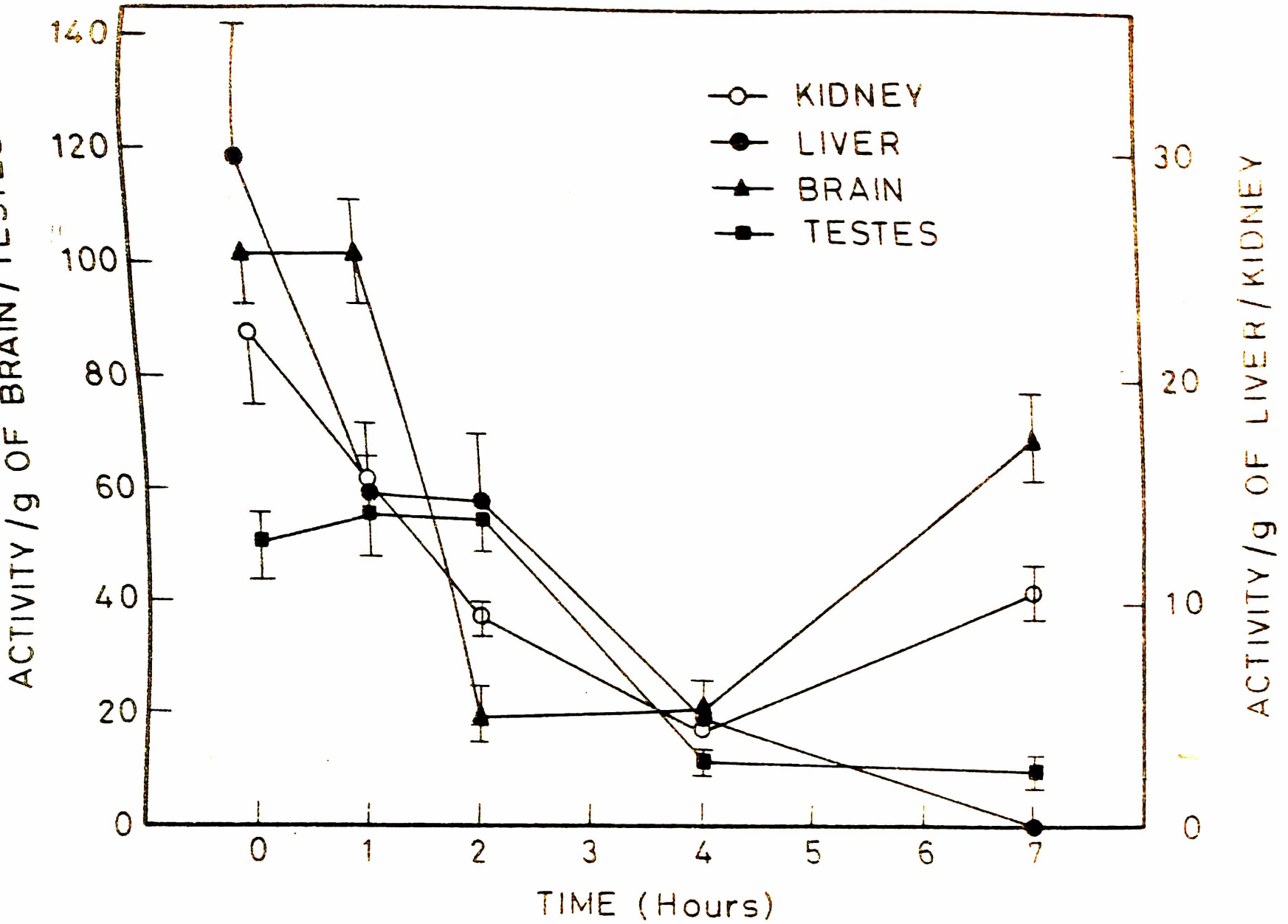


Fig.4 EFFECT OF ACUTE CS_2 (400 mg/Kg body wt) TREATMENT ON ALCOHOL DEHYDROGENASE ACTIVITY IN RAT TISSUES

TABLE - VIII : Peak effects of acute CS_2 (400 mg/kg body wt.) and SDDTC (1.2 g/kg body wt.) on Alcohol dehydrogenase activity (u/g tissue ± SE) in rats in seven hour observations (Figures in bracket with enzyme activity indicate hour of observation).

Tissue	Control	CS_2 (a)	SDDTC (b)	p between a & b
No. of Rats	10	10	10	
Brain	101.6 ± 9.04	19.5 ± 0.572 (2,4) p < 0.001	19.5 ± 5.04 (7) p < 0.001	NS
Liver	29.9 ± 6.45	ND (7)	ND (2)	-
Kidney	22.0 ± 7.00	4.30 ± 0.32 (4) p < 0.001	0.100 ± 0.07 (4) p < 0.01	< 0.001
Testes	49.5 ± 6.01	9.70 ± 2.60 (7) p < 0.001	75.3 ± 6.02 (1) p < 0.01	-
			29.3 ± 4.05 (4) p < 0.02	< 0.001

p - Significance level

ND - Not detectable

NS - Not significant

the Figure 5 that the enzyme activity progressively decreases in brain after SDDTC treatment upto 7th hour observation. The enzyme activity also decreases in first two hours in kidney and liver followed by increase in its activity in 4th hour onwards. The alcohol dehydrogenase activity in testes again shows a biphasic response towards SDDTC i.e. the enzyme activity showed an initial increase in 1st hour followed by progressive decrease upto 4th hour and almost recovered by 7th hour.

Table VIII shows that significant changes in the alcohol dehydrogenase activity occurred in brain and testes in response to the acute dose treatment with both CS_2 and SDDTC.

(b) Chronic Treatment:

Observations on the chronic treatment of rats with CS_2 and SDDTC (Table IX, Figure 6) reveals that there is a significant decrease in alcohol dehydrogenase activity in brain and liver. The significance level of decrease in brain activity being $p < 0.05$ for both CS_2 and SDDTC treatments, whereas in liver $p < 0.02$ and $p < 0.01$ for CS_2 and SDDTC respectively. The decrease in alcohol dehydrogenase activity was not

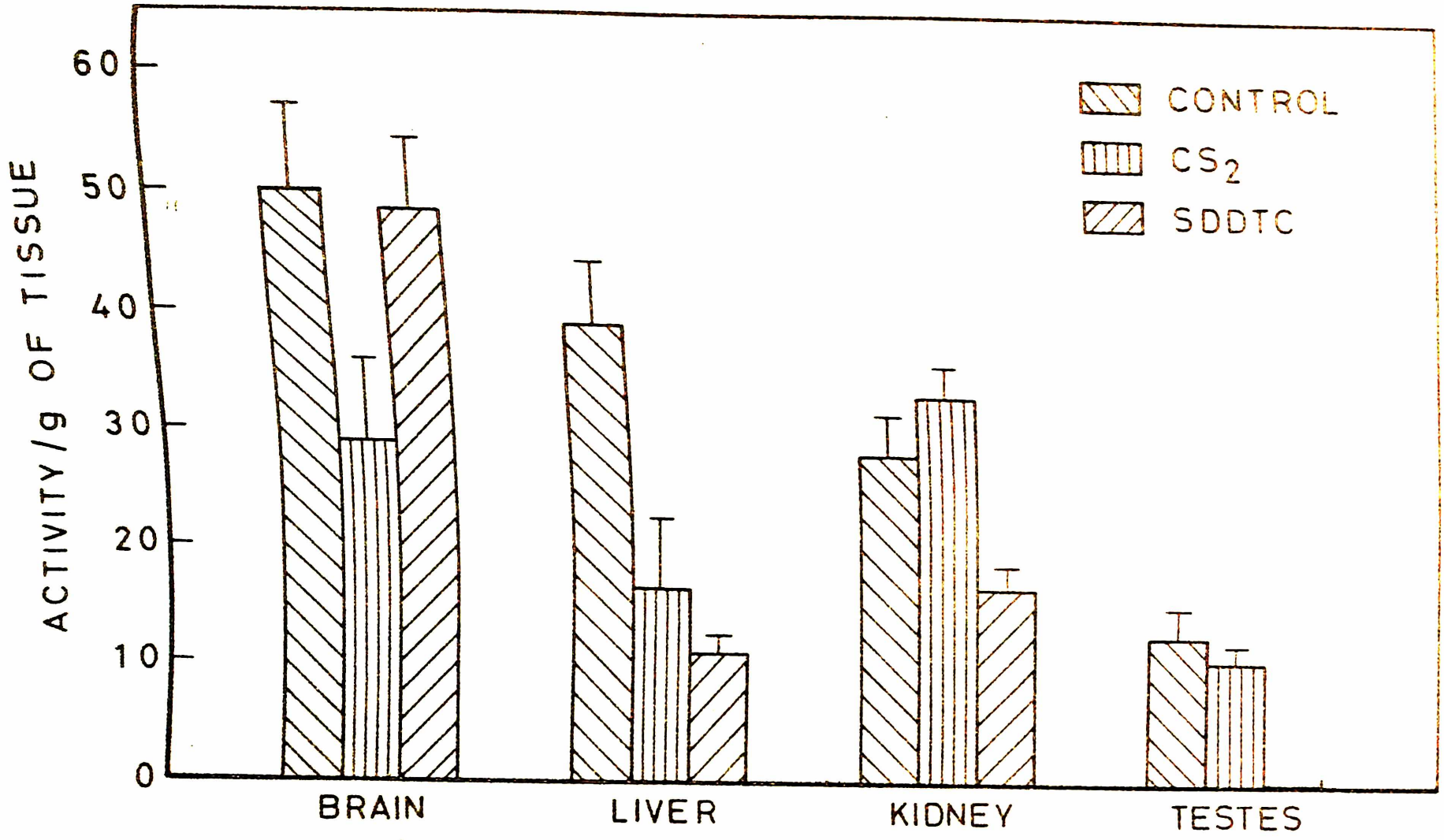


Fig.6 EFFECT OF CHRONIC CS_2 (40 mg/Kg body wt) AND SDDTC (120 mg/Kg body wt) TREATMENT ON ALCOHOL DEHYDROGENASE ACTIVITY IN RAT TISSUES

TABLE - IX : Effect of CS_2 (40 mg/kg body wt.) and SDDTC (120 mg/kg body wt.) chronic treatment on tissue Alcohol dehydrogenase (u/g tissue ± SE) in rats.

Tissue	Control	CS_2 (a)	SDDTC (b)	p between a & b
No. of Rats	10	10	10	
Brain	49.99 ± 6.789	29.00 ± 6.994 p <0.05	48.52 ± 6.034 NS	< 0.05
Liver	38.88 ± 5.648	16.67 ± 6.415 p <0.02	11.11 ± 1.483 p <0.001	NS
Kidney	27.78 ± 3.456	33.22 ± 2.77 NS	16.67 ± 1.856 p < 0.02	< 0.001
Testes	12.26 ± 2.698	10.52 ± 1.415 NS	ND	-

p - Significance level

ND - Not detectable

NS - Not significant.

significant in CS_2 treatment for kidney and testes but was significant in case of SDDTC treatment (Table X and Figure 6).

3. Alkaline Phosphatase:

(a) Acute Treatment:

The rats treated with acute dose of CS_2 did not show any significant change in the alkaline phosphate activity in brain, kidney and testicular tissues but the liver tissue showed a decrease in enzyme activity on 2nd hour followed by restoration in activity by 7th hour (Table X and Figure 7). As compared to CS_2, the SDDTC acute dose resulted in variable effects on brain, liver, kidney and testes (Table X; Figure 8). It can be seen from the Table X and Figure 8 that the alkaline phosphatase activity was completely stopped in liver and slightly elavated in brain. The kidney and testes showed an opposing effect on alkaline phosphatase activity, i.e. the enzyme activity became depressed on 2nd and 4th hour observation in testes and became normal by the 7th hour while the opposite is true for kidney (Figure 8). Table X reveals a significant change in enzyme activity at $p < 0.001$ level in brain but not in other tissues.

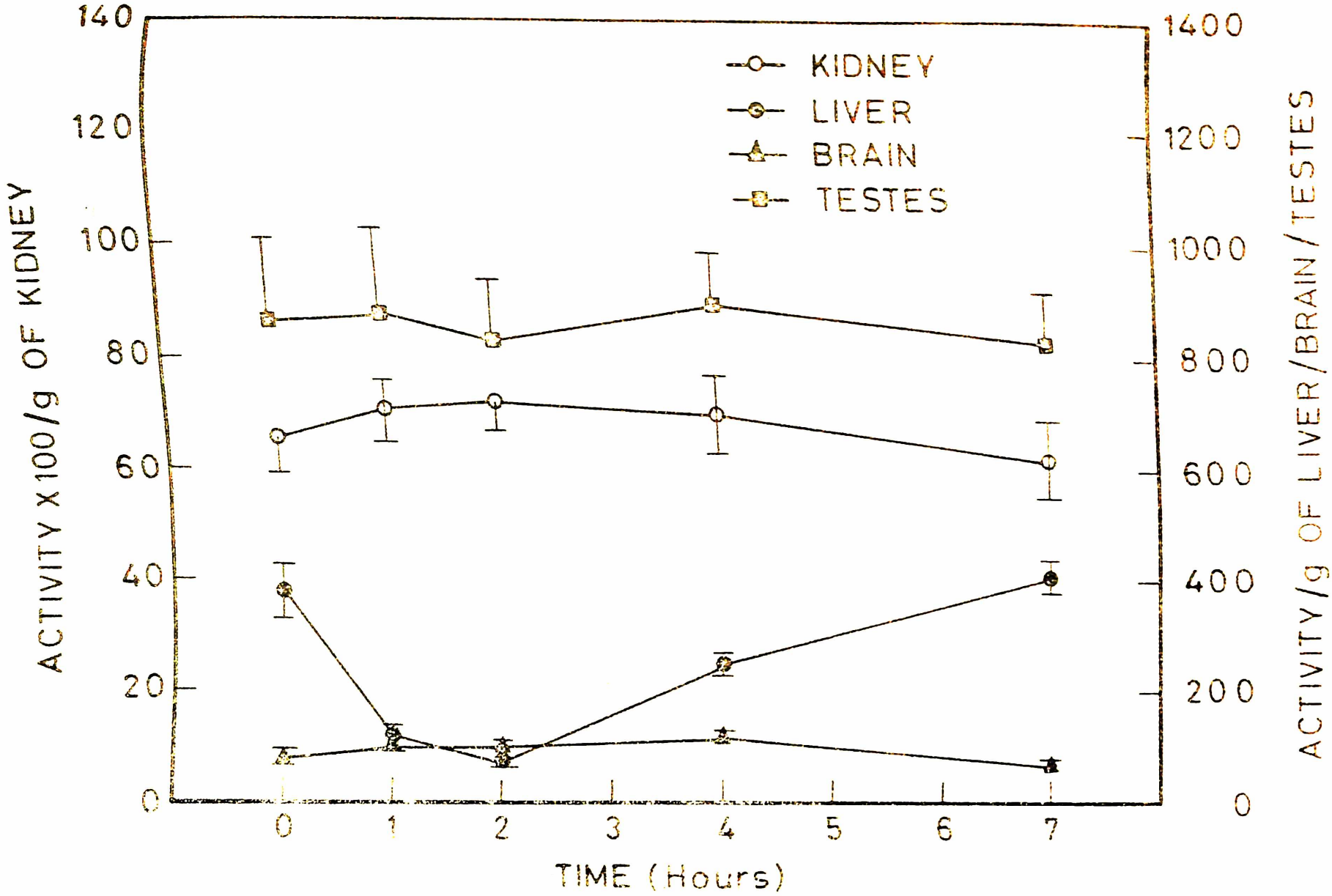


Fig.7 EFFECT OF ACUTE CS_2 (400 mg/Kg body wt) TREATMENT ON ALKALINE PHOSPHATASE ACTIVITY IN RAT TISSUES

TABLE - X : Peak effect of acute CS_2 (400 mg/kg body wt.) and SDDTC (1.2 g/kg body wt.) on Alkaline phosphatase activity (u/g tissue) in rats in seven hour observations. (Figures in brackets with enzyme activity indicate hour of observation).

Tissue	Control	CS_2 (a)	SDDTC (b)	p between a & b
No. of Rats	10	10	10	
Brain	83.4 ± 8.46	114.5 ± 12.45 (4) NS	140.9 ± 6.78 p <0.001(4)	NS
		71.5 ± 7.31 (7) NS		-
Liver	376.8 ± 51.24	83.4 ± 12.3 (2) p < 0.001	ND (7)	<0.001
Kidney	6600.0 ± 734.3	7200 ± 61.0 (2) NS	7100 ± 830.3(2,4) NS	-
		6200 ± 39.0 (7) NS	5700 ± [illegible] NS	-
Testes	869.0 ± 146.3	826.0 ± 94.5 (7) NS	601.0 ± 95.9(2,4) NS	-

p - Significance level ND - Not detectable NS - Not significant.

(b) Chronic Treatment:

The chronic treatment of rats with both CS_2 and SDDTC lead to significant decrease in alkaline phosphatase activity as is evident from Table XI and Figure 9. The Table XI shows that the alkaline phosphate activity decreased at significance of $p < 0.001$ in brain, liver and kidney tissues in CS_2 treated as well as SDDTC treated rats. Though the enzyme activity decreased at $p < 0.001$ in testes of SDDTC treated rats but the level of significance was only $p < 0.01$ in CS_2 treated rats. Further, Figure 9 shows that decrease in activity in brain, liver and kidney tissues was greater than 45% whereas in testes the decrease was less than 50%.

V. COPPER DEPENDANT ENZYMES:

The effect of acute and chronic treatment of rats with CS_2 and SDDTC have been recorded in Tables 12-17 and Figures 10-18 on copper dependant enzymes viz. histaminase, tyrosinase and uricase, in brain, liver, kidney and testes.

1. Histaminase:

(a) Acute Treatment:

The effects of administration of acute dose of carbondisulphide are shown in Table XII and Figure 10.

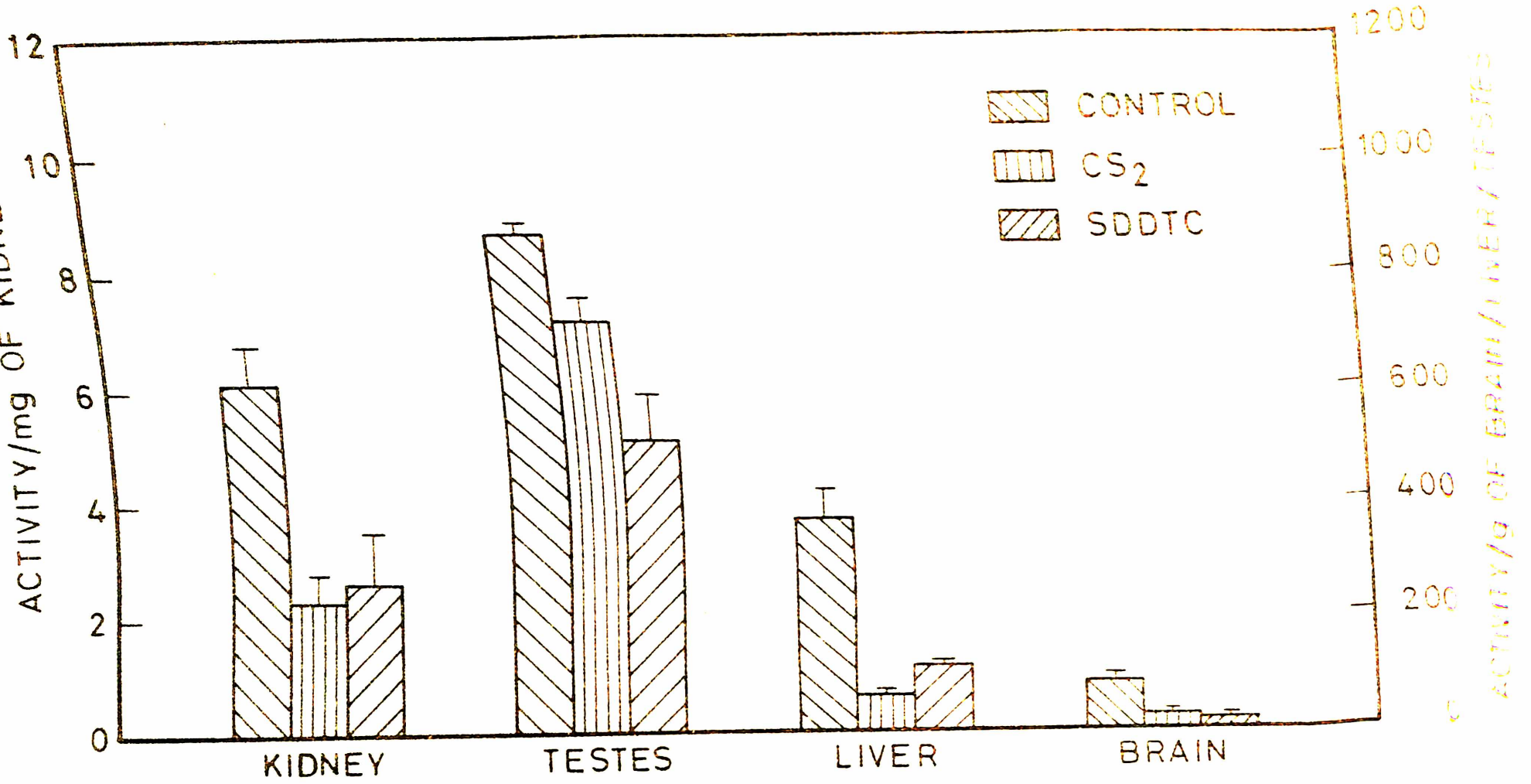


Fig.9 EFFECT OF CHRONIC CS_2 (40 mg/Kg body wt) AND SDDTC (120 mg/Kg body wt) TREATMENT ON ALKALINE PHOSPHATASE ACTIVITY IN RAT TISSUES

TABLE - XI : Effect of CS_2 (40 mg/kg body wt.) and SDDTC (120 mg/kg body wt.) chronic treatment on tissue Alkaline phosphatase activity (u/g tissue) in rats.

Tissue	Control	CS_2 (a)	SDDTC (b)	p between a & b
No. of Rats	10	10	10	
Brain	82.74 ± 10.23	19.21 ± 1.478 p < 0.001	14.78 ± 4.179 p < 0.001	NS
Liver	378.2 ± 46.75	66.35 ± 3.503 p < 0.001	113.4 ± 9.434 p < 0.001	<0.001
Kidney	6146.0 ± 698.3	2305.0 ± 487.4 p < 0.001	2671.0 ± 88.30 p < 0.0 1	NS
Testes	869.0 ± 14.52	723.4 ± 42.94 p < 0.01	508.3 ± 78.64 p < 0.001	<0.05

p - Significance level

NS - Not significant.

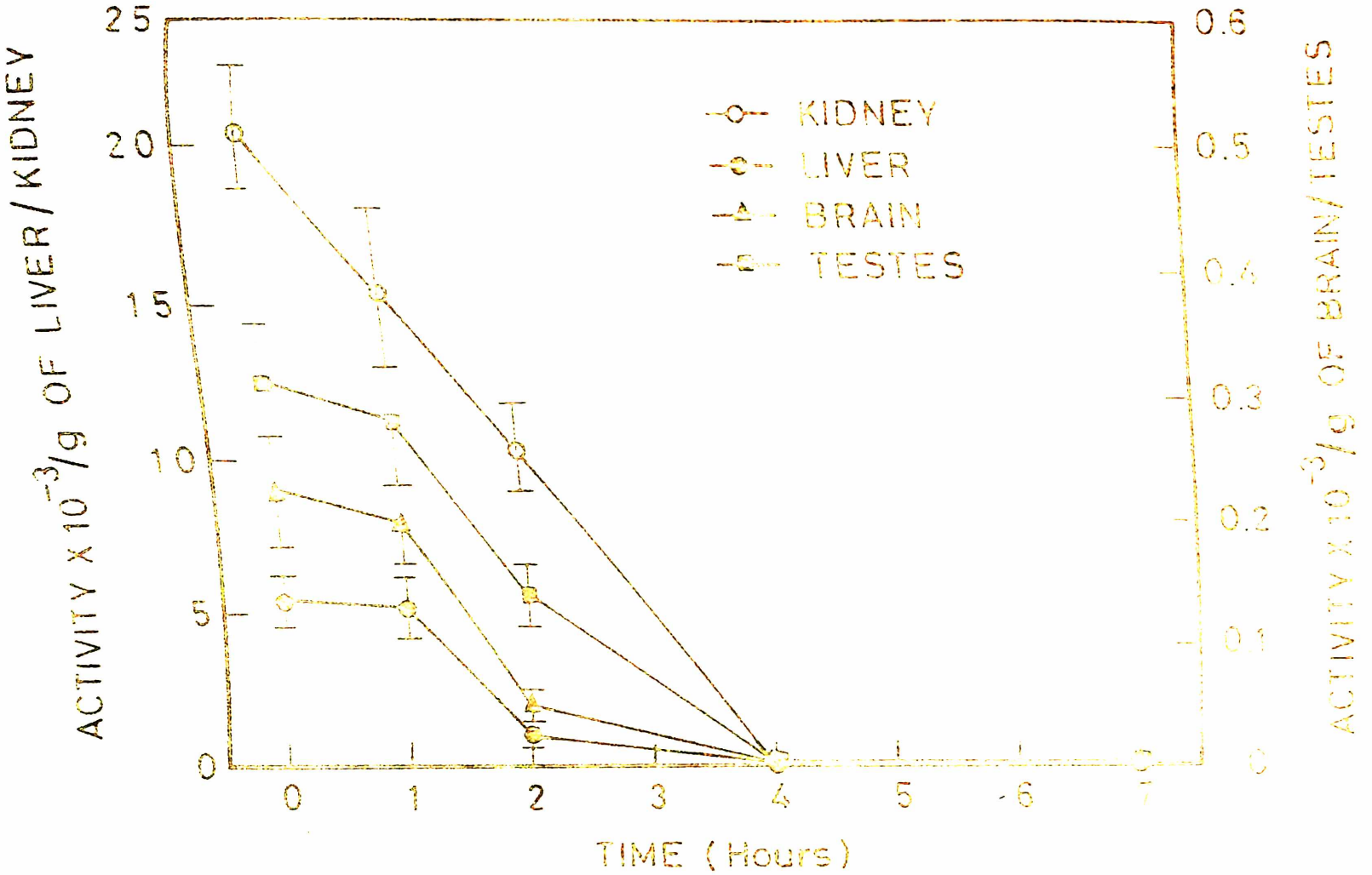


Fig.10 EFFECT OF ACUTE CS_2 (400 mg/kg body wt) TREATMENT ON HISTAMINASE ACTIVITY IN RAT TISSUES

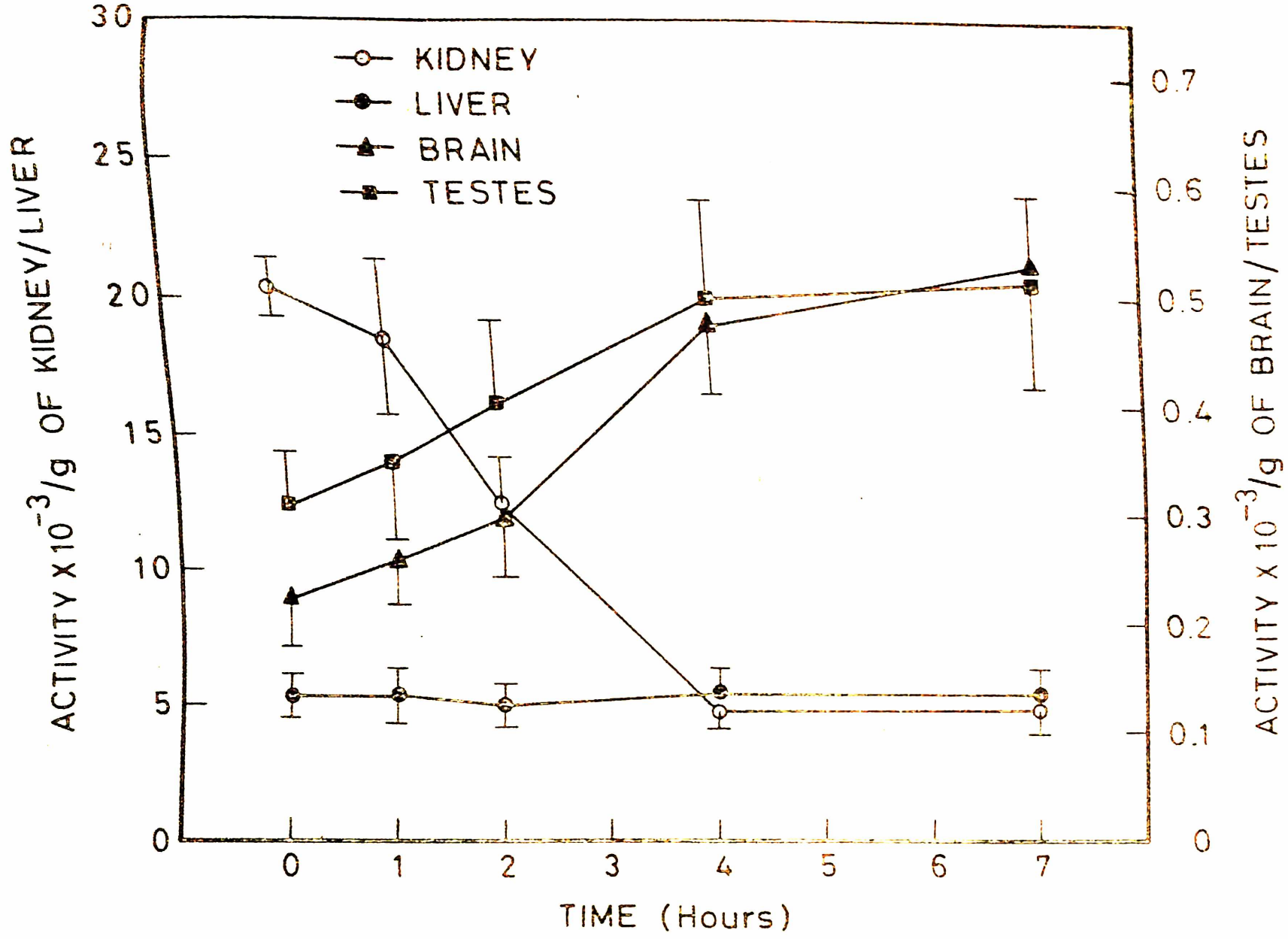


Fig.11 EFFECT OF ACUTE SDDTC (1.2 g/Kg body wt) TREATMENT ON HISTAMINASE ACTIVITY IN RAT TISSUES

TABLE - XII : Peak effect of acute CS_2 (400 mg/kg body wt.) and SDDTC (1.2 g/kg body wt.) on Histaminase activity (u x 10^{-3}/g tissue) in rats in seven hour observations. (Figures in bracket with enzyme activity indicate hour of observation).

Tissue	Control Activity ± SE	CS_2 (a) Activity ± SE	SDDTC (b) Activity ± SE	p between a & b
No. of Rats	10	10	10	
Brain	0.2245 ± 0.0436	ND (4)	0.5218 ± 0.0748(7) $p < 0.01$	-
Liver	5.364 ± 0.8461	ND (4)	5.012 ± 0.8135(2) NS	-
Kidney	20.40 ± 1.876	ND (4)	4.893 ± 0.7828(7) $p < 0.001$	-
Testes	0.3086 ± 0.0491	ND (4)	0.5143 ± 0.0927(7) NS	-

p - Significance level ND - Not detectable NS - Not significant.

Both the Table XII and Figure 10 show that the histaminase activity was completely lost after 4 hours of the acute dose in all the four tissues investigated. The effect of acute SDDTC dose was variable on the four tissues (Table XII; Figure 11). Progressive decrease in enzyme activity was observed in kidney (Figure 11) by 7th hour after treatment at a significance of $p < 0.001$. There was no significant change in liver. The brain and testes showed an increase in histaminase activity progressively upto 7 hours of observation (Figure 11). The increase was found to be at level of $p < 0.01$ in brain but was not significant in testes.

(b) Chronic Treatment:

Table XIII shows that chronic treatment of rats with CS_2 did not have any significant effect on the histaminase activity, in brain and liver though there was a slight increase in the enzyme activity. The enzyme activity showed significant decrease in kidney at $p < 0.001$ and complete inhibition in testes (Table XIII and Figure 12). The chronic SDDTC treatment decreased the histaminase activity in kidney only ($p < 0.001$), whereas in brain and testes the overall

Fig.11 EFFECT OF CHRONIC CS_2 [illegible]
[illegible] TREATMENT ON [illegible]

TABLE - XIII : Effect of CS_2 (40 mg/kg body wt.) and SDDTC (120 mg/kg body wt.) chronic treatment on tissue Histaminase activity (u x 10^{-3}/g tissue) in rats.

Tissue	Control Activity ± SE	CS_2 (a) Activity ± SE	SDDTC (b) Activity ± SE	p between a & b
No. of Rats	10	10	10	
Brain	0.2245 ± 0.0436	0.2348 ± 0.0312 NS	0.3131 ± 0.0512 NS	NS
Liver	5.364 ± 0.8461	6.152 ± 0.5801 NS	4.917 ± 0.8745 NS	NS
Kidney	20.40 ± 1.876	3.563 ± 0.7843 $p < 0.001$	3.054 ± 0.6012 $p < 0.001$	NS
Testes	0.3086 ± 0.0491	ND	0.4115 ± 0.0774 NS	-

p - Significance level ND - Not detectable NS - Not significant.

activity of enzyme showed an increase while slight decrease was noted in liver activity (Table XIII and Figure 12).

2. Tyrosinase:

(a) Acute Treatment:

The CS_2 treated rats showed an overall decrease in tyrosinase activity in all the four tissues. The enzyme activity was not detectable in brain, kidney and testes but in case of liver the decrease is not significant (Table XIV; Figure 13). Figure 13 shows that activity in kidney and testes became undetectable by 4th hour and in brain by 7th hour. Table XIV shows that acute treatment with SDDTC had a similar effect on tyrosinase activity in kidney and liver. However, in case of brain and testes, the tyrosinase activity decreases by 2nd hour but thereafter increases upto 7th hour. (Figure 14). The change in activity is significant at $p < 0.05$ in brain and $p < 0.001$ in liver. The decrease in tyrosinase activity in liver in response to SDDTC treatment was more severe than CS_2 at a significance level of $p < 0.001$.

(b) Chronic Treatment:

The effect of chronic treatment of rats with CS_2 and SDDTC are as shown in Table XV and Figure 15.

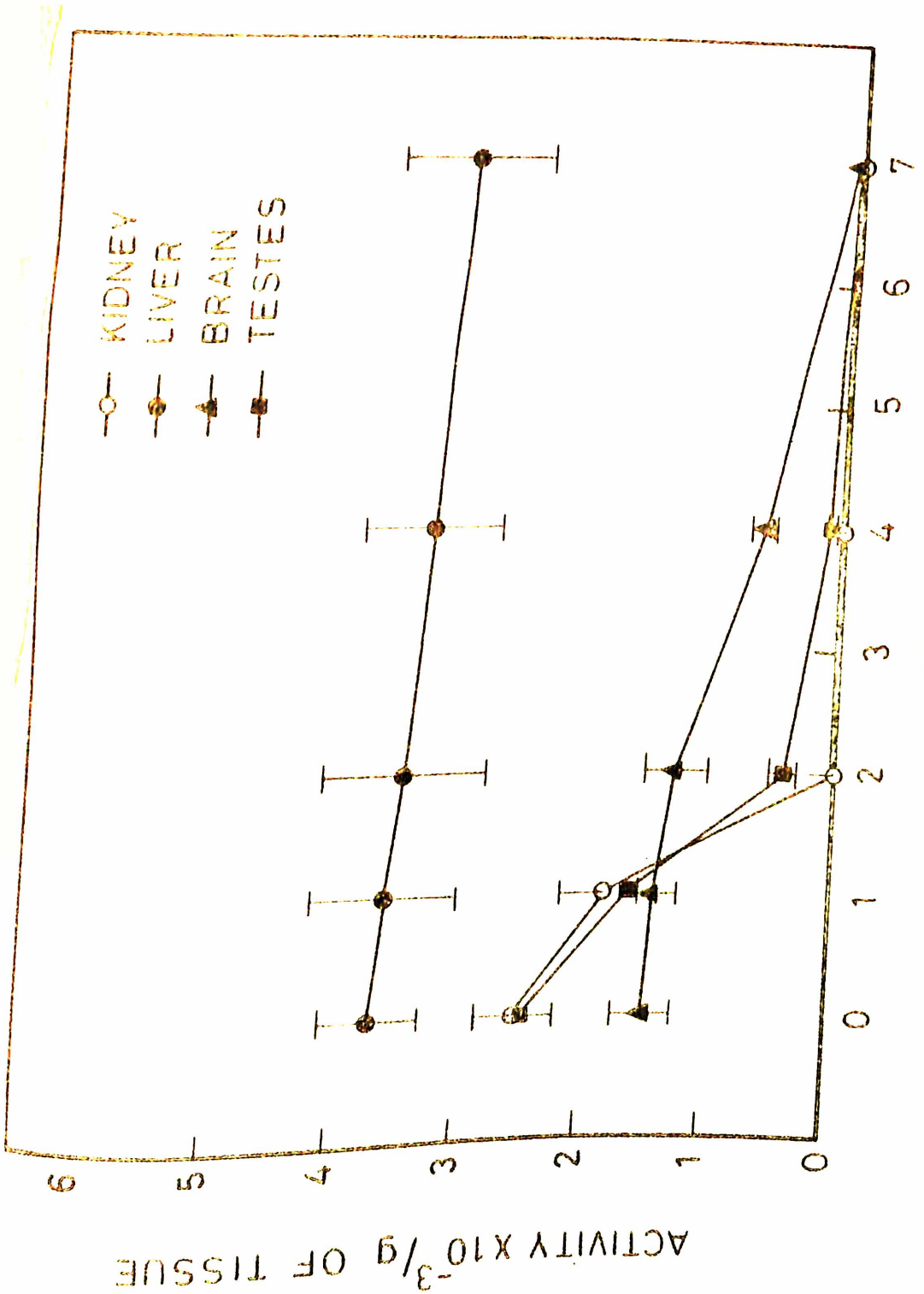


Fig.13 EFFECT OF ACUTE CS_2 (400 mg/Kg body wt) TREATMENT ON TYROSINASE ACTIVITY IN RAT TISSUES

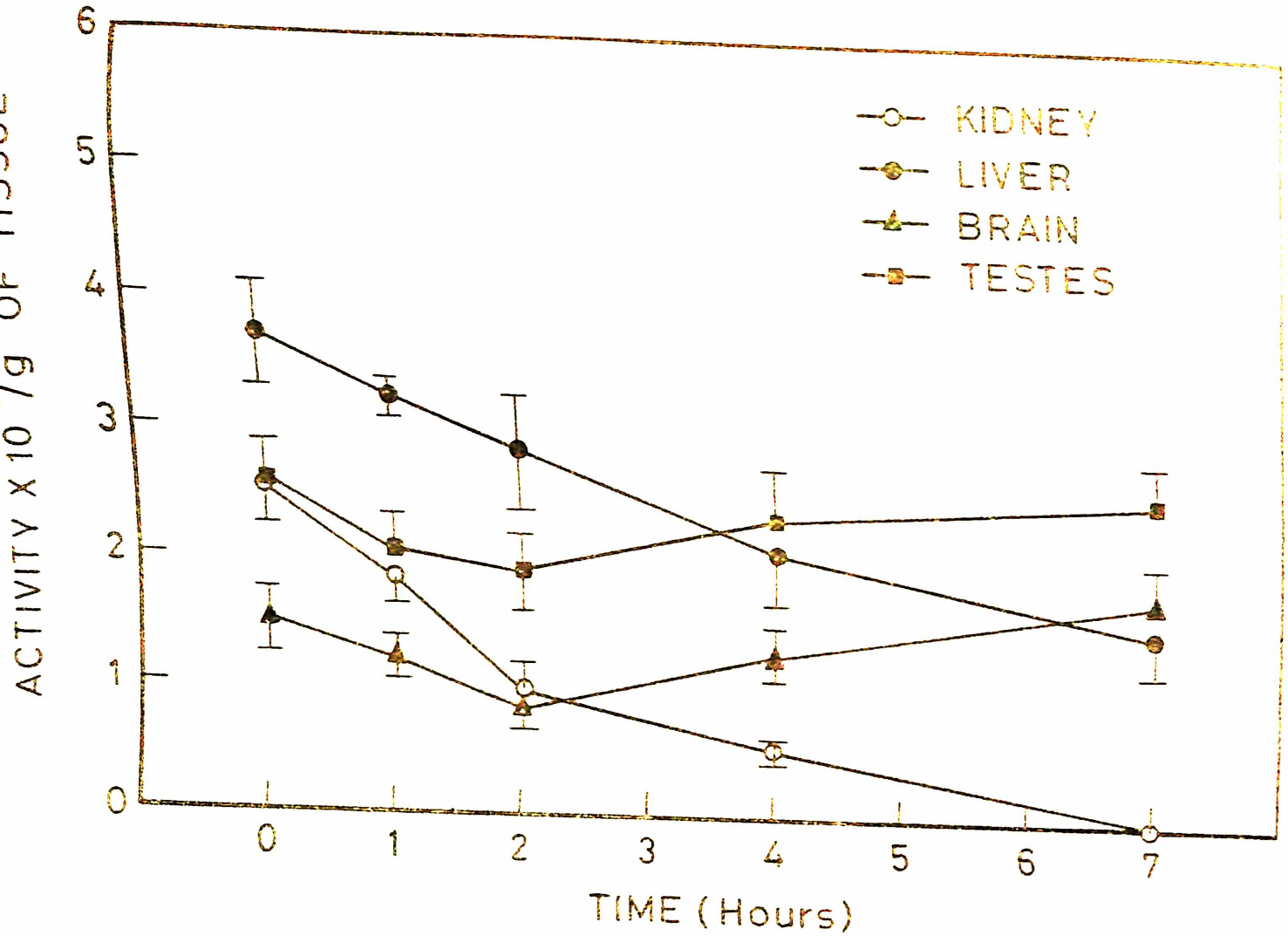


Fig.14 EFFECT OF ACUTE SDDTC (1.2 g/Kg body wt) TREATMENT ON TYROSINASE ACTIVITY IN RAT TISSUES

TABLE - XIV : Peak effect of acute CS_2 (400 mg/kg body wt.) and SDTC (1.2 g/kg body wt.) on Tyrosinase activity (u x 10^{-3}/g tissue) in rats in seven hour observations. (Figures in brackets with enzyme activity indicate hour of observation).

Tissue	Control Activity ± SE	CS_2 (a) Activity ± SE	SDTC (b) Activity ± SE	p between a & b
No. of Rats	10	10	10	
Brain	1.522 ± 0.2341	ND (7)	0.862 ± 0.1382 (2) $p < 0.05$	-
Liver	3.725 ± 0.3816	3.166 ± 0.5960(7) NS	1.490 ± 0.2816 (7) $p < 0.001$	< 0.001
Kidney	2.545 ± 0.3134	ND (2)	ND (7)	-
Testes	2.572 ± 0.369	ND (7)	1.875 ± 0.3216 (2) NS	-

p - Significance level ND - Not detectable NS - Not significant.

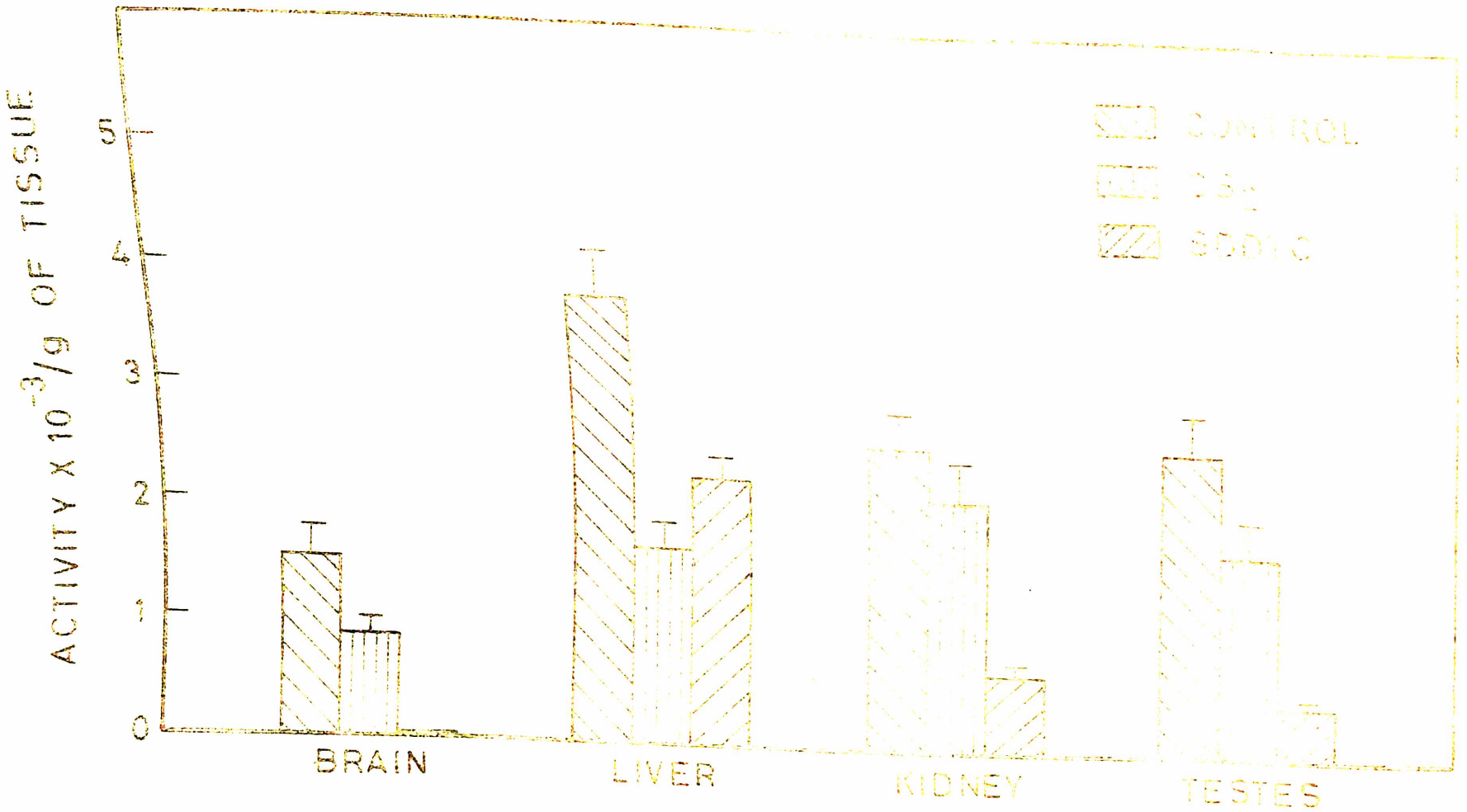


Fig.15 EFFECT OF CHRONIC CS_2 (40 mg/kg body wt) AND SDDTC (120 mg/kg body wt) TREATMENT ON TYROSINASE ACTIVITY IN RAT TISSUES

TABLE - XV : Effect of CS_2 (40 mg/kg body wt.) and SDDTC (120 mg/kg body wt.) chronic treatment on tissue Tyrosinase activity (u x 10^{-3}/g tissue) in rats.

Tissue	Control Activity ± SE	CS_2 (a) Activity ± SE	SDDTC (b) Activity ± SE	p between a & b
No. of Rats	10	10	10	
Brain	1.522 ± 0.2341	0.8697 ± 0.1625 $p < 0.05$	ND	-
Liver	3.725 ± 0.3816	1.676 ± 0.1872 $p < 0.001$	2.235 ± 0.2012 $p < 0.01$	NS
Kidney	2.545 ± 0.3134	2.121 ± 0.2806 NS	0.6454 ± 0.1025 $p < 0.001$	< 0.001
Testes	2.572 ± 0.3679	1.714 ± 0.3011 NS	0.4286 ± 0.0581 $p < 0.001$	< 0.001

p - Significance level ND - Not detectable NS - Not significant.

The CS_2 treatment causes a decrease in the tyrosinase activity in brain and liver at significance level of $p < 0.005$ and $p < 0.001$ respectively, but decrease in kidney and testes is not significant (Table XV). The effect of SDDTC treatment caused complete loss in enzyme activity in brain. The decrease in other tissues activity has a significance level of $p < 0.01$ in liver, $p < 0.001$ in kidney and $p < 0.001$ in testes (Table XV). SDDTC caused more severe decrease in tyrosinase than CS_2 in all the tissues investigated except liver.

3. Uricase:

(a) Acute Treatment:

The effect of acute dose of CS_2 as well as SDDTC caused significant decrease in the uricase activity in brain, liver, kidney and testicular tissues (Table XVI), Figures 16 and 17). Table XVI shows that the uricase activity was completely lost after acute CS_2 treatment. The enzyme activity also decreased in brain, liver and kidney at significance level of $p < 0.001$, $p < 0.01$ and $p < 0.001$ respectively (Table XVI). Only the liver shows partial recovery in the activity by 7th hour (Figure 16).

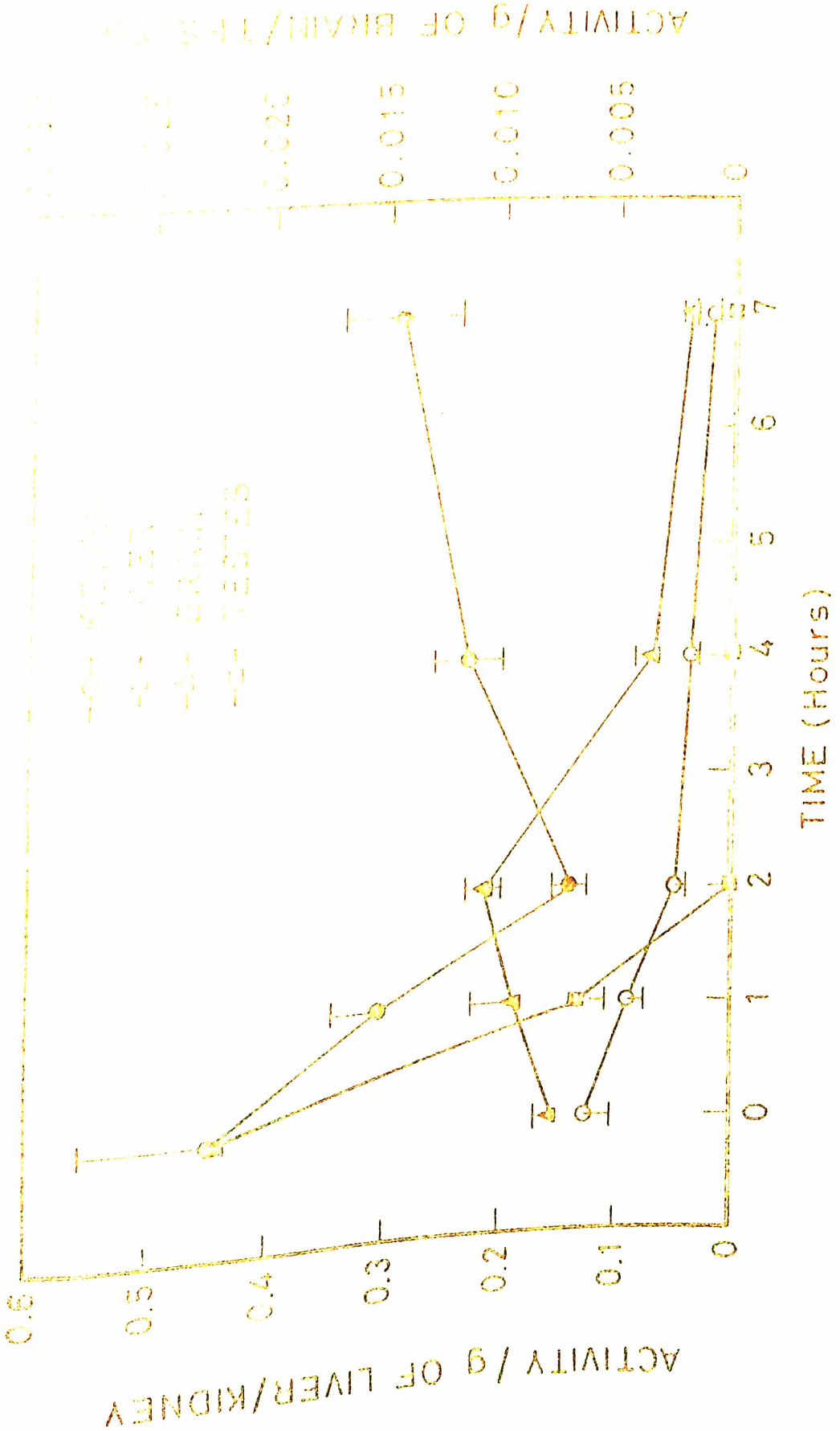


Fig.16 EFFECT OF ACUTE CS_2 (400 mg/Kg body wt) TREATMENT ON URICASE ACTIVITY IN RAT TISSUES

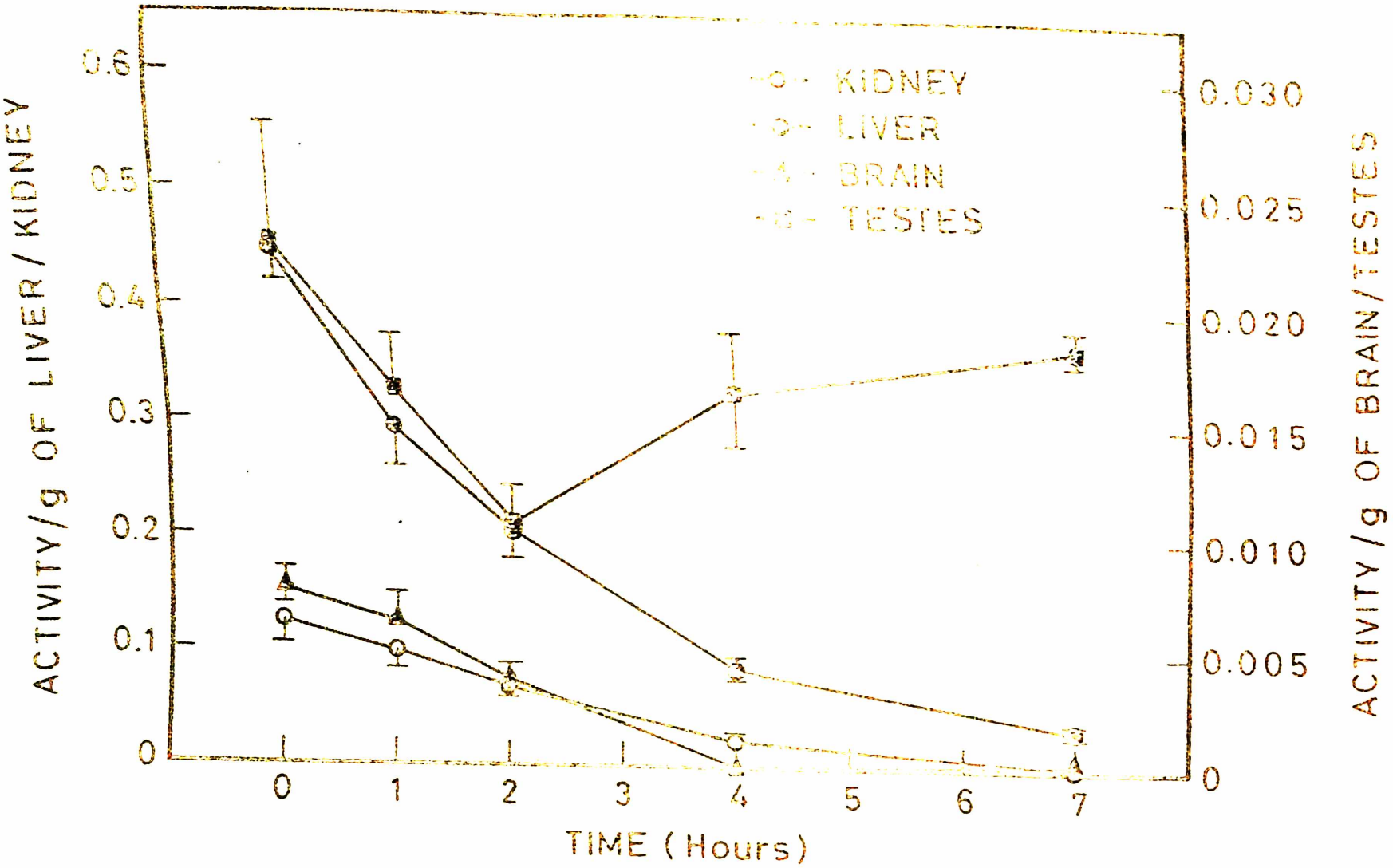


Fig.17 EFFECT OF ACUTE SDDTC (1.2 g/Kg body wt) TREATMENT ON URICASE ACTIVITY IN RAT TISSUES

TABLE - XVI : Peak effect of acute CS_2 (400 mg/kg body wt.) and SDDTC (1.2 g/kg body wt.) on Uricase activity (u x 10^{-3}/g tissue) in rats in seven hour observations. (Figures in brackets with enzyme activity indicate hour of observation).

Tissue	Control Activity ± SE	CS_2 (a) Activity ± SE	SDDTC (b) Activity ± SE	p between a & b
No. of Rats	10	10	10	
Brain	7.70 ± 0.760	2.050 ± 0.1250(7) $p < 0.001$	ND (4)	-
Liver	449.5 ± 105.7	140.5 ± 1.25 (2) $p < 0.01$	33.00 ± 5.280(7) $p < 0.001$	< 0.001
Kidney	122.5 ± 21.20	19.00 ± 3.230 (7) $p < 0.001$	ND (7)	-
Testes	22.5 ± 0.750	ND (2)	10.40 ± 1.768(2) $p < 0.001$	-

p - Significance level

ND - Not detectable.

The SDDTC treatment of rats resulted in the complete loss of uricase activity in brain as well as in kidney by 7th hour after the treatment (Figure 17). The decrease in uricase activity in liver and testes is also highly significant at $p < 0.001$ level (Table XVI). Further, the decrease in the enzyme activity in liver in response to SDDTC is significantly ($p < 0.001$) more than that due to CS_2.

(b) Chronic Treatment:

The chronic treatment with CS_2 was found to have no significant effect on the uricase activity in brain, liver and kidney. There was decrease in the uricase activity in brain, liver and kidney, on the contrary the enzyme activity increased ($p < 0.001$) in testes (Figure 18; Table XVII). The effects on uricase activity in response to SDDTC treatment are as shown in Table XVII and Figure 18. It can be seen from figure 18 that the enzyme activity increases in brain and testes at $p < 0.001$ level whereas decreases in liver and kidney are insignificant. The decrease in activity in liver and kidney is found to be insignificant. Further, the increase in uricase activity due to SDDTC in comparison to that of CS_2 was found to be highly significant at $p < 0.001$ level in brain and testes.

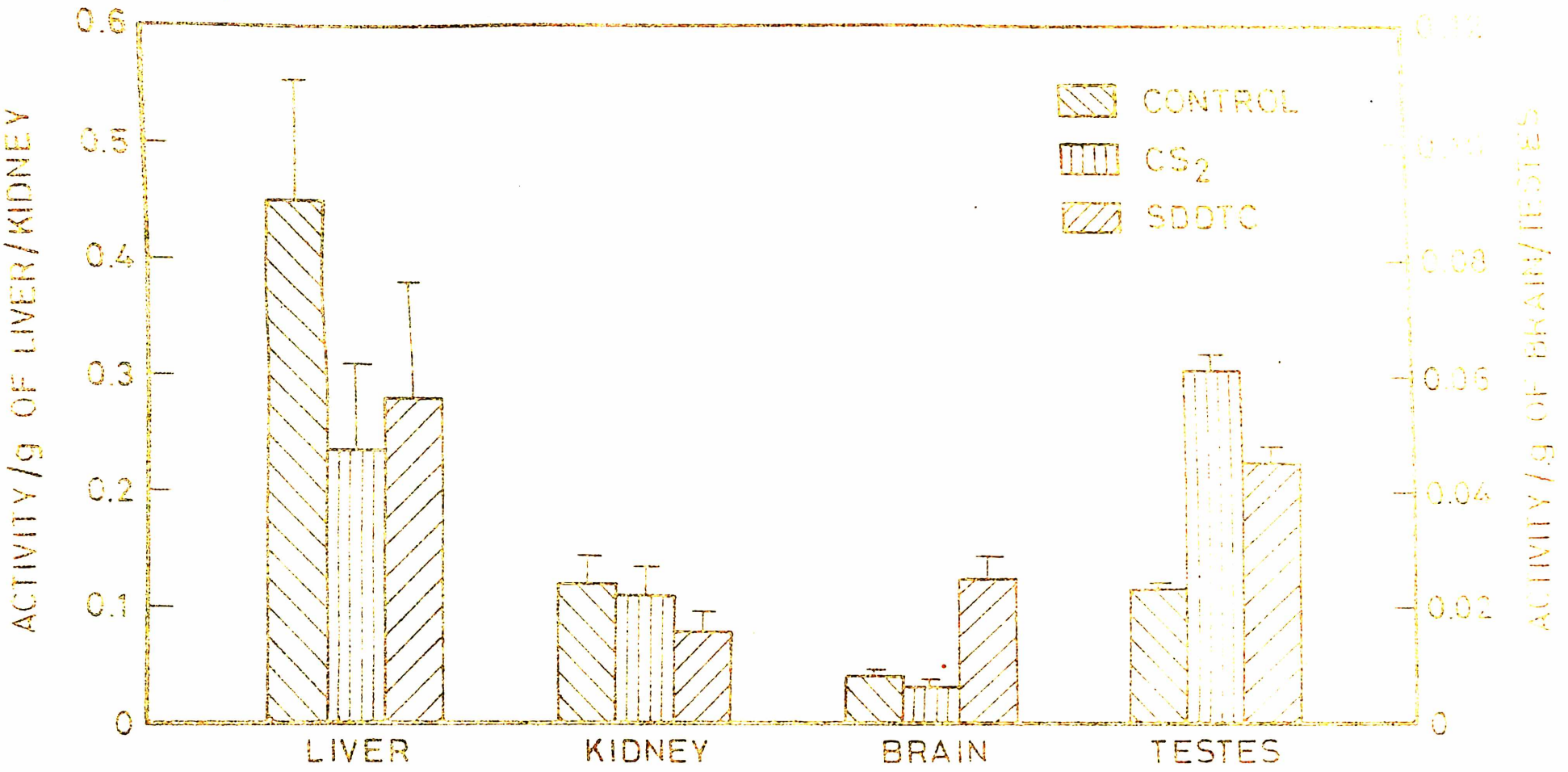


Fig.18 EFFECT OF CHRONIC CS_2 (40 mg/Kg body wt) AND SDDTC (120 mg/Kg body wt) TREATMENT ON URICASE ACTIVITY IN RAT TISSUES

TABLE - XVII : Effects of CS_2 (40 mg/kg body wt.) and SDDTC (120 mg/kg body wt.) chronic treatment on tissue Uricase activity (u x 10^{-3}/g tissue) in rats.

Tissue	Control Activity ± SE	CS_2 (a) Activity ± SE	SDDTC (b) Activity ± SE	p between a & b
No. of Rats	10	10	10	
Brain	7.660 ± 0.7700	6.000 ± 1.200 NS	24.50 ± 3.500 $p < 0.001$	< 0.001
Liver	449.5 ± 105.7	233.0 ± 76.00 NS	280.0 ± 99.80 NS	NS
Kidney	122.5 ± 21.20	109.5 ± 26.60 NS	80.00 ± 16.00 NS	NS
Testes	22.50 ± 0.7500	61.0 ± 2.500 $p < 0.001$	45.00 ± 3.0 $p < 0.001$	< 0.001

p - Significance level NS - Not significant.

VI. EFFECTS OF CS_2 AND SDDTC ON METAL LEVELS:

Since the enzyme selected for present investigations are Zn and Cu dependant, it was essential to observe the effect of CS_2 and its metabolic analogue SDDTC on the levels of Zn and Cu in the tissues investigated. The effects of CS_2 and SDDTC on the levels of Zinc and Copper in brain, liver, kidney and testes are recorded in Tables XVIII and XIX and Figures 19 and 20.

1. Zinc:

The level of Zn increases in all the four tissues investigated in response to the chronic treatment of both CS_2 and SDDTC as is evident from Table XVIII and Figure 19. Further the increase in Zn level is more against CS_2 treatment in brain and testes whereas liver and kidney showed increase in Zn level against SDDTC treatment (Figure 19). The increase in Zn level is highly significant at $p < 0.001$ in brain, liver and kidney in response to CS_2 while insignificant in case of testes. Similarly chronic SDDTC treatment changed the Zn level significantly ($p < 0.001$) in brain and kidney and at $p < 0.02$ in liver but the change was insignificant in testes (Table XVIII).

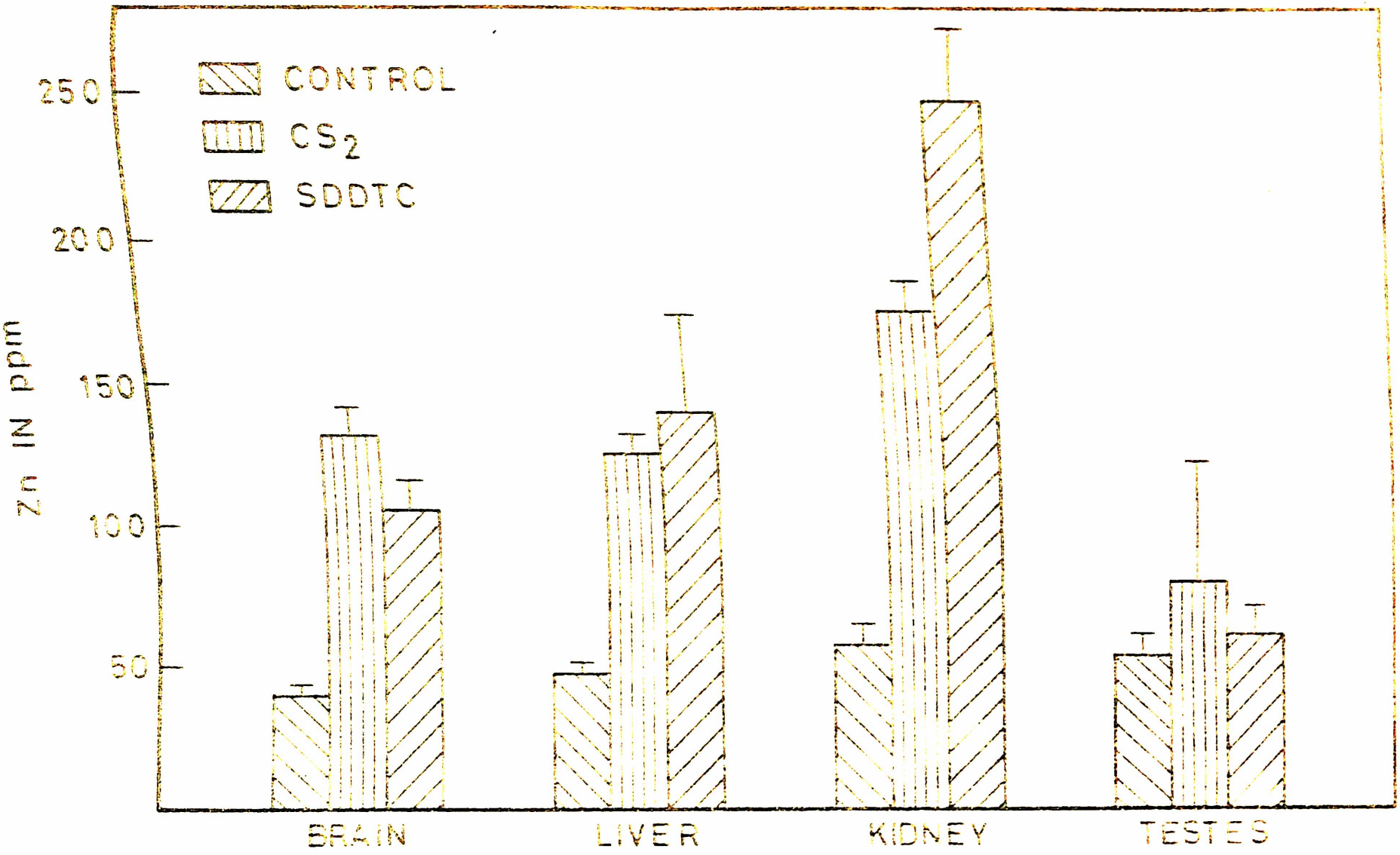


Fig.19 EFFECT OF CHRONIC CS_2 (40 mg/Kg body wt) AND SDDTC (120 mg/Kg body wt) TREATMENT ON Zn LEVELS IN RAT TISSUES

TABLE - XVIII : Effect of chronic treatment of rats with CS_2 (40 mg/kg body wt.) and SDDTC (120 mg/kg body wt.) on Zn concentration (ppm) ± SE in various tissues.

Tissue	Control	CS_2 (a)	SDDTC (b)	p between a & b
No. of Rats	10	10	10	
Brain	39.37 ± 3.270	131.8 ± 9.910 p < 0.001	105.4 ± 9.230 p < 0.001	NS
Liver	47.7 ± 3.980	126.9 ± 4.890 p < 0.001	140.2 ± 34.41 p < 0.02	NS
Kidney	57.6 ± 8.070	176.3 ± 9.430 p < 0.001	247.7 ± 24.77 p < 0.001	< 0.02
Testes	54.34 ± 7.560	80.74 ± 44.10 NS	62.34 ± 9.190 NS	NS

p - Significance level

NS - Not significant.

2. Copper:

The copper concentration in brain, liver, kidney and testes showed highly significant increase after the chronic treatment of both CS_2 and SDDTC (Figure 20). It can be seen from the table XIX that increase in concentration is more than three times the control. The increase in metal level is highly significant at $p < 0.001$ level in all the four tissues investigated in response to both CS_2 and SDDTC (Table XIX). Further, the increase in Cu concentration was highly significant in chronic treatment with SDDTC as compared to that of CS_2 ($p < 0.001$) in brain and kidney. The above result shows that there is a significant accumulation of Cu in all the tissues studied in response to chronic treatment with both CS_2 and SDDTC.

VII. IN VITRO EFFECT OF Zn AND Cu SUPPLEMENTATION ON ENZYMES INHIBITED WITH CS_2 AND SDDTC:

The effect of Zn and Cu supplementation on the activity of liver enzymes of rats treated with acute and chronic doses of CS_2 and SDDTC are shown in Tables XXa and XXI . The supplementation of 4M $ZnSO_4$ and 0.33 $CuSO_4$ to 10% liver homogenate separately did not show any effect on the activity of aldolase,

FIG. 20 EFFECT OF CUPRIC Cu_2 (40 mg/kg body wt) ANT ESTRO (120 mg/kg body wt) TREATMENT ON Cu LEVELS IN RAT TESTES

TABLE - XIX : Effect of chronic treatment of rats with CS_2 (40 mg/kg body wt.) and SDDTC (120 mg/kg body wt.) on Cu Concentration (ppm ± SE) in various tissues.

Tissue	Control	CS_2 (a)	SDDTC (b)	p between a & b
No. of Rats	10	10	10	
Brain	4.370 ± 0.5800	20.04 ± 1.36 p < 0.001	31.88 ± 1.15 p < 0.001	< 0.001
Liver	6.770 ± 0.7100	19.12 ± 2.62 p < 0.001	20.80 ± 0.55 p < 0.001	NS
Kidney	6.470 ± 1.360	22.84 ± 0.71 p < 0.001	31.87 ± 1.89 p < 0.001	< 0.001
Testes	3.470 ± 0.930	23.37 ± 0.71 p < 0.001	22.82 ± 0.60 p < 0.001	NS

p - Significance level

NS - Not significant.

alkaline phosphatase, histaminase and uricase. Hence the results are not presented here. On the contrary, the Zn and Cu supplementation gave some significant change in the enzyme activity under inhibition, in case of alcohol dehydrogenase and tyrosinase as shown in table XXb and XXIb.

1. Alcohol Dehydrogenase:

In vitro observations on the pure alcohol dehydrogenase (ADH) activity in presence of CS_2 and SDDTC revealed that CS_2 does not affect the normal activity of this enzyme whereas the activity of the enzyme was inhibited with SDDTC (Figure 21). Since the ADH activity became inhibited with SDDTC, the rate constant and inhibition constant for the pure enzyme and in presence of 0.1 mM SDDTC were calculated by using Lineweaver-Burk plot (Figure 22) and are as shown in Table XXa. From Figure 22 and Table XXa, it is evident that the inhibition is non-competitive type.

When $ZnSO_4$ was added to the reaction media containing inhibited ADH with 0.1 mM SDDTC, there was a recovery of more than 45% activity of this enzyme with reference to the normal activity (Figure 23).

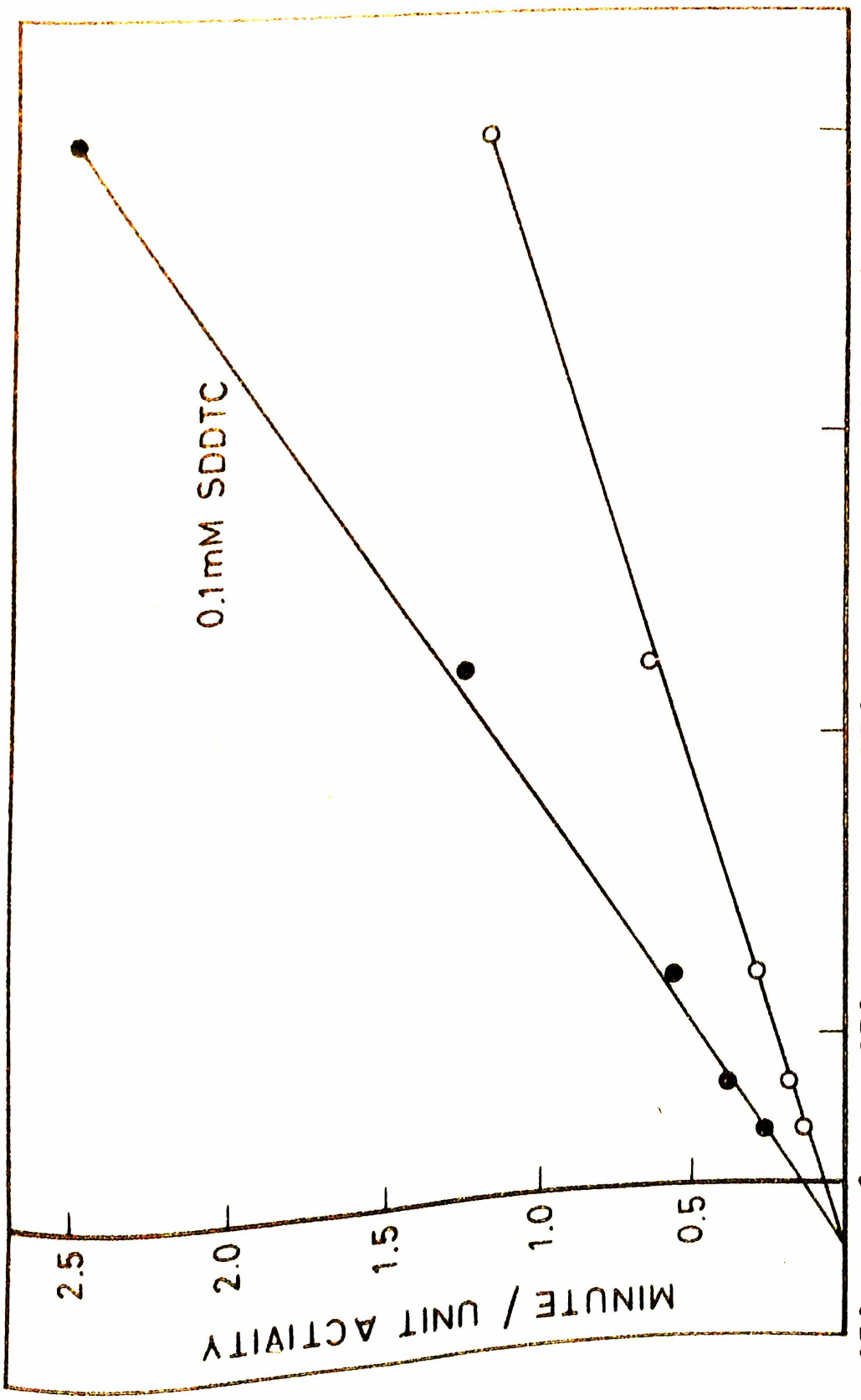
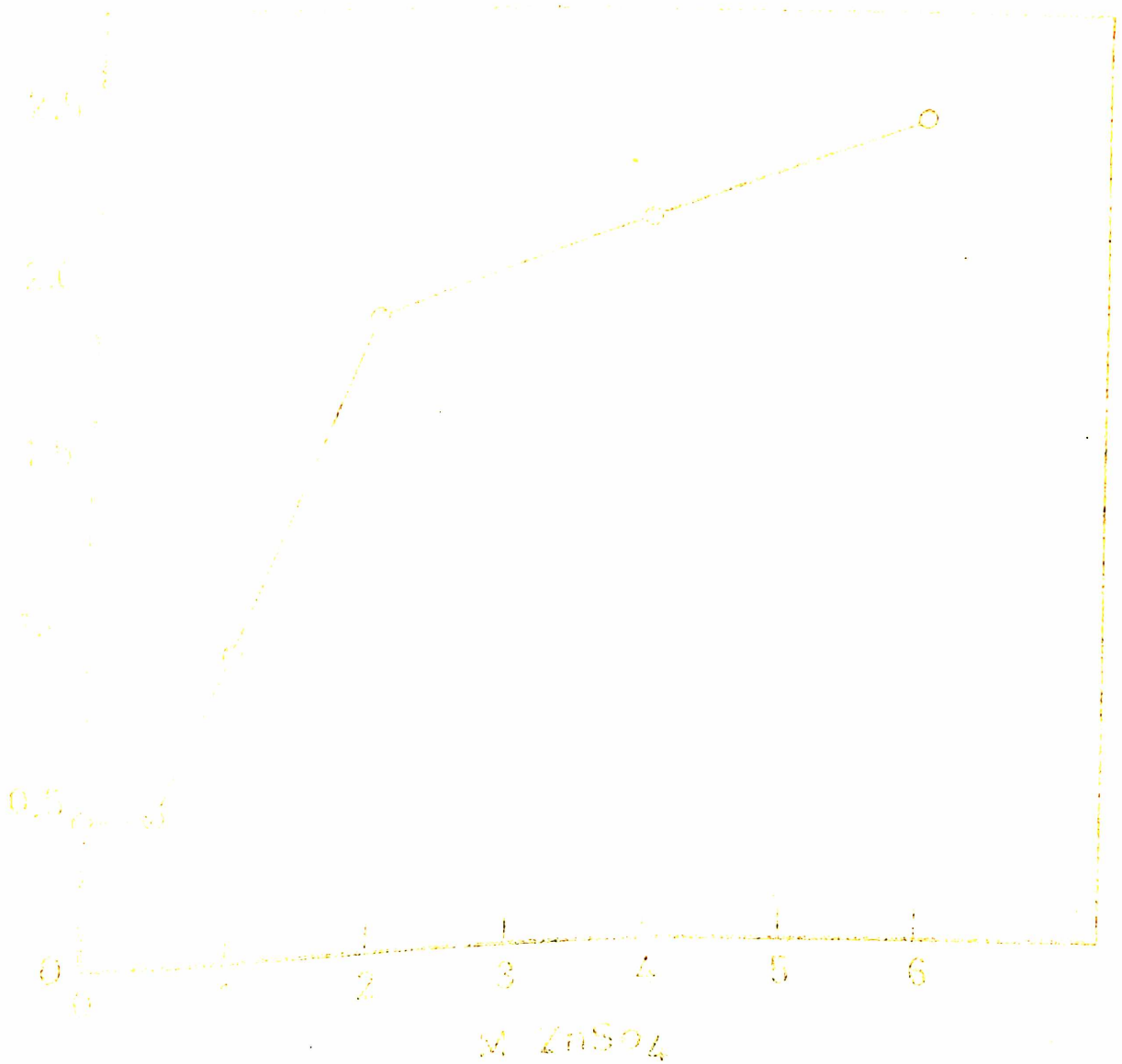


Fig.22 EFFECT OF 0.1 mM SDDTC ON LINEWEAVER-BURK PLOT OF ALCOHOL DEHYDROGENASE

TABLE - XXa : Alcoholic dehydrogenase enzyme constants and SDDTC inhibition constant.

Alcohol dehydrogenase control	:	k_m	= 9.226 mM
		V_m	= 14.316 u/min
Alcohol dehydrogenase in 0.1 mM SDDTC	:	k_p	= 8.053 mM
		V_p	= 6.341 u/min
Inhibition constants	:	$k_{0.1\ mM}$	= 0.1030 mM
		k'_m	= 7.120 mM


2
3
4
5
6
M ZnSO4

When the effect of CS_2 and SDDTC was observed on the ADH in 10% liver homogenate, it was found that liver homogenate from both CS_2 and SDDTC treated rats, did not have any detectable ADH activity in case of acute dosing. The addition of 4M $ZnSO_4$ however resulted in about 13% and 31% recovery in the activity respectively (Table XXb).

The chronic treatment with CS_2 and SDDTC of rats revealed about 41% and 28% ADH activity respectively in 10% liver homogenate as compared to the control. When 4M $ZnSO_4$ was added to their liver homogenates there was a restoration of activity from 41% to about 49% in case of CS_2 inhibited and from 28% to 44.5% in case of SDDTC inhibited (Table XXb).

2. Tyrosinase:

Results in vitro and in liver homogenate study on the effects of CS_2 and SDDTC, and effect of copper supplementation on the enzyme inhibition are given in Table XXI a & b and Figures 24 - 26.

In vitro addition of CS_2 and SDDTC to the reaction media containing pure tyrosinase revealed that CS_2 did not affect the normal enzyme activity whereas SDDTC

TABLE - XXb : Effect of 4M $ZnSO_4$ on CS_2 and SDDTC treated rat liver alcohol dehydrogenase activity (u) per 0.1 ml of 10% liver homogenate.

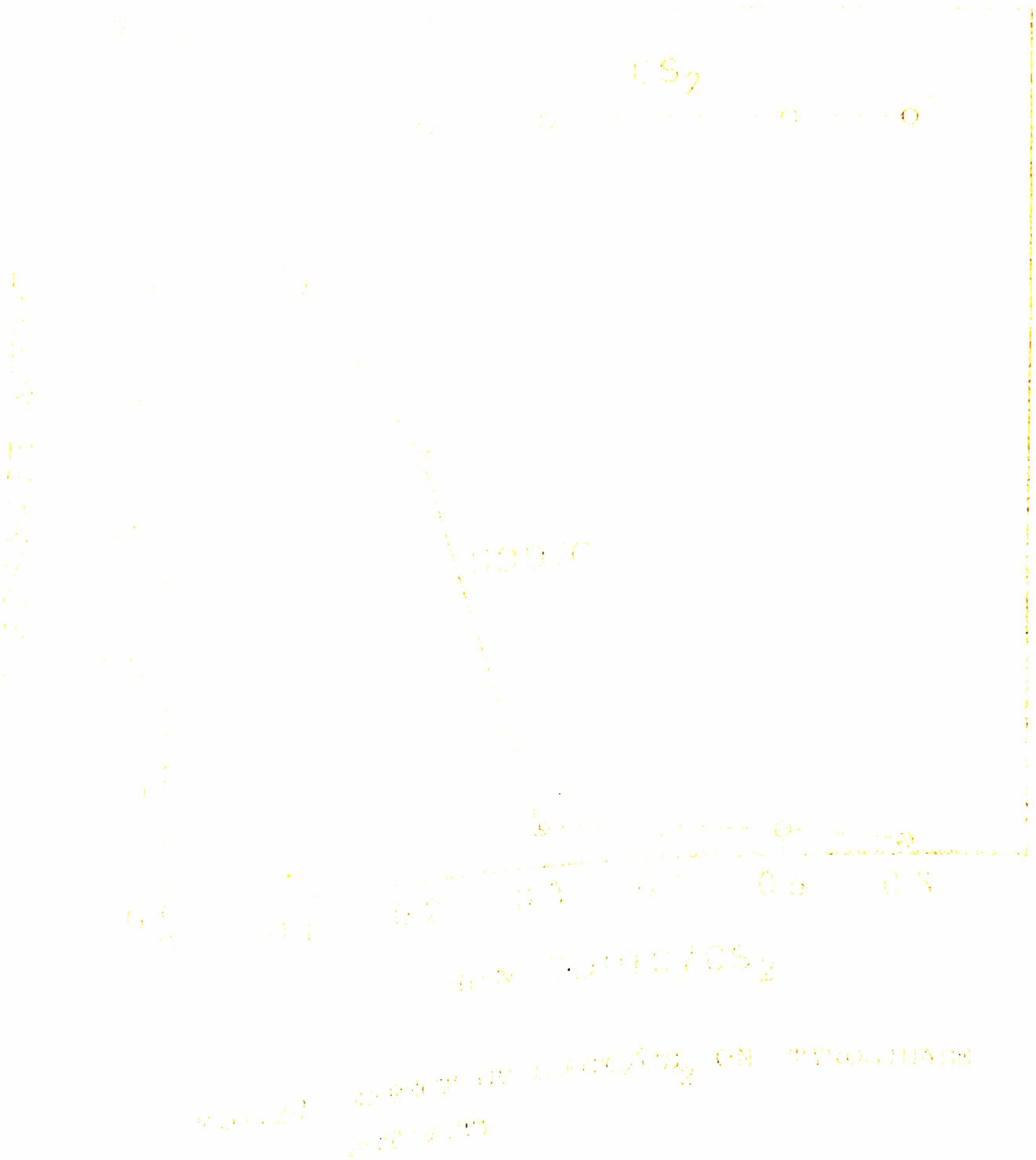
Mode of $ZnSO_4$ Treatment.	Liver Alcohol Dehydrogenase Activity				
	Control	Acute treatment		Chronic treatment	
		CS_2	SDDTC	CS_2	SDDTC
Without $ZnSO_4$	3.88	ND	ND	1.67	1.11
With 4M $ZnSO_4$	4.00	0.53	1.24	1.99	1.78

ND - Not detectable.

inhibited the enzyme activity. The degree of inhibition was maximum in 0.3 mM to 0.6 mM concentration of SDDTC, (Figure 24). When the rate constant and the inhibition constant of the enzyme tyrosinase and in presence of 0.1 mM SDDTC were calculated it was found that the inhibition is of competitive type as is evident from Figure 25 and Table XXIa. When the inhibited enzyme was subjected to variable concentration of $CuSO_4$, it was found that the enzyme activity showed a maximum recovery with 0.33 mM $CuSO_4$ concentration. However, any further increase in the concentration of $CuSO_4$ resulted in inhibition of tyrosinase activity proportionate to increase in the concentration (Figure 26).

The effect of optimal $CuSO_4$ concentration (0.33 mM) on tyrosinase activity in 10% liver homogenate from rats subjected to acute and chronic treatment with CS_2 and SDDTC is shown in Table XXIb.

The table shows that the supplementing copper to the enzyme did affect the enzyme activity. 59% inhibition was caused by acute SDDTC dose which was reduced to 47.5% on addition of $CuSO_4$; thus restored the enzyme activity by 11.5% (Table XXIb). The results of $CuSO_4$ supplementation to the 10% liver



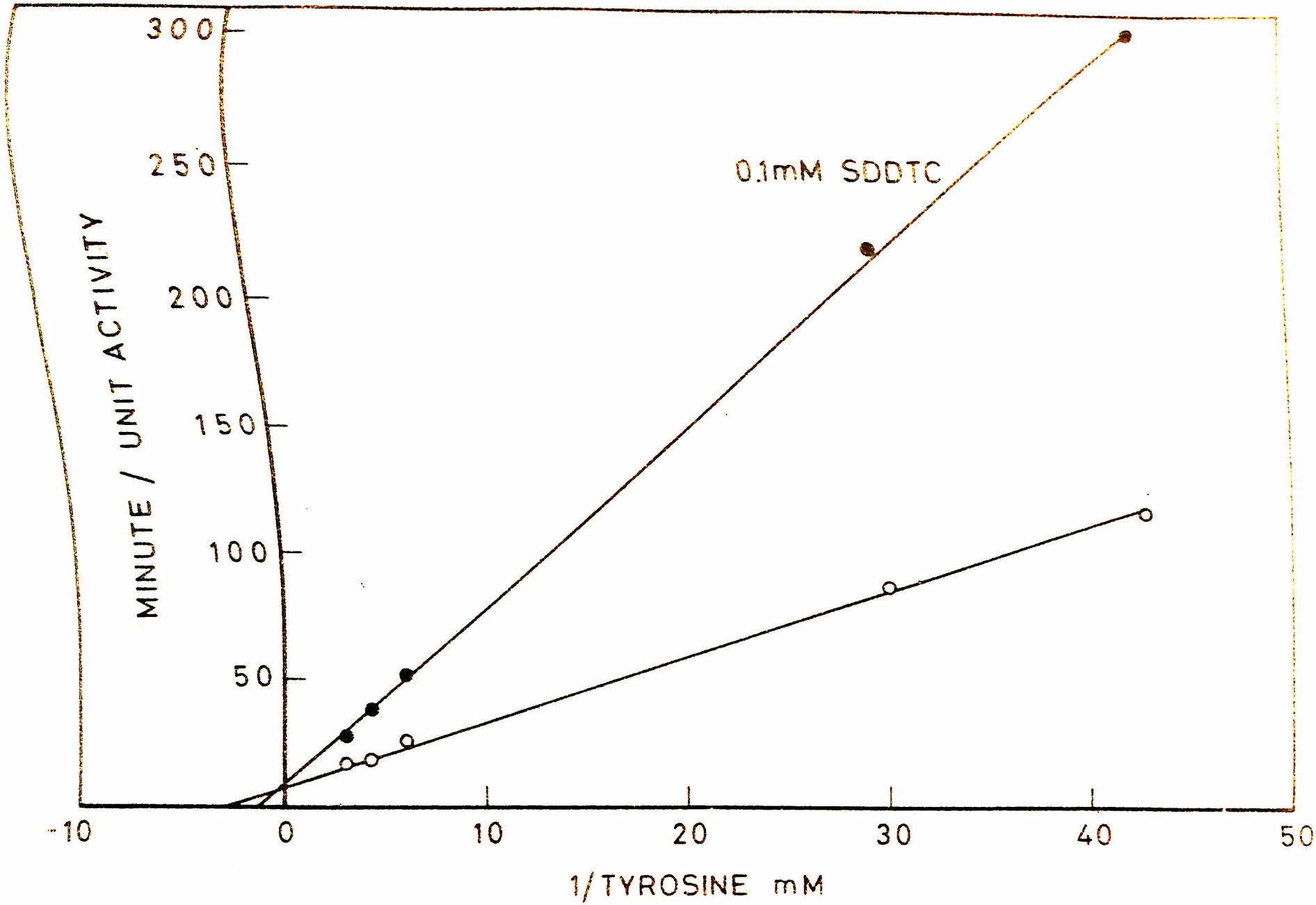


Fig.25 EFFECT OF 0.1 mM SDDTC ON LINEWEAVER-BURK PLOT OF TYROSINASE

TABLE - XXIa : Tyrosinase enzyme constants and SDDTC inhibition constants.

Tyrosinase control	: k_m	= 0.3249 mM
	V_m	= 0.1242 u/min
Tyrosinase in 0.1 mM SDDTC	: k_p	= 6.670 mM
	V_p	= 0.1194 u/min
Inhibition constants	: $k_{0.1\ mM}$	= 4.913×10^{-3} mM
	k'_m	= 164.5 mM

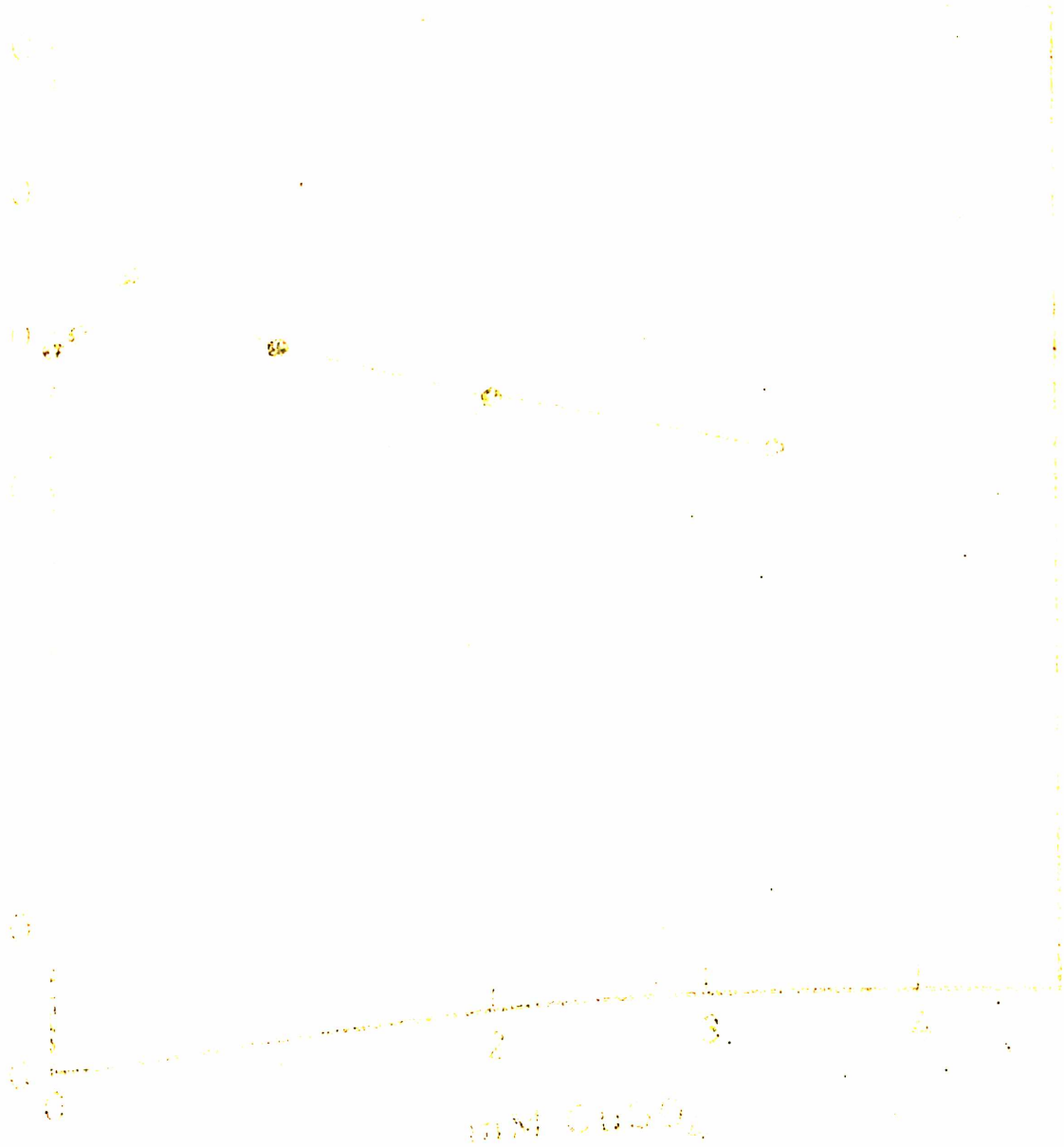


TABLE - XXIb : Effect of 0.33 mM $CuSO_4$ on CS_2 and SDDTC treated rat liver Tyrosinase activity (u) per 0.1 ml of 10% liver homogenate.

Mode of $CuSO_4$ Treatment.	ALiver Tyrosinase Activity				
	Control	Acute treatment		Chronic treatment	
		CS_2	SDDTC	CS_2	SDDTC
Without $CuSO_4$	0.37	0.32	0.15	0.16	0.22
With 0.33 mM $CuSO_4$	0.40	0.33	0.21	0.19	0.24

homogenate from chronically treated rats were not as impressive as that of acute treatment. In case of chronically treated rats, carbondisulphide treatment showed about 57% decrease in the tyrosinase activity which became restored to 52% of the initial activity thus showing a recovery of about 5% activity. However liver homogenate from chronically treated rats with SDDTC did not show any change on degree of inhibition (Table XXIb).

C H A P T E R - V

DISCUSSION

Carbon disulphide (CS_2) due to its fat solvent properties, is extensively used in the manufacture of viscose rayon, rubber products, xanthates, resins, plywood adhesives and also in process like cold rubber vulcanization and froth floatation (Davidson et al, 1972). Because of its low boiling point CS_2 impregnates the working environment with its vapour, thereby causing the problem of pollution and serious health hazards (Ayer, 1975). The CS_2 toxicity is known to cause behavioural and neurological disorders starting from initial restlessness to coma (Davidson et al,1972). Similar effects were observed with acute CS_2 dosing in rats under present investigation. However, the chronic CS_2 administration caused only restlessness immediately after its injection. The chronic SDDTC showed effects similar to CS_2 except that its chronic treatment caused nasal bleading indicating severe damage to the nasal epithelium. The present observation that the rats showed loss of apetite and body weight, is in conformity with the earlier reports (Gondzik, 1971; Davidson et al, 1972; Sharma, 1980; etc.).

It is well documented that the CS_2 toxicity is centered around the interaction between CS_2 or its metabolite dithiocarbamate compounds (DTC) with proteins, carbohydrates, fats, coenzymes and trace-metals (Dinman, 1978; Eneanya et al, 1981; Neal and Halpert, 1982). According to Dinman (1978) approximately 90% of inhaled CS_2 is metabolized in the body. Various mechanisms have been put forward for CS_2 metabolism in the body. Pergal (1972) states that CS_2 while entering in the body avidly combines with amino groups of proteins, peptides and free amino acids to produce dithiocarbamate (DTC) which further gets converted to thiazolidines through cyclisation. The thiazolidines being strong metal-chelating agents, sequester essential metals. Many workers (Cohen etal, 1959; Sandell, 1959; Afonova, 1965; Eneanya et al, 1981; etc.) describe the CS_2 interaction with amino groups of peptides and proteins, leading to the production of DTC which is a potent chelator for Zn, Cu and other diavalent metals. According to McKenna and Distefano (1977) CS_2 produces DTC by interacting with biogenic amines in the tissue and during the process utilises available Zn and Cu.

The primary aim of the present investigation was to study the effects of CS_2 on the Zn and Cu metalloenzymes. The enzyme selected for present investigation were alcohol dehydrogenase and alkaline phosphatase as Zinc-enzymes; histaminase, tyrosinase and uricase as cuproenzymes, aldolase as non-metalloenzyme from mammals. Since the CS_2 become metabolized to DTC, which is a strong metal-chelating agent, in the actual investigation sodium diethyl dithiocarbamate (SDDTC) was also included along with the CS_2. Experimental researches using laboratory animals have concluded acute damage to brain, liver, kidney and testes (Bond et al, 1969; Gondzik, 1971; Davidson et al, 1972; Dalvi et al, 1974; Sharma, 1980, etc.). Therefore, the present investigation was centered around the comparative screening of the effects of acute and chronic CS_2 and its metabolic analogue SDDTC in the brain, liver, kidney and testes.

EFFECTS ON ALDOLASE:

Scrutton (1973) divided aldolase in two general types (i) zinc metalloenzyme in microbes, and (ii) aldolase without zinc in higher plants and animals. In the non-zinc containing aldolase the ϵ $-NH_2$ group of

a lysine residue forms the active catalytic site. The present investigations reveal that the acute CS_2 dosing results in an initial increase followed by a decrease in liver and testes aldolase activity. On the contrary, the brain aldolase shows an initial decrease followed by a recovery of its activity. The kidney aldolase did not show any significant change in its activity. The acute and chronic SDDTC as well chronic CS_2 treatments of rats have been found to progressively inhibit the aldose activity in all the four tissues investigated presently. Since CS_2 on entering the tissue cells first become converted into DTC, it does not effect aldolase initially till the DTC is formed. The later binds first with metal ions present in high concentration in liver and testes (Scheinberg and Sternlieb, 1976; Prasad, 1976) and later with the ϵ-NH_2 group in the active site of aldolase as a secondary preference. The fact that inhibition of aldolase activity by DTC complexes is a secondary effect, is further supported by the observations that the chronic CS_2 and SDDTC administration results in progressive inhibition of aldolase in all the four tissues.

EFFECTS ON METALLOENZYMES:

Some metalloenzymes contain concrete metal binding sites that are important for their catalytic function and structural stability (Kirchgessner and Roth, 1980). The Zn and Cu metalloenzymes selected for the present investigation fall in the catagories of enzymes requiring metal ions for their catalytic action.

ZINC METALLOENZYMES:

Alcohol dehydrogenase (ADH) and alkaline phosphatase (AP) were the Zn containing metalloenzymes selected for the present investigation. Both these enzymes contain 4 g atom Zn/mole, of which two are essential for their catalytic activity while additional two Zn atoms stabilize the protein structure (Drum et al, 1967; Vallee, 1978). In experimentally caused Zn dificiency, both these enzyme show quick loss of their activity (Riordan and Vallee, 1976; Kirchgessner and Roth, 1980; etc.).

Alcohol Dehydrogenase:

The assignment of two Zn atoms as structural stabilizer for ADH is based on the fact that they are

completely surrounded by protein and are inaccesible to chelating agents, whereas the catalytic Zn atoms are bound to catalytic site in proximity to the co-enzymes binding site. According to Vallee (1978) the strong metal chelating agents like 1,10-phenonthroline binds to the catalytic Zn and eventually forms complex with ADH at neutral pH, thereby abolishing the catalytic function of the enzyme. The present observation reveals that both CS_2 and SDDTC lead to initial inhibition of ADH in all the four tissues investigated, but followed by a partial recovery of ADH activities in case of acute CS_2 dosing. The decrease followed by partial recovery in ADH activity may possibly be due to unstable catalytic Zn-CS_2 complex formation. The Zn-CS_2 later dissociates by further reaction with biogenic amines releasing catalytic Zn thereby restoring ADH activity as suggested by McKenna and Distefano (1977) in case of cuproenzymes. The inhibition of ADH followed by its activation in case of SDDTC acute dosing also follows the same mechanism suggested by McKenna and Distefano (1977). According to these authors the DTC compound form an unstable complex with catalytic metal ion and undergoes rapid dissociation in presence of already formed stable metal-DTC

complexesand coenzymes, leading to the partial reactivation of enzyme. The present investigation further reveals that the ADH inhibition is more intense in case of chronic CS_2 and SDDTC treatment.

Alkaline Phosphatase:

Like ADH, alkaline phosphatase (AP) also contain 4 g atom Zn/mole of which two are structure stabilizer and two have catalytic function (Kirchgessner and Roth, 1980). The catalytic Zn is easily complexed and removed by chelating agents like DTC (Eneanya et al, 1981). The catalytic Zn ions are bound to tyrosine residue located in close proximity to phosphorus binding serine residue (Scrutton, 1973). The substrate molecule binds to catalytic Zn thereby becomingoriented for enzymatic attack.

In the present investigation, the AP activity does not show any significant change after acute CS_2 administration but becomes inhibited after acute SDDTC dosing in all the four tissues studied. It appears that in case of AP the CS_2 does not complex with Zn as such but its metabolic product DTC forms chelate with the catalytic zinc, hence inhibits AP activity. This is further supported by the fact that AP activity

becomes inhibited by chronic CS_2 and SDDTC dosing, where CS_2 formed DTC or injected SDDTC perpetually forms stable Zn-DTC complex by removing the Zn from catalytic site (Kneanya et al, 1970). The mechanism of action of CS_2 thus appears to be through DTC formation, which inturn removes the catalytic zinc to form stable complex.

The in vitro studies in the present investigation reveals that CS_2 and SDDTC caused irreversible inhibition of AP and non-competitive inhibition of ADH. The addition of Zn^{++} to CS_2 and SDDTC inhibited ADH and AP, reactivates ADH activity by more than 45% but not of AP. This observation further support the earlier observations that the inhibition of AP is due to the removal of catalytic Zn by DTC formed from CS_2 or SDDTC to form a stable complex thus making the Zn unavailable for enzyme activity. On the contrary, the restoration of ADH activity by adding supplementary Zn indicates different mechanism of action by CS_2 product DTC and SDDTC. The possible mechanism appears to be that catalytic Zn is firmly bound to catalytic site of ADH and DTC or SDDTC forms an enzyme-Zn-DTC unstable complex,on addition of supplementary Zn^{++}, the DTC dissociates from enzyme-Zn-DTC complex and forms stable complex with free Zn ions, hence ADH activity is restored.

COPPER METALLOENZYMES:

Malkin (1973) in his review on copper containing oxidases, describes three forms of copper in the cuproenzymes: (1) Cu(I) giving intense blue colour to the enzyme, Cu(II) having low visible extinction and Cu(III) electron paramagnetic resonance non-detectable. According to this author, the blue-cuproenzymes have atleast 4 g atom Cu/mole of enzyme whereas non-blue cuproenzymes or oxidases contain 1 or 2 g atom Cu/mole of enzyme. According to this classification the three enzymes selected for present investigations, are non-blue cuproenzymes as the copper in histaminase is 2 g atom/mole, in tyrosinase is 1 g atom/mole and in uricase is 1 g atom/mole. The effects of acute and chronic exposures of both CS_2 and SDDTC on these three enzymes are varied and so also their reactivation with copper supplementation <u>in vitro</u>. Therefore the effects of CS_2 and SDDTC on these enzymes have been discussed separately.

<u>Histaminase (Diamine oxidase)</u>:

Histaminase or diamine oxidase, catalyses the oxidation of diamine and also of some monoamines (Bradsley et al, 1970). It contains copper ions which

do not change valancies as oxidation proceeds and require pyridoxal phosphate for substrate binding. According to Bradsley et al (1970), histaminase has a carbonyl group proton acceptor site responsible for substrate binding and a copper containing electron acceptor site for substrate oxidation. The histaminase becomes completely inhibited by a carbonyl group reacting agent which block substrate binding site or a strong metal chelating agent removing Cu^{2+} metal (Bradsley, 1970; Malkin, 1973; Mondovi et al, 1967; Sharma, 1980; etc.).

The present investigations have revealed that the histaminase activity becomes inhibited with the acute treatment of CS_2 in all four tissues. On the contrary, the enzyme activity shows a slight increase in brain and testes after acute treatment with SDDTC. However, the liver histaminase activity does not show any significant change after acute SDDTC treatment. Earlier McKenna and Distefano (1977) reported that CS_2 forms dithiocarbamate compound by interacting with biogenic amines in the tissues utilizing Cu metal in the process. The present observation that CS_2 inhibit tissue histaminase activity may be explained on the basis that when acute dose of CS_2 is given, it is

metabolized to dithiocarbamate by utilizing Cu^{2+} at oxidizing site of histaminase, thus inhibiting the histaminase activity. The same may hold true for the inhibition of histaminase in kidney and testes during the chronic CS_2 treatment. This is further supported by the fact that both chronic and acute SDDTC treatments do not inhibit brain, liver and testes histaminases as the catalytic Cu^{2+} cannot be sequestered by DTC (Mondovi et al, 1967). However, the inhibition of histaminase in kidney cannot be explained. Earlier, Mondovi et al (1967) described that the interaction between dithiocarbamate (DTC) and histaminase does not lead to removal of Cu and the DTC-Cu binding could be reversed if already formed DTC-Cu complex is removed. From the above it can be concluded that when SDDTC enters in the body tissues it forms SDDTC-Cu complex with Cu available from non-histaminase source and the so formed Cu-SDDTC complex does not allow the SDDTC in presence of complex forming factor to remove copper from the oxidizing site of histaminase. This fact is further supported by the *in vitro* studies, where the complex forming factors are absent, the addition of SDDTC and CS_2 in pure enzyme does not affect the enzyme activity.

Tyrosinase:

Tyrosinase (phenol o-monooxygenase) catalyses two different reactions (i) hydroxylation of monophenols to o-diphenols (cresolase activity) and (2) oxidation of o-diphenols (catacholase activity). The catalytic activity of this enzyme is due to the coordinated copper at the active site (Martell and Khan, 1973). The copper in the tyrosinase can be easily removed by the strong metal chelating agents like DTC (Livitt et al, 1965; Mondovi et al, 1967; Truhaut et al, 1971; Stripp et al, 1969; Goodchield, 1969; Malmstron et al, 1975; McKenna and Distefano, 1977; Massoud et al, 1977).

The present investigation reveals that the tyrosinase is completely inhibited by acute and significantly by chronic intake of CS_2. It appears that CS_2 inhibits the tyrosinase activity by two methods, (i) by removing Cu^{2+} from tyrosinase during DTC formation (Stripp et al, 1969; Massoud, 1977) and (ii) by reacting with pyridoxamine and making it unavailable for synthesis of pyridoxal phosphate (Vasak and Kopecky, 1967; Dussault and LePage, 1976; Eneanya et al, 1981) a coenzyme required for tyrosinase activity. This conclusion is further supported by the fact that though acute SDDTC

dosing significantly decreases tyrosinase activity but the inhibition is not complete as in case of CS_2. The effect of chronic CS_2 dosing is similar to that of acute and chronic SDDTC treatment except in case of brain and testes. The brain and testes though show a significant decrease in tyrosinase activity at second hour after SDDTC treatment but the activity is partially restored later on. The non-recovery of tyrosinase activity in case of chronic CS_2 and SDDTC treatment may be explained on the basis that CS_2 formed DTC and SDDTC by forming complex with catalytic Cu^{2+} inactivate tyrosinase (Truhaut et al, 1971; McKenna and Distefano, 1977; Malmstron et al, 1975; Stripp et al, 1969; Massoud, 1977; etc.) but some of the inhibition is reversed by the presence of excess DTC-Cu complex formed and the pyridoxal phosphate present in the tissue (Mondovi et al, 1967).

<u>In vitro</u> studies, in the present investigation, reveals that SDDTC inhibition of tyrosinase activity is competitive and the enzyme activity can be restored partially by adding Cu^{2+} ions. The present results are in agreement with the reports of Mondovi et al (1967) and Malmstron et al (1975) in that the reactivation of enzyme activity is obtained by the addition

of suitable amount of Cu^{2+}. Further the recovery of tyrosinase activity by seventh hour after the acute SDDTC treatment, can be explained on the basis of conclusion drawn by Mondovi et al (1967). According to these workers DTC-Cu binding is partially reversed if the DTC-Cu complex was not removed and also in presence of pyridoxal phosphate (Malmström et al 1975).

Uricase:

Uricase catalyses the conversion of uric acid to allantoin and CO_2 without forming any free radical intermediate (Mahler, 1963; Martell and Khan, 1973). The enzyme contains Cu which is essential for the catalytic activity (Mahler et al, 1955) and does not undergo redox reaction. However, Malmström et al(1975) have expressed doubts regarding the universal presence of Cu in this enzyme. Reports on the effect of metal chelating substances on the uricase are scanty.

Mahler et al (1955) reported that diethyl dithiocarbamate though having high chelation affinity for Cu, does not inhibit uricase and even enhances the activity of certain preparations. The present investigation also agree with observation of Mahler et al (1955)

in case of *in vitro* studies and the effects of chronic dosing of CS_2 and SDDTC that these two compounds do not inhibit uricase activity but enhances the activity in brain and testes. On the contrary, the present results do not agree with the above authors in case of acute dosing of CS_2 and SDDTC which certainly leads to the highly significant decrease or complete loss of uricase activity in the four tissues studied under the present investigation. This suggest that DTC-Cu binding is highly reversible at low concentration of CS_2 or SDDTC as was the case in the chronic treatment. However, at higher concentration of chelating agent, enzyme activity becomes inhibited possibly because of the inhibition of factors for the reversion of DTC-Cu complexes.

Zn AND Cu LEVEL:

Bruin (1976) in his review on the effect of CS_2 on mineral metabolism has described the accumulation of Zn and Cu in response to experimental CS_2 exposure in brain, liver and kidney (Wronska-Szpakowa, 1961; Lukas et al, 1974; Djuric et al, 1967).

According to Scheel et al (1960) the chelation of metal ions with DTC results in displacement

of essential metals among the tissues resulting in diminished availability of these ions for essential cellular metabolic reactions this metabolic disfunction (See Bruin, 1976). The present investigation also shows an overall increase in the concentrations of Zn and Cu in the tissues of brain, liver, kidney and testes simultaneous to the inhibition of Zn and Cu metalloenzymes. These findings are inagreement with the earlier reports mentioned above. Further, the accumulation of Zn and Cu should be in the form of metal-DTC complexes whose rate of translocation from inside the cells to out side is very slow (Bruin, 1976).

The present investigation leads to the conclusion that CS_2 inhibits enzyme activity by three mechanisms, viz. (1) by binding with the active site of an enzyme directly as in case of aldolase, (2) by binding directly with metal located at catalytic site of a metalloenzyme to form DTC compound as in case of ADH and (3) by producing DTC, which being strong Zn and Cu chelating agent, removes metal ions from catalytic site to form metal-DTC complex as in case of AP, histaminase, tyrosinase and uricase. The nature of inhibition by CS_2 or DTC

varies according to the binding of metal at catalytic site. The inhibition may be either irreversible as in case of AP, histaminase and uricase or reversible as in case of ADH and tyrosinase. Further, the reversible inhibition may be either competitive like tyrosinase or non-competitive as in ADH.

C H A P T E R - VI

SUMMARY

Carbon disulphide (CS_2) a highly toxic compound used in many industries, impregnates the working environment with vapour due to its highly volatile nature. These vapours cause serious problems of pollution and health hazards. The CS_2 toxicity leads to behavioural, physiological and clinical disorders. A survey of literature reveals that this compound causes extensive damage to brain, liver, kidney and testes. It is well documented that CS_2 upon entering into the body reacts with amino group of proteins, polypeptide and free amino acids and produces dithiocarbamates (DTC). The DTC being strong metal chelating agent, removes Zn and Cu from the tissue system and forms stable complexes. Since Zn and Cu forms the catalytic components of many important metalloenzymes, these enzyme become inactivated due to removal and unavailability of these metals.

The present investigation was aimed to study the effect of CS_2 and sodium diethyldithiocarbamate (structural analogue of DTC) on one non-metalloenzyme, two zinc metalloenzymes and three cuproenzymes in brain, liver, kidney and testes. The enzymes selected were

aldolase (known to contain Zn in microbes but not in mammals), two zinc metalloenzymes alcohol dehydrogenase and alkaline phosphatase, and three cuproenzymes histaminase (diamine oxidase), tyrosinase (diphenol o-mono-oxygenase) and uricase. The investigation was divided into three parts viz. (1) evaluation of the effects of acute and chronic exposure of CS_2 and sodium diethyldithiocarbamate (SDDTC) on the six selected enzymes in albino rats, (2) determination of Zn and Cu level in the four tissues of chronically treated rats selected in relation to altered activities of the above mentioned six enzymes, and (3) to determine whether the Zn and Cu supplementation restores the activity of their respective metalloenzymes <u>in vitro</u>.

Analysis of the effects of CS_2 and SDDTC exposures has lead to the following results:

(1) <u>Aldolase:</u> The acute CS_2 treatment of rats leads to inhibition of aldolase activity in liver and testes. The brain aldolase shows an initial inhibition followed by recovery of its activity. The kidney aldolase remain unaffected. The chronic CS_2 as well as acute and chronic SDDTC treatment results in inhibition of aldolase in all the four tissues studied. The result

indicates that dithiocarbamates formed from CS_2 binds with ϵ NH_2 group present in the catalytic site. The aldolase inhibition is irreversible.

(2) Alcohol dehydrogenase: The acute and chronic CS_2 and SDDTC treatment inhibits the alcohol dehydrogenase (ADH) activity in all the four tissues selected but the activity becomes partially restored in case of acute treatment with both CS_2 and SDDTC and forms unstable complex with Zn at catalytic site and dissociates again to form DTC in case of CS_2 and stable complex with Zn (from other source) in case of SDDTC. The inhibition of ADH is non-competitive and is partially reversed when supplementary Zn^{2+} is added *in vitro*.

(3) Alkaline phosphatase: The alkaline phosphatase (AP) activity does not alter significantly by acute CS_2 treatment but becomes inhibited on acute treatment with SDDTC. The chronic treatment of rats with both CS_2 and SDDTC results in inhibition of AP. The inhibition is irreversible. It appears that CS_2 does not react directly with catalytic Zn of AP but its metabolic product DTC removes the Zn from catalytic site and forms stable Zn-DTC complex.

(4) Histaminase: The treatment of CS_2 inhibit histaminase in all the four tissues. On the contrary, SDDTC does not induce any significant change in histaminase activity. This differential action may be due to the fact that CS_2 binds directly to catalytic Zn to metabolise into a Zn-DTC complex directly. However the dithiocarbamate compounds are not able to form a stable complex with catalytic Cu in presence of Cu-DTC complex formed from non-histaminase source.

(5) Tyrosinase: Both acute and chronic CS_2 exposure inhibit tyrosinase. The SDDTC treatment also inhibit tyrosinase activity significantly. The inhibition with CS_2 is almost complete as compared to SDDTC. The inhibition pattern indicates that the CS_2 inhibits tyrosinase activity in two ways (1) by removing Cu^{2+} from catalytic site through Cu-DTC complex formation, and (2) by reacting with pyridoxamine to inhibit pyridoxal phosphate synthesis essential as co-enzyme for tyrosinase. The inhibition of tyrosinase activity is competitive and is reversed on addition of supplementary Zn *in vitro*.

(6) Uricase: Uricase activity is not inhibited in case of chronic CS_2 and SDDTC treatment as well as *in vitro* studies. On the contrary, acute CS_2 and SDDTC exposures inhibit uricase. The experimental results indicate that lower concentrations of CS_2 and SDDTC do not inhibit uricase. However, the acute concentration of CS_2 and SDDTC induce irreversible inhibition.

(7) Zn and Cu Levels: The present investigation shows that there is accumulation of Zn and Cu in brain, liver, kidney and testes. This accumulation may be attributed to the formation of metal-DTC complexes which inturn become bound to fatty compounds due to their high solubility in fats.

C H A P T E R - VII

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