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STUDIES ON
THE PATHOLOGICAL CHANGES IN
SHEEP POISONED WITH
CARBON TETRACHLORIDE

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY,
BIHAR

IN PARTIAL FULFILMENT OF THE

REQUIREMENTS FOR THE
DEGREE OF
MASTER OF SCIENCE(VETERINARY)

BY

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1975

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This is to certify that the work embodied in this Thesis entitled "STUDIES ON THE PATHOLOGICAL CHANGES IN SHEEP POISONED WITH CARBON TETRACHLORIDE" is the bonafide work of Shri J.P. Singh and was carried out under my guidance and supervision.

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CERTIFICATE

Certified that the research work incorporated in this THESIS have not been published in part or in full in any other journal.

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The work embodied in this Thesis was carried out in the Department of Pathology, Bihar Veterinary College, Patna, under Junior Fellowship awarded by the Rajendra Agricultural University, Bihar. The author is grateful to the Vice-Chancellor for the financial help.

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A C K N O W L E D G M E N T

The author is extremely grateful to Dr. C.D.N.Singh, B.V.Sc.& A.H.,M.Sc.(Vet.), F.R.C.V.S. (Sweden), Professor and Chairman, Post-graduate Department of Pathology,Bihar Veterinary College,Patna, for constant supervision, valuable guidance, helpful advice and encouragement.

The author is also thankful to Dr. L.P.Singh,M.Sc.(Vet), Professor and Head, Department of Anatomy, for worthy suggestion, keen interest and help during the tenure of the study and research work.

Thanks are also due to Dr. B.K.Sinha,M.V.Sc., J.A.R.O., Postgraduate Department of Pathology for his sincere help throughout the study and research work.

I wish to place an record my sincere gratitude to Dr.R.L. Kausal, Advisor to the Vice-Chancellor and ex-Dean,Faculty of Veterinary Science and Animal Husbandry, Rajendra Agricultural University,Bihar, for giving invaluable advice in carrying out this study.

My sincere thanks are also due to Dr. K.N.Tiwary, ex-Principal,Bihar Veterinary College,Patna for providing all necessary facilities for the completion of this work.

The author is greatly indebted to Dr. R.N.Singh,Principal, Bihar Veterinary College,Patna, for his kind help.

The author also expresses his gratefulness to Dr.Md. Murtuja,M.V.Sc.,Lecturer and Dr. T.Prasad,B.Sc.,M.V.Sc.,P.G., Dip-in-Biochem. & Physiol.(Copenhagen),Assistant Professor and

Head, Department of Biochemistry, for extending their help in completing Biochemical analysis of blood and providing all sorts of laboratory facilities in their department.

Grateful acknowledgments are also due to Dr.G.J.Jha, Dr. P.N.Sinha, Dr. Lala Naresh Prasad as well as to the staff of the department of Pathology, for their whole hearted cooperation during the period of study.

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DEDICATED

TO

MY

PARENTS

C O N T E N T S

I N T R O D U C T I O N

Sheep occupies a remarkable place in the economy of our nation through exporting its wool, skin and their different products. The origin of the domesticated sheep is lost in antiquity and possibly several races of wild sheep were fore runners of our present day domestic sheep.

At present there are about 44 million sheep in our country and it is the sixth largest population of the world. But the productive capacity of the sheep is very low as compared with the other sheep raising countries. There are no organised farms except a few Government ones and most of sheep are kept under village condition and their existence is based on grazing in the fields. The flock lead more or less nomadic life. The flock cannot be economic unless they are kept in healthy condition. There are many diseases threatening the sheep industry . Sheep are very much susceptible for parasitic infestations. Parasites are of great importance to veterinarian and sheep raiser.

Minett (1949) conducted a survey on mortality on sheep and goat in India and came to the conclusion that helminthic disease is an outstanding source of mortality while the effect of environment, nutritional and climatic in this connection is also emphasized. The death rate from helminthic disease in sheep seems to be at its worst from six months to two years. As parasites are proved to be serious impediments in successful breeding programme chiefly by impairing the nutrition of the stock and damaging their vital tissues resulting in morbidity, inanition or malnutrition, underdevelopment or stunted growth

there by affecting normal performance of the animal.

To guard against this menacing problem of parasitic diseases, many anthelmintics are in practice and carbon tetrachloride is the commonest drug among them. Faulty administration of the carbon tetrachloride (over dosing to sheep) at times causes high mortality in them. Highly susceptible sheep succumbed to even small doses of carbon tetrachloride.

Hall (1921) found carbon tetrachloride is nearly hundred percent effective in removing hook worm from dog. Since then carbon tetrachloride has been used extensively as an anthelmintic in various species of animals against different kinds of parasites such as blood sucking worms in all species, notably haemonchosis in ruminant, strongylosis in horses and ankylostomiasis in dogs, liver fluke in cattle and sheep and ascarides in all species except swine.

Carbon tetrachloride is a general protoplasmic poison. It passes through the stomach unchanged and is absorbed to some extent from intestine and is concentrated in the liver. Highest liver concentration of carbon tetrachloride is reached 3-5 hours after drenching.

Excretion occurs quickly and only traces of carbon tetrachloride can be found in the liver 24 hours after dosing. Much of carbon tetrachloride is excreted through the lungs and the irritant irritation may be so great as to cause severe pulmonary oedema. Some of the drug is also excreted through kidneys and causing some damage to them.

The idea behind the present research work is to throw light on pathologic changes occurring in different organs of sheep in acute carbon tetrachloride poisoning. Carbon tetrachloride is very commonly used parasiticidal in this country and the accidents leading to fatal ends due to its unskilledful administration are very much frequent. The finding on various histopathological changes and blood disorders will form guide lines to future research worker and clinician engaged in treatment of various sheep diseases.

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REVIEW OF LITERATURE

Information on the pathologic changes in carbon tetrachloride poisoning in sheep in greater details ia not available in literature. Only histopathological studies have been mostly made by several workers in sheep. No work seems to have been done so far on carbon tetrachloride poisoning in sheep in India.

Rose (1932) gave an account of the observation made on 18 Australian sheep died after normal drenching with carbon tetrachloride. He reported that the incubation period was four days and there was loss of wool which increased due to inclement weather condition at the time of administration of this drug.

Shaw (1937) could not succeed to disturb the liver function in sheep through changes in the diet in an attempt to induce susceptibility to carbon tetrachloride poisoning.

Winterhalter (1942) studied the action of carbon tetrachloride on the liver of sheep by administering two capsules containing 2.7 ml. of carbon tetrachloride per head in the animals of two groups of ten sheep. They were kept without food and water. They were slaughtered 1-10 days later.

Silva Leitao (1945) studied experimentally the toxicity of carbon tetrachloride in lambs. The author found that one lamb had survived a dose of 55 ml. of carbon tetrachloride on being followed by calcium gluconate injection. Other lambs survived doses of 15 to 20 ml. without calcium therapy.

Blackemore and McDougall (1946) reported fatty

degeneration and necrosis of liver with congestion of the abomasum of ewes (under general unthriftness). They died after administration of 1 ml. carbon tetrachloride per head in capsule. Authors observed that blood serum of these sheep had normal calcium level whereas guanidine like substances had increased in it.

Walker (1947) described the effect of carbon tetrachloride poisoning in sheep with a rising plan of nutrition.

Fairfax (1948) reported that administration of carbon tetrachloride produced poisoning in a mob of 100 lambing ewes of which 15 died. He also observed that similar mob of ewes were unaffected by carbon tetrachloride which had remained on natural pasture.

Sloan (1950) found no toxic effect in sheep given therapeutic doses of carbon tetrachloride at monthly intervals for a period of nine months.

Staskiewicz et al.(1955) estimated the calcium,inorganic phosphorus, glucose and cholesterol level of blood in sheep following subcutaneous injection of carbon tetrachloride.Authors reported the fall in the levels of calcium, glucose and a rise in cholesterol following subcutaneous injection of 2-3 ml. of carbon tetrachloride in fluke free 10 sheep. Calcium level soon returned to normal but the changes in cholesterol and glucose continued for further eight days.

Frunder et al.(1956) described primary metabolic lesions in the liver in carbon tetrachloride poisoning. The main feature was the depletion of liver glycogen and marked reduction

of glucose content due to disturbance of mitochondrial function.

Setchell (1959) studied the effect of carbon tetrachloride on kidney and liver function in the sheep. He administered a mixture of 50 ml. carbon tetrachloride and 100 ml. liquid paraffin to 5 adult sheep. Three of them died and two remained clinically normal. There was severe liver dysfunction in all sheep and severe kidney dysfunction in three sheep which died due to carbon tetrachloride poisoning.

Downey (1960) reported a fall in serum calcium level of 13 percent after 96 hours of giving carbon tetrachloride to ewes but no significant change in the serum magnesium level, was noticed.

Muth (1960) studied the pathological changes of carbon tetrachloride poisoning in eleven ewes kept on low selinium diet. Author concluded that the characteristic lesion of carbon tetrachloride poisoning was hepatic centrilobular haemorrhagic necrosis.

Delak and Ilijas (1961) reported the influence of hyaluronidase on absorption of carbon tetrachloride from subcutis and muscle. The pure carbon tetrachloride was absorbed much more rapidly than the carbon tetrachloride suspended in liquid paraffin. They found that absorption of pure carbon tetrachloride was enhanced by simultanious injection of 300-400 I.U.(International unit) of hyaluronidase preparation.

Gallagher (1961) discussed in detail the pathogenesis and prophylaxis of carbon tetrachloride poisoning in rats and sheep. The lipid solvent action of carbon tetrachloride initially damaged the cell structure leading to loss of essential

materials from the cell. Liver cells lost the cytoplasmic constituents within 1,3 and 10 hours after drenching with carbon tetrachloride. Necrosis of the cells followed due to failure of respiration and loss of essential co-enzymes.Author pastulated two effective methods of prophylaxis by stimulating the synthesis of pyridine nucleotide and by preventing the loss of pyridine nucleotide from mitochondria.

Kees et al.(1961) undertook the investigation to elucidate the interrelation of fatty degeneration, mitochondrial damage and hepatic necrosis in carbon tetrachloride toxic liver injury by studying the time relation of the various phenomena.

Gallagher (1962) studied the effect of drenching technique on poisoning of sheep with carbon tetrachloride. He also described the postmortem changes of sheep died due to carbon tetrachloride administration into mouth, pharynx and larynx.

Gallagher et al.(1962) concluded that intraperitoneal injection of 2 g. nicotinic acid 2 days before administration of carbon tetrachloride to sheep gave better protection against carbon tetrachloride poisoning.

Gallagher et al.(1962) studied the susceptibility of carbon tetrachloride poisoning in sheep under different environmental, climatic and dietary conditions.

Setchell (1962) pastulated that oxalate containing plants and cold weather might increase the susceptibility of carbon tetrachloride poisoning in sheep.

Setchell (1962) analysed the blood serum of sheep poisoned with anthelmintic doses of carbon tetrachloride .

He observed that both kidney and liver functions were impaired in most sheep died in 1-3 days but calcium level was not altered in most cases.

Kondas et al.(1963) studied experimentally the toxicity and anthelmintic effect of carbon tetrachloride given by intramuscular or intraruminal route.

Setchell and Little Johns (1963) described the histopathology of liver of sheep died due to anthelmintic dose of carbon tetrachloride poisoning. Centrilobular necrosis of sheep liver was common when died in 1-3 days but rare when animal died after 4 days. Fatty degeneration or cloudy swelling were the usual findings.

Setchell et al.(1964) found that the degree of liver damage were greater in those sheep which were kept on stinkwart plant and then drenched with carbon tetrachloride.

Kondas and McClymont (1965) reported that toxicity of carbon tetrachloride in sheep was enhanced when sheep were kept on high protein diet.

Kondas and McClymont (1966) reported that cold stress increased the intoxication of sheep by carbon tetrachloride.

Winterhalter and Vulinec (1969) also studied experimentally the effect of carbon tetrachloride in therapeutic doses in liver cell function in 7 sheep. They concluded that carbon tetrachloride had stimulated hepatic cells in 3 sheep, damaged them in another 3 sheep but produced no marked change in last sheep.

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MATERIALS AND METHODS

Experimental sheep and its maintenance:

The present research was conducted on 12 sheep of three years of age of the same sex of a nondescript local breed. Of these, three were kept in control group. All the sheep were tagged with number and kept in the same environment, management and on same food throughout the research. The standard ration comprises grams,bhusi,salt and greens. They used to graze in the field and received adlib supply of water. The sheep were kept in clean, dry and airy sheds which were cleaned daily and disinfected once in a week.

The sheep were examined for internal parasite and also checked for normal health. Only sheep free of parasitic infection and other diseases were selected for the experimental work.

Blood Collection and Examination:

About 5 cc of blood was collected asceptically with sterilised syringe from jugular vein of all the sheep in the morning before giving any food. One drop of blood was dropped on a clean microslide and uniform smear was made for differential count of leucocytes and rest of the blood was transferred into a clean test tube containing a suitable quantity of Wintrobes isotonic amonium and potasium oxalate mixture. Wintrobes anticoagulant was evaporated to dryness in the tubes on water bath on a previous day. The tubes were shaken for a few minutes to ensure a thorough mixing of blood and anticoagulant.

This blood was used for the examination of the total count of R.B.C., W.B.C., Haemoglobin percentage, Erythrocyte sedimentation rate and packed cell volume.

For chemical analysis of blood for urea and calcium, blood was collected in separate tubes.

Total Erythrocyte count:

Double Neubaur's counting chamber was used for total Erythrocyte and Leucocyte counts. Physiological saline containing 0.85 percent saline was used as diluent in preference to Hayem's and Cower's solution for R.B.C. count according to "Uscar Wschalm" Veterinary Haematology, 1961. As agglutination of Erythrocyte used to occur with Hayem's solution, the physiological saline was used in the present experiment.

Total Leucocyte count:

The same procedure and technique were followed as described by "Uscar Wschalm" in Veterinary Haematology, 1961.

Preparation of staining of blood smears:

Properly spread uniform blood films were made on polished and absolutely clean slides. The smears thus made were stained by Leishman's staining technique. Two hundred leucocytes were counted in each slide following the "battlement" system (1 mm down, 1 mm across and 1 mm above).

Haemoglobin determination:

Haemoglobin percentage was obtained by Sahli-Haden Haemoglobinometer following the direction for its use.

Erythrocyte sedimentation rate:

This test was done with the help of Wintrobe's Haematocrit Method within an hour of collection of oxalated blood.

Clean Wintrobe's Haematocrit tubes with a uniform 3 mm bore and a double 10 cm scale caliberation with milimeter divisions were used. The scale on the left was read from top to bottom while the scale on the right was in reverse. Tubes were filled with oxalated blood upto 0 mark on the left hand scale and were allowed to stand vertically. Only one reading was taken at the end of one hour.

The Wintrobe's tubes were afterward centrifuged to determine the Packed Cell Volume at the speed of 3000 pm for 50 minutes and then for 20 minutes. The results were recorded as the number of ml. of cell per 100 ml. of blood. Speed and time of centrifugation were not changed in any case.

Blood analysis for urea,and calcium:

Estimation of blood urea nitrogen:

Method for colorimetric determination of blood urea:- All the reagent required for this test made by Bharat Laboratories had been purchased from local market. The procedure and technique were followed as per instructions supplied with the reagent.

Reagent: - 1. Solution A - Sulfuric acid.

2. Solution B - Sodium Tungstate solution.

3. Solution C - Sodium Acetate buffer.

4. Solution D - Urea Nitrogen standard stock solution containing 1.5 mg of urea nitrogen per 5 ml.

From the stock standard working standard solution was prepared by diluting 5 ml to 100 ml of distilled water. Five ml. working urea nitrogen standard contained 0.075 mg urea nitrogen.

5. Solution E - Nesseler's solution.

6. Urease powder.

PROCEDURE:

1. In a clean test tube, 7 ml. of distilled water and 1.0 ml. of solution A were taken.

2. 1 ml. of blood was added after shaking the tube well.

3. 1 ml. of solution B was also added to it.

4. It were mixed well, kept for 5 minutes and then filtered.

5. Three test tubes were taken and each of these was graduated at 25 ml and marked as S (standard), T(test), B (reagent blank) tubes.

6. In the tube marked, 'S' 5 ml of diluted standard solution was taken.

In the tube marked 'T' 5 ml of protein free filterate was taken.

In the tube marked 'B' 5 ml dilstilled water was taken.

7. To each of the three tubes a pinch (about 20 mgs) of urease powder and 0.5 ml. of solution C was added.

8. All the three tubes were then incubated in a water bath at 50°C for ten minutes.

9. These tubes were cooled under cold running water.

10. In each of the three tubes about 10 ml. of distilled water and 2.5 ml. of solution E were taken.

11. The content of the tubes then diluted to 25 marks with distilled water and mixed well.

12. Read in the colorimeter using blue filter and recorded percentage transmission in each blank to 100 per cent transmission.

CALCULATION:

$$\text{Mgm. of urea nitrogen per 100 ml. of blood.} = \frac{\text{Optical density of test.} - \text{Optical density of reagent blank.}}{\text{Optical density of standard.} - \text{Optical density of reagent blank.}} \times 15$$

Determination of calcium:

About 5 ml. of blood from jugular vein of the sheep was drawn and kept in a test tube.After a few hrs. serum had been separated.

The procedure and technique for determination of calcium was followed as described by "Clark-Collip Modification of the Kramer - Tisdall Method" in HAWK'S PHYSIOLOGICAL CHEMISTRY (FOURTHENTH EDITION,1965).

The statistical analysis of different blood attributes before and after poisoning by 'T' test (Snedecor and Cochran, 1967)was done.

CARBON TETRACHLORIDE ADMINISTRATION:

All the experimental sheep showing normal haematological values were given same dose of 50 ml. carbon tetrachloride per sheep by stomach tube. Blood was collected from the affected sheep for different haematological studies after eight hours of drenching.

Post-mortem examination:

When the animals died due to poisoning, a thorough postmortem examination was conducted. First the animals were examined externally. Colour of external mucous membrane of the body was noted. The carcasses were opened and various parts of body starting from subcutaneous tissue and muscle to visceral organs were examined. The size, colour and consistency of all the visceral organs were noted.

Histopathology:

Pieces of organs which showed the macroscopic lesions were collected in 10 percent formalin for histopathological examinations. These pieces were also collected in 40 percent alcohol for histochemical examination. After fixation for a few days, the small pieces of tissues were washed in running water for 20 hours, dehydrated in ascending grades of prepared alcohol, cleared in benzene and blocks in paraffin were prepared. Sections were cut at 5 micron to 6 micron in thickness and stained by Haematoxylene and Eosin (Lillie, 1954) and studied under microscope.

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RESULTS

The artificial poisoning by administering carbon tetrachloride (B.D.H.) to 9 sheep in the age group of three years was produced. The disease appeared in them in acute form. No subacute, subclinical or chronic case would be seen. The disease was recognisable from half an hour after poisoning and none of the sheep survived beyond 36 hours after poisoning. The dead sheep were necropsied soon after death. Haematological studies in the ailing sheep were performed. Symptoms of poisoning marked by excessive excitement and stretching of head and neck were noticed in all the sheep even after half an hour of poisoning. Naked eye lesions were predominantly present in liver, kidney, lung and brain and emphysematous and atelectic changes were very prominant in lungs, and these were very much outstanding lesions. Haematological studies were also carried on control of three sheep and the necropsy performed further in them did not reveal any lesion in their organs.

Clinical symptoms.-

The symptoms appeared in all the sheep after about half an hour of its administration. The poisoned sheep stretched their head and neck and their heads were more inclined towards back. They started trembling and were seen to perform quivering of muscles around the nares. Peculiar chewing like movements of the lips and jaws were observed in them. They became dull and depressed and all the four limbs were seen in abducted position (Fig.1). They were found to be respiring deeply and excessive nasal discharges were also observed. Temperature did not show any variation and it was

102°F after half an hour. The sheep voided deep yellow coloured urine. They stood motionless and resisted attempt of making them move. The sheep some times fell on the ground but when they were made to stand on their feet, they preferred to remain on the feet till they fell to the ground. There was shallow abdominal breathing. They lied on the ground on any side of the body and stretched all their limbs and remained in commatosed condition. They remained in such a recumbency and passed profound watery discharge from mouth. The limbs trembled violently with jerky movements at times. The sheep were seen to take laboured respiration suffered from dyspnea and finally they breathed their last in such recumbency.

Pathologic changes.-

Gross changes

Liver .- The liver of the sheep was moderately swollen with rounded edges. There were slight flow of blood on the cut surfaces of liver. The gall bladder contained yellowish white viscid bile. There were a few focal greyish white subcutaneous foci about 2 mm. in diameter in the liver. The liver was slightly soft to touch. There were firm dull red depressed areas just below the level of surrounding tissue.

Kidney.- The kidneys were pale and swollen. The capsules were easily stripped off. There was presence of deep yellow urine in the urinary bladder.

Spleen.- Spleen had rounded edges and there was slight flow of blood.

Intestine.- There were slight reddening of the duodenal mucosa. Abomasal and jejunal mucosae showed a few patechae.

Lungs.- Cut pieces of lungs sank in water. The cut surfaces presented almost dry appearance. There were pink or pale grey areas just below the level of the surrounding tissue (fig.2). Such areas contained air which could be easily compressed by fingers. There was slight flow of blood on cut surface of lung.

The trachea and bronchi of lungs contained froathy material. There were slight blood tinged material in small bronchi.

Thyroid.- The thyroid glands were hyperaemic and when cut, blood ran down on its cut surface.

Brain.- The meninges were slightly hyperaemic. The cut surface of the brain was slightly moist.

Heart.- It did not reveal any gross changes.

Microscopic changes.-

Liver.- The epithelial cells of liver were highly eosinophilic in most of the lobules and the cells in the hepatic cords had lost end to end contact and had also pyknotic nuclei mostly in the periacinar zone (fig.3). The central vein was very much distended and contained homogeneous eosinophilic material with a few erythrocytes (fig.4). The vessels in the portal tract were severely engorged with blood and very much distended to form round structures containing eosinophilic homogeneous material alongwith some red cells and lymphocytes. The liver cells were

very much disorganised and their nuclei were hyperchromatic and reduced in sizes. Many epithelial cells had lost their nuclei to form almost homogeneous eosinophilic mass (fig.5). Such changes were visible throughout the lobules and the sinusoid opening into the vein were distended and also empty. Nuclei were also broken into pieces at places (Karyorrhexis). Necrotic changes were more marked in the epithelial cells around the central vein of the lobules. There were several empty vacuoles in the epithelial cells in the periportal areas of the lobules (fig.6). There were areas with marked degenerative changes in the periacinar zone. Haemorrhages were also present in the dilated inter lobular spaces (fig.7). Many of the nuclei of epithelial cells had lost their staining ability (chromatolysis) and individualised epithelial cells were more prominent around the central vein (fig.8). There were several empty vacuoles at the margin of the homogeneous material lying in the blood vessels in the portal tracts.

Kidney.- There was increase in size of Bowman's capsule. The epithelial cells lining the tubules had almost been fused with faintly visible nuclei and tubular lumen contained eosinophilic granular material (fig.9). The interstitial blood vessels were engorged with blood. The epithelial cells lining the tubules were desquamated to form cellular clumps in the lumen. The cells were highly eosinophilic. There were cellular casts in tubules. The intertubular spaces contained granular eosinophilic material mixed with highly pyknotic nuclear mass. The epithelial cells lining the tubules were very much swollen and granular to leave behind very narrow slits at places (fig.10). The cells of glomeruli were highly pyknotic. The glomeruli had been increased to

occupy almost whole Bowman's capsular space in a few cases. The cells lining the tubules were almost homogeneous and eosinophilic and had pyknotic nuclei. The epithelial cells in a few cases lining the tubules were highly eosinophilic and had almost filled the tubules. There were areas of necrosis where the tubules were almost fused to loose their individual identity and formed almost homogeneous material mixed with very faintly staining nuclei. There were areas of focal haemorrhage in the interstitium of the kidneys in a few cases (fig.11). The cells lining the tubules were swollen, granular and had dark nuclei with vacuolated cytoplasm in the medullary rays. There were focal almost hyalinised areas alongwith faintly staining material. These hyalinised areas were also containing indistinguishible debris in the lumen. The interstitium was very emphasized due to haemorrhage at places. The blood vessels were hyperaemic and filled with erythrocytes and there was excess of pink stained eosinophilic material around the blood vessels (fig.12).

Spleen.- No significant histological changes were noticed in the spleen. There was slight increase of erythrocytes in red pulp. The splenic corpuscles were some what depleted of lymphocytes.

Intestine.- There was pink stained material just below the muscularis mucosae of small intestine (oedema). The glands in the mucosae had pyknotic nuclei and had been desquamated to fall into the lumen as cellular clumps. The cytoplasm in the cells lining the crypts of liberkuihn's was highly eosinophilic

The smooth muscle bundles were separated from each other by empty spaces. The cell had both pyknotic nuclei. Lymphoid patches had dark stained nuclei and were mostly depleted of their cells. The Brunner's gland contained more goblet cells than usual. The squamous epithelial cells lining the ruminal villi had highly eosinophilic cytoplasm. The lamina epithelialis and villi of the small intestine were lost at places (fig.13).

Lungs.- There were areas of collapse and emphysema. In the areas of collapse the alveolar walls were very much approximated to each other leaving behind almost narrow slits (fig.14). There were also areas of emphysema where the walls of the alveoli were broken and the alveoli intercommunicated with each other. There were knobs at the broken ends of alveolar walls. There was pink stained homogeneous material in the distended alveolar spaces (oedema) in a few cases. The lumens of bronchi were dilated and contained eosinophilic material. The blood vessels in the peribronchial areas were hyperaemic. At places, the capillaries in the alveolar walls were also hyperaemic. In some cases, the interstitium was very much emphasized and was found to be thickened due to infiltration of lymphocytes and monocytes with empty alveolar spaces in one case. Bronchial lumen contained cellular debris

alongwith eosinophilic materials.

Thyroid.- The vessels in the interstitium between the follicles of thyroid were severely engorged with blood (fig.15). Follicles had been distended with colloid. The blood vessels in parathyroids were hyperaemic. There was presence of pink stained homogeneous material in the parathyroid dissimilar to homogeneous colloid found in the thyroid follicles. There were abnormally large empty spaces between thyroid and parathyroid.

Brain.-

The nuclei of neurons were pyknotic and dark in colour. There was empty areas around neurons. There were empty spaces (areas of liquefaction) containing mostly eosinophilic materials (fig.16). There was several empty spaces or malacic foci with irregular frayed borders in cerebral cortex. They were of different shapes and sizes. There was moderately increased space around the blood vessels (perivascular oedema). The blood vessels were engorged and contained hyaline material alongwith a few erythrocytes. At places nuclei of neurons had peripheral position and these were pyknotic and had got clustering microglia around them(fig.17). Many of the neurons were shriveled, hyperchromatic and reduced in sizes.

Heart:- The muscle fibers had lost their striations and there were areas of focal haemorrhages in between the muscle fibers (fig.18).

Haematology:

Different haematological values were estimated in all the sheep before and after poisoning. The statistical analysis of the blood revealed that only blood urea and blood calcium differed highly significantly before and after poisoning. In the case of blood urea the value after poisoning became higher whereas in the case of blood calcium the values were significantly lowered after poisoning (table I). None of the other blood attributes viz, Red blood count, White blood count, Haemoglobin percentage, packed cell volume, Mean corpuscular volume, Mean corpuscular haemoglobin, Mean corpuscular haemoglobin concentration, Neutrophil, Lymphocyte, Monocyte and Eosinophil percentage showed any significant difference before and after poisoning.

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TABLE I

Statistical analysis of different blood attribute before and after poisoning of sheep.

	Before poisoning			After poisoning			Difference between mean.
	n	Mean ± S.E.	C.V. percentage	n	Mean ± S.E.	C.V. percentage	
RBC(million cmm.)	9	9.22 ± 0.28	9.22	9	9.32 ± 0.48	15.34	0.10 NS
WBC(thousand cmm)	9	10.64 ± 0.27	7.52	9	10.67 ± 0.27	7.69	0.03 NS
Hb (gm.%)	9	8.68 ± 0.18	6.11	9	8.77 ± 0.17	5.82	0.09 NS
PCV(%)	9	23.44 ± 0.38	4.82	9	23.67 ± 0.33	4.22	0.23 NS
MCV(cu / micron)	9	25.39 ± 0.98	11.66	9	25.62 ± 0.47	5.58	0.23 NS
MCH(micro micro gm.)	9	9.54 ± 0.20	6.49	9	9.52 ± 0.19	6.19	0.02 NS
MCHC(%)	9	36.79 ± 0.28	2.34	9	37.03 ± 0.26	2.13	0.24 NS
Neutrophil (%)	9	38.6 ± 0.15	1.11	9	38.00 ± 0.86	6.82	0.60 NS
Lymphocyte (%)	9	54.67 ± 0.11	0.61	9	55.89 ± 0.42	2.27	1.22 NS
Monocyte(%)	9	2.11 ± 0.35	49.76	9	2.00 ± 0.17	25.00	0.11 NS
Eosinophil (%)	9	4.33 ± 0.85	58.89	9	4.00 ± 0.71	53.00	0.33 NS
Blood urea (%)	9	20.11 ± 0.78	3.88	9	24.44 ± 0.29	3.60	4.33**
Blood Ca(%)	9	12.24 ± 1.44	35.29	9	7.97 ± 0.30	11.29	4.27**

n = number of observation., S.E. = standard error., CV = coefficient variation., NS = not significant., ** = highly significant.

DISCUSSION

As already stated under introduction the carbon tetrachloride (CCl_4) poisoning was produced in a group of local nondescript breed of sheep of three years of age to study the symptoms and pathological picture of the disease. All the sheep in this group were given same dose of 50 ml. of carbon tetrachloride per os using stomach tube.

The symptoms observed in them were trembling, stretching their head and neck, peculiar chewing like movement of lips and jaw, laboured breathing and voilent jerky movement of the limbs. Gallagher et al.(1962) described that the animals under carbon tetrachloride poisoning exhibited signs of central nervous disfunction. In the animals of the study group administered 50 ml. carbon tetrachloride by stomach tube into the rumen, postural and locomotor abnormality were present alongwith dyspnea. They reported total death in their study groups to be eight of eight sheep (i.e.100%).

In the present study all the sheep administered 50 ml. carbon tetrachloride by stomach tube into the rumen died (i.e. cent percent mortality). Symptoms of nervous and locomotory disturbances were also noticed after half an hour of carbon tetra-

chloride administration. Dyspnea was also noticed in them.

The main haematological changes in all poisoned sheep were found to be increased in the values of blood urea and fell in that of serum calcium. Changes in other blood values (R.B.C. count, W.B.C. count and Haemoglobin percentage etc.) were not observed.

Setchell (1959) pointed out that hepatic dysfunction was accompanied by almost complete renal failure in carbon tetrachloride poisoning in sheep. Setchell (1962) reported the increased level of blood urea of the sheep dying after drenching with therapeutic doses of carbon tetrachloride. High level of blood urea in the present experimental group is clearly indicative of liver dysfunction. It has been further supported by the presence of necrotic changes in hepatic lobules. However, Setchell(loc.cit.) did not find any alteration in serum calcium concentration.

Staskiewicz et al.(1955) reported fall in the level of calcium in the blood of sheep given subcutaneous administration of 2-3 ml. of carbon tetrachloride. It appears from aforesaid observations that blood calcium level in the sheep given carbon tetrachloride is dependent upon the distrubances of doses of the drug. However, no significant changes were noticed in other blood values.

The histopathological changes in the liver were accorded

with those of Setchell (1961). Centrilobular necrosis were noticed in the liver of sheep dying less than three days after drenching carbon tetrachloride. In present study there was coudy swelling and empty spaces in the liver cell in the periportal area whereas the cell in the centrilobular zone were highly eosinophilic with pyknotic nuclei. Gallagher (1961) examined rat liver 24 hours after lethal dose of carbon tetrachloride and noticed centrilobular necrosis and fatty changes. As pointed out by Jubb and Kennedy (1963) anoxia resulting from reduced blood flow and lowered oxygen tension of the blood appeared to be of paramount importance in the development of periacinar (centrilobular) necrosis. Carbon tetrachloride is well known powerful hepatotoxin. In the present study hyperemia was noticed in the liver.

Gallagher (1961) described the lipid solvent action of carbon tetrachloride of the cell structure in the liver of animals and attributed cell death due to loss of mitochondrial component. Hyperemia of the liver and hepatotoxic effect of carbon tetrachloride are believed to cause more pronounced degenerative changes in periacinar zone of hepatic lobules. In the peripheral zone cells were mostly swollen, granular and had empty vacuoles.

Gallagher et al.(1962) mentioned as to whether the dyspnea

is in part of atleast in central origin. They noticed severe pulmonary oedema in two sheep which died soon after carbon tetrachloride poisoning. In the present study the most marked changes in the lungs of sheep usually died after 12 to 36 hours of carbon tetrachloride poisoning were those of collaps and emphysema. Even in the sheep which died after 12 hours of carbon tetrachloride poisoning there was marked collaps of emphysema. This supports the suspicion of Gallagher et al. about the role of central nervous system in producing dyspnea and respiratory distress in the sheep. Hyperemia and oedema could be noticed in only two sheep which died after 36 hours of carbon tetrachloride poisoning. According to Colebatch and Halmagyi(1961); Halmagyi and Colebatch (1961) the atlectasis and dyspnea were probably due to the collaps of alveoli by reflex closure and by the accumulation of fluid and foam in the alveoli. In the sheep dying 36 hours of carbon tetrachloride poisoning with gross lesions of hyperemia and oedema, the atlectesis and respiratory distress could be attributed to the presence of fluid (oedema) in the alveoli and air passage of the sheep.

According to Smith and Jones(1970) the degenerative changes are found in liver and kidneys in poisoned animal.

Setchell (1962) reported impairment of both kidney and liver function in most of the sheep dying after drenching with carbon tetrachloride poisoning. Gallagher (1962) described degeneration of epithelium lining the tubules and presence of hyaline cast in the tubules. The kidney of sheep dying at different hours of carbon tetrachloride administration showed hyperemia, degenerative changes, necrosis and focal haemorrhages.

Histopathology of brain in carbon tetrachloride poisoning in sheep is not available in the literature. But in the present study group neuronophagia, satellitosis and malacic foci were seen in the brain of sheep. Nervous symptoms can very well attributed to such changes in the brain.

In the intestine degenerative changes in the glands of liber-kuihns, presence of pink stained material in submucosa (oedema) and pyknotic changes in the lamina epithelialis were the most marked changes.

Only hyperemia were noticed in thyroid of sheep in carbon tetrachloride poisoning.

No significant changes were noticed in heart and spleen.

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S U M M A R Y

Sheep of non-descript breed were experimentally poisoned by administering carbon tetrachloride into the rumen through stomach tube. Pathologic changes in different organs of sheep dying to acute carbon tetrachloride posoning have been described. The dose of carbon tetrachloride and route of administration were not changed in any sheep of this experimental group. During the course of the disease locomotor and nervous disturbances were noticed in them. Dyspnoea, stretching of head and neck and peculiar quivering movements of lips and jaw were the main symptoms in them. Affected sheep were observed to remain standing on their feet till they sank to the ground.

Haematological studies were carried out in sheep given toxic doses of carbon tetrachloride. There was highly significant rise in the level of blood urea and fall in that of blood calcium.

Degenerative changes were the most prominent lesions in liver and kidneys in fatal cases of carbon tetrachloride poisoning. Collapse and emphysema were noticed in sheep dying after 12-24 hours of carbon tetrachloride poisoning. In the brain of these sheep degenerative changes were also noticed.

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BIBLIOGRAPHY

Blakemore,F. and McDougall,E.I. (1946) Vet.Rec., 58: 400.

Delak, M. and Ilijas,B. (1961) Vet.Arhiv., 31: 44-52.

Downey, N.E. (1960) Vet.Record., 72: 598-599 and 600.

Fairfax, R.E. (1948) Yearb.Inst.Insp.Stk., N.S.W.(1948). p.73.

Frunder, H.,Fischer, W. and Boring,H. (1956) Hoppe. Seyl. Z., 306; 112-119.

Gallagher,C.H. (1961) Aust. Vet.J., 37: 131-134.

Gallagher,C.H. (1962) Aust.Vet.J., 38: 575-579.

Gallagher,C.H., Kondos, A.C. and Southcott, W.H. (1962) Aust.Vet.J., 38: 409-411.

Gallagher,C.H., Kondos,A.C. and Southcott,W.H. (1962) Aust.Vet.J., 38: 406-408.

Hall,M.C. (1921) J.Agric.Res., 21: 157-175.

Jubb, K.V.F. and Kennedy Peter,C. Pathology of domestic animals, Academic Press, New York,London, 1963.

Kees, K.R., Sinha, K.P. and Spector,W.G. (1961) J.Path.and Bact., 81: 107-118.

Kondos, A.C., Southcott, W.H. and McClymont, G.L.	(1963)	Aust. J. Agri. Res., 14: 852-866.
Kondos, A.C. and McClymont, G.L.	(1965)	Aust. Vet. J., 41 : 349-351.
Kondos, A.C. and McClymont, G.L.	(1966)	Aust. J. Agri. Res., 17: 363-373.
Minett, F.C.	(1950)	Indian J.Vet.Sci., 20(1): 69-103.(Received for publication on 14th September, 1949).
Muth, O.H.	(1960)	Amer.J.Vet.Res., 21 : 86-87.
Rose, A.L.	(1932)	Aust. Vet. J., 8: 122-137.
Setchell, B.P.	(1959)	Nature, Lond., 184: Suppl.no.16, 1242-1243.
Setchell, B.P.	(1962)	Aust.Vet.J., 38: 487-494.
Setchell, B.P.	(1962)	Aust.Vet.J., 38: 580-582.
Setchell, B.P. and Little Johns, I.R.	(1963)	Aust. Vet. J., 39:49-50.
Setchell, B.P., McInnes, P. and Christie, D.G.	(1964)	Aust. Vet. J., 40: 30-31.
Shaw, J.N.	(1937)	J. Parasit., 23 : 536.

Silva Leitao, J.L.	(1945)	Repos. Lab. Pat. Vet. Lisboa., 6: 153-157.
Sloan, J.E.N.	(1950)	Vet. Rec., 62 : 380-381.
Snedecor, G.W. and Cochran, W.G.	(1967)	Statistical Methods, Sixth ed., Iowa State University Press, Ames, Iowa.
Staskiewicz, G., Juszkiewicz,T. and Romanowska, M.	(1955)	Med. Vet. Varsovie, 11: 673-678.
Walker, D.J.	(1947)	Yearb.Inst. Insp. Stk.,N.S.W., 1947, pp. 57-58.
Winterhalter, M.	(1942)	Tierarzti. Rdsch., 48: 315-318.
Winterhalter, M. and Vulinec, M.	(1969)	Vet.Arch., 39: 45-50.