T H E S I S

SOME BIOCHEMICAL STUDIES OF CARBONDISULFIDE TOXICITY IN RATS

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SUBHASH CHANDER SHARMA

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BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE, PILANI (RAJASTHAN).

C E R T I F I C A T E

This is to certify that the thesis entitled "SOME BIOCHEMICAL STUDIES OF CARBONDISULFIDE TOXICITY IN RATS" and submitted by SUBHASH CHANDER SHARMA ID No. 76S87501, for the award of Ph.D. degree of the institute, embodies original work done by him under my supervision and guidance.

Pilani.

Date :

(S.M. CHOPRA)
Assistant Professor.
Department of Pharmacy.

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LIST OF PUBLICATIONS

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ABBREVIATIONS

CS_2	Carbondisulfide
CA	Catecholamines
DA	Dopamine
NE	Norepinephrine
5-HT	5-hydroxytryptamine
MAO	Monoamine Oxidase
HMP	Hexose monophosphate Shunt
de-dtc	diethyl- dithio carbamate
dm-dtc	dimethyl dithio carbamate
edtc	ethyl dithio carbamates
G-SH	Reduced glutathione
FDPase	Fructose, 1-6 diphosphatase
ATPase	Adenosine Triphosphatase
SDH	Succinic dehydrogenase
ANSA	Alpha amino napthyl - sulfonic acid
TTC	2,4,6 - Triphenyl Tetrazolium Chloride

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In the biosphere, pollution is caused by substances that exist in nature i.e the whole range of semisynthetic or synthetic substances like molasses, polymers, industrial solvents etc. The industrial pollutants are spread over through deliberate or careless handling of raw materials or waste products. Although nature is a readymade laboratory to deal fully with the first category of substances spread over by physical and natural resources, the other category of pollutants pose a complex problem since nature cannot deal fully to remove them. The rapid introduction of more and more new compounds due to industrialization has further complicated the problem. The ecotoxicological problems arising out of this situation have demanded scientific probe in this direction.

Carbondisulfide (CS_2), an inorganic compound capable of dissolving many organic substances, is a very useful solvent. Its main use in the industry is as a chemical intermediate in the xanthation of cellulose in the viscose rayon manufacturing and as a fumigant. Besides, it is encountered most frequently in the chemical laboratories where it is extensively used as a solvent. The low boiling point of CS_2 impregnates the working environment with its vapours, causing thereby the problem of pollution and a serious health hazard to mankind. The main risks of CS_2 are by vapour inhalation and fire explosion. Carbondisulfide is reputed for

for its central nervous system depressant effects i.e. reduction of motor activity, lethargy etc. Coma and death follow excessive inhalation, though instances of such a serious consequence are very rare. However, persistent alteration of central and peripheral nervous system functions have been reported to occur as a result of single massive over exposure. A series of less severe exposures (Teisinger, 1972), may also result in similar alterations. Symptoms range from headache, fatigue and listlessness to loss of memory, anorexia and reduction in sexual function. In more severe cases, cardiovascular disorders, hallucinations and other symptoms resembling psychosis have been reported. Of these, the more interesting manifestations of chronic CS_2 intoxication is loss of reflexes; giving first indication of nerve damage. (Lieben, 1974).

The workers who are exposed to very high concentrations of CS_2 have been reported to manifest similar neurological symptoms and cardiovascular disorders (Seppalainen et.al. 1966 ; Tiller et.al. 1968). These symptoms and signs have been known to be directly related to the intensity and period of exposure to the intoxicant. Only few attempts have been made so far to elucidate the mechanisms of CS_2 hazards in a living system. These studies have also not clearly shown any relationship between the mechanism of CS_2 intoxication and biochemical picture of an animal. Therefore, In the present

work, some biochemical modifications due to CS_2 toxicity have been studied to correlate the mechanism of CS_2 toxicity with carbohydrate metabolism abberrations. In the present work various biochemical parameters dealt with, using rats as test organisms are :

1. Alteration in carbohydrate metabolism and its enzymes in liver and brain.

2. Alterations in tissues biogenic amines level under CS_2 toxicity.

3. Interrelationship of tissues biogenic amines with mono-amine oxidase activity and reduced glutathione (G-SH).

4. Alterations in trace element metabolism under the stress of carbondisulfide.

The thesis is divided into six chapters. First chapter deals with the review of the existing literature and the second with the materials and methods adopted for carrying the biochemical studies. The third chapter includes the results and observations while the fourth chapter is devoted to discussion. The fifth chapter includes a summary of the work and conclusions drawn from this study. The sixth chapter lists relevant references pertaining to the present work.

1.1 EPIDEMIOLOGICAL STUDIES OF CARBONDISULFIDE TOXICITY :-

The problem evolved on the human health from carbondisulfide toxicity gained recent extensive scientific impetus from the presentation of toxicological data of carbondisulfide at the International Symposium at Prague (1966). A major recommendation of the Symposium was to conduct the comprehensive epidemiological studies on the morbidity and mortality of workers exposed to toxic vapours in the viscose rayon industry for several years (Brieger, 1967; Viglaini, 1967).

Cavalleri et al (1966), on the endocrinological findings in the young workers exposed to carbondisulfide, reported an increased urinary excretion of 17- ketosteroids and 17-hydroxy-steroids. Savic (1967) reported pathological changes in the eyes caused by carbondisulfide. Tolonen et al (1975) found the disturbed occular microcirculation and suggested that occupational exposures to carbondisulfide were not the basis of the intensity of physiological abnormalities but the severity of manifestations of vasculopathy was related to the quantity of CS_2 in environment. The safety margin of CS_2 recommended by American Conference of Governmental Industrial Hygeinists is 20 ppm. The incidences of death by coronary heart disease in males due to exposure of carbondisulfide were higher than that deaths in unexposed men due to this abnormality and other unexposed workers of the same

industry (Tiller et al, 1968; Herneberg et al, 1970; Tolonen et al , 1975). In an another study Herneberg and Norman (1973) confirmed the finding in both sexes. The risk of death rose with increasing exposure. These findings confirmed the earlier mortality studies, and strongly supported the hypothesis of causal relationship between CS_2 exposures and coronary heart diseases.

Martinez and Farina (1969), in a clinical examinations of seventy five workers exposed to high levels of CS_2 for many years in viscose factories reported 83 percent workers having vasculopathy in encephalic region, 22 percent having disturbance of renal activity, 5 percent having renal hypertension, and 7 percent of diabetes mellitus. Higher incidences of angina pectoris and hypertension with electrocardio graphic abnormalities were also observed by Herneberg et al (1970). The incidence of diabetes mellitus among workers exposed to the risk of carbondisulfide poisoning were more than the normal persons (Ferrero 1969). Szott, et al (1969) estimated the urinary excretion of uropepsion and reported an increase of uropepsin from 46-92 units per hour, the latter value being close to that observed in cases with pepsin ulceration. These studies further, showed some correlation between uropepsin elimination and duration of exposure to carbondisulfide.

Hanninen (1971), performed a battery of psychological tests to three different groups of workers, one with carbondisulfide poisoning, second exposed to carbondisulfide but without known poisoning and the third group consisted of unexposed workers. A prominent and significant reduction in performances involving speed vigilance, mannual dexterity and intelligence was noticed in the first two groups. The group exposed to only low CS_2 concentration showed impairment but the changes were less severe in comparison to poisoned group. Further, in this work the poisoned group could reliably be distinguished from the exposed workers in two ways i.e. latent and manifest. The latent and manifest poisoning differed not only in intensity but also in quality as, latent poisoning was characterised by traits indicative of depressive mood, slight motor disturbances and intellectual impairments whereas clinically manifested poisoning resulted in lowered vigilance, diminished intellectual activity, diminished rational control, retarted speed and motor disturbances. Seppalainen et al (1974) also reported the neurotoxicity of long term exposure to CS_2 by measuring the maximum motor conduction velocity and conduction velocity of slower motor fibers.

Herneberg et al (1969) studied plasma and erythrocyte changes in zinc and magnesium levels in Finish and Norwegian workers exposed to CS_2 to study the racial variations. The

magnesium levels were slightly reduced in erythrocytes whereas plasma magnesium level increased in exposed Finnish workers while, zinc concentration is lowered slightly. No such differences in plasma and erythrocyte magnesium and zinc were found in Norwegian men by these workers. Carbondisulfide also caused depletion of serum zinc by an increase in rate of zinc excretion, and an increase in all serum protein fractions. The effects were temporary and improved on cessation of exposure (EI' Gazzar et al 1973).

Herneberg et al (1971) examined blood lipids, glucose tolerance, and plasma creatinine in viscose rayon industry workers as a part of planned study of diseases. This study included workers exposed to an average available CS_2, 20-40 ppm in 1950's and 10-30 ppm in 1960's. The only difference reported between the exposed workers and matched controls were in fasting glucose; the differences bear a direct correlation with the exposure time and exposure index and in mean plasma creatinine which was higher in exposed group.

Goto et al. (1971) selected exposed workers from 11-Japanese viscose rayon plants and reported a higher blood sugar levels trend in advance age group of exposed workers. A corticosteroid priming glucose tolerance test indicated a subclinical defect in carbohydrate metabolism resembling a mild diabetogenic action in some apparently healthy carbondisulfide exposed workers.

The incidences of prevalence of retinal microaneurisms which depended on the exposure to carbondisulfide were also reported to be increased. Higher blood sugar levels with increasing grade of microaneurisms had been reported. Unfortunately no information to the intensity of carbondisulfide exposure and its relation to microaneurisms has been reported.

Mancuso and Locke (1972) studied social aspects of workers in CS_2 environment at one of the rayon factory and found an excess of suicides in workers first employed between the years 1938 and 1948. In addition to an increased cases of recorded suicides, there were a number of deaths recorded due to other causes which strongly resembled suicides.

Sakurai (1972) reported temporary increase of blood pressure in a Japanese viscose rayon plant for more than ten years and found reduction of blood pressure, when workers were removed from carbondisulfide environment to an improved environment. Prevovska and Zvolsky (1973), reported that continuous exposure to carbondisulfide could make the persons as invalid and deaths had been due to organic complications of vascularsclerosis. They therefore, interpreted CS_2 as a possible atherogenic agent.

Murashko (1974) observed the deleterious effect of carbondisulfide on digestive organs of workers at viscose fibre plant and suggested a special diet for workers exposed to carbondisulfide.

Herneberg and Tolonen (1976) reported that the altered conditions with the excess of CS_2 could bring about reversible risk for future fatal attacks of coronary heart diseases which were minimized by removing the persons from CS_2 exposure. This study, therefore, reflected that CS_2 exert its direct toxic effect on myocardium. Andrazewska et al. (1976) examined visual space localization for upper and lower extermeties and the trunk in man. The neuropathological action of CS_2 on these organs and parts of the body was attributed again to its direct manifestations in these body organs. Sugimoto et al. (1976) reported temporary retinopathy due to CS_2 in workers exposed for a long duration which disappeared or improved after cessation from CS_2 environement.

The incidence of angina and the increase of blood pressure i.e., the diastolic and systolic, were significantly higher among the exposed workers and race dependant because it was shown in this study Finish workers were more prone to CS_2 toxicity than Japanese. The E.C.G. studies showed that the left ventrical ejection time was shortened and isovolumic contraction time was prolonged in workers exposed industrially to carbondisulfide for a period of eighteen years (Franco, 1976). The possible mechanism of this cardiac defect was attributed to the interference of CS_2 with Kreb's Cycle or with catecholamine metabolism.

Gullko and Pikisllskaya (1977) revealed further, that prolonged contact with CS_2 promoted the development of cardiovascular disorders like unstable arterial pressure with tendency to hypertension, an increase of propagation rate of the pulse wave in elastic blood vessels, dilation and intensification of the aortal shadow. Disorders of the lipid metabolism are a possible precondition for the development of early atherosclerosis. Therefore, they suggested that prolonged effect of carbondisulfide may lead to the development of early atherosclerosis. Tarlov et al. (1977) reported that cardiac disorders depended on the functional condition of the nervous system and were nonspecific. They also found typical neurotic functional cardiopathy and dystrophic changes in 521 workers of viscose rayon factory, exposed to CS_2. For these patients they recommended, beta-blocking agents and tranquilizers.

1.2 EXPERIMENTAL STUDIES OF CS_2 TOXICITY :-

1.2.1 HISTOLOGICAL STUDIES OF CS_2 TOXICITY :-

Carbondisulfide poisoning achieved by administering CS_2 in vegetable oil for three weeks did not produce much deformation but a considerable restorative process in somatic tissue was observed (Klishov et al. 1970). However, Barilyerk and Vasileva (1975) studied the effect of small concentration of CS_2 and hydrogen sulfide on intrauterine development of rats. They reported that the exposure of female rats to CS_2 before and

during gestation was lethal to embryos at the pre and post implantation stages. The effect of CS_2 on embryonic stage was also noticed when male rats were inhaled with CS_2 before mating with untreated females. The embryonic abnormality due to CS_2 was suggested to the defect caused in the genitourinary and bone systems of embryo. Distrubances in ossification, blood formation and dystrophic changes in liver and Kidneys were also observed.

Gondzik (1976) reported irreversible changes in microstructure of gonads and in seminiferous tubules of testis by CS_2. However, he observed that the concentration of CS_2 is an important factor as there were no alterations in the testicles and kidneys of the rat after exposure to lower concentration vapors of air for 20 weeks and in man working in an atmosphere of CS_2 containing 0.02- 0.06 mg CS_2/ liter.

Seppalainen et al. (1976) examined the electrophysiological findings in rat given chronic carbondisulfide inhalation. The development of neuropathy was monitored biomonthly by measurement of motor conduction velocity in sciatic nerve. A slight but statistically significant slowing of motor conduction velocity was observed on second week. No further reduction occured during the next three weeks but after 8 to 10 weeks the difficulties in voluntary control of hind limbs

with marked slowing of mortor conduction velocity was demonstrated. However, permanent alteration in motor conduction velocity did not occur in animals exposed to carbondisulfide for five weeks.

Greenberg (1977) reported the ulcer formation at the site of injection of CS_2 subcutaneously, which increased in size after the last injection. The lesion seen with the first or second dose caused the infiltration of neutrophils in the epidermis around the ulcer and lateral dermal invasion from ulcer margin by hyperplastic epidermal cell masses.

Zajaczek et al (1977) explored the effects of CS_2 on chromosomes structure in vitro and mitotic capability of leukocytes in man and rat. A complete inhibition of mitosis in the cultures of peripheral blood leukocytes and some structural disturbances in chromosomes were observed. Solecka et al . (1977) reported a significant increase in the agglutination test of leukocytes in rabits breathing carbondisulfide vapors for many days. Mihalache et al.(1977) reported the myocardial lesions similar to those in myocardial eschemia without vascular lesions whose severity was directly related to the increased exposure time. This experiment therefore, suggested that the lesions may result from a direct toxic action of carbondisulfide on myocardial fibres or from inhibition of myocardial circulation.

Juntunen Japani et al. (1977) showed demonstrable acetylocholinesterase activity by CS_2 intoxication which was distributed in myoneural junctions of both exposed and unexposed rats. In both groups, intense enzyme activity was localised at the level of postsynaptic membrane of myoneural junction. No enzyme activity has been seen outside the zone of myoneural junctions. In the terminal axons, signs of various degrees of degeneration were also reported to be present with widened synaptic clefts because of schwann cells interposition. Apparently, CS_2 poisoning primarily altered the presynaptic structures of myoneural junctions whereas postsynaptic side remained relatively intact.

Dietzmann & Lass (1977) reported myelin sheath disruptions within longitudinal track systems of the spinal cord, distructions of individual gangalion cells in all brain regions, and elective parenchymal necrosis in frontal and parietal cerebral cortices. The histochemical assays of monoamine oxidase, ATPase, glucose phosphatase, acetylcholineesterase and succinic dehydrogenase activities in the entire central nervous system revealed no change in the enzymes activities however, succinic-dehydrogenase and acetylcholinesterase revealed reduction in the activities within elective parenchymal necrotic region.

Wronska-Nofer et al. (1978) reported four fold elevation of total (free and esterified) serum cholesterol

with a simultaneous rise in phospholipids. In rabbits exposed to carbondisulfide and fed with atherogenic diet increase in cholesterol was 40 to 100 percent and triglyceride was 100 percent. Gross atheromatosis of the aorta and enchanced histo-coronarny lesions were observed as a result of CS_2 intoxication. Further, evidence was presented of an enhanced susceptibility of arterial wall to the atherogenic diet under the influence of chronic CS_2 intoxication with special reference to the individual regional susceptibility of arterial coronary vessels. But Verigin et al. (1978) reports on the development of atherosclerotic process under experimental conditions, showed that CS_2 did not alter blood serum cholerterol, phospholipids or triglycerides level in rabbits maintained on either normal or cholesterol supplemented diet. No morphological changes indicative of atheroscelerotic process were observed after CS_2 treatment and normal diet.

1.2.2. BIOCHEMICAL STUDIES OF CARBONDISULFIDE TOXICITY :-

1.2.2.1 ENZYMATIC CHANGES DUE TO CS_2 TOXICITY :-

In the recent years much of the interesting toxi-cological studies have been directed to the biochemical studies to underline the cause of various disturbances produced by carbondisulfide. Hockin et al. (1968) observed reversible biochemical changes in lens and retina of rabbit eyes which was related to the period of exposure to CS_2

of the animals. The ATP content of retina was reduced by 8 percent and 22 percent after 7 days and 14 days respectively. In the lens the activity of aldolase,glucose 6- phosphate dehydrogenase, hexokinase and glucokinase were decreased and those of malate dehydrogenase, glutamate- pyruvic transminase, sorbitol dehydrogenase were increased shortly after CS_2 treatment. However, the activities of malate dehydrogenase, sorbitol dehydrogenase had returned to normal level after 14 days of treatment. The irreversible changes had been suggested to be important for the genesis of cataract.

Bond and Matteis (1969) reported that 1 ml/kg oral dose of CS_2 persistently suppressed the O- dealkylation of p-nitroanisole and hydroxylations of phenobarabital and aniline in rats with rapid loss of hepatic cytochrome P- 450 content which resulted in an increased liver size and protein content. They proposed that the prolongation of phenobarbital sleeping time by CS_2 was due to suppression of this enzyme.

Bond et al. (1969) studied the interference of drug metabolism by CS_2 exposure. They suggested that a metabolite probably a dithiocarbamate, rather than free CS_2 itself, could be the causative agent for the liver microsomal depressions. Prior treatment with phenobarbital allow CS_2 to be metabolised more rapidly in animals and potentiation of hepatotoxicity of CS_2 was observed.Freundt and Dreher (1969) noticed the depression of N- demethylase and nitroreductase following CS_2

vapor inhalation at low concentrations independently of morphological changes in the liver. Prior equilibration of rats to air levels of CS_2 significantly prolonged hexobarbital induced hypnosis and depressed the microsomal oxidation or demethylation of drugs which was suggestive by the reduced urinary elimination of metabolities like 4- amino antipyrine and trichloroethylene after administration of antipyrine.

Mack and Freundt (1970) stated that inhibition of drug metabolising enzymes activity by CS_2 was reversible, regression of the depressed enzyme activity and excretion deficits occurred soon after the cessation of exposure. Small amount of inhaled CS_2 also produced depression in the rate of aminopyrine N- demethylation, as reflected by reduced 4- amino antipyrine excretion.

Caligami et al. (1972) reported that approximately 45% of labelled CS_2 (S^{35}) bound to the microsomes incubated with CN, and was released in a form which appeared identical to SCN. Incubation of microsomes with unlabelled CS_2 followed by incubation with CN^{14} also resulted in the recovery of what appeared to be C^{14} labelled SCN. A portion of 'S' released in microsomal metabolism of CS_2 had been suggested with- SH groups of cysteine residues in microsomal proteins to form hydrosulfide.

Murasko & Gubskis (1976) reported the modification of liver and small intestine enzymes in rats chronically poisoned with carbondisulfide. A decrease in the activities of alpha-keto- glutaric dehydrogenase and succinic dehydrogenase in liver mitochondria with disrupted membrane digestive function of small intenstine was indicated by reduction in dipeptidase activity and glucose absorption. The reduction in liver and intestinal enzymes activity were more severe with higher concentrations of CS_2 inhaled.

Tereshchenko et al. (1976) reported a decrease in erythrocyte--SH group level in persons poisoned by CS_2, whereas erythrocytic -SH group content increased in healthy workers, if exposed to CS_2 for a long time.

Ol'khovskaya and Kazan (1976) found the change in the activity of blood enzymes in patients with chronic CS_2 poisoning, especially in those with polyneuritis symptoms. The serum and erythrocyte levels of cholinesterase, aldolase, ceruplasmin and. total and reduced glutathione decreased as compared with those of normal subjects.

Sidorowicz et al.(1977) found a reduction in haemoglobin concentration, osmotic resistance and ATP level where as AMP, ADP and 2,3 diphosphoglycerate levels increased corroborating the haemolytic effect of CS_2 in human beings exposed chronically to

CS_2. The disturbances in the activity of key enzymes of anaerobic glycolytic cycle and decrease in ATP level caused hemolysis. The increased levels of 2,3 diphosphoglycerate showed a profound tissue hypoxia which was suggested to be a compensating mechanism. The dysfunction of anaerobic glycolysis was observed to reduce the erythrocyte function which was contemplated with neurological disturbances and premature atheromatosis.

Savolainen and Jarvisalo (1977), reported the specific binding of CS_2 metabolities to microsomal proteins in rat liver. Binding of CS_2 by microsomal proteins of phenobarbitone pretreated and untreated rats following an intraperitoneal injection of radiolabelled CS_2 indicated that the principal binding microsomal protein of CS_2 after its oxidative decomposition was cytochrome P- 450.

EL - Dessoukey et al. (1977) examined the serum enzyme changes associated with CS_2 hepatotoxicity in experimental animals. Rats treated with CS_2 showed elevation of serum GPT, GOT and serum alkaline - phosphatase. Rats treated similarly showed a slight to moderate rise in serum lactate dehydrogenase.

Jarvisalo and Savolainen (1977) found that the binding of S^{35} was considerably higher than that of C^{14}, three hour after injection. There was a significant fall in P- 450 whereas microsomal epoxide hydrolase activity of UDP glucuronyl transferase increased significantly in phenobarbitone pretreated rats.

They suggested that changes in P- 450 results from the binding of metabolities of CS_2 to cytochrome and its subsequent degradation. The increase in UDP glucuronyl transferase resulted probably from activated perturbation in the structure of microsomal membranes by metabolism of carbondisulfide.

Wagner et al. (1977) examined the levels of alpha-I antitrypsin in children from areas of different air pollution. They had found that lowering of alpha- I antitrypsin activity was associated mainly with high levels of SO_2 and CS_2.

Murashko et al. (1977), studied the effect of inhaled CS_2 on digestive enzymes. Carbondisulfide was found to disrupt enterokinase formation by small intestine, inhibition of enterokinase and alkaline phosphatase activity in large intestine which caused the appearance of mucus and nonalimentary proteins in the feces and disrupted regeneration process in the large and small intestines. Also it disrupted glucose absorption and inhibited the intestinal enzymes responsible for the hydrol ysis of disaccharide and polysaccharides.

Dalvi and Howell (1978), reported no alterations in the concentration of P- 450 cytochrome from liver microsomes by CS_2 malathione and parathione in phenobarbitone pretreated animals. But inhibition of oxidative desulfuration was recorded by the insecticides by microsomes from CS_2 treated animals was attributed to the prior binding with P- 450 of 'S' released from CS_2 as

confirmed from the results of spectral binding of SKF 525- A, parathione and malathione. Thus CS_2, parathione and malathione undergoes oxidative desulfuration in an analogous manner and the metabolism of the insecticides is impaired in the liver previously exposed to CS_2.

Merkur'eva, et al. (1978) observed the lysosomal and cytoplasmic enzyme changes in liver, kidneys, brain, aorta and blood serum, and carbohydrate levels in rabbits exposed to CS_2 for six weeks. These changes were indicative of possible neurotropic and hepatotropic activity of carbondisulfide.

Jarvisalo et al . (1978) concluded that the accelerated liver heme conversion to bile pigments caused by CS_2 and other sulfur containing drugs is related to the increased metabolism by oxidative desulfuration and ensuring the microsomal toxicity. The damage of the apoprotein of cytochrome P- 450 might have attained a reduced affinity for heme, thus, causing more liver heme being available for degradation.

1.2.2,2. LIPIDS AND CS_2 TOXICITY :-

Wronska- Nofer (1966) studied the role of CS_2 in inducing the disturbances in lipid metabolism. Rats were exposed to CS_2 following forced feeding of 0.2 mg of cholesterol C^{14}. The blood cholesterol levels at six, twelve, and twentyfour hours later were higher in CS_2 exposed rats. Differences were evident at 12 weeks and twice as great after 30 weeks. Carbondisulfide

increased the absorption of cholesterol from intestinal tract, and concomitantly speeded up its excretion via the bile.

Makarova et al. (1974) examined the effect of sulfur containing organic compounds on lipid metabolism in presence of CS_2. The level of cholesterol and beta-lipoproteins was higher in the blood of workers exposed to CS_2 but methionine, 2-mercapto-benzathiazole, tetramethyl-thio-carbamoyl disulfide caused the protection to the increased cholesterol level.

Wronska Nofer (1976) studied the aortic phospholipid synthesis in rat administered CS_2 toxic dose. The rate of incorporation of phosphate precursor into phospholipids was investigated in normal and in CS_2 intoxicated group of rat. The specific activity of P^{32} in phospholipids was measured in plasma and the aortic wall at various times after intravenous administration of 0.2 mcu phosphate. In CS_2 intoxicated rat the specific activity of aorta phospholipids was more than the normal rat. According to the suggestion of these workers, rise of aortic phospholipid activity probably, was not derived from increased blood phospholipid concentration but was attributed to an increased rate of phospholipid synthesis in aorta tissue.

Wronska- Nofer (1977) reported slight reduction in C^{14} steroid excretion after 4-C^{14} cholestenol administration at the end of seven months. This was primarily due to the decrease

of C^{14} bile acid excretion. However, $4C^{14}$ - cholesterol in feces was 35% higher in the exposed animals. But $1\text{-}C^{14}$ was higher in liver and blood of exposed rats after $1\text{-}C^{14}$ cholesterol administration. Laurman et al. (1977) also reported loss of body weight, increased cholesterol levels in aortic wall and skeletal muscles but no change in liver cholesterol level was observed.

Jarvisalo et al. (1977) commenting on the deleterious effects of subacute CS_2 exposure on mouse liver thought that liver copper and phospholipid levels were increased. They reported interestingly, that microsomal enzyme activities were partially or totally restored inspite of continued exposure to CS_2. The diene conjugation of liver phospholipids enhanced which indicated, therefore, that the CS_2 exposure damaged the membrane lipids continuously. The return of the enzyme activities to normal values had been attributed to decreased sensitivity to CS_2 metabolites or to increased synthesis of enzymes. The increased diene conjugation of liver phospholipids indicated that the lipid environment of membrane bound enzymes changed during subacute exposure to carbondisulfide.

Cunningham & Holt (1977) reported a small but significant decrease in the rate of utilization of plasma free fatty acid (FFA). This decreased availability of FFA probably led to the increased catabolism of aminoacids and a large increase in urea

production. Wesolowska et al. (1978) observed the changes in the content of free and esterified cholesterol in serum and aorta. These changes were similar as observed in experimental sclerosis.

Balabaeven and Tabakova (1979) reported that during the entire period of pregnancy, carbondisulfide inhalation raised the level of FFA in the liver of mothers and decreased the level of triglycerides and phospholipids both in mother and fetus . They concluded that in mothers the level of FFA was most sensitive to carbondisulfide whereas in fetus the level of triglycerides was more labile than cholesterol.

1.2.2.3. PROTEINS AND CARBONDISULFIDE TOXICITY :-

Woyke (1969) reported that DNA and RNA content of human liver was decreased in the cytoplasmic fraction and nuclei fractions by CS_2 injection. But there was a rise of DNA with the fall of RNA in neutrophils and lymphocytes.

Savolainen and Jarvisalo (1977) noticed the increased activity of rat brain acid proteinase. It accompanied with the changes in leucine C^{14} turnover as well as in RNA content from 1 to 4 hour , after exposure. The changes were, however, more conspicuous in cerebellum than in other parts of the brain. The activities of creatinkinase, nonspecific cholinesterase, displaced only subtle changes as seen in cerebral homogenates and serum. These reports, thus, are indicative for the transient changes of brain protein metabolism with a single dose of carbondisulfide.

Jarvisalo et al. (1977) observed that there was an initial C^{14} - leucine uptake inhibition in phenobarbitone pretreated rat. This decreased C^{14} - leucine uptake was accompanied by decreased RNA levels and led them to conclude that CS_2 affected protein synthesis as well as protein turnover .

EL'Dessoukey et al. (1977) reported a state of hypoaminoacidemia and hyperaminoacid urea in various groups of rat treated with toxic dose of CS_2 for various weeks. However, this effect was transient as the cessation of CS_2 intoxication led to the improvement of amino acid patterns of urine and blood in rat.

1.2.2.4 CARBOHYDRATES AND CS_2 TOXICITY :-

There is a paucity in the literature about the full evidences regarding carbondisulfide effect on carbohydrate metabolism.However, Minden et al. (1970) reported a typical condition of tissue acidosis in CS_2 poisoning with hypolacticemia. Kurzinger and Freundt (1969) reported an increase in the hepatic lactate and glycogen diminution.

Cunningham and Holt (1977) studied the turnover of blood glucose in the rat after acute CS_2 intoxication and reported a reduction in blood glucose concentration in CS_2 treated rats as compared to control. These studies made them to suggest that the rate of gluconeogenesis was unchanged in CS_2 treated but starved rats while lowering of blood glucose in CS_2 treated non-starved rats is due to an increase in futile cycling at the triosephosphate stage of glycolysis, rather than increased clearance of

glucose from blood.

1.2.2.5. BIOGENIC AMINES AND CS_2 TOXICITY :-

Aleksander et al. (1966) studied the behaviour of certain biogenic amines in subjects exposed to CS_2 and recorded an increase in elimination of 5- hydroxyindole acetic acid in urine of those workers exposed to toxic levels of CS_2. They also reported an increase of the blood levels of various catecholamines.

Maj and Vetulani (1970) studied the pharmacological properties of N-N disubstituted dithiocarbamates and their effect on brain catecholamines and reported a reduction in the brain norepinephrine (NE) content and spontaneous activity. Dimethyldithiocarbamate (dmdtc) and diethyldithiocarbamate (dedtc) elevated the brain dopamine level whereas dibutyldithiocarbamate (dbdtc) depressed it. Dimethyldithiocarbamate and dedtc potentiated the convulsive effect of pentalenetetrazole whereas edtc exerted a protective effect showing thereby that ethyldithiocarbamate(edtc) is not formed during CS_2 poisoning.

Maj Jerzy et al. (1970) also studied the effect of disulfiram, de-dtc and dm-dtc on serotonin and 5 HIAA levels in rat brain and reported that at lower dose of de-dtc and dm-dtc did not change the levels of serotonin and 5 HIAA signficantly in brain. However, higher dose of de-dtc increased the 5 HIAA content by 143% without effecting the serotonin content but still

higher dose of de-dtc decreased the serotbnin content without effecting the level of 5 HIAA . Disulfiram and dm-dtc decreased serotonin and 5-HIAA in the animals. Ethyldithio-carbamate decreased the serotonin content of hippocampus by 74% while it increased the serotonin content in hypothalamus by 72%. They concluded that all the sulfur containing substances effect the serotonin level in different ways and pattern.

Magos and Jarvis (1970) reported that rats exposed to 2 mg CS_2/litter of air for two days showed a reduction in their brain NE and elevation in DA concentration. Exposure of ten days further, decreased the NE level but DA concentration returned to control level. Dopamine concentration in adrenals after 10 days of exposure were 67% higher than control animals suggesting a reduction in DA beta-hydroxylase enzyme. Exposure to CS_2 did not effect tyrosine level and MAO-activity in the brain,whereas, they found that sod-de-dtc inhibited the MAO activity as well as increased the tyrosine level.

Magos and Jarvis (1970) in another work studied the effects of diethyldithiocarbamate and CS_2 on brain tyrosine levels. They found that de-dtc increased the level of DA and tyrosine whereas decreased NE level in brain. Tyrosine level like that of DA was maximal 1 hour after the injection of de-dtc and the increase was followed by a rapid decrease to 80% of the control value. A similar two phased change in brain

level of tyrosine was also observed after repeated exposure to CS_2. The increased DA level in the Ist phase of their action might be due to slowed conversion of tyrosine to dopa suggesting due to stimulation of feed back mechanims, resulting in a subsequent decrease in brain NE and DA levels.

Magos (1971) reported further, the amphetamine induced stereotype in CS_2 exposure and diethyl dithiocarbamate treated male rats. CS_2 exposure increased brain DA levels and decreased the NE levels. Pretreatment of Sod. diethyl dithiocarbamte increased the duration but not the intensity of subsequent amphetamine induced behaviour.

Diliberto and Distifano (1973) examined the effects of 2-mercaptoethyl guanidine and other compounds on NE synthesis by adrenal medullary granules. This compound like reserpine phosphate inhibited NE formation from 1-C^{14} labelled DA by bovine adrenal medullary granules. Reserpine prevented DA uptake by granules whereas 2- mercapto ethylguanidine inhibited dopamine beta-hydroxylase. The presence of copper was found to stimulate dopamine beta-hydroxylase. Diliberto and Distefano (1973) studied the mechanism and kinetics of the inhibition of dopamine beta-hydroxylase (DBH) by 2- mercaptoethyl guanidine and suggested that 2- mols of inhibitor combines with 2 Cu^{++} ions in the active site and thus inhibit DBH. The intramolecular distance between anionic site of inhibitor and beta-hydroxylation site of dopamine was coinciding with the intramolecular distance between the

positive charge of the inhibitor and the site of binding of dopamine beta-hydroxylase.

Andrea and Guido (1974) reported the effect of chronic environmental stress on 5-HT receptors of smooth muscles and suggested that exposure of newly born animals to a chronic stress induced a faster maturation of 5-HT recptors sensitivity with reduced activity of MAO.

Stankovic et al. (1975) studied the effect of CS_2 and found that the amount of 5-HIAA in the urine of exposed workers was about 4 to 6 times higher than in the control group. It was therefore, concluded by these workers that CS_2 exert its marked effect on tryptophan metabolism.

Michael and Victor (1977) proposed a mechanism for the action of carbondisulfide according to which inhibition of DBH activity by CS_2 was mediated by the action of dithiocarbamates formed from the endogenous amino acids and catechol amines.

1.2.2.6 TRACE ELEMENTS AND CS_2 TOXICITY :-

Djuric et al (1968) reported the significant increased excretion of zinc in the urine of persons exposed to carbon-disulfide in viscose rayon industry.

Massoud (1977) reported the effect of CS_2 intoxication on the levels of serum copper and ceruplasmin. Intramuscular injection of CS_2 in rat slightly increased serum copper and ceruplasmin

levels but prolonged treatment of CS_2 caused these values to be decreased due to intoxication caused by the loss of appetite in animals. After the cessation of CS_2 administration, animals regained their appetites and showed a slight reversal of serum copper and ceruplasmin though the levels were still below normal which suggested local action rather than the effect on serum values.

EL'Dessoukey and Awadallah (1977) reported that serum zinc, iron, calcium, magnesium levels decreased, while serum potassium levels increased under the effect of CS_2 intoxication. The reduction in serum zinc, iron, calcium and magnesium levels had been due to a decrease in dietry minerals because of appetite loss and elevated potassium levels caused by tissue destruction. They reported these changes to be reversible.

Michael and Victor (1977) studied the binding of dithiocarbamates with copper of the enzyme dopamine- beta-hydroxylase, while these dithiocarbamates are formed by the reaction of CS_2 with free amino groups available in cellular environment. They stated that it is the complexing of copper with dithiocarbamates which caused the inhibition of copper containing enzymes.

1.2.2.7. PROPOSED MODE OF ACTION OF CARBONDISULFIDE :-

Stripp et al. (1969) reported that CS_2 undergoes partial metabolic conversion into dithiocarbamates. The suspicion that microsomal inhibition by CS_2 depended on the formation of these

metabolities has been strengthened by the fact that dithiocarbamates or related substances behaved as active microsomal inhibitors thereby inducing diminished tolerance to many drugs.

Tessinger (1971) discussed the mechanism of CS_2 toxicity in relation to pyridoxine or vitamin B_6 deficiency. They stressed that CS_2 toxicity caused the difficiency of Vitamin B_6 and its imparied metabolism caused the inhibition of monoamineoxidase and related enzymes.

Matteis and Seawright (1976) reported the liver toxicity of CS_2 and possible significance of its metabolism by oxidative desulfuration. They concluded that reactive sulfur liberated during the oxidative desulfuration of CS_2 might bound to cellular components and initiated toxic changes in the liver. This accounted for the loss of cytochrome P- 450 and also for the centrilobular hydrophic degeneration which was also seen in vivo. Savolainen and Vainio (1976) examined the high binding of sulfur of CS_2 in spinalcord axonal fraction. The sulfur was found to be bound to spinal cord axons in larger fractions than by spinal cord homogenate. In contrast, the carbon from CS_2 was not significantly bound by axons. So they reported that toxic effect of CS_2 may be due to sulfur containing intermediates formed in the nervous system.

Savolainen and Jarvisalo (1977) studied the binding of CS_2 in CNS of control and phenobarbitone pretreated rats.

They found that more sulfur atoms were bound to rat brain. The phenobarbitone pretreatment increased the cerebral binding of sulfur and decreased that of carbon. This finding suggested that a considerable amount of CS_2 is retained in the nervous system and phenobarbitione pretreatment may also alter the brain metabolism of carbondisulfide.

Meckenna et al (1977) described the metabolism of inhaled CS_2 in rat. They found that elimination of CS_2 from the rat during the post exposure period was rapid and occurred primarily through kidneys. Accumulation of these acid labile metabolites upon repeated exposures and their presence in all tissues suggested that they may play a role in the toxicity of carbondisulfide.

Savolainen and Vainio (1977) reported the CS_2 binding to protein fraction of spinal neurofilaments of rat. So the binding of CS_2 to neurofilaments may contribute to the development of neural manifestations in chronic CS_2 poisoning.

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CHAPTER - 2

MATERIALS AND METHODS

Albino rats of either sex (Haffkins strain) weighing between 150-200 grms. were employed throughout this study. The selection of the animals was made at random basis. These animals were fed with pellet diet supplied by Hindustan Levers, Bombay, alongwith cereals and green vegetables. The access to water supply to animals was not restricted at any stage during this work.

GROUPING OF RATS :- For each of the experiments the animals were divided into two groups, i.e. the first group constituted carbondisulfide treated animals and other group severed as control. Two types of carbondisulfie treatments were made in this work. 1. Acute 2. Chronic. In each of the treatments, six to seven animals formed a group.

ACUTE TREATMENT :- Five different groups of animals consisting of 6 to 7 animals in each group were taken for this treatment. The carbondisulfide (AR-grade) in a dose of 1890 mg/kg, body weight was administered intraperitonealy in CS_2 treated group and the control group was injected with equivalent volume of vehicle. The physical observations on the gait, activity, and other bodily activities were made at an interval of 1,2,3 and 4 hours after CS_2 treatment. A group which was not sacrificed, died after five hours. Brain, liver, heart and blood was taken immediately and preserved in the deepfreeze for biochemical studies.

CHRONIC TREATMENT :- Rats were treated for four weeks., 6 days per week with carbondisulfide emulsion in groundnut oil 25 mg/kg intrapertonealy whereas control group was injected with equivalent volume of simple groundnut oil. Carbondisulfide emulsion was made by dissolving 1.0 ml of carbondisulfide in 100 ml of groundnut oil (Postman Brand). The animals were sacrificed by puncturing intrajagular vein and the blood was collected in an oxalated tubes, then the head was separated from the body. The brain, liver, heart , kidneys, pancreas, lungs and testis were taken in a dried and clean beaker to be preserved in a deepfreeze at $-7^{\circ}C$ ($\pm 2^{\circ}C$) till these were used for further processing for various biochemical parameters. The following behavioural observations and biochemical methods of analysis were done for each of the parameters.

2.1. BEHAVIOURAL OBSERVATIONS :-

Rats were trained in a jumping box for conditional avoidance response for two weeks. After CS_2 treatment, conditional avoidance response was noted. In addition to this the eye movements, limb movements , gait activity and breathing movements were observed in acute as well as chronically treated rats.

2.2. GLYCOGEN ESTIMATION :-

Glycogen estimation in liver and brain was done by the method originally used by Pfluger (1905) and modified by Good ,et.al.

(1933). The process depends on the digestion of tissues in hot concentrated KOH, precipitation of glycogen with acid, and determination of glucose in the hydrolysate as a reducing sugar.

Procedure :- About 0.5 to 1.0 Gram. of exactly weighed tissue was put in a 15.0 ml pyrex centrifuge tubes and reduced it to fine particles in a tissue homogeniser. After delivering the sample in a test tube, the tube was kept in a boiling water bath for 30 minutes to dissolve the tissue. 0.5 ml of saturated sodium sulfate was added and the glycogen was precipitated by the addition of 1.1 to 1.2 vol of 95% ethanol. The contents were strirred with a rod, and the rod washed with a small quantity of 60% ethanol. The tubes and contents were heated on a water bath again until the mixture was boiled, thereafter it was cooled and centrifuged at 3000 rpm. The supernatant was decanted and the aliquots were allowed to drain off. The precipitated glycogen was dissolved in water and the total volume was made up to 250 ml in a volumetric flask in each case. 0.5 ml of this solution and 4.5 ml of water was transferred to a set of tubes. In a separate tube, was introduced 5.0 ml of glucose standard containing 100 ug of glucose. To another tube was added 5.0 ml of water which served as a blank.

The tubes were submerged in the ice cold water. 10.0 ml of 0.2% anthrone reagent in (95%) H_2SO_4 was added in each tube, and the reactants were mixed on vortex mixer. These cold tubes

were covered with glass marbles and heated for 10 minutes in a boiling water bath. They were then cooled immediately in a bath containing ice cold water and read on a spectrophotometer Spectronic-20 at 620 nm, after adjusting with the blank.

Calculations :-

$$\text{Amount of glycogen in aliquot (ug)} = \frac{100 \times U}{1.11 \times S}$$

U = optical density (O.D.) of unknown test solution.

S = O.D. of Standard glucose 100 ug/ml.

1.11 = It is the factor determined by Morris (1948), for the conversion of glucose to glycogen.

2.3. BLOOD GLUCOSE, ESTIMATION :-

Blood glucose determination depends upon the reducing nature of glucose, which reduces cupric ions to cuprous ions, that in turn reduce phosphomolybdic acid to phosphomolybdous acid of blue color.

Procedure :- Determination of blood glucose was done by the method of Folin & Wu (1926). Folin- Wu protein free filtrate was prepared by adding 9 volumes of the mixture of 8 parts N/12 H_2SO_4 and one part 10% sodium tungustate directly to the blood. After mixing it was filtered. The clear filtrate was taken in Folin- Wu tube. Two ml of alkaline copper solution was added in all the tubes. The tubes were kept in a boiling

waterbath for six minutes. After cooling, 2.0 ml of phosphomolybdic acid solution was added to all the tubes. The contents were diluted up to 25.0 ml and optical density was read at 420 nm, on Spectronic- 20 . 2.0 ml of standard glucose solution (1 mg/ 1 ml of glucose) and a blank containing 2.00 ml of distilled water were run alongwith the test samples.

Calculations :-

$$\frac{\text{OD of Test}}{\text{OD of Standard}} \times \text{mg of glucose in standard}$$

$$\times \frac{100}{2} = \text{mgs. of glucose per 100 ml of blood.}$$

2.4. PROTEIN ESTIMATION :-

Procedure :- (i) Total plasma proteins : These were determined by the method of Lowry and Rosenberg (1951). 1.0 ml of plasma was diluted to 500 ml in a volumetric flask. 1.0 ml of diluted plasma was added in 5.0 ml of alkaline copper sulfate solution and allowed to stand for 10 minutes at room temperature. Similarly, in tubes marked standard, 1.0 ml of freshly prepared bovine serum albumin solution (100 ug/ml) was taken, and in blank 1.0 ml of distilled water was added. In both, standard and blank, 5.0 ml of alkaline copper sulfate solution was added and allowed to stand for 10 minutes. After this, 0.5 ml of folin cicalteau reagent was added in all tubes and mixed on a vortex mixer. All the set was allowed to stand for half an hour. The optical density was read at 740 nm on Spectronic-20

(using red lamp).

(ii) For tissue proteins :- 10% homogenate of brain and liver was prepared in distilled water and further diluted 20 times and the above method described for plasma was followed.

Calculations :-

$$\frac{\text{Optical density of Test}}{\text{Optical density of standard}} \times \text{ug of proteins in standard}$$

$$\times \frac{\text{Dilution factor}}{\text{Vol of plasma or tissue extract}} = \text{ug of proteins/ ml. - of test solution}$$

2.5. SERUM -ALPHA- AMYLASE :-

Alpha amylase is an enzyme secreted by pancreas and salivary glands. It hydrolyses starch to disaccharides, maltose. Starch but not the maltose forms a blue colloidal complex with iodine in solution, and the intensity of this color is directly proportional to the concentration of the starch. The blue color produced by the starch substrate when combined with iodine, is measured after incubation with serum and compared with blank. The decrease in color is proportional to the amylase activity. Iodometric method by Carway (1959) depending on this principle was followed to determine the activity of serum alpha-amylase.

Reagents :-

(i) Stable buffered starch substrate pH 7.0 :- 13.3 gm of anhydrous disodium phosphate and 4.3 gm of benzoic acid in about 250 ml of water was dissolved and was boiled 0.20 gms. of Merck's solution starch was mixed in 5.0 ml of cold water and it was added to the boiling mixture. Boiling was continued for one minute. After cooling to room temperature, pH was adjusted to 7.0. Then the contents were diluted to 500.0 ml with distilled water.

(ii) Stock solution of Iodine 0.1 N :- 3.56 gm Potassium iodate and 45 gm of potassium iodide was dissolved in 800 ml of water. To this was added slowly, with mixing 9.0 ml of concentrated HCl (12 N) and diluted to one liter with water.

(iii) Working Iodine Solution :- (0.01 N):-59.0 gm of sodium fluoride was dissolved in 350.0 ml of water. To this was added 50.0 ml of stock iodine solution and diluted to 500 ml mark in a volumetric flask.

Procedure :-

5.0 ml of starch substrate was pipetted in to 50 ml graduated tubes, marked test and blank. All the tubes were placed in water bath at 37°C for 5 minutes to warm up the contents. 0.1 ml of serum was pipetted in the bottom of the tubes labelled test. Allow the reaction to proceed for exactly

7½ minutes. No serum was added in the blank but instead 0.1 ml of distilled water was added. After 7½ minutes the tubes were removed from water bath and the volume was made up to 40.0 ml mark by addition of water. Immediately, 5.0 ml of working iodine solution to each of the tube was added and again the content was made up to 50.0 ml mark with water. Mixed well by shaking. Optical density was measured of tests and blank without delay against water at 660 nm on Spectronic- 20.

Calculations :-

$$\frac{\text{Optical density of Blank - Optical density of test}}{\text{Optical density of Blank}} \times 800$$

= amylase units 100 ml.

800 indicates that complete hydrolyses of starch would correspond to serum amylase activity of 800 units/100 ml. One amylase unit is the amount of enzyme that will hydrolyse 10.0 mg of starch in 30.0 minutes to a stage at which no color is given by iodine.

2.6. PHOSPHORYLASE ASSAY :-

Phosphorylase activity was determined by measurement of the rate of liberation of inorganic phosphate from glucose-1-phosphate in presence of glycogen i.e. phosphorylase activity was measured in the direction of synthesis of polysaccharides by the method of Sutherland (1951).

Procedure :-

The reaction was started by the addition of 1.0 ml of glucose-1-phosphate, glycogen and sodium fluoride to 0.5 ml of enzyme 5-AMP- NaF solution. The 0.5 ml of enzyme mixture contained 0.1 ml of 5-AMP (0.02 M), enzyme dilution (liver and brain, 10% homogenate), and 0.1 M sodium flouride to the volume of 0.5 ml. The assay mixture was incubated for 10 minutes at 37°C. The reaction was stopped by the addition of 10% trichloroacetic acid. In-organic phosphate was determined by the method of Fiske and Subha Row, as adopted for the Klett Summerson photometer at 660 nm.

Units and specific Activity :- One unit of enzyme was defined as that amount which caused the liberation of 1.0 mg of inorganic phosphate in 10 minutes when the percent conversion of glucose-1-phosphate was in the range of 12 to 22 percent.

2.7. PHOSPHOGLUCOMUTASE :-

The measure of phosphoglucomutase activity is based on the different chemical properties of two hexose phosphates. Glucose-1-phosphate is a nonreducing sugar with acid labile phosphate group. By contrast, glucose-6-phosphate has acid stable phosphate and reduces copper reagents.

For measuring enzyme activity, glucose-1-phosphate is used as the substate because of the favourable equilibrium toward the forward reaction. The rate of reaction which is

dependent on the amount of enzyme present can be followed by measuring the reduction in hydrolysable phosphate. This principle was employed by Victor et.al. (1954).

Procedure :- 0.1 ml of Mg SO_4 (6 x 10^{-2} M), 0.1 ml glucose -1-phosphate (2 x 10^{-2} M, pH 7.5) and 0.1 ml of cysteine (0.1 M, pH 7.5), were pipetted in a test tube and placed in a water bath at 30°C. After temperature equiliberation, the reaction was started by adding 0.1 ml of the enzyme dilution. After incubation for 5 minutes, the reaction was stopped by the addition of 1.0 ml of 5 N H_2SO_4. The volume was made up to 5.0 ml with water, and the reaction tubes were placed in a boiling water bath for 3 minutes to hydrolyse the remaining glucose-1-phosphate. To the sample 1.0 ml of molybydate solution followed by 0.5 ml of 1-amino-2-napthol- 4 -sulfonic acid (ANSA) solution were added according to Fiske & Subba Row (1925). The volume was made up to 10.0 ml, and the intensity of color produced was measured at 660 nm in Klett Summerson photometer. The amount of stable phosphate formed is the difference between the hydrolysable phosphate added and that remaining at the end of 5 minutes.

Unit Activity :- One unit of enzyme is defined as that which, under the conditions described above, catalyses the formation of 1.0 mg., acid stable phosphate.

2.8. FRUCTOSE 1-6 DIPHOSPHATASE :-

The method is that of Pogell and McGilvery (1955) in

which the inorganic phosphate liberated from Fructose 1-6-Diphosphate (FDP) is determined.

Procedure :- 0.1 ml of FDP (0.05 M, pH 7.4), 0.4 ml of borate buffer (pH 9.5), 0.1 ml of Mg SO_4 (.05 M), and 0.1 ml of $MnCl_2$ (.005 M), were pipetted in centrifugation tubes. After warming 0.2 ml of enzyme was added to the tube, followed immediately by 0.1 ml of cysteine (.05 M, pH 9.5).

Then this mixture was incubated at 38°C for twenty minutes and to it was added 1.0 ml of trichloroacetic acid and inorganic phosphate was determined by the method of Fiske and Subba Row (1925).

Unit Activity :- One unit of enzyme is defined as the amount which will liberate 1 mg of inorganic phosphate with linear kinetics of 20 minutes under the above conditions.

2.9. ADENOSINE TRIPHOSPHATASE :-

The assay of adenosine triphosphatase is most simply carried out by estimating colorimetrically the inorganic phosphate liberated by the enzyme from ATP under the specified conditions as stated by (Seth et.al. 1966).

Procedure :- 0.2 ml of Tris buffer (0.5 M,pH 8.0), 1.5 ml of water, 0.1 ml of 10% tissue homogenate, 0.2 ml of ATP (55mg/10 ml) was pipetted in the centrifugation tube. The assay mixture was incubated at 37°C for 15 minutes. The reaction

The reaction was stopped by adding 1.0 ml of 10% trichloracetic acid. The mixture was centrifuged and to 1.5 ml of supernatant 1.0 ml ammonium molybdate (2.5 % in 10 N H_2SO_4) 10 N H_2SO_4 was added followed by the addition of 7.1 ml of water and 0.4 ml of ANSA and optical density was noted at 660 nm.

One unit of enzyme is that which liberates 1.0 mg of inorganic phosphates in 15 minutes.

2.10.SUCCINIC DEHYDROGENASE :-

The activity of Succinic Dehydrogenase (SDH) was determined by the method of Kun and Abowd (1949), by its ability to oxidize succinic acid and reducing equivalents liberated in turn reduce the 2,4,6 triphenyl tetrazolium chloride,(TTC).

Procedure :- To a 15.0 ml caliberated centrifuge tubes, 0.5 ml of 0.1 M phosphate buffer (pH 6.7) and 1.0 ml of 10% tissue homogenate were pipetted. Finally, 1.0 ml of freshly prepared 0.1% TTC solution was poured in. After shaking, the tubes were placed in a water bath at 38°C for a period of exactly 30 minutes. Immediately, after removal of tubes from water bath, 3.0 ml of acetone was added and the tubes were stoppered and shaken vigorously. Then tubes were centrifuged and the clear supernatant was read at 420 nm on spectronic-20. Sodium - dithionite (1%) is used as standard reducing substance and mili-gram of TTC dye reduced is calculated.

2.11. BIOGENIC AMINES :-

The monoamines, dopamine (DA), norepinephrine (NE) and 5-hydroxytryptamine (5-HT) were estimated on Aminco-Bowman spectrofluorometer. DA and NE were extracted and oxidized by the method of Chang et.al.(1964) and 5-HT was extracted and estimated by the method of Curzon and Curzon (1970).

Reagents :-

(i) Acidified butanol :- Analytical grade of n-butanol was prepared by adding 0.85 ml of concentrated HCl per liter of n-butanol.

(ii) 0.1 M EDTA :- 37.2 G of disodium ethylene diamine tetra-acetate (EDTA) was dissolved in 1 M sodium acetate solution and diluted to one liter. The pH of the resultant solution was adjusted between 6.7 to 7.0.

(iii) Alcoholic Iodine Solution :- 1.27 G iodine dissolved in 100 ml of absolute alcohol.

(iv) Alkaline sulfite reagent :- 1 ml of 25% Na_2SO_3(anhydrous) solution diluted with 9.0 ml of 5 N NaOH. This solution was always made fresh immediately before use.

(v) Standard solutions :- 100 ug/ml. solutions of NE, DA and 5-HT in 0.01 N HCl were kept at low temperature in a refrigerator. The stock standard was diluted when needed,with .02 M acetic acid to a concentration of 5 ug/ml. for DA and NE and 1 ug/ml for 5-HT.

Extraction of DA and NE :-

Whole rat brain, a weighed portion of liver, whole heart, a weighed portion of lungs were homogenised separately in acidified butanol using glass homogenising tubes and a motor driven teflon pestle. 10% solution of homogenates were prepared and were centrifuged for 10-15 minutes at 4000 rpm at - 7°C (± 2°C).

To 5.0 ml of supernatant, was added 10.0 ml of n-heptane (AR Grade) and 5 ml of water. These were shaken for 2 minutes on vortex mixer. 5.0 ml of aqeous phase was taken, to that was added to 0.2 gm alumina. Adjusted the pH between 7 to 7.5 by adding 1.0 ml of 2 M sodium acetate to each tube. Again the tubes were shaken for 5 minutes and centrifuged. DA and NE were adsorbed on activated alumina. The alumina bound catecholamines were eluted by shaking with 3.0 ml of 0.1 M acetic acid. The pH of eluate was always adjusted around 4.0 to 4.3.

1.0 ml of eluate was used for oxidation of DA and NE. To a 0.1 ml of eluate, 0.2 ml of EDTA reagent was added and the mixture was adjusted to a pH of 6.5. To the resultant solution, 0.1 ml of 0.1 N alcoholic iodine solution was added to oxidise catecholamines (CA).Exactly after two minutes, 0.2 ml of alkaline sulfite solution was added to stop the reaction. Again after 30 minutes, pH of the solution was adjusted to about 5.4 by addition of 0.2 ml of 5 N acetic acid.

Tissue Blank :-

To 1.0 ml of acidified eluate 0.2 ml of EDTA reagent was added and the mixture was adjusted to a pH of 6.5. The iodine solution and alkaline sulfite reagent were added in a reverse manner in contrast to standard i.e. first 0.2 ml alkaline sulfite reagent was added followed by 0.1 ml of iodine solution. The solutions of standard catecholamines were prepared by taking 0.1 to 2 ug of standard solution, oxidized as described above.

Internal standard solutions equivalent from 20 to 200 ng of NE and DA were added to the tissue supernatant and were carried through extraction and oxidation procedures as described above. Therecovery of exogenous amines added to the tubes prior to the first shaking step was 90 to 95% for 5-HT and 75- 80% of NE and DA. These solutions were heated in a boiling water bath for 30 minutes, cooled and read in aminco bowman's spectrofluorometer as follows :-

For NE- excitation of the solution at 395 mu and emission read at 485 mu. Band pass was kept at 2, slit width at 2. For DA,excitation of the solution at 345 mu end emission read at 410 mu. Band Pass was always kept at 2 with slit width at 2.

2.12. EXTRACTION OF 5-HYDROXYTRYPTAMINE :-

10% homogenate prepared in acidified butanol as prepared

for CA extraction,was taken. To 2.5 ml of supernatant was added 5.0 ml of n-heptane, 0.4 ml of 0.1% cysteine in 0.1 N HCl. The contents were mixed thoroughly and centrifuged. To the aqeous phase 0.6 ml of .004 % ortho pthalaldehyde, prepared in 10 N HCl, was added. The contents were kept in boiling water bath for 15 minutes and then cooled in water. The fluorescence was read on Aminco-bowman Spectrofluorometer by activation wavelength at 295 nm and emission wavelength at 470 nm. Band Pass was kept at 2, and slit width at 4. (Curzon and Curzon 1970).

2.13. MONOAMINE OXIDASE :-

The activity of monoamine oxidase (MAO) was determined by its action on benzylamine hydrochloride which resulted in the formation of benzaldehyde and the extinction of benzaldehyde at 250 nm was used to determine the activity of this enzyme (Tabor et.al. 1955).

Procedure :

0.4 ml of phosphate buffer (0.5 M, pH 7.2) was pipetted into a centrifugation tube followed by addition of 1.3 ml of water. 0.2 ml of 10% homogenate of tissues were poured, followed by addition of 0.1 ml of benzylamine-hydrochloride (0.1 M). This assay mixture was incubated for 30 minutes at 37°C. The reaction was stopped by addition of 1.0 ml of 10% perchloric acid. 3.0 ml of water was added, then centrifuged and read at 250 nm on U.V. Spectrophotometer, (Unicam SP-500). Extinction

of benzaldehyde at 250 nm is 11,300.

2.14. GLUTATHIONE- REDUCED : (G-SH) :-

The method of Ellman (1959) was used to assay sulfhydral groups of reduced glutathione.

Procedure :-

To 10% homogenate was added equal volume of 10% trichloro-acetic acid (TCA) and then centrifuged. To 0.1 ml of supernatant 0.4 ml of 5-5' dithiobis 2-nitrogenzoic acid (DTNB) was added which was prepared as 5 mg per 100 ml of 0.5 M phosphate buffer (pH 8.0). The optical density was taken at 412 nm.

2.15. ANALYSIS OF ZINC, COPPER AND MANGANESE :-

Analysis of these trace elements in various organs of rat in CS_2 treated and in control was done by atomic absorption spectrophotometer (Perkin-Elmer) Model- 303.

Sample Preparation :-

Sample preparation was done by wet ashing method. Placed about 0.5 gm (accurately weighed) of the tissues in a 25 ml erlenmeyer flask, having glass beads and 2.5 ml of deionised water. Added 1 ml of a mixture (1:1) of concentrated HNO_3 and $HClO_4$. The sample was boiled until the solution was clear. The final volume of the mixture was made to 10 ml with deionized water. This extract was used for analysis of zinc, copper and manganese on specified conditions and settings of

the instrument for each element, are described below.

Standard Conditions for Zinc :-

Stock Standard Solution :- Zinc 500 ug/ml. 0.5 gm of Zinc metal was dissolved in minimum volume of (1+1) HCl, and diluted to one liter with 1% (v/v) HCl.

Operating Parameters :-

Wavelength was set at 214 nm, slit was kept at 4 (.7 nm). Light source was Hollow cathode lamp. Air - acetylene oxidising flame (lean and blue) was used.

Linear Working Range :-

Zinc was linear upto concentrations of approximately 1 ug/ml in aqueous solution. A standard zinc solution containing 0.5 ug/ml. showed an absorbance reading of about 0.12 absorbance i.e. about 25% absorption.

Standard Conditions for Copper :-

Stock Standard Solution :- (Copper, 1000 ug/ml) Dissolved 1.0 gm of copper metal in a minimum volume of (1+1) HNO_3 and diluted to one liter with 1% (v/v) HNO_3.

Operating Parameters :-

Wavelength was set at 325 nm and slit was set at 4 (0.7 nm). Light source used for estimatimation was Hollow cathode lamp and air acetylene flame when employed showed a linear working

range upto concentrations of approximately 5 ug/ml in aqeous solution.

Standard Conditions for Manganese :-

Stock Standard solution :- (Manganese 1000 ug/ml) Dissolved 1.0 gm of manganese metal in a minimum volume of (1+1) HNO_3 and diluted to one liter with 1% (v/v) HCl.

Operating Parameters :-

Wavelength was set at 279 nm. Slit was set at 3(0.2 nm). Light source was Hollow cathode lamp and air acetylene oxidizing flame was used.

The working range for manganese was linear upto concentrations of approximately 3 ug/ml in aqeous solution. A standard containing 2 ug/ml manganese was specified, as it gave an absorbance reading of about 0.16 absorbance units i.e. about 31% absorption.

****

CHAPTER - 3.

RESULTS AND OBSERVATIONS

3.1. BEHAVIOURAL OBSERVATIONS :-

A. Acute Treatment :- The animals exhibited an intense stimulation immediately after the administration of CS_2. There was itching at the site of injection and the spontaneous activity was reduced very much, afterward the animal became sedated and the eyelid size became slightly less. The animals acquired mild to intense rigidity and became placcid and akinesic which increase in intensity and ultimately passed on to comma and death within 5 hours of administration of CS_2.

B. Chronic Treatment :- The animal showed a slight stimulation immediately after the injection of CS_2. But after some time the animals became normal. After a few weeks of treatment there body hair became rough and eye pupil dilated. There was no significant changes in physical observation in these rats.

3.2. EFFECT OF SINGLE DOSE OF CARBONDISULFIDE (ACUTE TREATMENT) IN RAT ON SOME BIOCHEMICAL PARAMETERS :-

The following changes in various parameters were noticed after an acute dose of carbondisulfide.

3.2.1. Tissue Glycogen level :-

There was an appreciable fall in the liver glycogen content as after one hour of the injection the liver glycogen

decreased by 30.65%, at two hours it was decreased by 83.80% at three hour it was decreased by 89.43% and after four hours glycogen was almost completely depleted from the liver, (97.31%) (Table-1; Fig.1). CS_2 treated rats showed an initial rise in the brain glycogen content, as after the first and second hour of the injection the glycogen content was increased by 8.33% and 52.38% respectively, whereas brain glycogen decreased by 12.0% and 17.36% in third and fourth hour after the injection of CS_2 (Table-2; Fig.2).

3.2.2. Tissue Protein Level :-

A significant reduction in the total proteins of liver was found in CS_2 treated group of rat when compared to control group (untreated). The liver total proteins at various intervals after the CS_2 injection have been shown in Table-3; Fig.3. Liver proteins continuously reduced after 1,2,3 and 4 hour of CS_2 injection by 19.22%, 45.10%, 54.9% and 70.0% respectively (Table-3; Fig.3). In Table 4, Fig.4, the effect of CS_2 on brain protein level at various intervals is shown. Total brain proteins pattern varies quite in contrast to that of liver proteins. There is an initial rise in brain total proteins, in first hour after CS_2 injection, (56.5%). After 2,3 and 4 hours the brain proteins increased but not significantly as the rise in comparison to controls was only 14.3%, 11.5% and 2.7% respectively. One can say that brain proteins increased in first hour and then decreased in 2,3 and 4th hour gradually.

3.2.3. Plasma Proteins, Blood Glucose and Serum Alpha-Amylase :-

A marked effect of acute carbondisulfide treatment on plasma proteins was observed because a significant decrease in plasma proteins have been found in first, 2nd, 3rd and 4th hour after the injection of CS_2. (Table-5, Fig.5) shows the decrease of plasma proteins level in CS_2 treated rat by 19.13%, 41.4%, 48.84% and 65.89% respectively in comparison to control group. Blood glucose is also severely affected by CS_2 intoxication. First hour after the CS_2 injection blood glucose increased highly and rose from 140.5 mg % to 234.37 mg % .
But from second hour to fourth hour, glucose level reduced gradually by 28.8%, 49.02% and 60.24% in comparison to control values (Table-6; Fig.6).

In Table-7 Fig.7, the effect of CS_2 on serum alpha-amylase activity has been given. In treated rat the serum alpha amylase activity was found to be increased very appreciably as compared to untreated rat. In first hour after treatment, there was 86.40% rise, in second 250.35%, in third 205.94% and in fourth hour the rise was 246.05%. So in the first two hours there was a steep rise in serum alpha-amylase activity after which it did not rise but was similar to that of second hour level.

The changes in the weight of the organs after one month treatment with CS_2 is also represented in Table-8; Fig.8. The liver weight has increased significantly without any significant change in the weight of other organs.

3.3. EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT ON BIOCHEMICAL PARAMETERS :-

3.3.1. Tissue Glycogen :-

After one month treatment with CS_2, the glycogen level of liver was 74.88% higher than the control group. Similar was the case of glycogen level in rat brain which was 52.2% higher (Table-9; Fig.9).

3.3.2. Tissue Proteins :-

Table 10;Fig.10, shows the effect of chronic treatment on protein level of liver and brain. In liver a fall of protein level was observed by 15.93%, but brain protein level increased by 55.39% when compared to control group. So one can assume that after CS_2 treatment protein accumulate in brain to a greater extent.

3.3.3. Plasma proteins, blood glucose and serum alpha-amylase activity :-

Plasma protein level decreased in CS_2 treated rat by 41.5% whereas blood glucose level increased in treated group by 44.19% as compared to untreated group. Serum alpha-amylase activity was similarly found to be higher in treated group by 49.3% as compared to normal untreated rat (Table-11; Fig. 11).

3.3.4. Effect of Chronic Carbondisulfide Treatment on some Enzymes :-

The results of various estimations of enzymes, related to carbohydrate metabolism, are described below :

Enzymes activity was estimated both in liver and brain which have been found to be the main metabolic organs in this study.

3.3.4.1. Phosphorylase :- The liver phosphorylase activity was decreased by 58.43% in treated group as compared to untreated group, whereas brain phosphorylase is not effected significantly which decreased only by 9.99% (Table-12; Fig.12).

3.3.4.2. Phosphoglucomutase :- Phosphoglucomutase is an important enzyme of glycogen biosynthesis and its alteration observed due to CS_2 has been represented in Table-13; Fig.12. Liver phosphoglucomutase activity increased steeply by 248.59% in the CS_2 treated group in comparison to control group. Brain phosphoglucomutase also showed an upward trend as it increased by 34.61% as compared to untreated group. (Table-13;Fig.12).

3.3.4.2. Fructose 1-6, Diphosphatase :- Fructose- 1-6 diphosphatase (FDPase) an enzyme of importance in gluconeogenesis was also observed to be altered by carbondisulfide treatment for one month, as shown in Table -14; Fig.12. Liver FDPase activity is decreased by 42.02% and brain FDPase activity is decreased by 21.60% when compared to untreated group. The changes are statistically significant ($P < .05$, $< .025$)

3.3.4.4. Adenosine triphosphatase (ATPase) :-

Aderosinetriphosphatase is significantly effected by chronic CS_2 toxicity. Table-15;Fig.13, shows the ATPase alteration in different tissues. Brain ATPase activity was found to be decreased by 24.73% while liver ATPase activity decreased by 48.24%.

3.3.4.5. Succinic Dehydrogenase :-

Succinic dehydrogenase,an important enzyme of Kreb's cycle, was also found to be altered by carbondisulfide toxicity as shown in Table-16; Fig.13. Liver succinic dehydrogenase decreased by 36.80% as compared to control and the brain it decreased by 28.54% when compared with control group. The decrease is significant in both the organs.

3.3.5. EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT ON BIOGENIC AMINES, MONOAMINE OXIDASE AND GLUTATHIONE (G.S.H.):-

Chronic treatment of carbondisulfide showed a severe alterations in the metabolism of neurotransmitters in various tissues. Following are the variations observed after CS_2 treatment.

3.3.5.1. Dopamine :- Dihydroxyphenylethylamine (Dopamine) level is changed in all the tissues studied as shown in Table-17; Fig.14. Brain, heart and liver of treated group

showed a rise of dopamine by 161.24%, 412.65% and 262.94% respectively, where as the concentration of dopamine is decreased by 71.89% in lungs. So dopamine metabolism is effected variently but sharply by CS_2 toxicity.

3.3.5.2. Norepinephrine (NE) :-

Norepinephrine, an important neurotransmitter and an important metabolic regulatory hormone is effected by carbondisulfide toxicity. In brain a rise of 86.37% in heart a rise of 138.2% in liver a rise of 56.69% and in lungs a rise of 78.57% of norepinephrine content in treated group was observed as compared to nontreated group. (Table-18;Fig.14-16) These changes in NE tissue concentration may be responsible for many metabolic alterations in the living system.

3.3.5.3. Serotonin (5-Hydroxy Tryptamine):-

Serotonin, a metabolite of tryptophan metabolism and a neurotransmitter, altered by carbondisulfide toxicity as shown in Table-19;Fig.14-16. There was a significant decrease in brain serotonin by 45.88%. But 5-HT level of heart,liver and lungs increased by 41.95%, 240.0% and 367.1% respectively.

3.3.5.4. Monoamineoxidase :-

Monoamine-oxidase is an enzyme which is responsible for the metabolism of neurotransmitters. Carbondisulfide shows

a different effect in different organs on monoamine oxidase activity. There was a marked fall of MAO activity in brain, which was observed to be 42.57% lesser than control value. However, MAO inlungs decreased by 4.21% which is not a significant change. However, a marked rise of 47.18%,71.25% was observed in heart and liver MAO activity (Table-20;Fig.17).

3.3.5.5. Reduced Glutathione (G-SH) :-

Glutathione is an important molecule responsible for the covalent binding of various toxicants. Carbondisulfide was found to effect the level of glutathione (G-SH) appreciably in various organs (Table-21; Fig.18). Glutathione was observed to be decreased in all organs studied in treated group as in brain there was a decrease of 17.91%, in heart, 90.87%, in liver 14.30% and in lungs 86.18 %.

3.3.6. EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT ON TISSUES ZINC, MANGANESE AND COPPER LEVEL.

Carbondisulfide toxicity is found to altered the various trace elements in the living system. Following is the observed changes in Zinc, Manganese and Copper levels in various tissues i.e. , liver pancreas, kidneys, testis and brain.

3.3.6.1. Zinc :-

Zinc concentration was observed to be altered by CS_2

toxicity in various tissues as shown in Table-22;Fig.19. Zinc concentration in liver was decreased by 26.43%, in pancreas by 33.73% in testis by 34.87%, in brain 17.77% where as it is not significantly changed in Kidney (2.97% decrease only).

3.3.6.2. Manganese :-

Manganese distribution was also altered by CS_2 treatment in various tissues as shown in Table-23;Fig.20. Manganese concentration was increased in liver by 34.81% in pancreas by 150.38% and in Kidneys by 18.42%. But in testis the concentration of manganese was decreased by 36.90% which was signficant, (P-.005). Change in brain was N.S.

3.3.6.3. Copper :-

Copper concentration of liver is increased a little i.e. about 14.5% by CS_2 treatment whereas copper concentration in pancreas was altered markedly i.e. 157.83% more than the control group (Table-24 ; Fig. 21). Kidney, Testis and Brain Copper concentration was decreased by 30.32%, 59.79% and 27.22% respectively.

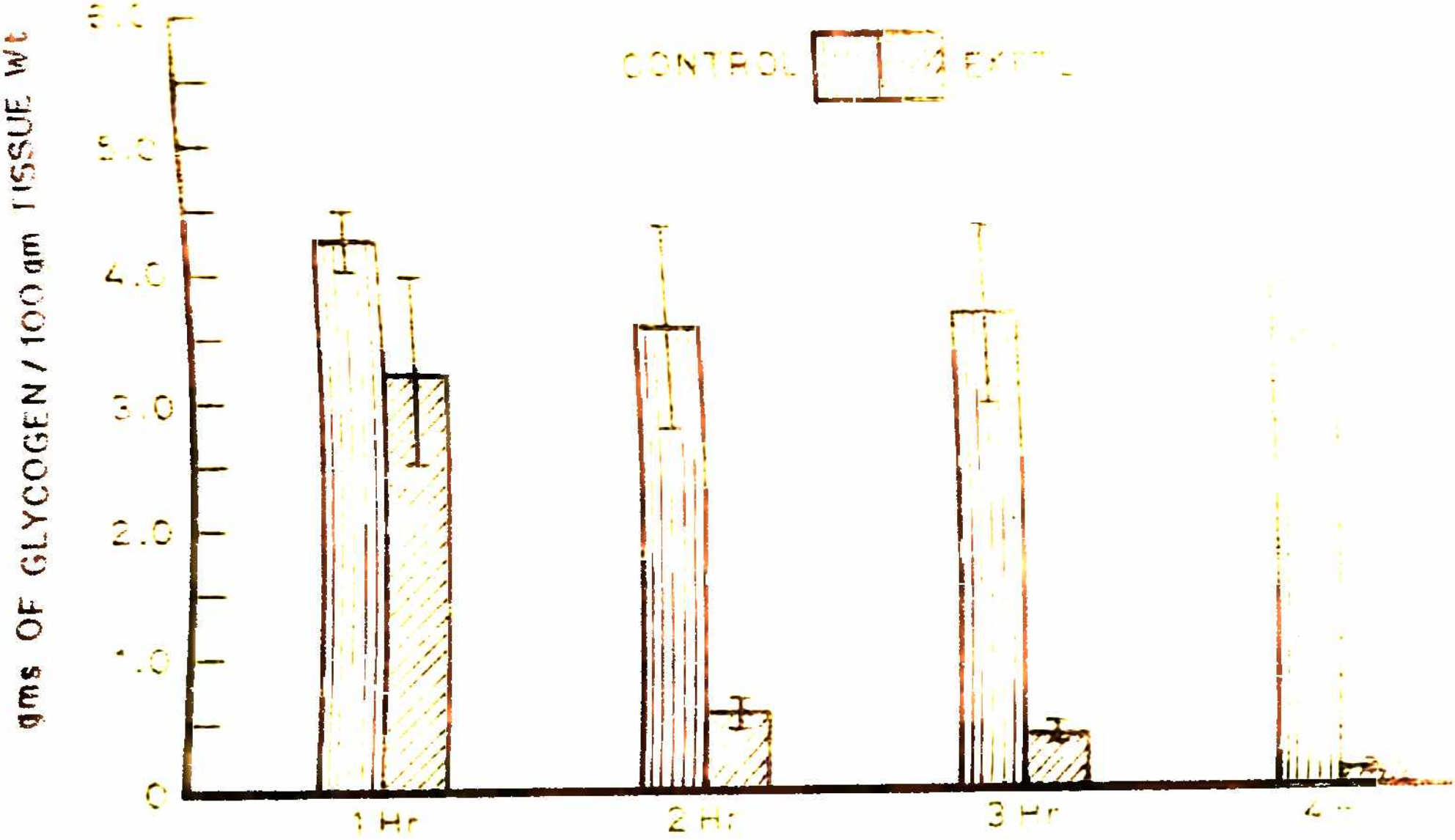
TABLE : 1

EFFECT OF SINGLE ACUTE DOSE OF CARBONDISULFIDE (1890 mg/kg body weight) ON GLYCOGEN LEVEL (G/100 G tissue weight) OF LIVER.

Hours After Injection	No. of rats	Control	CS_2 Treated	Percentage Change	Significance Level.
1	5	4.37 ± 0.21	3.28 ± 0.70	-30.65	< .05
2	5	3.52 ± .87	0.57 ± .10	-83.80	< .005
3	5	3.58 ± .67	0.41 ± .07	-89.43	< .005
4	5	3.93 ± .58	.107 ± .056	-97.30	< .005

+ Shows a n increase , - Shows a decrease.

All experimental values are , Mean ± Standard error (S.E.)



LIVER GLYCOGEN

FIG : 1

TABLE : 2

EFFECT OF SINGLE ACUTE DOSE OF CARBONDISULFIDE (1890 mg/kg body weight) ON GLYCOGEN LEVEL (G/100 G tissue weight) OF BRAIN.

Hours After Injection	No. of rats	Control	CS_2 Treated	Percentage Change	Significance Level
1	5	0.24 ± .04	0.26 ± .04	+ 8.33	N.S.
2	5	0.21 ± .06	.32 ± .07	+ 52.38	N.S.
3	5	0.25 ± .047	0.22 ± .03	- 12.0	N.S.
4	5	0.19 ± .02	.157 ± .02	- 17.36	N.S.

+ Shows an increase, - Shows a decrease.

FIGURE: 2

SHOWS THE EFFECT OF ACUTE DOSE OF CARBONDISULFIDE ON GLYCOGEN LEVEL OF BRAIN AFTER 1 HOUR, 2 HOUR, 3 HOUR AND 4 HOUR OF INTRAPERITONEAL INJECTION.

TABLE - 3

EFFECT OF SINGLE ACUTE DOSE OF CARBONDISULFIDE (1890 mg/kg body weight) ON TOTAL PROTEIN LEVEL (mg/G Tissue weight) OF LIVER.

Hours After Injection	No. of rats	Control	CS_2 Treated	Percentage Change	Significance Level.
1	5	320.40 ± 20.56	259.24 ± 39.88	-19.12	<.05
2	5	356.42 ± 32.16	194.66 ± 16.96	-45.10	<.005
3	5	338.90 ± 44.84	152.73 ± 26.06	-54.9	<.005
4	5	308.24 ± 18.86	92.48 ± 8.45	-70.00	<.005

+ Shows an increase , - Shows a decrease.

FIGURE: 3

SHOWS THE EFFECT OF ACUTE DOSE OF CARBONDISULFIDE ON TOTAL PROTEIN LEVEL OF LIVER AFTER 1 HOUR, 2 HOUR, 3 HOUR AND 4 HOUR OF INTRAPERITONEAL INJECTION.

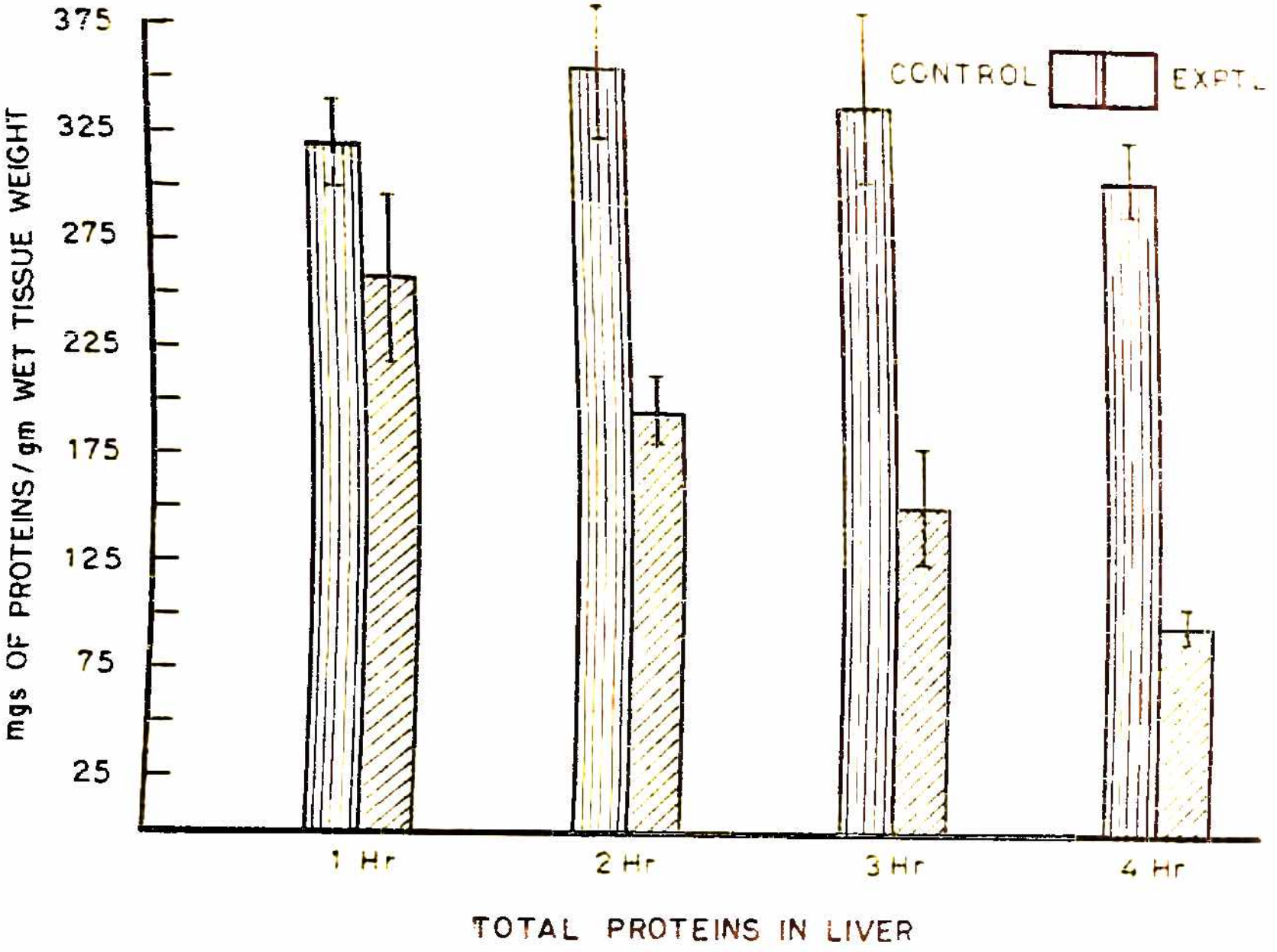

375
325
275
225
175
125
75
25
mgs OF PROTEINS / gm WET TISSUE WEIGHT
CONTROL
EXPT.
1 Hr
2 Hr
3 Hr
4 Hr
TOTAL PROTEINS IN LIVER

FIG: 3

TABLE : 4

EFFECT OF SINGLE ACUTE DOSE OF CARBONDISULFIDE (1890 mg/kg body weight) ON TOTAL PROTEIN LEVEL (mg/G Tissue weight) OF BRAIN.

Hours After Injection	No. of rats	Control	CS_2 Treated	Percentage Change	Significance Level.
1	5	72.70 ± 8.60	113.81 ± 13.11	+ 56.5	< .01
2	5	85.38 ± 14.60	107.74 ± 8 .02	+ 14.3	N.S.
3	5	82.42 ± 8.83	91.94 ± 7.15	+ 11.5	N.S.
4	5	86.10 ± 7.32	88.49 ± 6.01	+ 2.7	N.S.

+ Shows an increase , - Shows a decrese.

FIGURE: 4

SHOWS THE EFFECT OF ACUTE DOSE OF CARBONDISULFIDE ON TOTAL PROTEIN LEVEL OF BRAIN AFTER 1 HOUR, 2 HOUR, 3 HOUR AND 4 HOUR OF INTRAPERITONEAL INJECTION.

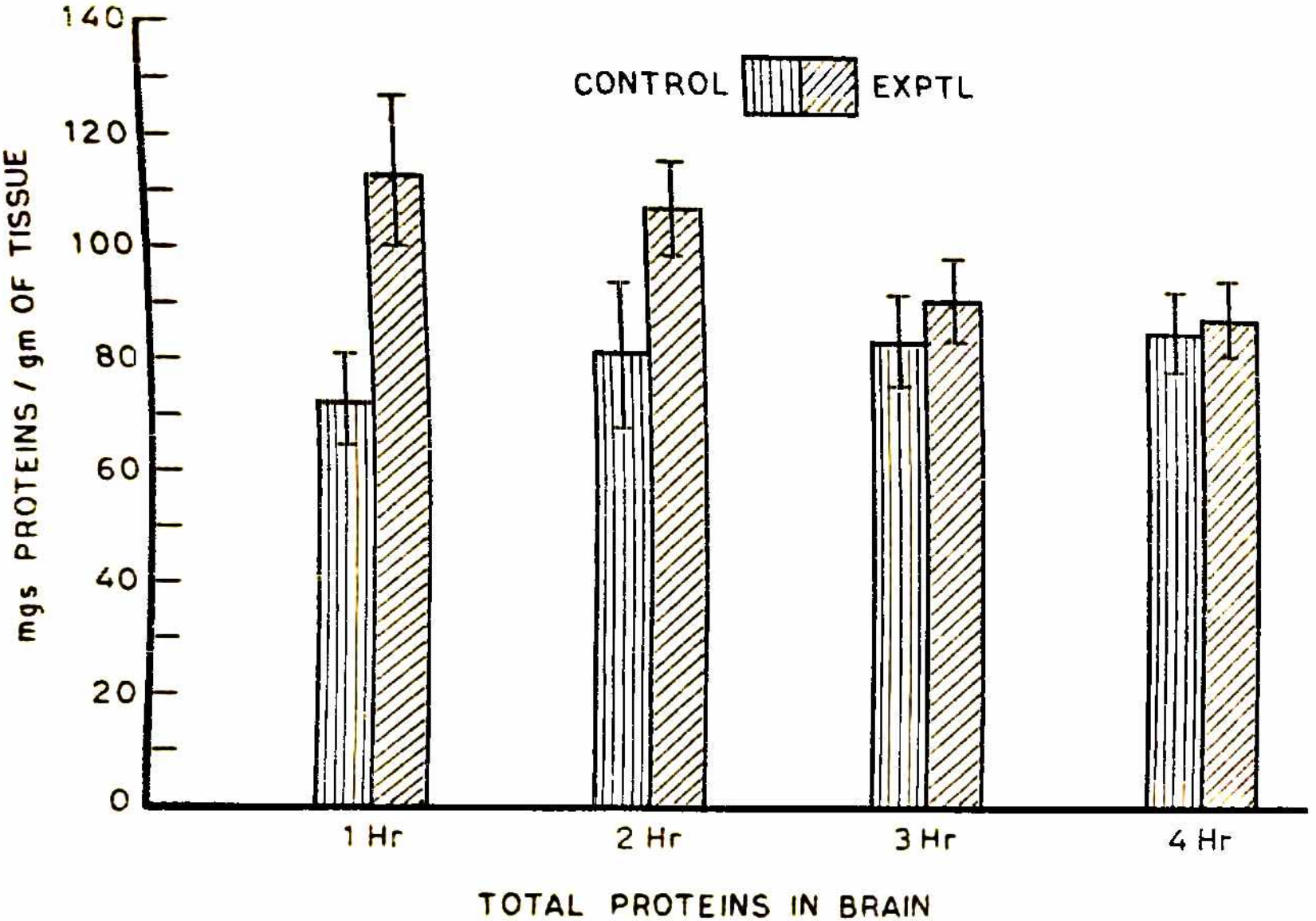

140
120
100
80
60
40
20
0
mgs PROTEINS / gm OF TISSUE
CONTROL
EXPTL
1 Hr
2 Hr
3 Hr
4 Hr
TOTAL PROTEINS IN BRAIN

FIG : 4

TABLE : 5

EFFECT OF SINGLE ACUTE DOSE OF CARBONDISULFIDE (1890 mg/kg body weight) ON TOTAL PLASMA PROTEIN LEVEL (G/100 ml) .

Hours After Injection	No. of rats	Control	CS_2 Treated	Percentage Change	Significance Level.
1	5	11.76 ± .92	9.51 ± .74	-19.13	<.025
2	5	10.00 ± .61	5.86 ± .55	-41.4	<.005
3	5	10.40 ± .84	5.32 ± .69	-48.84	<.005
4	5	9.56 ± .38	3.26 ± .53	-65.89	<.005

+ Shows an increase, - Shows a decrease.

FIGURE: 5

SHOWS THE EFFECT OF ACUTE DOSE OF CARBONDISULFIDE ON TOTAL PLASMA PROTEIN LEVEL AFTER 1 HOUR, 2 HOUR, 3 HOUR AND 4 HOUR OF INTRAPERITONEAL INJECTION.

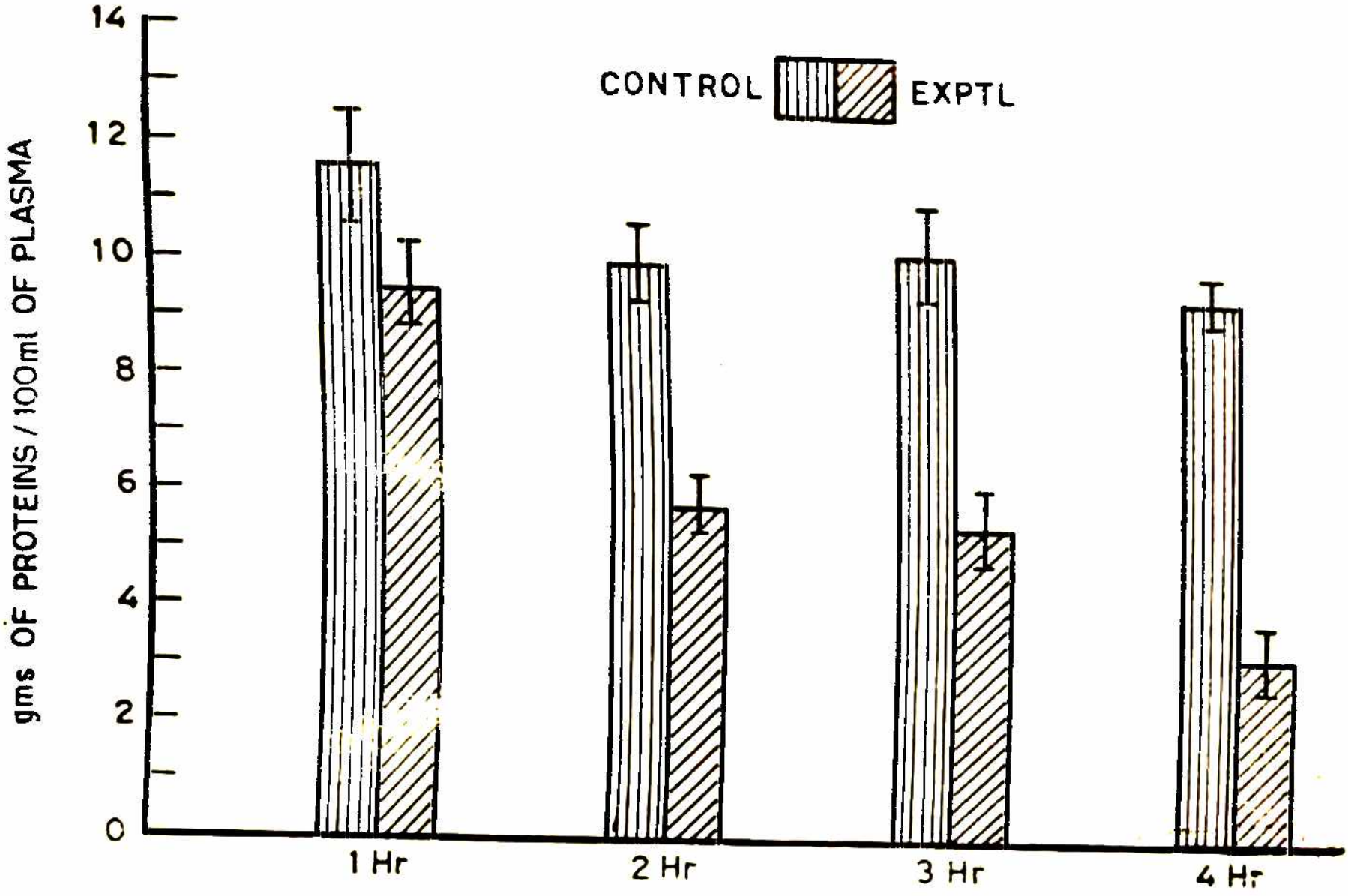

14
12
10
8
6
4
2
0
gms OF PROTEINS / 100ml OF PLASMA
CONTROL
EXPTL
1 Hr
2 Hr
3 Hr
4 Hr
TOTAL PLASMA PROTEINS

FIG: 5

TABLE : 6

EFFECT OF SINGLE ACUTE DOSE OF CARBONDISULFIDE (1890 mg/kg body weight) ON BLOOD GLUCOSE LEVEL (mg/100 ml).

Hours After Injection	No. of rats	Control	CS_2 Treated	Percentage Change	Significance Level.
1	5	140.5 ± 15.5	234.37 ± 45.83	+ 66.8	<.025
2	5	135.5 ± 25.7	96.42 ±12.57	- 28.84	<.01
3	5	115.30 ± 9.50	58.78 ± 13.4	- 49.02	<.005
4	5	129.1 ± 14.4	51.58 ± 6.66	- 60.24	<.005

+ Shows an increase , - Shows a decrease.

FIGURE: 6

SHOWS THE EFFECT OF ACUTE DOSE OF CARBONDISULFIDE ON BLOOD GLUCOSE LEVEL AFTER 1 HOUR, 2 HOUR, 3 HOUR AND 4 HOUR OF INTRAPERITONEAL INJECTION.

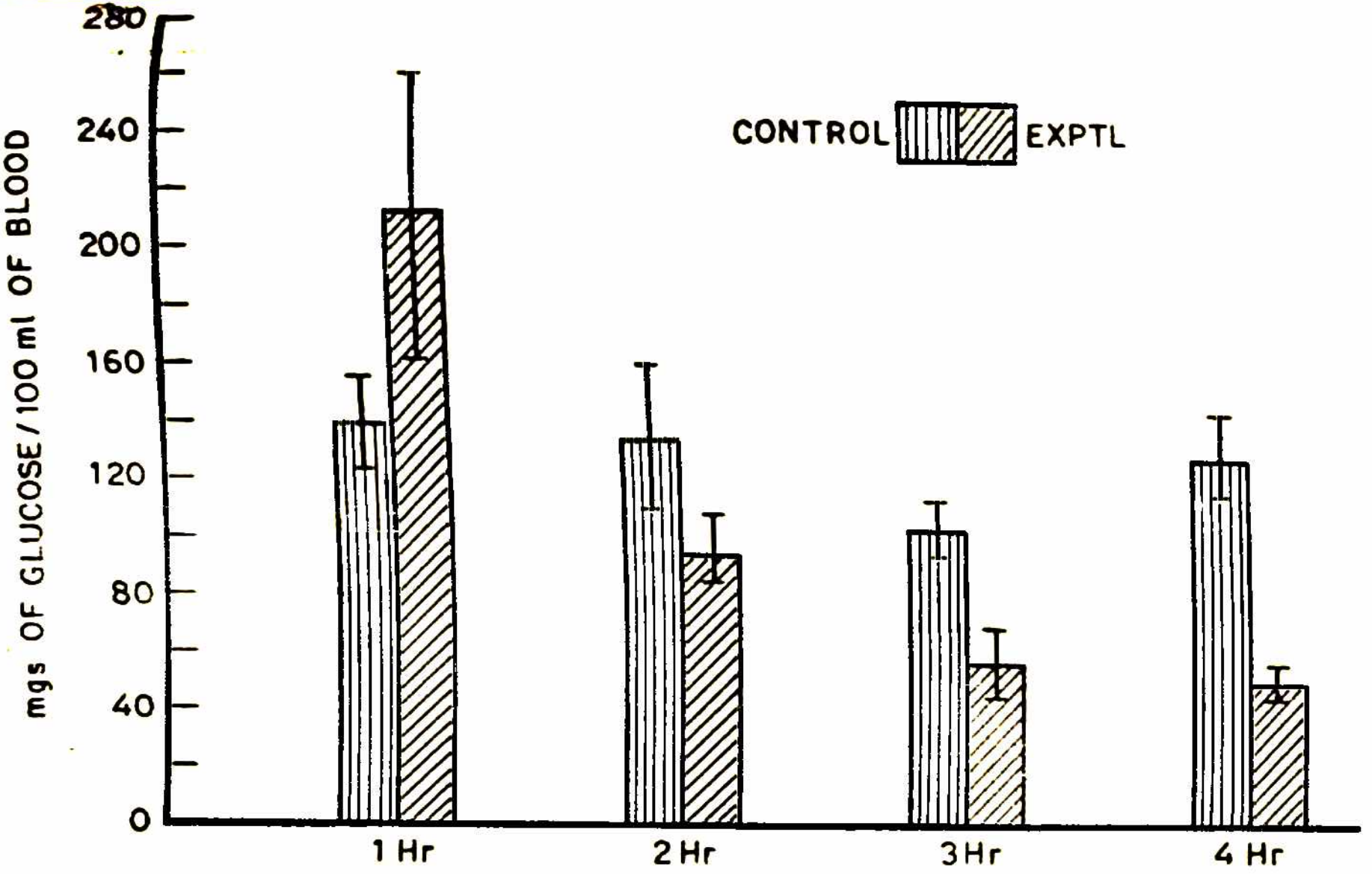

280
240
200
160
120
80
40
0
mgs OF GLUCOSE / 100 ml OF BLOOD
CONTROL
EXPTL
1 Hr
2 Hr
3 Hr
4 Hr
BLOOD GLUCOSE

FIG : 6

TABLE : 7

EFFECT OF SINGLE ACUTE DOSE OF CARBONDISULFIDE (1800 mg/kg body weight)
ON SERUM ALPHA AMYLASE ACTIVITY. (*Units/100 ml.)

Hours After Injection	No. of rats	Control	CS_2 Treated	Percentage Change	Significance Level.
1	5	295.6 ± 43.20	551.00 ± 184.61	+ 86.40	< .1
2	5	274.5 ± 38.30	961.72 ± 133.51	+250.35	< .005
3	5	354.7 ± 62.5	1085.20 ± 72.30	+ 205.94	< .005
4	5	393.4 ± 74.3	1361.4 ± 245.08	+ 246.05	< .005

+ Shows an increse , - Shows a decrese.
*Amylase Unit :- Is the amount of enzyme that will hydrolyze 10.0 mg of Starch in 30.0 minutes to a stage at which no color is given by iodine.

FIGURE: 7

SHOWS THE EFFECT OF ACUTE DOSE OF CARBONDISULFIDE ON SERUM ALPHA AMYLASE ACTIVITY (UNITS/100 ML) AFTER 1 HOUR, 2 HOUR, 3 HOUR AND 4 HOUR OF INTRAPERITONEAL INJECTION.

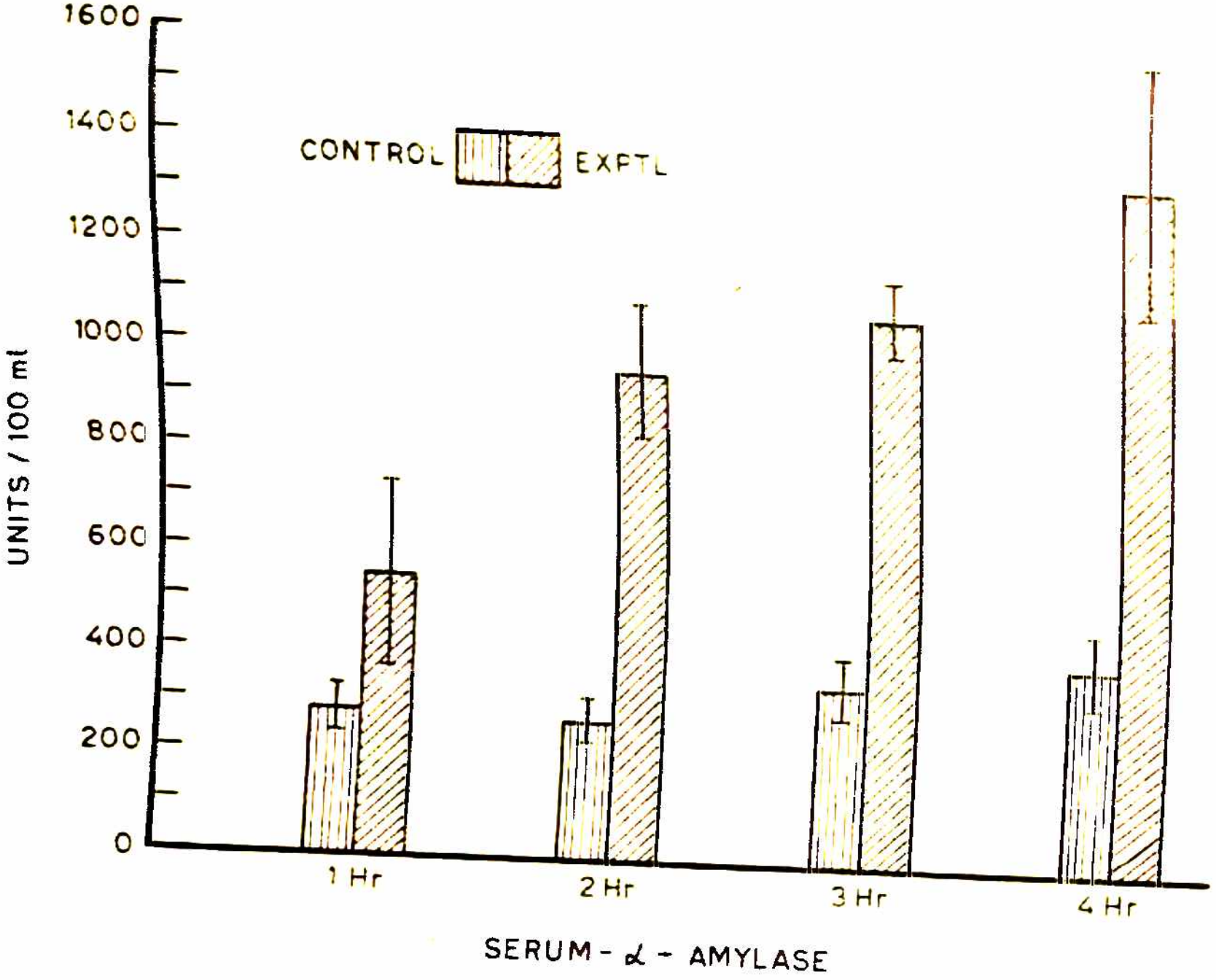

1600
1400
1200
1000
800
600
400
200
0
UNITS / 100 ml
CONTROL
EXPTL
1 Hr
2 Hr
3 Hr
4 Hr
SERUM - α - AMYLASE

FIG: 7

TABLE : 8.

EFFECT OF CARBONDISULFIDE TREATMENT (25.0 mg/kg body weight) FOR ONE MONTH ON THE WEIGHT OF ORGANS (in g ms.)

Orgns	Control	CS_2 Treated	Percentage Change	Significance Level.
Liver	5.351 ± .277	6.585 ± .279	+ 23.06	< .005
Brain	1.523 ± .107	1.597 ± .086	+ 4.84	< N.S.
Heart	.616 ± .033	.625 ± .080	+ .113	N.S.
Kidneys	.967 ± .04	1.142 ± .260	+ 18.08	N.S.
Lungs	1.077 ± .127	1.292 ± .129	+ 1.44	N.S.

FIGURE: 8

SHOWS THE EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT FOR ONE MONTH ON THE WEIGHT OF THE ORGANS OF RAT.

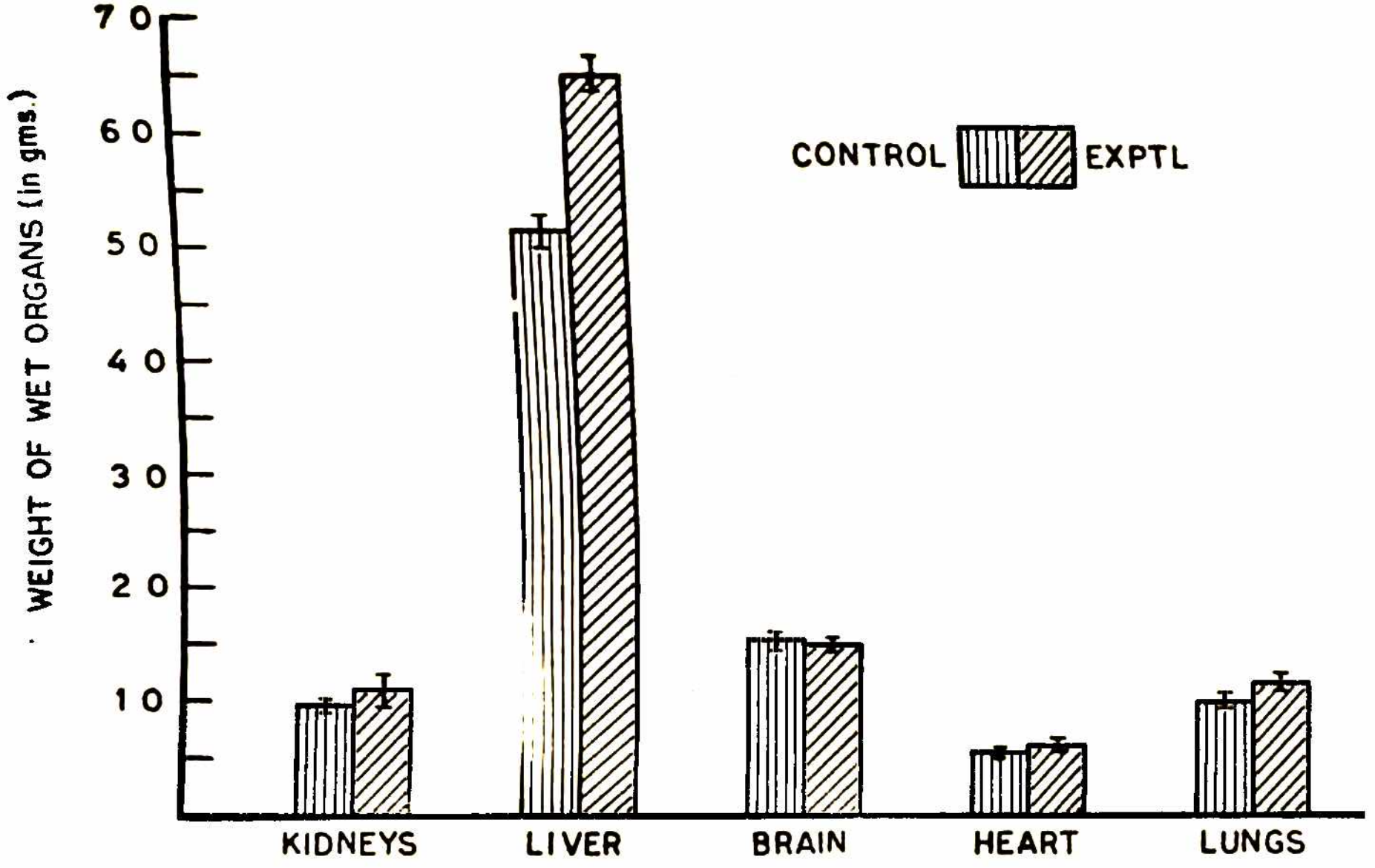

WEIGHT OF WET ORGANS (in gms.)
70
60
50
40
30
20
10
CONTROL
EXPTL
KIDNEYS
LIVER
BRAIN
HEART
LUNGS
ORGANS

FIG: 8

TABLE: 9.

EFFECT OF CARBONDISULFIDE TREATMENT (25.0 mg/kg body weight) FOR ONE MONTH ON GLYCOGEN LEVEL(G/100 G tissue weight) OF LIVER.

ORGANS	No. of Rats	Control	CS_2 Treated	Percentage Change	Significance Level.
LIVER	6	2.102.04 ± .327	3.676.12 ± .327	+ 74.88	< .005
BRAIN	6	.977. ± .177	1.586 ± .251	+ 52.2	< .025

FIGURE: 9

SHOWS THE EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT ON THE GLYCOGEN LEVEL OF LIVER AND BRAIN.

TABLE : 10.

EFFECT OF CHRONIC TREATMENT (25.0 mg/kg body weight) FOR ONE MONTH ON TOTAL PROTEIN LEVEL (miligrams of Proteins/ gm tissue weight) OF LIVER AND BRAIN.

ORGANS	No. of Rats	Control	CS_2 Treated	Percentage Change	Significance Level.
LIVER	6	136.25 ± 4.71	114.543 ± 4.29	- 15.93	< .005
BRAIN	6	55.454 ± 7.653	86.171 ± 22.613	+ 55.39	< .1

FIGURE: 10

SHOWS THE EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT FOR ONE MONTH ON THE TOTAL PROTEIN LEVEL OF LIVER AND BRAIN.

TABLE : 11.

EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT (25.0 mg/kg. body weight) FOR, ONE MONTH, ON SERUM ALPHA AMYLASE ACTIVITY, BLOOD GLUCOSE LEVEL, PLASMA PROTEIN.

Parameter	No. of rats	* Control Units/100 ml.	CS_2 Treated *Units/100 ml.	Percentage Change	Significance Level.
Serum Alpha Amylase Activity	6	534.66 ± 31.32	798.33 ± 18.81	+ 49.31	< .005

Parameter	No. of rats	Control mgs/100 ml.	CS_2 Treated mgs/100 ml	Percentage Change	Significance level.
Blood Glucose	6	90.50 ± 5.60	132.50 ± 6.20	+ 44.19	< .005
		gms/100 ml.	gm/100 ml.		
Plasma Proteins	6	10.10 ± .61	5.90 ± .54	- 41.5	< .005

*Amylase Unit :- Is the amount of enzyme that will hydrolyze 10.0 mg of Starch in 30.0 minutes to a stage at which no color is given by iodine.

FIGURE : 11

SHOWS THE EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT FOR ONE MONTH ON THE BLOOD GLUCOSE LEVEL AND SERUM ALPHA ANYLASE ACTIVITY.

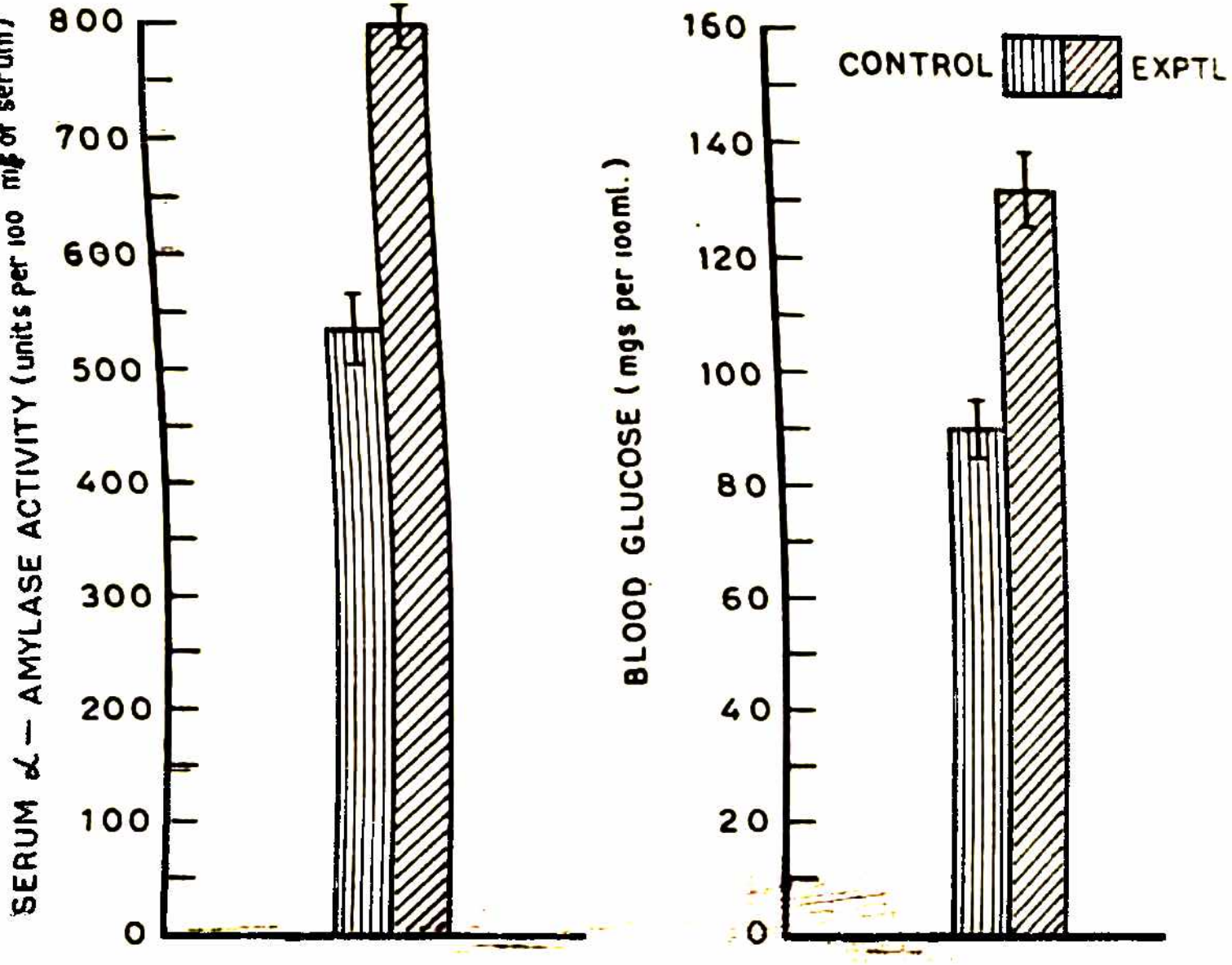

SERUM α – AMYLASE ACTIVITY (units per 100 ml of serum)
800
700
600
500
400
300
200
100
0
BLOOD GLUCOSE (mgs per 100ml.)
160
140
120
100
80
60
40
20
0
CONTROL
EXPTL

FIG : 11

TABLE : 12 .

EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT (25.0 mg/kg, body weight), FOR ONE MONTH ON ENZYME, PHOSPHORYLASE IN LIVER AND BRAIN.

ORGANS	No. of Rats	Control *Units.	CS_2 Treated *Units.	Percentage Change	Significance Level.
LIVER	5	1.201 ± .042	0.499 ± .0522	- 58.43	<.005
BRAIN	5	1.196 ± .101	1.076 ± .057	- 9.99	<.15 (N.S.)

One*Unit of enzyme is that while caused the liberation of 1.0 mg of inorganic phosphrorous per gm. of tissue weight in 10 minutes at 37°C.

TABLE: 13.

EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT (25.0 mg/ gm. body weight) , FOR ONE MONTH ON ENZYME, PHOSPHOGLUCOMUTASE ACTIVITY IN LIVER AND BRAIN.

ORGANS	No. of rats	*Control Units.	CS_2 Treated	Percentage Change	Significance Level.
LIVER	6	0.489 ± 0.266	1.707 ± 0.270	+ 248.59	< .005
BRAIN	6	0.719 ± .170	1.100 ± 0.202	+ 34.61	< .1

* Units = miligram of acid stable inorganic phosphorous liberated per gram of tissue weight per 30 minutes at 30°C.

TABLE : 14.

EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT (25.0 mg/kg, body weight) FOR ONE MONTH ON ENZYME FRUCTOSE 1-6 DIPHOSPHATASE, IN LIVER AND BRAIN.

ORGANS	No. of rats	Control *Units	CS_2 Treated *Units.	Percentage Change	Significance Level
LIVER	6	0.476 ± 0.115	0.252 ± .080	- 42.02	<.05
BRAIN	6	0.231 ± 0.019	0.181 ± 0.015	- 21.60	<.025

*Units = miligram of inorganic phosphorous liberated/gm of tissue weight per 30 minutes at 37°C.

FIGURE: 12

SHOWS THE EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT FOR ONE MONTH ON THE ACTIVITY OF PHOSPHORYLASE, FRUCTOSE, 1-6 DIPHOSPHATASE (FDPASE) AND PHOSPHO-GLUCOMUTASE IN LIVER AND BRAIN.

PHOSPHORYLASE UNIT : One unit of enzyme is that which caused the liberation of 1.0 mg of inorganic phosphorous per gram of tissue weight in 10 minutes at 37°C.

FDPase Unit : Unit activity is defined as miligrams of inorganic phosphorous liberated per gram of tissue weight in 20 minutes at 37°C.

Phosphoglucomutase Unit : Unit is defined as miligrams of acid stable inorganic phosphorous liberated per gram of tissue weight in 30 minutes at 30°C.

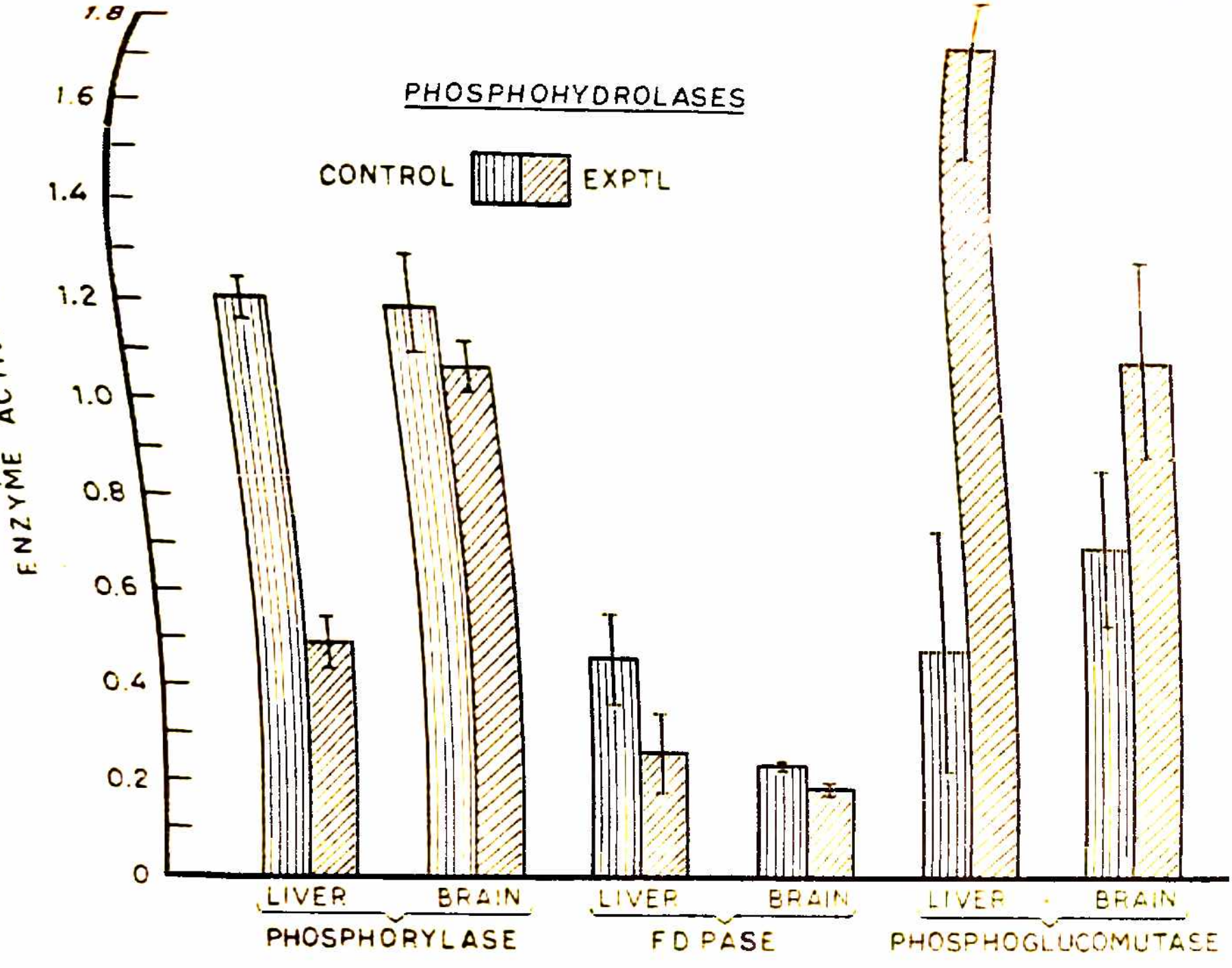

PHOSPHOHYDROLASES
CONTROL
EXPTL
ENZYME ACTIVITY (UNITS)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
LIVER
BRAIN
PHOSPHORYLASE
LIVER
BRAIN
FD PASE
LIVER
BRAIN
PHOSPHOGLUCOMUTASE

FIG: 12

TABLE : 15.

EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT (25.0 mg/kg, body weight) FOR ONE MONTH ON ENZYME ADENOSINE TRIPHOSPHATASE IN LIVER AND BRAIN.

ORGANS	No. of rats	*Control Units.	CS_2 Treated *Units.	Percentage Change	Significance Level.
BRAIN	6	0.975 ± 0.171	0.734 ± .040	- 24.73	< .1
LIVER	6	1.083 ± .234	0.5606 ± .072	- 48.24	< .025

* ATPase Unit : miligram of inorganic phosphorous liberated per gram of tissue weight in 15 minutes at $37^{o}C$.

TABLE : 16.

EFFECT OF CARBONDISULFIDE TREATMENT (25.0 mg/kg, body weight) FOR ONE MONTH ON SUCCINIC DEHYDROGENASE ACTIVITY IN LIVER AND BRAIN.

ORGANS	Control *Units.	CS_2 Treated *Units.	Percentage Change	Significance Level.
LIVER	8.458 ± 1.28	5.345 ± .787	- 36.80	< .05
BRAIN	1.137 ± .258	.812 ± .108	- 28.54	< .1

* Unit Activity is expressed as mgs of the dye (TTC) reduced per 30 minutes at $37^{\circ}C$.

FIGURE: 13

SHOWS THE EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT FOR ONE MONTH ON THE ACTIVITY OF ADENOSINETRIPHOSPHATASE (ATPase) AND SUCCINIC DEHYDROGENASE (SDH) IN LIVER AND BRAIN.

ATPase Unit : Unit is defined as miligrams of inorganic phosphorous liberated per gram of tissue weight in 15 minutes at 37°C.

SDH Unit : Unit activity is expressed as miligrams of the dye (TTC) reduced in 30 minutes at 37°C.

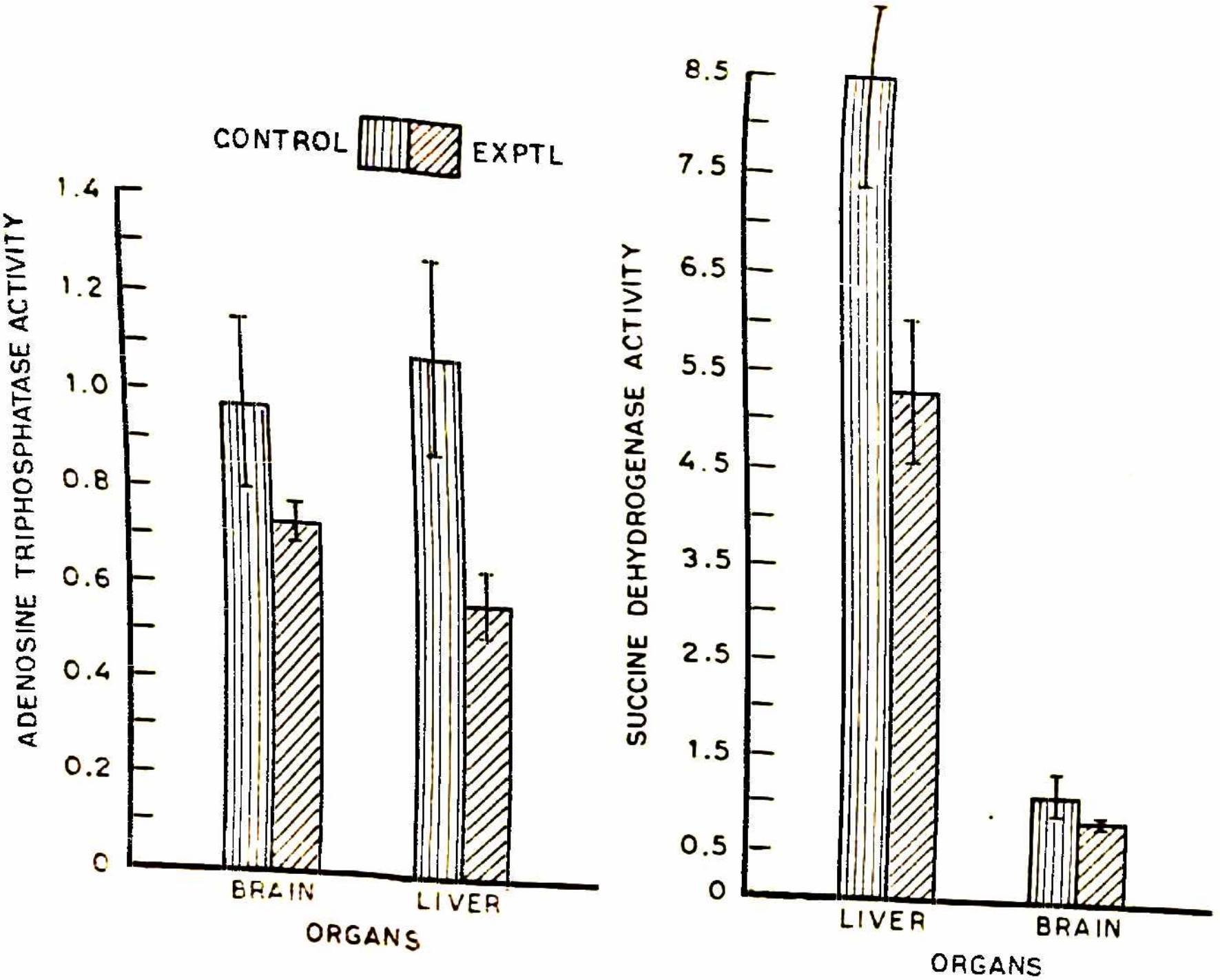

CONTROL
EXPTL
ADENOSINE TRIPHOSPHATASE ACTIVITY
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
BRAIN
LIVER
ORGANS
SUCCINE DEHYDROGENASE ACTIVITY
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
0
LIVER
BRAIN
ORGANS

FIG : 13

TABLE : 17.

EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT (25.0 mg/kg body weight)FOR ONE MONTH ON DOPAMINE LEVEL (ug/G. Tissue weight) OF BRAIN,HEART,LIVER AND LUNGS.

ORGANS	No. of rat	Control	CS_2 Treated	Percentage Change	Significant Level.
BRAIN	5	3.094 ± .057	8.082 ± .111	+ 161.24	<.005
HEART	5	1.264 ± .089	6.48 ± .074	+ 412.65	<.005
LIVER	5	2.24 ± .045	8.13 ± .048	+ 262.94	<.005
LUNGS	5	7.542 ± .043	2.12 ± .028	- 71.89	<.005

TABLE : 18.

EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT (25.0 mg/kg, body weight) FOR ONE MONTH, ON NOREPINEPHRINE LEVEL (ug/G. Tissue weight) OF BRAIN, HEART, LIVER AND LUNGS.

ORGANS	No. of rats	Control	CS_2 Treated	Percentage Change	Significant Level.
BRAIN	5	1.116 ± .081	2.08 ± .049	+ 86.37	<.005
HEART	5	0.89 ± .036	2.12 ± .321	+ 138.2	<.005
LIVER	5	1.136 ± .0811	1.78 ± .068	+ 56.69	<.005
LUNGS	5	0.84 ± .08	1.50 ± .054	+ 78.57	<.005

TABLE : 19.

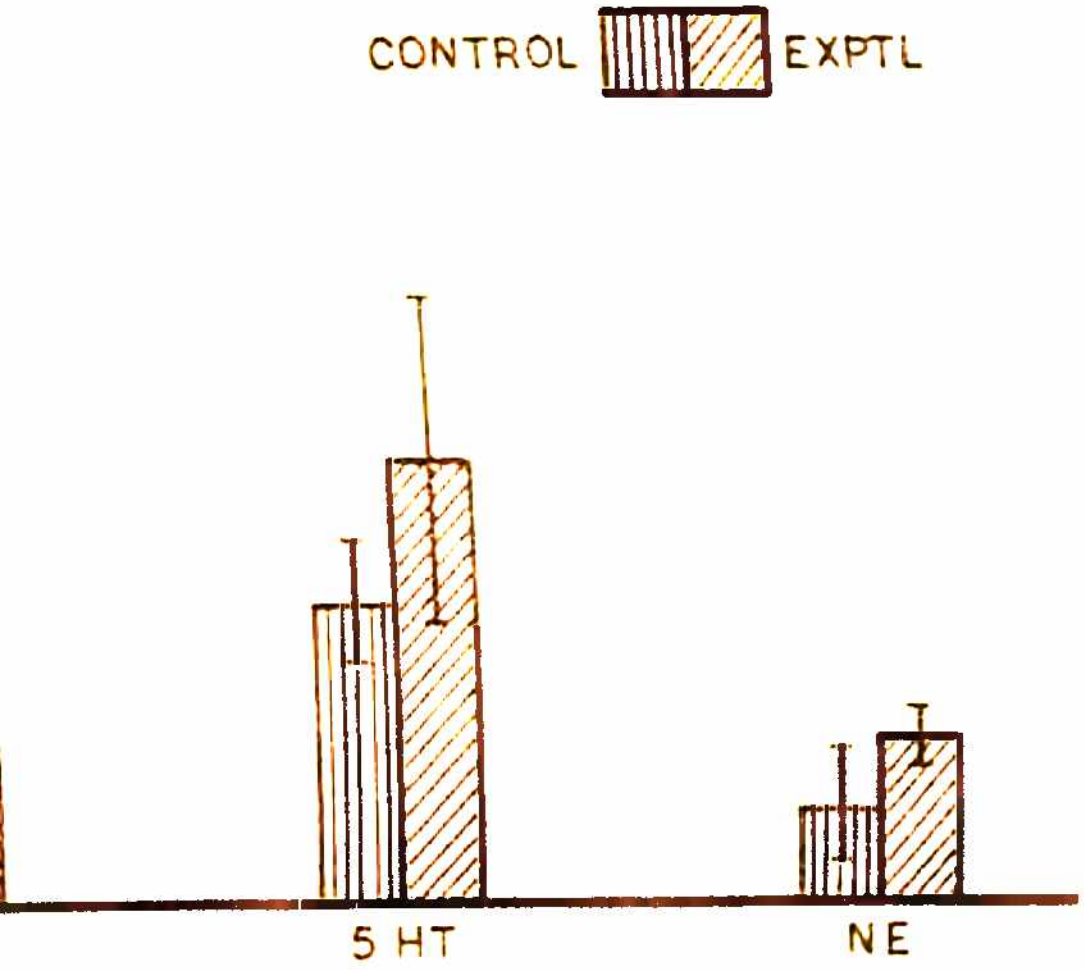
EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT (25.0 mg/kg, body weight) FOR ONE MONTH ON 5-HYDROXYTRYPTAMINE LEVEL (ug/G.Tissue weight) OF BRAIN , HEART, LIVER AND LUNGS.

ORGANS	No. of rats	Control	CS_2 Treated	Percentage Change	Significance Level.
BRAIN	5	3.77 ± .08	5.50 ± .26	+ 45.88	<.005
HEART	5	2.86 ± .037	4.06 ± .185	+ 41.95	<.005
LIVER	5	.60 ± .178	2.04 ± .272	+240.0	<.005
LUNGS	5	2.20 ± .167	6.24 ± .87	+367.1	<.005

[illegible]

12.0
10.0
8.0
6.0
4.0
2.0
0
µg/gm WET TISSUE Wt.
DA

BRAIN



BIOGENIC AMINES

FIG : 14

[illegible]

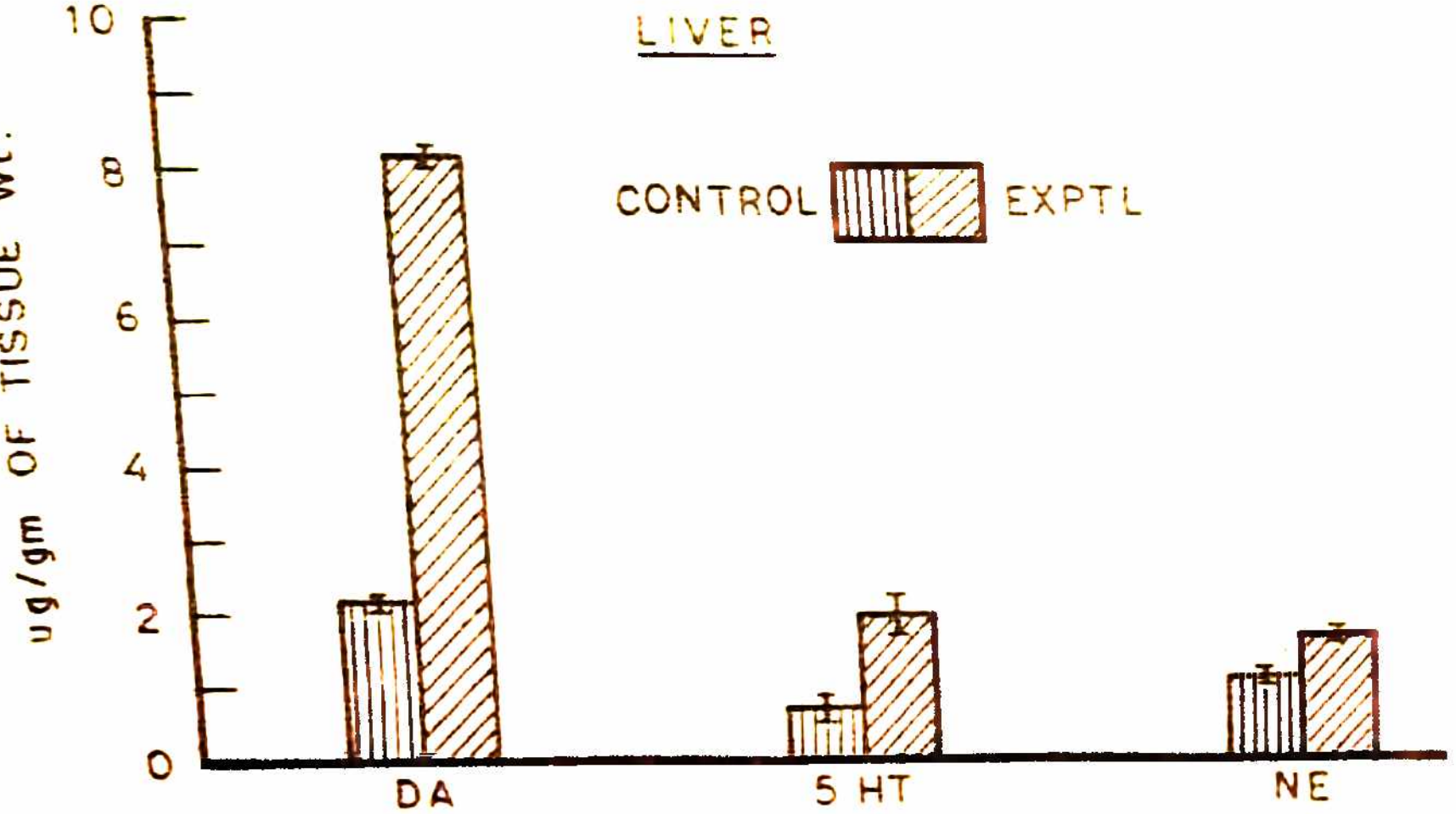

LIVER
CONTROL
EXPTL
ug/gm OF TISSUE Wt.
10
8
6
4
2
0
DA
5 HT
NE
BIOGENIC AMINES

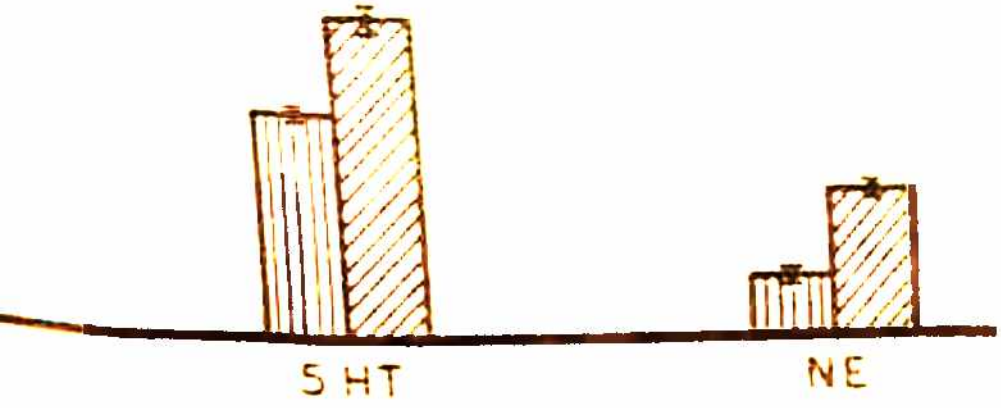
FIG : 15

FIGURE : 16

SHOWS THE EFFECT OF CHRONIC CARBON TETRACHLORIDE TREATMENT FOR ONE MONTH ON DA, 5-HT, AND NE LEVELS OF HEART & LUNGS

10
8
6
4
2
0
ug/gm OF TISSUE Wt.
DA

HEART



BIOGENIC AMINES

FIG: 16 A

TABLE : 20.

EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT (25.0 mg/kg, body weight) FOR ONE MONTH, ON MONOAMINE-OXIDASE ACTIVITY (MAO) OF BRAIN, HEART, LIVER AND LUNGS.

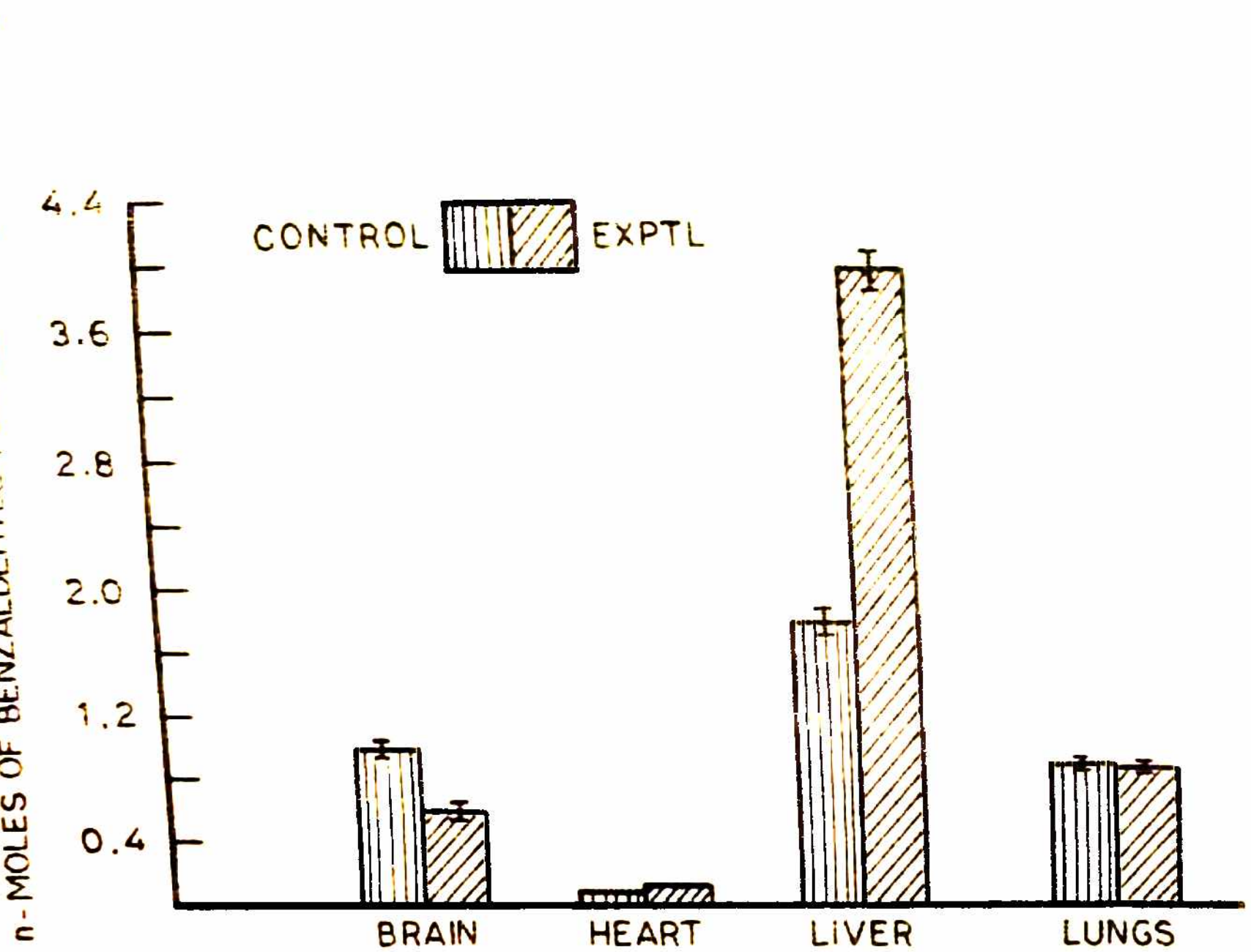
ORGANS	No. of rats	Control *Units	CS_2 Treated *Units.	Percentage Change	Significance Level.
BRAIN	5	1.01 ± .030	0.58 ± .028	-42.57	< .005
HEART	5	.061 ± .003	.091 ± .004	+ 49.18	< .005
LIVER	5	2.35 ± .085	4.024 ± .092	+71.23	< .005
LUNGS	5	0.95 ± .036	.91 ± .040	- 4.21	N.S.

* MAO Activity nanomoles of benzaldehyde release / gm of tissue weight/ per minute.

FIGURE: 17

SHOWS THE EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT FOR ONE MONTH ON MONOAMINE OXIDASE ACTIVITY OF BRAIN, LIVER, LUNGS AND HEART.

Monoamine Oxidase (MAO) Unit : Monoamine Oxidase Unit is defined as nano-moles of benzaldehyde produced per gram of tissue weight per minute.



MONO AMINE OXIDASE

FIG: 17

TABLE : 21.

EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT (25.0 mg/kg, body weight) FOR ONE MONTH ON GLUTATHIONE (ug/G.Tissue weight) LEVEL OF BRAIN, HEART, LIVER AND LUNGS.

ORGANS	No. of rats	Control	CS_2 Treated	Percentage Change	Significance Level.
BRAIN	5	2.680 ± .054	2.20 ± .028	- 17.91	<.005
HEART	5	.570 ± .034	.052 ± .0036	- 90.87	<.005
LIVER	5	8.460 ± .045	7.250 ± .070	- 14.30	<.005
LUNGS	5	.948 ± .087	.131 ± .005	- 86.18	<.005

FIGURE : 18

SHOWS THE EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT FOR ONE MONTH ON GLUTATHIONE LEVEL OF BRAIN, LIVER, LUNGS AND HEART.

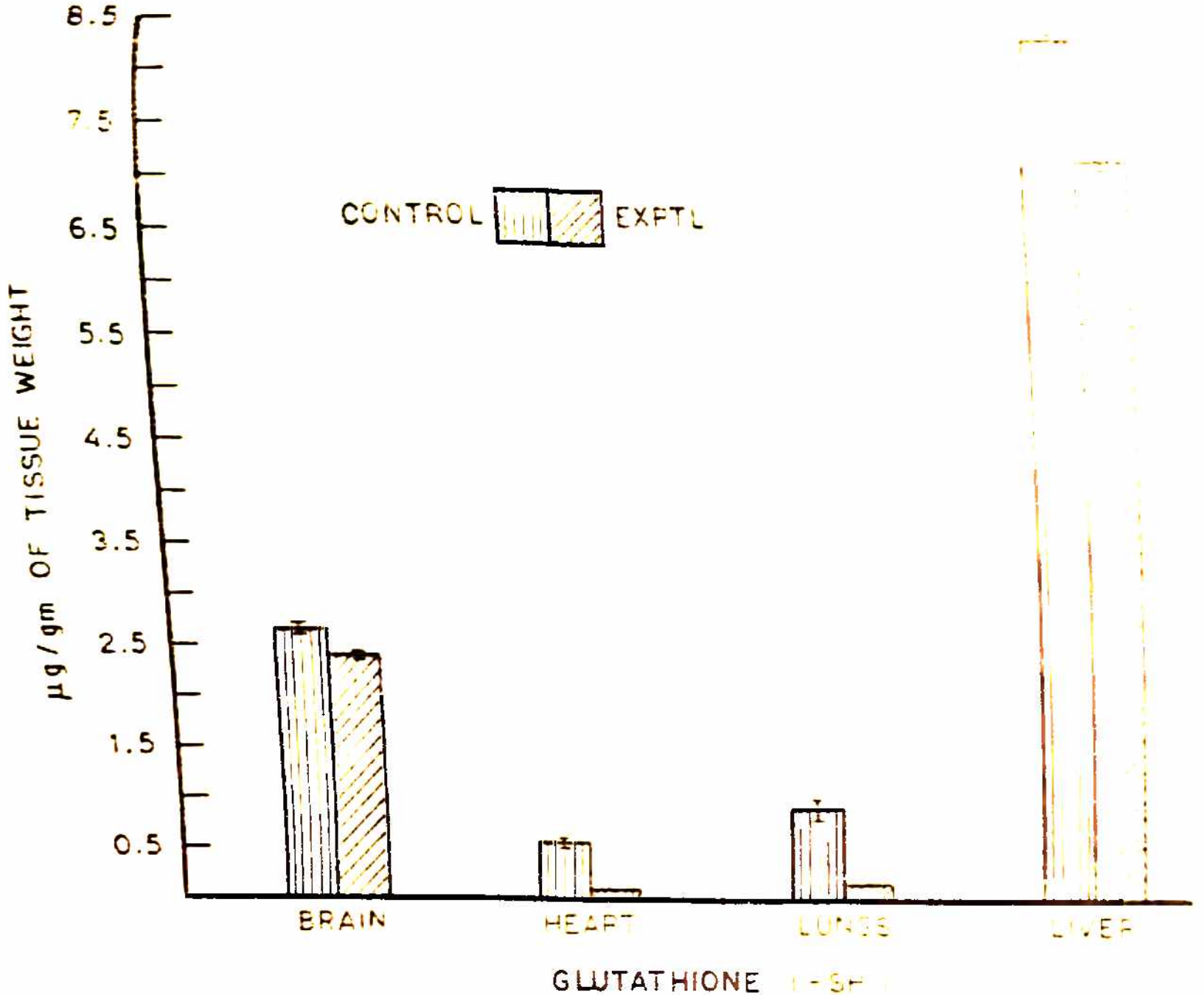

8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
µg / gm OF TISSUE WEIGHT
CONTROL
EXPTL
BRAIN
HEART
LUNGS
LIVER
GLUTATHIONE (-SH)

FIG : 18

TABLE : 22.

EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT (25.0 mg/kg, body weight) FOR ONE MONTH ON ZINC LEVEL (ug/G. Tissue weight) OF LIVER, PANCREAS, KIDNEY, TESTIS, BRAIN.

ORGANS	No. of rats	Control	CS_2 Treated	Percentage Change	Significance Level.
LIVER	5	22.02 ± .139	16.20 ± .066	- 26.43	< .005
PANCREAS	5	8.24 ± .0667	5.46 ± .0509	- 33.73	< .005
KIDNEY	5	12.76 ± .088	12.38 ± .090	- 2.97	N.S.
TESTIS	5	25.12 ± .082	16.36 ± .095	- 34.87	< .005
BRAIN	5	25.32 ± 1.32	20.82 ± 1.39	- 13.77	< .05

FIGURE : 19

SHOWS THE EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT FOR ONE MONTH ON ZINC LEVEL OF LIVER, BRAIN, TESTIS, PANCREAS AND KIDNEYS.

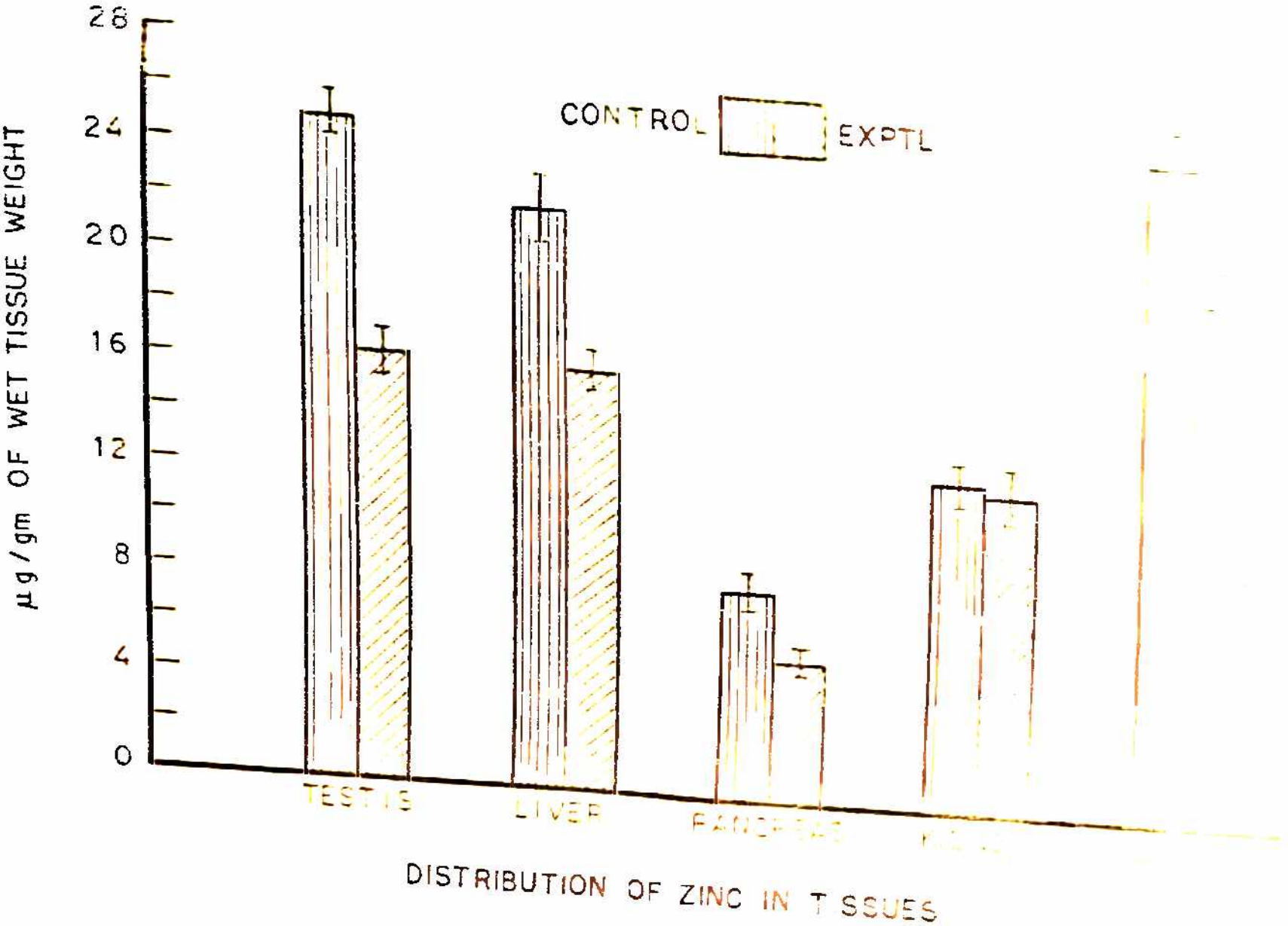

µg/gm OF WET TISSUE WEIGHT
28
24
20
16
12
8
4
0
CONTROL
EXPTL
TESTIS
LIVER
PANCREAS
DISTRIBUTION OF ZINC IN TISSUES

FIG: 19

TABLE : 23.

EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT (25.0 mg/kg, body weight) FOR ONE MONTH ON MANGANESE LEVEL (ug/G. Tissue weight) OF LIVER, PANCREAS, KIDNEY, TESTIS, BRAIN.

ORGANS	No. of rats	Control	CS_2 Treated	Percentage Change	Significance Level.
LIVER	5	1.35 ± .079	1.82 ± .035	+ 34.81	< .005
PANCREAS	5	0.65 ± .035	1.4 ± .254	+150.38	< .005
KIDNEY	5	1.20 ± .42	1.26 ± .035	+ 18.42	N.S
TESTIS	5	2.092 ± .126	1.32 ± .04	- 36.90	< .005
BRAIN	5	.50 ± .06	0.44 ± .05	- 12.0	N.S.

FIGURE : 20

SHOWS THE EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT FOR ONE MONTH ON MANGANESE LEVEL OF LIVER, BRAIN, TESTIS, PANCREAS AND KIDNEYS.

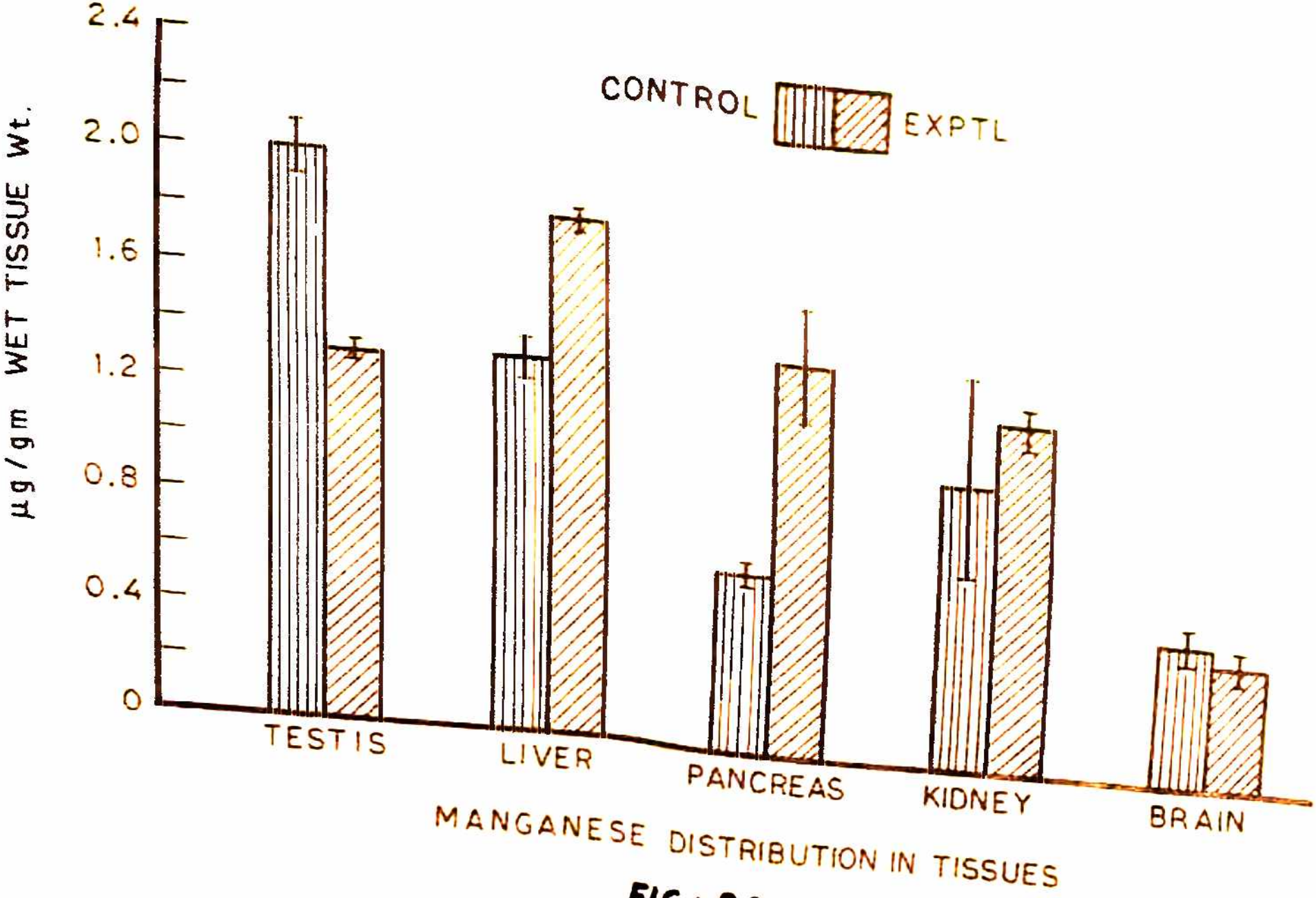

2.4
2.0
1.6
1.2
0.8
0.4
0
µg/gm WET TISSUE Wt.
CONTROL
EXPTL
TESTIS
LIVER
PANCREAS
KIDNEY
BRAIN
MANGANESE DISTRIBUTION IN TISSUES

FIG : 20

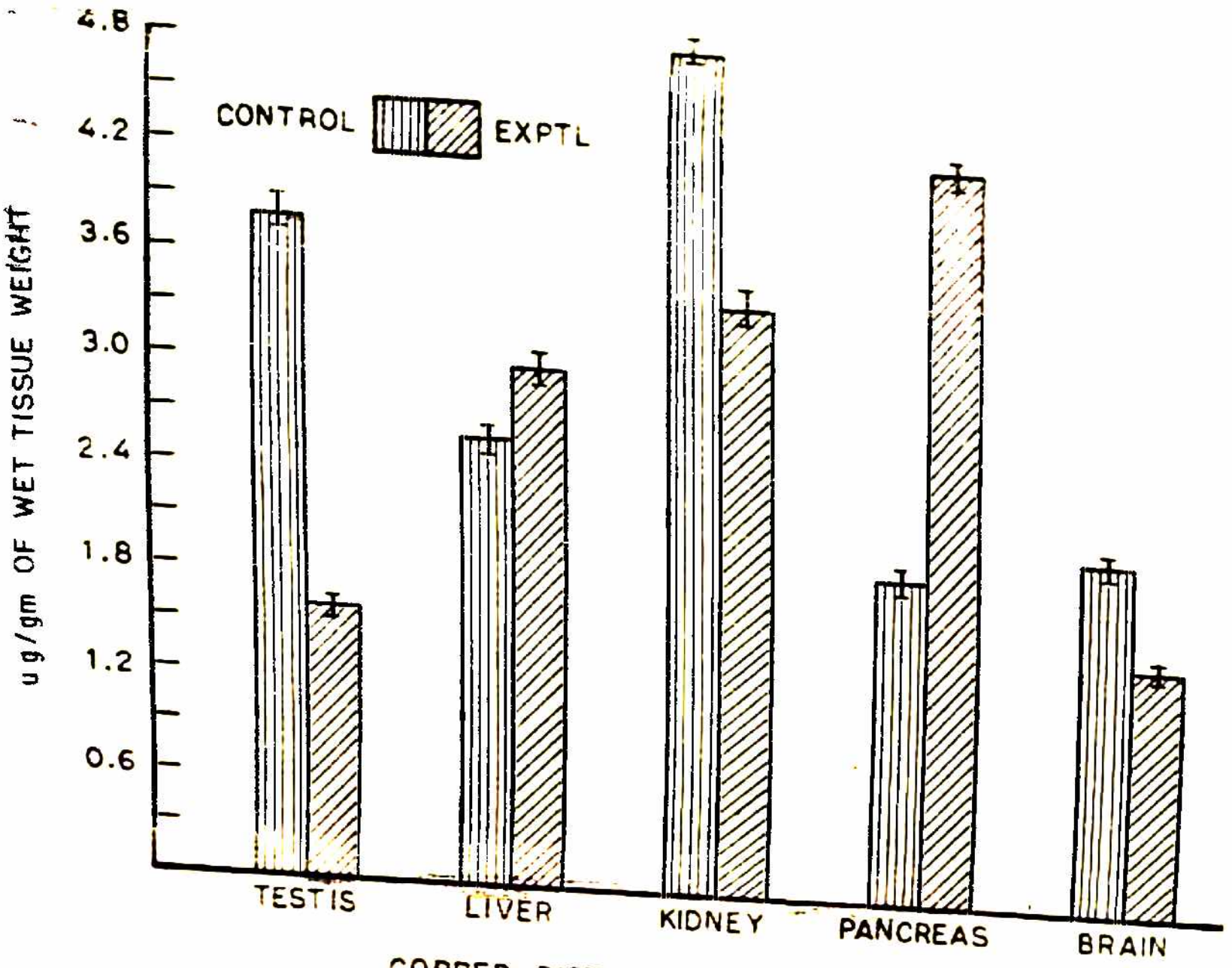
TABLE : 24.

EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT (25.0 mg/kg, body weight) FOR ONE MONTH ON COPPER LEVEL (ug/G. Tissue weight) OF LIVER, PANCREAS, KIDNEY, TESTIS, BRAIN.

ORGANS	No. of rats	Control	CS_2 Treated	Percentage Change	Significance Level.
LIVER	5	2.62 ± .076	3.00 ± .085	+ 14.50	< .005
PANCREAS	5	1.66 ± .073	4.28 ± .060	+157.83	< .005
KIDNEY	5	4.88 ± .066	3.40 ± .088	- 30.32	< .005
TESTIS	5	3.88 ± .063	1.56 ± .055	- 59.79	< .005
BRAIN	5	2.02 ± .056	1.47 ± .048	- 27.22	< .005

FIGURE : 21

SHOWS THE EFFECT OF CHRONIC CARBONDISULFIDE TREATMENT FOR ONE MONTH ON COPPER LEVEL OF LIVER, BRAIN, TESTIS, PANCREAS AND KIDNEYS.



COPPER DISTRIBUTION IN TISSUES

FIG : 21

CHAPTER- 4

DISCUSSION

4.1 EFFECT OF ACUTE CARBONDISULFIDE TREATMENT :-

4.1.1. GLYCOGEN :-

The acute damage to liver and brain have been considered to be the important manifestations of acute CS_2 poisoning (Seppalainen, 1977 ; DeMatteis and Seawright,1976; Jarvisalo and Gibbs, 1978; Mckenna and Victor, 1977). The damage to the liver appeared to be more intensive because of apparent sudden release of stored glycogen in the animals from 1 to 4 hour after CS_2 injection ($P < .005$). This finding support the observations of Kurzinger and Freundt (1969), who reported a sharp decline in liver glycogen by CS_2 intoxication.

The brain glycogen has been, however, found to be unaffected during the period of observation, which reflects that brain glycogen might be bound more firmly and CS_2 cannot affect the bound glycogen. Kacew and Singhal (1973) have also demonstrated that administration of p - p', DDT produced a marked decrease in concentration of hepatic glycogen. The acute administration of CS_2 in a large dose appears to produce the damage to the adrenals and thus results in the release of catecholamines. It is, therefore, possible that these endogeneously liberated catecholamines have produced the breakdown of liver glycogen.

4.1.2. BLOOD GLUCOSE :-

Blood glucose is effected drastically by acute CS_2 treatment as it significantly increased in first hour ($P < .005$) The fall of glucose level which has its onset from second hour persisted till the fourth hour, confirmed the earlier reports of the fall in blood glucose level by CS_2 toxicity in rat (Cunningham, 1975), Cunningham and Daphne (1977), also studied the turnover of blood glucose under CS_2 intoxication. Katz et.al. (1974) concluded that fall in blood glucose has been at triosephosphate level and gluconeogenesis is not stimulated. The initial rise in blood glucose in first hour may therefore, be due to imbalance of released glucose and insulin to rapid hepatic glycogen breakdown as have been suggested earlier. But in the later hours i.e. from second hour onward perhaps, glucose utilisation have increased or muscular glycogen deposition has enhanced due to increased insulin in blood.

4.1.3. SERUM ALPHA AMYLASE :-

Serum alpha amylase activity has been found to be increased sharply after CS_2 treatment ($P < .005$). This increased serum alpha amylase activity has been a proof of the damage to pancreas, which is involved in carbohydrate metabolism (Carway, 1959). Therefore, the sharp decline in blood glucose during acute intoxication alongwith parallel rise in serum alpha amylase activity confirms our assumption of the sudden release of insulin from pancreatic storage granules. Hence,

this mechanism is responsible for higher circulating level of insulin and increased glucose turnover (Cunningham et.al. 1976).

4.1.4. PROTEINS :-

Total protein level in tissues, as well as in the plasma showed a marked fall during first hour of acute CS_2 intoxication. This finding confirms the earlier suggestion of Bond and De Matteis (1969) that CS_2 intoxication decreased the synthesis of liver proteins or depletion of proteins along-with hyperaminociduria and hyperaminoacidemia (EL'Dessoukey et.al. 1977) or the increase turnover of proteins (Jarvisalo 1977); or higher level of urea in the plasma (Cunningham, 1975). These observations tend this study to propose that protein degradation is enhanced under acute CS_2 toxicity, which may be providing fuel for energy and gives evidence for weight loss in man and animals exposed to CS_2.

The acute studies of CS_2 toxicity therefore, lead to suggest that carbohydrate metabolism is immediately affected. The liver appears to be the main site of action of CS_2 and liver glycogen gets depleted rapidly which in turn lead to hyperglycemia. The hypoglycemia alongwith glycogen depletion from liver indicates the rapid utilization of glucose which could be attributed to the observed rapid and faster respiration rate. Further, the mobilization of glycogen from liver to muscles

might also play some role. The increased serum alpha amylase activity is an indicator of the pancreatic damage which caused the excessive release of insulin and hence may increase peripheral utilization of glucose,. The decreased liver proteins and plasma proteins also support the assumption of mobilization of proteins from liver to muscles. Further, this study has provided evidence that CS_2 might be responsible for the enhanced degradation of proteins in liver for energy metabolism, and probable rise of blood urea (Cunningham et.al. 1975).

4.2. EFFECT OF CHRONIC CARBON DISULFIDE TOXICITY :-

4.2.1. GLYCOGEN :-

Glycogen of liver ($P < .005$) and brain ($P < .025$) increased significantly with one month chronic treatment of CS_2. This observation, however, are in contrast to the effect of p-p'. DDT observed by Kacew and Singhal (1973), who observed a decrease in hepatic glycogen after its chronic treatment. Therefore, the sites of hepatic toxicity of CS_2 and p-p'. DDT could be different and the rise in glycogen in CS_2 poisoning might be due to increased glycogen synthesis or/and decreased glycogen breakdown as is revealed by the study of enzymes described further in this work.

4.2.2. BLOOD GLUCOSE :-

The permanent rise in blood glucose may be due to

the uniformly decreased peripheral utilization of glucose or inhibition of glycolysis in liver and muscles as also observed with p-p'- DDT by Kacew and Singhal (1973). This could be due to inhibition of glucose metabolising enzymes and increased serum alpha amylase activity. The increase in enzyme activities has been attributed to pancreatic damage, which probably is responsible for decreased glucose utilization for the energy production and increased level of glucose in blood. These observations, therefore, tend to give some clue to the observed higher blood glucose and thus diabetogenic tendencies in workers exposed to CS_2 for long duration of time (Gotto et.al. 1971; Hernberg et.al. 1977).

4.2.3. TOTAL PROTEINS :-

The chronic treatment of CS_2 is not found to effect the liver and brain proteins significantly whereas plasma proteins have been found to be decreased significantly, $P < .005$ (Jarvisalo et.al. 1977; Cunningham et.al. 1975). The reduction in plasma protein level was related to the increased turnover of proteins and higher urea excretion and urea concentration in plasma by earlier workers (Cunningham et.al. 1977 ; Jarvisalo et.al. 1977). These observations also confirm our previous contention that liver is more susceptible than brain in CS_2 toxicity.

FIGURE: 22

SHOWS THE PATHWAY OF GLYCOGEN SYNTHESIS AND STIMULATION CAUSED BY CARBONDISULFIDE OF PHOSPHOGLUCOMUTASE AND INHIBITION OF PHOSPHORYLASE AND FRUCTOSE, 1-6 DIPHOSPHATASE IN LIVER.

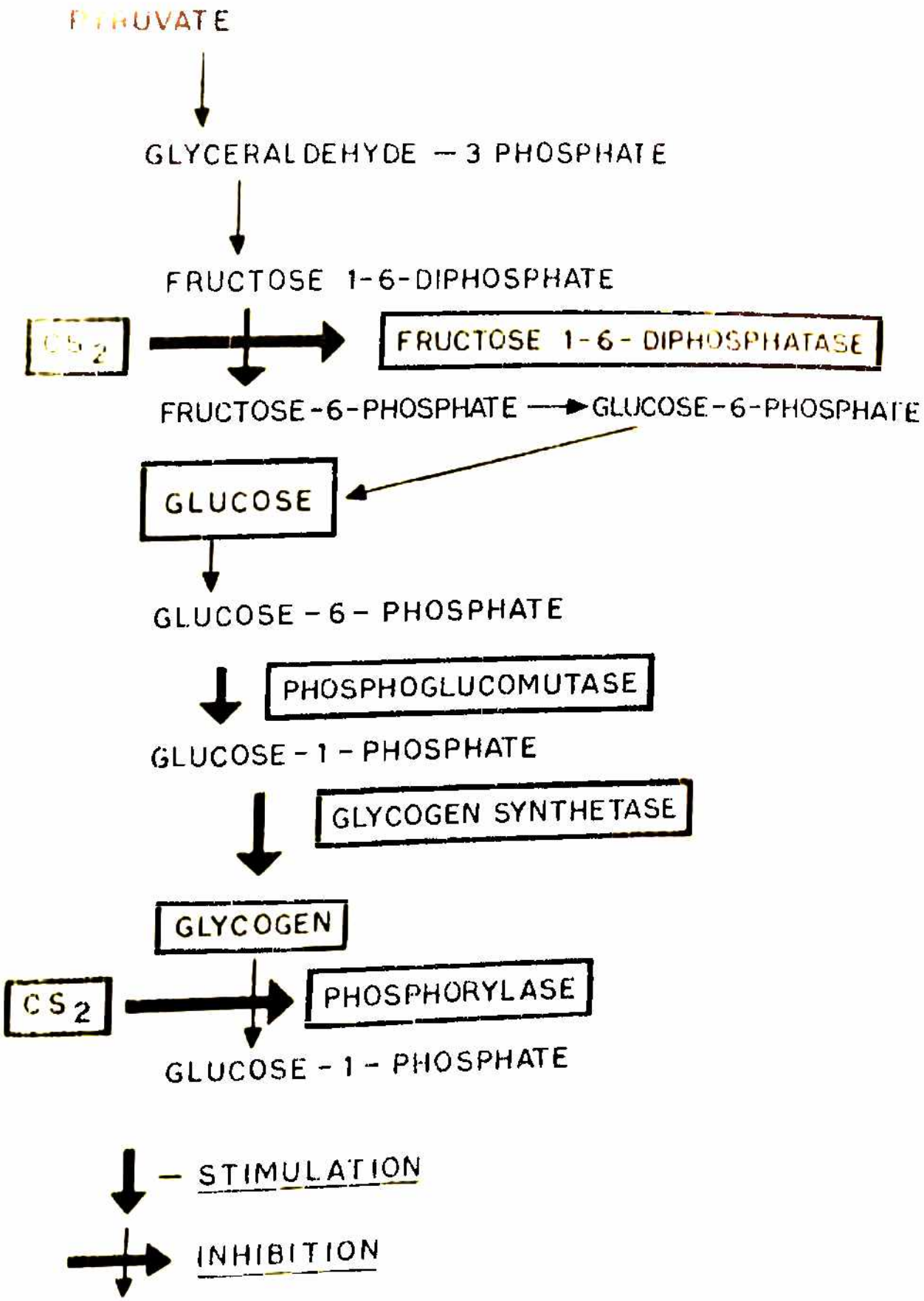

PYRUVATE
GLYCERALDEHYDE — 3 PHOSPHATE
FRUCTOSE 1-6-DIPHOSPHATE
CS_2
FRUCTOSE 1-6-DIPHOSPHATASE
FRUCTOSE-6-PHOSPHATE
GLUCOSE-6-PHOSPHATE
GLUCOSE
GLUCOSE-6-PHOSPHATE
PHOSPHOGLUCOMUTASE
GLUCOSE-1-PHOSPHATE
GLYCOGEN SYNTHETASE
GLYCOGEN
CS_2
PHOSPHORYLASE
GLUCOSE-1-PHOSPHATE
— STIMULATION
INHIBITION

FIG: 22

4.2.4. PHOSPHORYLASE :-

The reduction of liver phosphorylase (P < .005), supported the earlier observations of Chandra et.al. (1972), who too observed the reduction of phosphorylase histochemically. The fall in phosphorylase activity leading to retardation of glycogen degradation may therefore be responsible for the accumulation of glycogen in the liver. The brain phosphorylase activity again, observed to be unaffected which confirms further, that the increase in brain glycogen is due to increased glycogen synthesis rather than decreased breakdown as observed in liver.

4.2.5. PHOSPHOGLUCOMUTASE :-

Glucose is converted to glucose 6-phosphate and then to glucose 1- phosphate by phosphoglucomutase, which then get transformed to glycogen by glycogen synthetase system. Hockin et.al. (1968) reported a decrease in the activity of glucose 6-phosphate dehydrogenase, hexokinase and glucokinase in rabbits after CS_2 exposure. These results showed that there is a decrease in catabolism of glucose by glycolysis and hexose monophosphate shunt (HMP) pathway. Therefore, the present observation of decreased phosphorylase and enhanced phosphoglucomutase with chronic CS_2 treatment tempts one to assume that more of glucose is channelised for the formation of glycogen rather than its normal degradation. Thus, the incrased glycogen content in liver and brain can be attributed to the rise in phosphoglucomutase.

4.2.6. ADENOSINETRIPHOSPHATASE :-

Sidrorwicz et.al. (1977), while studying the effect of CS_2 on red cell metabolism in viscose rayon workers, reported a decreased level of ATP and increased level of AMP, ADP. The present study has also observed a decrease in ATPase, thus leading to the reduction of oxidative phosphorylation phenomena. This observation may contribute an explanation to hypoxia observed by Sidrorwicz et.al. (1977) by CS_2 toxicity. The ATPase inhibition by CS_2 has been found to affect the membrane transport of metabolites and thus be responsible for the disturbance in metabolic pathways (Srivastava et.al. 1975).

4.2.7. FRUCTOSE, 1-6 DIPHOSPHATASE (FDPase) :-

Ol'Khovaskaya (1976) found the inhibited activity of aldolase due to inhibition of glycolysis in erythrocytes in CS_2 toxicity. The present study has revealed the fall in FDPase activity which, therefore, suggests the inhibition of gluconeogenesis (Cunningham and Holt, 1977). Therefore, the chronic treatment with CS_2 can be observed to reduce the glucose formation by gluconeogenesis from noncarbohydrate precursors. The inhibition of FDPase confirms that increased glucose level may be due to decreased glycolysis rather than increased gluconeogenesis.

4.2.8. SUCCINIC DEHYDROGENASE :-

Succinic dehydrogenase is a key enzyme of Kreb's cycle

and links with energy utilisation and CS_2 inhibits the energy metabolismng enzymes of liver and brain (i.e., brain and liver ATPase and succinic dehydrogenase). The SDH and ATPase are considered to be important since these are membrane bound enzymes (Murasko and Gubskis, 1976). The inhibition of these enzymes showed an alteration of membrane transport, which can be an interaction of CS_2 with mitochondrial membrane. This chemical intoxication may thus,lead to some kind of derangement in the membrane pattern of lipids of the mitochondria, which could lead to alteration in the energy metabolism of carbohydrates.

It may, therefore, be proposed from the study of chronic treatment that CS_2 inhibits the glucose breakdown caused by glycolysis or HMP pathway alongwith inhibition of gluconeogenesis. This phenomena has been due to the decreased glucose breakdown and causes the reduction in the synthesis of glucose. It could be the responsible phenomenon for the stimulation of the biosynthesis of glycogen and also reduction in glycogen breakdown. The membrane bound mitochondrial enzymes inhibition resulted in the reduced respiration rate and normal functioning of Kreb's cycle or electron transport chain. However, the energy production could be due to an increased protein breakdown rather than glucose metabolism.

FIGURE : 23

SHOWS THE RELATIONSHIP BETWEEN GLYCOGEN, PHOSPHORYLASE AND PHOSPHOGLUCOMUTASE UNDER THE STRESS OF CARBONDI-SULFIDE.

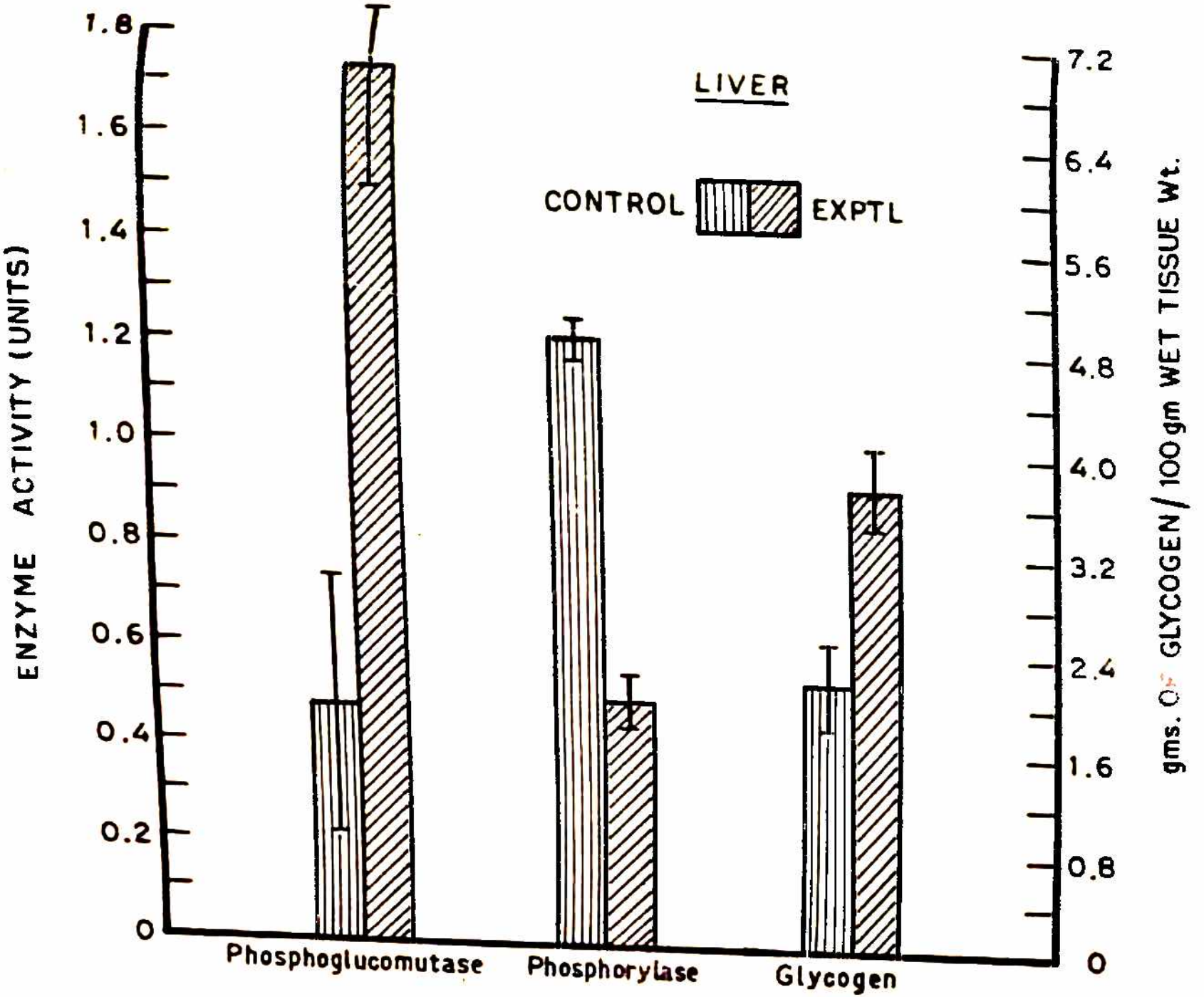

LIVER
CONTROL
EXPTL
ENZYME ACTIVITY (UNITS)
gms. GLYCOGEN / 100 gm WET TISSUE Wt.
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
7.2
6.4
5.6
4.8
4.0
3.2
2.4
1.6
0.8
0
Phosphoglucomutase
Phosphorylase
Glycogen

FIG : 23

4.2.9. ACUTE VS CHRONIC CS_2 TOXICITY :-

Chronic biochemical changes are more firm qualitatively and quantitatively in contrast to that of acute studies. The chronic studies revealed that CS_2 might be inhibiting glycolysis as well as gluconeogenesis which have been shown by the inhibition of FDPase, SDH and ATPase. But the rise in glycogen level is observed which is attributed to the increased phosphoglucomutase and decreased phosphorylase activity. In acute studies, the glycogen depletion is noticed alongwith hypoglycemia in later hours which suggested a quick mobilization of glycogen from liver to peripheral tissues. The glycolysis may be supposed not to be inhibited under acute toxicity because of lowering of blood glucose level which occur suddenly in response to insulin release from pancreas.

4.3. ALTERATIONS OF BIOGENIC AMINES BY CHRONIC CARBONDISULFIDE TREATMENT :-

4.3.1. CARBONDISULFIDE TOXICITY AND CATECHOLAMINES :-

It is well established that CS_2 forms dithiocarbamate with catecholamines in the tissues undergoing metabolic conversion. Most of the catecholamine metabolising enzymes are also reported to be inhibited by the action of these dithiocarbamates (Stripp et.al. 1969). A few of the studies on acute treatment

with CS_2 have revealed that brain NE level is decreased while observations with brain DA are varied (Stripp et.al. 1969). Maj and Vetulani (1970) found that N-N'-disubstituted dithiocarbamates synthesized in the nerve granules, by the action of catecholamines with CS_2 , have been responsible for the reduction of brain NE content and spontaneous activity in animals. Magos and Jarvis (1970) found that rats exposed to CS_2 for two days showed a decrease in NE and increase in DA, and found no change in monoamine oxidase (MAO) activity. This may be attributed to the increased DA uptake, (Mckenna and Distefano 1977). They suggested that the reduction in rat brain NE and DA level has been due to the formation of diethyldithiocarbamate complex formed by interaction of CA with CS_2 on chronic or prolonged treatment. But the present chronic treatment study could not confirm their contention as there has been an increase of DA level in chronic treatment which indicated that granular DA, more firmly bound, may not be available for complex formation. Further, the rise in brain NE concentration may be partially attributed to the decreased brain MAO activity ($P < .005$) and unaffected granular DA.

The simultaneous studies on the estimation of biogenic amines of liver and heart have focussed to the interesting observations of increased MAO activity alongwith an increase of both DA and NE. These observations suggest that dopamine

beta- hydroxylase of liver may not be inhibited with chronic CS_2 treatment as observed in plasma under stress (Schanberg and Krishner, 1976 ; Mckenna and Distefano 1977). This suggested that chronic CS_2 intoxication caused stress and does not appear to affect the dopamine beta-hydroxylase as much seen with acute treatment. This statement is further augmented by an observed increase of copper made available for DBH in liver.

Reduced glutathione (G-SH), a protecting agent against many toxicants has been known to prevent the covalent binding of toxic substances to the important enzymes and proteins. The glutathione was reported to be decreased appreciably in the present studies as observed by earlier workers. (Corsini, 1972; Read et.al. 1972; Jallow et.al. 1971; Glende, 1972). The reduction in G-SH may therefore, contribute for the protective action against the reduction in enzymes like dopamine beta-hydroxylase and MAO in liver and heart. It is hence possible that the increased available tyrosine is directed for the synthesis of biogenic amines and thus causing an increase in NE and DA synthesis. This hypothesis is based on the results of Teisinger (1971), who reported that chronic CS_2 poisoning caused the deficiency of pyridoxalphosphate, a coenzyme responsible for decarboxylation and transamination reactions, required for normal tyrosine metabolism.

On the similar basis the elevation of NE and reduction

FIGURE : 24

SHOWS THE EFFECT OF CARBONDISULFIDE ON THE PATHWAY OF DOPAMINE SYNTHESIS AND INTERRUPTION OF FEED BACK MECHANISM BY CARBONDISULFIDE.

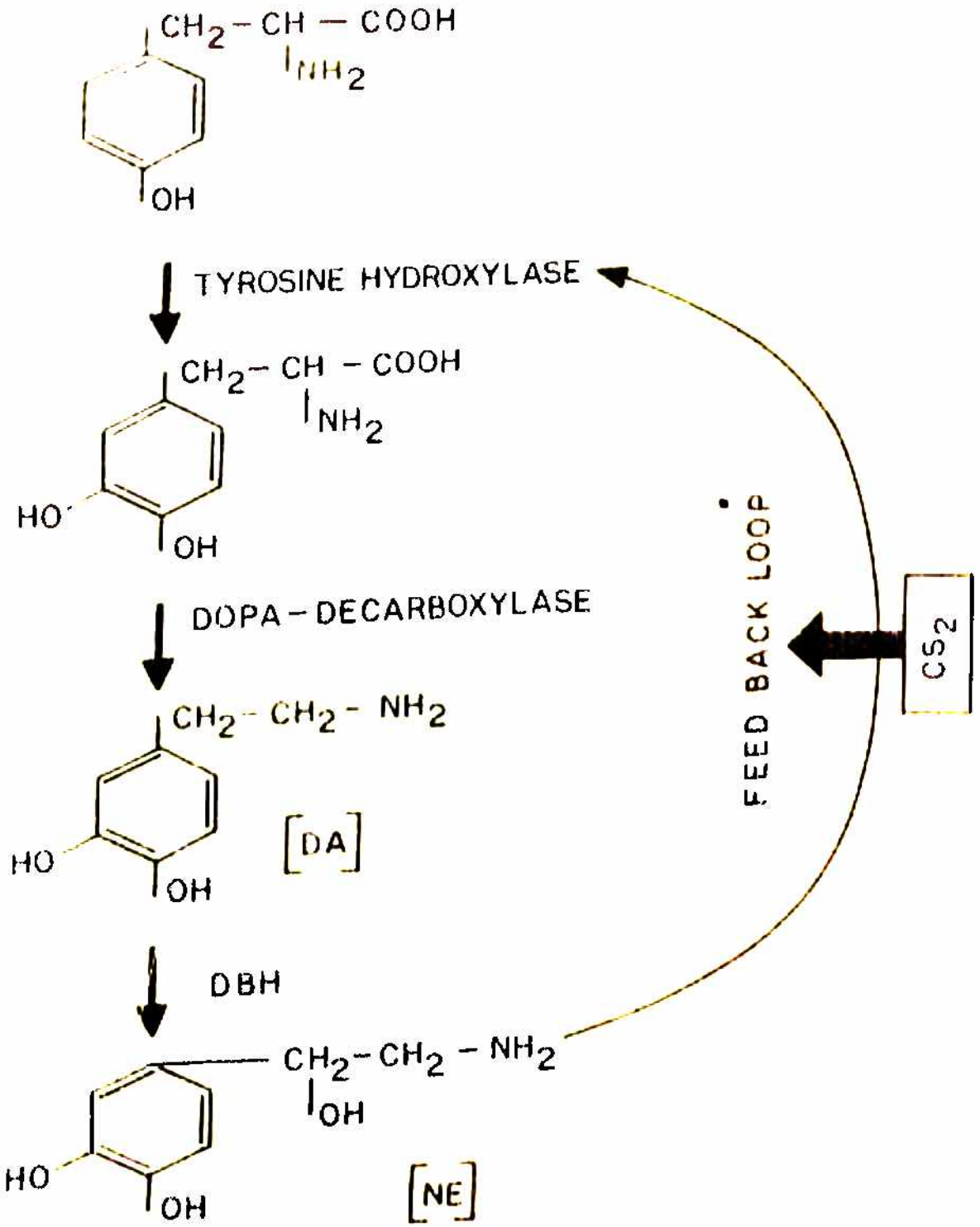

CH2 – CH – COOH
NH2
OH
TYROSINE HYDROXYLASE
CH2 – CH – COOH
NH2
HO
OH
DOPA – DECARBOXYLASE
CH2 – CH2 – NH2
HO
OH
[DA]
DBH
CH2 – CH2 – NH2
OH
HO
OH
[NE]
FEED BACK LOOP
CS2

FIG: 24

in DA levels in lungs after chronic CS_2 treatment may be responsible for the probable increase in the activity of dopamine beta-hydroxylase and decreased level of MAO activity ($P < .005$) observed in this study.

4.3.2. CARBONDISULFIDE TOXICITY AND 5 -HYDROXY-TRYPTAMINE :-

Aleksander (1966) and Meiner et.al. (1977) have reported an increased excretion of 5-HIAA in the urine of workers exposed to CS_2 and ozone respectively, and thought an increased degradation of 5-HT. Maj'Jerzy et.al. (1970) reported a rise in the level of brain 5-HT in toxicity studies with the disulfiram and diethyldithiocarbamate as observed in the present studies in brain, heart, liver and lungs of rats treated chronically with CS_2. However, the weight of lungs have not been increased which reflects that CS_2 does not effect either the tissues water retention as observed with other toxicant under similar conditions (Meiner et.al. 1977; Skillen et.al. 1961), or might not produce any injurious effect to kidneys to produce abnormal excretion of water. Therefore, the increase in the level of 5-HT can partially be explained on the basis of an increased tryptophan decarboxylase activity (Skillen et.al. 1961, with ozone). Besides, the rise in 5-HT level may also be in response to the inhibition of MAO and any MAO inhibitors is liable to cause an increase in the level of 5-HT in brain (Brodie and Shore 1957). In

FIGURE : 25

SHOWS THE EFFECT OF CARBONDISULFIDE ON THE PATHWAY OF TRYPTOPHAN CATABOLISM AND 5-HYDROXY TRYPTAMINE SYNTHESIS.

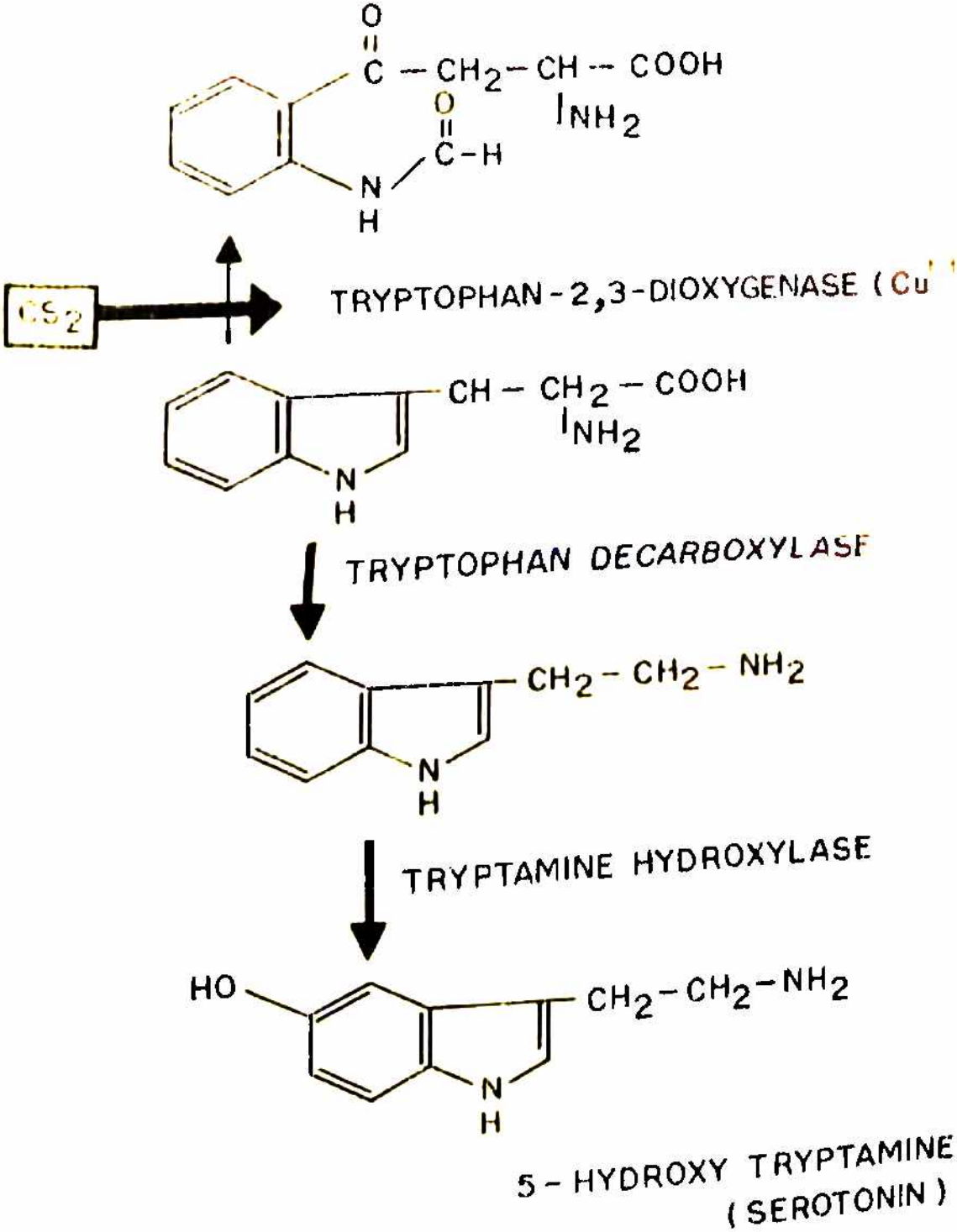

O
C – CH2 – CH – COOH
NH2
O
C – H
N
H
CS2
TRYPTOPHAN - 2,3 - DIOXYGENASE (Cu
CH – CH2 – COOH
NH2
N
H
TRYPTOPHAN DECARBOXYLASE
CH2 – CH2 – NH2
N
H
TRYPTAMINE HYDROXYLASE
HO
CH2 – CH2 – NH2
N
H
5 - HYDROXY TRYPTAMINE
(SEROTONIN)

FIG : 25

liver and heart, as there was not any significant inhibition of MAO, so the accumulation of 5-HT, may be wholly due to its increased synthesis. Normal tryptophan degradation pathway may, therefore, be assumed to be diverted for the increased synthesis of 5-HT because of presumed increase of tryptophan concentration. The increased synthesis of 5-HT leads to accumulation of 5-HT and the increased utilisation of this amino acid to the conversion of 5-HT. These findings lead one to think that tryptophan pyrrolase activity perhaps, is inhibited alongwith other enzymes of tryptophan degradation by CS_2 chronic treatment - of amines. The deficiency of pyridoxal phosphate caused by CS_2 toxicity may be another reason of 5-HT accumulation (Teisinger 1971). The decrease of tissue glutathione (G-SH) levels cannot also be overlooked since glutathione provides a defensive mechanisms to the body and various enzymes which react with CS_2 permanently.

Concludingly, we assume that simultaneous rise in DA and NE in most of the organs may be attributed to the increased activity of enzymes responsible for the catalysis of the conversion of Tyrosine to NE. From this it is suggested that feedback mechanisms have also been disrupted by chronic CS_2 toxicity which caused the rise in DA, NE and 5-HT in organs.

4.4. ALTERATION OF TRACE ELEMENT METABOLISM BY CARBONDISULFIDE TOXICITY :-

4.4.1. ZINC AND CARBONDISULFIDE TOXICITY :-

Earlier studies on zinc deficient rats have shown a reduced activity of various independent enzymes in testis bones, oesophagus and kidney (Prasad, 1966 ; Oberleas 1971). In the present work also, chronic CS_2 treatment causes a fall in SDH activity in liver and brain confirming the earlier view that this enzyme is a zinc dependent as it decreases with observed reduction of zinc. Therefore, supporting the earlier suggestion that SDH activity reduction causes focally occuring elective parenchymal necrosis in liver (Minder and Rothe, 1970). Similar fall of SDH activity in tissues of zinc deficient rat was also observed by Burch et.al. (1973) which is in support of our finding that CS_2 treatment causes zinc deficiency on the basis of another zinc metallo enzyme, aldolase study (Olkhovaskaya 1976). It can be proposed that CS_2 exposure can cause reduction of SDH and probably all other zinc dependent enzymes. Further, in this study the reduction in these enzymes activity bear a direct relationship to the decreased level of zinc and glycolysis in tissues.

The reduction of zinc in pancreas by chronic CS_2 treatment could be an indication of pancreatic dysfunction

because a considerable amount of zinc binds with insulin in pancreatic storage granules (Scott, 1934). On these basis, glucose tolerance of zinc deficient animals was reported to be decreased as compared to that of pair fed controls (Quarterman et.al. 1966; Boquis and Lernmark, 1969 ; Hendrick and Maheney, 1972; Huber and Gerschoff, 1973). Since zinc participates in the synthesis and storage of insulin in beta-cells of langerhans, therefore, the amount of insulin present in animals treated with CS_2 could be expected to be lowered, with reduced glucose tolerance which has been caused by an increased rate of insulin degradation (Hendricks and Mahoney,(1972) .

Theobserved rise in liver glycogen with an increased activity of phosphoglucomutase, shows that possibly, there is activation of the process of glycogen synthesis and reduction of glycolysis. This may partially or wholly be due to zinc deficiency caused by CS_2 treatment in liver, brain and pancreas. Hyper glycemia and decreased glucose catabolism in liver may, therefore, be due to the increased glycogen synthesis and its storage.

The depletion of total zinc of body by CS_2 chronic treatment may also be one of the reasons in the fall of protein level (Macpinlac et.al. 1968 ; Somers and Underwood, 1969 ; Sandstead et.al. 1971; Prasad et.al. 1971). Abnormality in protein metabolism pattern of plasma and tissues could indicate

that zinc deficiency can stimulate protein wastage by quick protein catabolism as suggested earlier (Fox and Hamson, 1965, Miller et,al. 1968 ; Tao and Hurlby, 1971 ; Macpinlac et.al. 1968; Somers and underwood, 1969). It has been indicated further, that impaired protein biosynthesis could also contribute to the deficiency of proteins in animals (Theuer and Hoekstra, 1968 ; Mills et.al. 1967). The decreased protein biosynthesis under zinc deficiency has been further related to lower RNA/ DNA ratios in liver, kidneys and pancreas (Prasad et.al. 1971) and higher non-protein nitrogen (Somers and Underwood,1969). The reduction of zinc in the body by CS_2 chronic treatment could be attributed to the reduction of its storage capacity of liver because of the degeneration produced by CS_2 (Unpublished observation, Subhash and Chopra, 1978).

4.4.2. MANGANESE AND CARBONDISULFIDE TOXICITY :-

Manganese homeostasis appears to be regulated at the excretory level rather than at the site of absorption (Maynard and Cotzias, 1958 ; Briton and Cotzias , 1966 ; Hughes et.al. 1966 ; Papavasiliou et.al. 1966; Berthicamps et.al. 1966). The regulation of bile from liver has been reported to be affected by manganese level, therefore, manganese has been considered to be an important element affected in disease and health.

A steep rise in manganese with an increase of liver

FIGURE : 26

SHOWS THE INTERRELATIONSHIP OF ZINC AND MANGANESE IN LIVER AND PANCREAS UNDER THE STRESS OF CARBONDISULFIDE.

and brain glycogen evolves a clue of involvement of certain manganese dependent enzymes responsible for carbohydrate metabolism. Most of glycosyl transferases involved in the transfer of sugar moiety from sugar nucleotide require manganese ion for the activity (Leach, 1971). The accumulation of manganese in liver, therefore, may have some influence on the increased glycogen biosynthesis which results due to an enhancement of the metalloenzyme, UDP- glycosyl transferase activity. This view supports the findings of Jarvisalo and Savolainen (1977) who also reported the increased activity of UDP- glucuronyl transferase of liver under CS_2 toxicity. An increase in tissue manganese and proposed increased in glycosyl transferase activity may cause increased phosphoglucomutase activity noticed in the present study. This could however, be explained only if the equilibrium is shifted in favour of the synthesis of glycogen in liver supporting further our earlier contention of increase in the synthesis of glycogen. Other alterations in carbohydrate metabolism related to manganese changes are (i) a rise of manganese in pancreas, (ii) a depletion of zinc in this tissue (iii) pancreatic necrosis and (iv) an increased serum alpha amylase activity, (Shrader and Everson 1968 ; Everson and Shrader 1968). The increased manganese level may therefore be considered as a protective action of the body against pancreatic damage showing thereby that manganese and zinc may have inverse relationship in liver and pancreas. The similarity between

the symptoms of chronic manganese poisoning and Parkinson's disease prompted that a study should be conducted to draw actual possible relationship between tissue manganese and biogenic amines. Mena et.al. (1970) have reported that the administration of L-dopa to patients with chronic manganese toxicity resulted in disappearance of rigidity and hypokinesia, improvement of postural reflexes and restitution of balance. Papavasiliou et.al. (1968) have proposed that cyclic AMP is the link between biogenic amines and manganese metabolism. They suggested that altered cyclic AMP increased manganese level and increased biogenic amines, as evidenced by increased liver retention of manganese and decreased biliary excretion, are interrelated. Mushtafa and Chandra (1975) reported reduction of DA and NE level while 5- HT level remained unaltered in animals fed with toxic dose of manganese. However, the present study with CS_2 toxicity depicted no true picture of biogenic amines in relation to manganese level because DA, NE and 5-HT levels were elevated while there was no significant change in brain manganese level. These observations with CS_2 could possibly be explained, on the basis that CS_2 alters blood brain barrier by producing drastic disturbances in physiological and biochemical picture of an animal and increasing the biogenic amines. This is supported further, by the observation of depletion of liver zinc which bear an inverse relation with manganese in all other tissues. A rise in liver manganese level alongwith simulataneous increase in biogenic

amines level of liver support our contention of brain biogenic amine and manganese level alterations. It may therefore, be suggested that CS_2 in rat brings about the changes in tissue manganese levels in various organs effecting carbohydrate metabolism and thereby brings about toxic influence on biogenic amines of brain and liver.

4.4.3. COPPER AND CARBONDISULFIDE TOXICITY :-

Copper has been advocated as an essential element for the elaboration of heme, a component of cytochrome oxidase, thus establishing that copper as a biochemical catalyst, The increase of copper in liver and pancreas has suggested that copper metalloenzymes quantities are increased in these organs thus causing increased metabolism. Simulataneously, CS_2 intoxication forms dithiocarbamates by the interaction with biogenic amines in these tissues by utilising the available copper in the process which results in the increase of the dithiocarbamates synthesis and thus CS_2 toxicity (Mckenna and Distefano, 1977). The interaction of CS_2 with copper and biogenic amines appears to be on the similar or same sites as observed with another inhibitor 2-mercaptoethyl guanidine an inhibitorof DBH (Diliberto and Distefano, 1973) because both the treatment caused increased DA level.

The fall of copper in brain, testis and kidneys

however, suggests that either there is no complexation of amines with CS_2 in these tissues or if the complex is being formed is not much dependent on copper. There may be other elements available in these tissues which chelates dithiocarbamates in these tissues and cause the inhibition of enzymes. The observation that the increase of copper level in liver and pancrease leads to suggest that CS_2 induced DBH inhibition can be due to its high lipid solubitlity, its ability to cross granular membrane and increased dithiocarbamate formation by binding the available biogenic amines in presence of enzymic copper (Mckenna and Distefano, 1977). However, the increase in DA and NE level seen in liver with an increase in copper level appears to be contrary to this hypothesis. Therefore, it can be speculated that liver CA granular storage may be different from brain CA storage sites.

The elevation of biogenic amines (DA, NE) is observed in all organs under CS_2 intoxication. The rise in DA level is in confirmity with the earlier findings (Mckenna and Distefano , 1977 ; Magos, 1971 ; Maj and Vetulani, 1970; Diliberto and Distefano, 1973; Magos and Jarvis, 1976). They attributed this rise of DA due to inhibition of DBH. The rise in brain NE may, therefore, be attributed only to the plausible inhibition of MAO, a cuproenzyme (Buffoni and Blaschko, 1964). The decrease of copper level in brain

further confirms the assumption that increased MAO activity of liver may be due to the increased copper level and the microsomal copper dependent enzyme system of liver. These enzymes may cause quick oxidation of biogenic amines and amino acids, viz. tyrosine and tryptophan. The increase of 5-HT in all organs may be related to inhibition of tryptophan pyrrolase, which is also a cuproenzyme and is essential for normal catabolism of tryptophan. The decreased protein synthesis may have diverted the tryptophan for the synthesis of 5-HT, which might be available for metabolism by increased MAO in the liver, thus confirming the existence of feed back mechanisms i.e. with the increased catabolising enzymes the biogenic amines level should be increased. Hence, the accumulation of 5-HT might be due to its increased synthesis and also decreased degradation in brain. (Maj and Maria, 1970).

Further, the reduction of copper in brain, kidney and testis also can be explained by considering the similar views of Massoud (1977), that useful copper in the living system becomes unavailable because of the firm interaction between copper and dithiocarbamates in liver. The reduction of copper in these tissues may lead to abnormal functioning of testis and kidneys, thereby giving an explanation for nephritis and infertility (Gondzik, 1976) in workers exposed to CS_2 environment. The decrease in usable copper with

decreased ceruplasmin has been found to alter the mobilisation of iron from reticuloendothelial cells (Rolser et.al. 1970) This might lead to decreased heme synthesis, increased heme degradation and increased heme oxygenase activity in CS_2 treated rats (Jarvisalo, et.al. 1978) and hence may explain the causes of anemia seen in patients from industry using carbondisulfide.

In the existing literature there is a pauscity of reports regarding the role of copper in carbohydrate metabolism. However, the observed changes in carbohydrate metabolism in the present study appear to be solely related to the zinc and manganese alterations but the role of copper level in various body organs and its relationship to carbohydrate metabolism cannot be ignored and needs further scientific probe in this direction.

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SUMMARY

The low health status and overall high susceptibility to various disorders of individuals exposed to CS_2 have caused considerable doubts about the safety of this substance. The interaction between biological systems and CS_2 has been a problem of only recent interest and few toxic manifestations in human beings and animals have been ascribed to it. The present work was undertaken to reveal the relationship, if any, between toxic manifestations of CS_2 with observed abnormality of carbohydrate metabolism in relation to various enzymes and biogenic amines in rats.

Acute and chronic CS_2 treatments caused a marked change only in liver glycogen rather than in the brain, which indicated that toxicity of CS_2 might be a result of liver damage. Liver phosphorylase was found to be inhibited significantly while in the brain it was altered to an insignificant extent only. However, phosphoglucomutase activity of liver varied markedly, i.e. about eight times, than in brain which showed that the deposition of glycogen is much higher in liver than in the brain. The indirect approach of studying fructose, 1-6, diphosphatase also showed an inhibition trend and revealed decreased gluconeogenesis. The inhibition of adenosine triphosphatase and succinic dehydrogenase activity in liver and brain provided the proofs for the altered membrane structure, decreased activity of Kreb's cycle and electron transport chain. This, further, showed a reduction

in glycolysis and increase in glycogen deposition indicating that all processes depending upon the transport mechanisms are affected by CS_2. Therefore, increased susceptibility to infections and diseases could be atributed to the membrane atructure alteration.

The depletion of plasma proteins and tissue proteins can be due to an increased utilisation of proteins by systems either for energy production or to overcome the stress induced by carbondisulfide.

The increase in brain, heart, liver and lungs biogenic amines i.e. dopamine, norepinephrine and 5-hydroxytryptamine (5-HT), provided further evidence of severe biochemical alterations by CS_2 in chronically treated rats. The increased level of these biogenic amines support the assumption of the loss of granular permeability of the vesicles. However, the synthesis of these essential amines, could not be altered as the accumulation of tryosine and tryptophan was found to be more than normal in these tissues. The simultaneous reduction in monoamine oxidase (MAO) activity in brain could explain further, the increase in the level of brain DA and NE. On the other hand, MAO activity in liver was increased which showed that the increase of biogenic amines in these organs may be due to an increase in their synthesis rather than inhibition

of catabolism. In liver, brain, heart and lungs there was a significant fall in the reduced glutathione which explains the protective action of glutathione against CS_2 in these organs. This shows that biogenic amines-synthesising enzymes might not be inhibited substantially, because glutathione must be sparing the enzymes for covalent binding with CS_2 and hence caused a rise in biogenic amines.

A study of trace elements metabolism showed that zinc was decreased in all these tissues. The pancreatic zinc reduction reflects pancreatic dysfunction or insulin reduction due to defective carbohydrate metabolism and in protein metabolism. The altered manganese concentration has been related to the increased glycogen biosynthesis and increased phosphoglucomutase activity in liver. Accumulation of manganese has been attributed to the increased level of DA and NE observed under CS_2 intoxication. The manganese studies, therefore, provide increased evidence of anemia and other cardiovascular disorders in the workers handling CS_2.

Increased level of copper in liver and pancreas might be considered as an index of increased biogenic amines which formed complex with CS_2 to give dithiocarbamates. On the other hand the decreased zinc level in testis, brain and kidneys may explain the mobilization of copper from these tissues to liver and pancreas so as to maintain Zn/Cu ratio

under the stress of CS_2 thus may afford to justify the lessening of the stress.

Therefore, it can be concluded from the present study that CS_2 toxicity causes a disturbance in carbohydrate metabolism of the body with alteration of paired trace elements "Zinc and Copper" and "Copper and Manganese". The paired elements have been found to bear an inverse relationship in liver and pancreas under the CS_2 stress. The increased liver glycogen is related to increased phosphoglucomutase activity and decreased phosphorylase activity. Increased liver manganese is thought to be responsible for glycogen accumulation by increasing glycogen synthesis activity indirectly. Decreased Zinc level may inhibit the enzymes of glycolysis and Kreb's cycle. Though no relationship between trace elements and biogenic amines was established in this study, the rise of liver manganese and biogenic amines are interrelated confirming the earlier studies.

From this work it may, further, be suggested that the diet supplemented with trace elements may alleviate some toxic effects of carbondisulfide in workers.

CHAPTER -6

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