Studies on Experimental Organophosphorus Compound (Malathion) Poisoning in Goats

A Thesis

Submitted to the Faculty of Veterinary Science

RAJENDRA AGRICULTURAL UNIVERSITY

in partial fulfilment of the requirment for the Degree of

MASTER OF VETERINARY SCIENCE

IN

MEDICINE

By

Yaresh Prasad Singh *B. V. Sc. & A. H.*

JUNIOR RESEARCH FELLOW R.A.U. BIHAR

POST - GRADUATE DEPARTMENT OF VETERINARY MEDICINE

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DEDICATED TO

My

PARENTS WHOSE AFFECTIONATE LOVE, SACRIFICES

AND CONSTANT ENCOURAGEMENT

ARE RESPONSIBLE FOR EVERY ACCOMPLISHMENT

OF MY LIFE.

Dr. S.S. Mishra,
B.V.Sc.& A.H.,P.G.(C.D.R.I.), Ph.D.(U.S.S.R.).
Professor & Chairman,
Post-graduate Department of Medicine,
Bihar Veterinary College,Patna-800014.
(Rajendra Agricultural University,Bihar).

P A T N A

Dated, the 11th May, 1978.

This is to certify that the work embodied in this Thesis entitled "STUDIES ON EXPERIMENTAL ORGANOPHOSPHORUS COMPOUND (MALATHION) POISONING IN GOATS" is the bonafide work of Dr. Paresh Prasad Singh and was carried out under my guidance and supervision.

11-8-78

(S. S. MISHRA)

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C E R T I F I C A T E

Certified that the research work encorporated in this Thesis has not been published in part or in full in any of the journal.

11/5/78

(P.P. Singh)

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C O N T E N T S

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INTRODUCTION

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INTRODUCTION

In the modern livestock and agricultural production programme the organophosphorus compounds are gaining much more importance. The recently introduced organophosphorus compounds used effictevely against ectoparasites, endoparasites and plant pests have gained a significant momentum in the modern livestock and agricultural programme. Their use in public health and in veterinary field is also not uncommon. Side by side it has introduced a serious and novel health hazard to livestock as well human beings (McGirr et al., 1953; Barnes, 1953; Younger et al., 1963).

However, the widespread application of these chemicals has also resulted in producing undesirable effects on living landscapes and environmental pollution. Many cases of poisoning have been reported in animals and man during manufacturing and their use even after great precautionary measures (Naresh Bhu et al., 1976).

Now a days goat farming is gaining popularity in our country. It is one of the meat and milk producing dual purpose animal, the rearing of which cost far less than that of cows or buffaloes. Different types of ectoparasites hamper the health and production of goats. Malathion (O,O-dimethylphosphorodithioate of diethyl mercaptosuccinate) is widely

and effectively used for the control of these ectoparasites as dip or spray. Some times it is also used orally as drench in weak concentration to control the endoparasites of different varities and species. Some times due to faulty concentration of dip, spray or drench they get poisoned. The grazing habit of goats many a times exposes them to this poisoning. The insecticides are generally used for better crop production, to overcome the insects and pests menace and if the goats get easy access, they very often suffer from poisoning.

Shortly before and during World War II, a comparatively new class of highly toxic chemicals, the organophosphates, was developed by Schrader of I.G. Frabenindustries, first as agricultural insecticide and later as potential chemical war fare agents (Goodman and Gilman, 1970). Since malathion possesses very low mammalian toxicity to man and animals it offers a wide range of margin of safety when used in crop fields, on food grains as well as in house holds, and in the maintenance of public and animal health programmes. Malathion is aptly described by the United States, Department of Agriculture as "one of the safest insecticides to handle". For the control of ectoparasites on dairy animals, it can be applied directly on the animal bodies as well as in the byres in dairy buildings and poultry pens. In fact under our Insecticides Act, Malathion is classified as "moderately toxic" with the label to have "bright blue colour indentifying

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triangle". Malathion was amongst first few of organophosphorus insecticides introduced in India. Extensive uses of Malathion during the last decade have amply shown that it has multipurpose use in agriculture, public health and animal health programmes.

Malathion is a synthetic organophosphorus compound having lower degree of anti-cholinesterase activity. It is non-systemic but exhibits powerful stomach and contact action accompanied by quick, initial knockdown effects. It also contain phosphorus radical in a combination which permits the compound to inhibit compitatively acetylcholinesterase. The biological action of this compound centres on the process and system of neuromuscular transmission in which an essential agent is acetylcholine or close analogues. Pharmacological effects are equivalent to an excessive accumulation of acetylcholine and certain other enzymes.

Malathion also has a group which can be broken off by esterases, resulting in phosphorylated enzyme instead of acetylated one. Phosphorylated enzyme becomes invactivated as it does not react with water, Excessive accumulation of acetylcholine at neuromuscular junction causes contineous stimulation of cholinergic fibre throughout the peripheral and central nervous system.

In our country, goats are having their monopoly in meat market as compared to their milk. Different types of

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REVIEW OF LITERATURE

toxicity of carbophenothion and methyltrithion in livestock and found that spray of carbophenothion (Trithion) at 0.5 and higher concentration was toxic to calves whereas spray by methyltrithion at 0.25 per cent and 0.5 per cent concentration did not produce signs of toxicosis but depression of cholinesterase activity was noted. Methyltrithion produced toxicity at 25 mg/kg body weight with cholinesterase depression. One per cent suspension spray of carbophenothion was toxic to cattle whereas no signs at 0.05 per cent and 0.1 per cent suspension spray of methyltrithion insecticide was noticed. They also observed that sheep tolerated 25 mg/kg of methyltrithion whereas poisoning occurred at 25 mg/kg with carbophenothion.

Madejski and Juszkiewicz (1966) studied the effect of ekatin (Thiometon) poisoning on some biochemical indices in chicks. They found that thiometon produced fatal poisoning in chicken given 12.5 mg/kg orally in a 25 per cent solution.

Marduel (1968) carried out the experimental intoxication study in horses by an organophosphrous insecticide and reported that blood cholinesterase activity was considerably depressed at first, but no other clinical, haematological or biochemical changes were noticed. There was no accumulation of products, but a gradual rise in cholinesterase activity from about 27 day suggested that some tolerance was established.

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REVIEW OF LITERATURE

Poisoning.

McGirr and Papworth (1953) studied the toxic hazards of dinitro and organophosphrous poisoning produced experimentally in man and animals. They reported that after normal spraying, lethal dose could be ingested by animals from few square yards of sprayed crops; however, breakdown resulting from certain weather condition was less than 4-5 weeks.

Bell *et al*. (1955) studied the toxicity of malathion and chlorthion in dogs and cats. Malathion in single oral dose ranging from 0.5-3.5 mg/kg or dipping 4 times at 4 days intervals in a 2 per cent solution, did not show any signs of toxicity except vomition 35 minutes after administration. Repeated dipping with 0.5-2 per cent solution at 4 days intervals of chlorthion proved more toxic to dogs and so was regarded as unsafe to apply but 0.25 per cent solution was safe for both dogs and cats.

Guarda (1960) studied the experimental parathion poisoning in fowls and reported that lethal parenteral dose of parathion for adult fowl was 2.26 mg/kg, whereas lethal oral dose was 3.13 mg/kg. He further reported that this dose rate was lower in younger birds.

Younger *et al*. (1963) made preliminary studies of the

McCarty et al. (1969) studied the oral toxicity of phorate, disulfoton, oxydimetonmethyl and azinophosmethyl. They concluded that non-toxic oral dose of phorate for new-born calves was 0.1 mg/kg, for yearling calves 0.25 mg/kg, for sheep 0.5 mg/kg and for goats 0.25 mg/kg. Dose of disulfoton for calves was 0.25 mg/kg, 0.5 mg/kg for yearlings and 0.1 mg/kg for sheep and goats. Dose of oxydimetonmethyl was 0.1 mg/kg for calves, 2.5 mg/kg for yearlings and 5 mg/kg for sheep and goats. Dose of azinophosmethyl for calves and sheep was 0.5 mg/kg and for yearlings and goats was 2.5 mg/kg.

Vadlamudi and Paul (1974) studied the subacute oral toxicity of summithion and malathion in buffalo-calves. They reported that 50 ppm of summithion and 20 ppm of malathion were safe to use, but 250 ppm of summithion showed diarrhoea between 8-15 days, with 100 ppm of malathion all calves died between 18-21 days after exhibiting depression, diarrhoea, weakness of the hind limbs and paralysis.

Korolyv (1976) studied the toxicity of valexon (Phoxim) for sheep when dipped against ticks, sheep dipped in 0.1% emulsion of phoxim at intervals of 14-15 days did not affect normal health or haematological values, with the exception of a blood cholinesterase activity by about 35 per cent. He further reported that dosage of 350 mg/kg body weight of phoxim produced acute poisoning and 400-450 mg/kg was lethal. He recommended for 45 days lapse between dippings and slaughter

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of the animal for human consumption.

Symptoms.

Radeleff (1954) studied the TEPP (Tetraethyl pyrophosphate) poisoning in twentynine heads of cattle of various age group. TEPP an insecticide intended for other than animal treatment, developed symptoms like dyspnoea, salivation, trembling, ataxia and paralysis terminating in death within 2-5 minutes in cattle.

Jolly (1957) studied the toxicity of organic phosphorus insecticides and described the symptoms of poisoning as seen in cattle, sheep, pig, dogs and poultry and concluded that blood cholinesterase estimation provided an indication of intoxication.

Radeleff et al. (1957) studied the toxicity of organophosphrous insecticides to livestock (cattle, sheep) by oral dose, spray and dip with twenty organophosphorus compounds. They found that in poisoned animals the first signs were excessive salivation, dyspnoea, restless wandering with stiff gait. Fasciculation of all skeletal muscles and exhaustion forced the animal to lie down. Death occurred due to suffocation and animal grunted softly. Convulsions were only seen in higher doses.

Gusev et al. (1962) studied the toxicity of dithiophos (Sulfotep) for cattle. They observed that two calves died

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from an oral dose of 3.8 or 4 mg/kg showing tremor, convulsions and copious salivation. One survived a dose of 4.5 mg/kg showing cholinesterase symptoms, leucocytosis and decreased cholinesterase activity. They also observed that intravenous injection of 1-3 mg/kg in cats caused a transient fall in blood pressure and increased respiration. These effects were prevented by atropine. They further reported that cattle grazing pasture sprayed with 0.1-0.25 per cent aquous emulsion had no clinical symptoms or blood changes.

Dimitrieve (1975) studied the clinical and pathological effects of DDVP (dichlorvos) poisoning in 30 hens aged 6-8 months, given orally in single dose of 30 or 15 mg/kg or as a repeated dose of 5 mg/kg daily for 20 days. He observed that a dose of 5 mg/kg was 100 per cent lethal after a period of 25 minutes to 3 hours. The clinical signs of poisoning, which appeared 2-4 minutes after the administration of 30 or 15 mg/kg included disturbance of nervous system function and of the haemopoietic organs and disruption of respiration and digestion. He opined that blood cholinesterase was practically abolished 6 hours after administration of a dose of 15 mg/kg it reappeared after six days. At the same time there were changes in the peripheral blood, characterised by an increase in leucocytes, erythrocytes and haemoglobin per cent, and the appearance of large number of pseudoeosinophils containing small coliform grannules. Administration of repeated non-toxic dose caused the hen to develop tolerance to the pesticide.

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Klee and Raake (1976) studied the primary symptom "Continuous salivation" in a herd of 20 cows, 14 young cattle, 1 bull and 7 calves which showed profuse salivation, bradycardia dyspnoea, and a reduction of cholinesterase activity in the blood. They treated all the animals with atropine at the dosage of 100 mg/kg body weight subcutaneously. Full clinical recovery was observed in all animals except one within 1-5 weeks. In view of the symptoms and response to atropine injection it was concluded that the vagotonia was caused by poisoning with a long acting phosphoric acid ester in the fodder.

Dobson (1977) reported an outbreak of suspected poisoning from feed medicated with trichlorofon in pig. A controlled experiment showed that toxicity could occur when trichlorofon was fed 3-5 days at levels greater than that recommended. Signs of inappetence, muscle tremor, ataxia and death were recorded.

Beck et al. (1977) studied the clinical signs, pathological and biochemical changes with triaryl phosphate in naturally (escaping from a gas pipeline compressor station) and experimentally (with a dose of 0.5-1 mg/kg body weight) poisoned cattle. Clinical signs such as posterior motor paralysis, dyspnoea, diarrhoea and agalactia were noticed in cases of natural poisoning whereas in experimental poisoning there was depression of cholinesterase and axonal degeneration in the spinal cord.

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Pathological changes.

Barnes and Denz (1953) studied the experimental demyelination with three organophosphorus compounds, bis-monoiso-propyl-aminofluorophosphinoxide (mipafox), di-isopropylfluorophosphate (D.F.P.) and tri-orthocresylphosphate (T.O.C.P.) and found demyelination in the peripheral nerves, particularly in spinal cord in stained section. They further concluded that the ability to produce demyelination was not solely or directly related to the activity of these compounds as inhibitors of cholinesterase.

Fenton (1955) studied the nature of paralysis in chicken and described the distribution of damage to the nervous system in fowls following D.F.P. poisoning. He opined that the distribution of the damage to the nervous system could be explained on the basis of a degeneration of the long nerve fibres and it was suggested that this inturn resulted from depression in metabolism of neurons. The general feature of paralysis resembled those of nutritional deficiency.

Fontanelli (1955) revealed petechial haemorrhage in viscera, muscles etc. with parenchymatous degeneration of liver and kidneys and congestion of mucous membrane during the courses of his investigation in two cows which had died suddenly. On analysis of viscera the presence of a thiophosphate acid ester known as parathion was established.

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Radeleff et al. (1957) studied the toxicity of organophosphorus insecticides to livestock (cattle, sheep) by oral dose, spray and dip with 20 organophosphorus compounds. They reported that in acute poisoning lesions were not outstanding or even might be absent. Lesions usually consisted of haemorrhage of varying degree on heart, lungs or G.I. tract Lungs were congested and oedematous, bronchi and trachea contained heavy froth.

Guarda (1959) studied lesions in the blood vessels of brain of fowls in acute and chronic poisoning with parathion. There was marked hyperaemia of capillaries and arterioles in the brain and small veins showed irregular dilatation, hyperaemia was more intense in the medulary substance of the cerebellum than in any other sector of brain, but there was no anaemia or ischaemia. He further opined that hyperaemia was the effect of complex physical phenomena immediately preceeding death.

Yasnova (1969) studied the pathological changes in chronic organophosphorus poisoning in twenty sheep and found blood stasis, multiple haemorrhage, necrobiosis and necrosis in visceral organs, regenerative cell proliferation in liver, myocardium and lungs, necrosis of the intestinal mucous membrane and purulent catarrhal pneumonia.

Hothi and Kwatra (1972) studied the experimental aldrin

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and malathion poisoning in buffalo-calves in relation to diagnosis, gross and microscopical changes. Toxicity was produced in buffalo-calves by daily oral administration of 5 mg/kg of either malathion or aldrin. Cholinesterase inhibition was produced 7 days after treatment with both the pesticides. Gross lesion consisted of haemorrhages on the meninges, epicardium, endocardium, thyroids, liver, kidneys, gall bladder, intestinal mucousae and lungs. Microscopic examination revealed retrogressive changes in central nutrons and glial nodules, haemorrhages, haemosiderosis and centrilobular necrosis of liver, severe congestion, extensive haemorrhages, haemosiderosis and instra-alveolar oedema of lungs, haemorrhages and congestive necrosis in kidneys.

Dimitriev (1975) studied the pathological effects of DDVP (dichlorvos) poisoning in 30 hens aged 6-8 months, given orally in single dose of 30 or 15 mg/kg or as a repeated dose of 5 mg/kg daily for 20 days. He observed that a dose of 30 mg/kg was 100 per cent lethal after a period of 25 minutes to 3 hours. Pathological changes in the tissues of poisoned hens consisted of infiltration of blood in the parenchymatous organs, pulmonary oedema, degeneration of the liver and kidneys and catarrh of the small intestine. He opined that these effects were reversible.

Kalinowska et al. (1976) studied the toxicological

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aspects of dermafos (fenchlorphos) in relation to pathological changes in hens given a single dose of 0.5 or 2.5 mg/kg body weight directly into the crop. They observed that no changes occurred in skeletal muscle, but dose related damage was seen in liver and spleen, where regeneration reactions started 16 days after administration of the drug. The residue in liver was estimated by thin layer chromatography, which persisted for a relatively long time, although a steady decrease occurred from the sixth day.

Biochemical changes.

Pankaskie et al. (1952) conducted the experiment on degradation and detoxification of parathion in cows. Parathion in the form of an excessively heavy experimental residue of approximately 14 ppm on baled alfa hay, was fed over a period of 61 days to five dairy cows in mid lactation. The average intake for all the cows was 166.9 mg of parathion/cow/day (0.33 mg/kg/day). They opined that neither parathion nor free p-nitrophenol, a probable hydrolytic product of parathion, was ever found in the milk or jugular blood of the experimental animals.

In the second series of experiment, parathion as a commercial wettable powder, was fed in capsules at increasing dose rate of 1-32 mg/kg/day. Here also no parathion, free p-nitrophenol or free p-aminophenol was ever found in samples of jugular blood, urine and milk taken during experimental

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period. They opined that the parathion might be hydrolysed in vivo to p-introphenol, reduced to p-aminophenol, conjugated with glucuronic acid and then excreted in urine as p-aminophenyl glucuronide. However, the fate of the thiophosphoric acid a portion of parathion molecule, was not determined.

O'Brien (1957) studied about the effect of malathion and its isomers on carbohydrate metabolism of mouse, cockroach and housefly. He concluded that it was effective inhibitor of pyruvate oxidation perhaps by interfearing with citrate oxidising system. He also concluded that the glycolytic and tricorboxylic acid cycle Pathways of carbohydrate metabolism in the mouse and cockroach were not substantially inhibited by these organophosphorus compounds in vitro. Further more there was generally smaller inhibition for insects than for mouse enzymes. Thus it was considered more toxic to insect than mammals.

Robbins et al. (1957) studied the metabolism and excretion of phosphorus-32-labelled diazinon in a cow administered orally at the dose rate of 20 mg/kg body weight. They concluded that it was rapidly metabolized and excreted and only a low level of unchanged toxicant were found in blood and milk samples. About 74 per cent of the dose excreted as polar degradation products in urine was accounted 36 hours after treatment.

Vigne et al. (1957) studied the metabolism of

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insecticide diazinon marked with radioactive phosphorus by feeding 236 mg orally in a goat. Measurement of the anti-cholinesterase activity and organic phosphorus content of the urine, faeces, blood and milk revealed that none of the insecticides get excreted in the milk and faeces and only 2 mg in urine. To account for the radioactivity of these materials, it was concluded that the insecticide was largely metabolized and the 32-p eliminated in the form of phosphate other than that of diazinon.

Mounter and Shipley (1958) demonstrated the inhibition of plasma by toxic phosphorus compounds and concluded that plasmin, the proteolytic enzyme of serum was inhibited by toxic organophosphorus compounds of the diisopropylfluorophosphate type. They further confirmed that differential inhibition provided additional distinction between plasmin and trypsin. It was further opined that the inactive precursor of plasmin was not affected by diisopropylfluorophosphate.

Goulding and Terriere (1959) carried out studies on malathion residues in milch cows treated for hornfly control and reported that the contamination was slight (0.01 ppm) 36 hours after the use of 4 per cent dust and moderate (0.03 ppm) 12-60 hours after using 10 per cent dust. 4 hours after treatment with 0.5 per cent spray only 0.5 ppm malathion was detected in the milk.

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Gaines et al. (1966) studied the liver metabolism of anti-cholinesterase compound in live rats in relation to toxicity. A direct inhibitor of cholinesterase isolan and dichlorvos or paraoxon an indirect inhibitor were infused into the intestinal or femoral veins of female rats. Parathion infused into the hepatic portal system were more toxic than when infused into the general circulation but isolan and dichlorvos underwent detoxification during hepatic passage. They found that dichlorvos was slightly more toxic by oral route than dermal in rats. Liver detoxification was only factor for lower toxicity of isolan by dermal route.

Gupta (1974) studied the blood sugar, plasma electrolytes and glycogen in rats after malathion poisoning. He observed that by intraperitoneal administration of malathion at two dose levels (50 and 500 mg/kg) in 12 hours fasted rats, there were no significant changes with lower dose but with higher doses greater changes were recorded within 30 minutes. Blood glucose was maximum at 2 hours and remained constant upto six hours of treatment whereas pl-asma sodium remained maximum upto six hours. No changes in plasma potassium level were observed. The glycogen content of liver, kidneys, heart and spleen was higher upto 6-24 hours. The brain glycogen content did not show any change.

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Diagnosis.

Hayes et al. (1954) studied the parathion poisoning in four dogs fed 2 or 4 mg/kg body weight for 2-3 weeks. The cholinesterase inhibition was measured in blood plasma and r.b.c's and it was assessed that there was no evidence of dogs developing a tolerance to parathion.

Gage (1955) studied the blood cholinesterase content of the r.b.c. and plasma in early diagnosis of exposure to organophosphorus insecticide in 19 normal human beings and it was measured at monthly intervals for a period of one year. The coefficient of individual variation was 12.8 for r.b.c. and 21.3 for plasma. It was further confirmed that there was no evidence of seasonal variation.

Obra et al. (1955) studied the brain cholinesterase activity in animals poisoned with parathion. They examined the various brain homogenates for cholinesterase activity before and after poisoning with parathion using a modified Hesterin's ferricacethydroxamic reaction for post-mortem diagnosis and suggested that the method was valid for 6-10 days after death.

Panciera (1955) studied about the diagnosis of acute fatal poisoning by organophosphorus insecticide using a histochemical technique to demonstrate this biochemical

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lesions and applied it to the diagnosis of poisoning by some anticholinesterase compounds. He collected the samples of intercostal muscles at various intervals after death and found that the enzyme activity in poisoned animals was markedly or completely inhibited and reactivation of enzymes occurred after death in tissue of all poisoned animals but at no time did reactivation resulted in maximal enzyme activity as in fresh tissues from control animals. Differentiation between acetylcholinesterase activity in tissues from poisoned animals and that in control animals was distinct in all samples collected upto 24 hours after death of the animals, even when subsequently preserved by refrigeration for a week or when frozen for 2 weeks.

Poloz et al. (1965) studied the prophylaxis and diagnosis of chronic poisoning in animals with organophosphorus compounds. They observed that animals which were fed spray contaminated forage with organophosphorus compound showed the symptoms of depression, muscular weakness, pallor of mucous membrane, loss of condition and diarrhoea after 25 days. Their milk contained 0.0008-0.001 mg of toxic organophosphorus compounds per litre. Calves fed with this milk also developed the same symptoms and 30 per cent of them died. They concluded that diagnosis was based on clinical picture, decreased cholinesterase activity of blood and determination of residues of toxic material in tissues, feed and water.

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Anderson et al. (1969) studied blood cholinesterase activity as an index of acute organophosphorus pesticide poisoning in sheep and cattle. The erythrocyte cholinesterase activity of 245 sheep and 299 cattle was determined and it was found that the seasonal variation in sheep was small. The effect of diazinon and crufomate on the activity in sheep and cattle was also estimated. They further concluded that the measurement of cholinesterase activity was useful in diagnosis of poisoning by organophosphorus compounds.

Roe (1969) reported about the whole blood cholinesterase and serum enzyme levels in cattle as indicators of exposure to organophosphorus compounds. After spraying with eithion or imidion (Phosmet) at different concentrations on 42 calves, the whole blood cholinesterase was greatly depressed, serum glutamate oxalate transminase (SGOT) and creatin phosphokinase showed no alteration even when acute toxicity was produced in some individuals.

Treatment.

Hutter and Kostial (1954) observed the effect of magnesium and calcium ions on the release of acetylcholine using profused superior cervical ganglion of the cat from the preganglionic nerve endings and found that magnesium ions in concentrations causing block of ganglionic transmission

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(12-25 m M) reduced the out put of acetylcholine whereas calcium ions (4-10 m M) releaved the block produced by magnesium ions and restored the put put of acetylcholine.

Bergner (1959) made study on the detection of fatal anticholinesterase in cadevors of rats and concluded that in rats, poisoned with diazinon or parathion or other cholinesterase inhibiting compounds, cholinesterase activity in muscle was still inhibited 2 hours after death but in muscle, removed 24 hours after death, it could be demonstrated histochemically by the copper thiocholine test. Inhibition of the enzyme was abolished by immersing muscle in a solution of an oxime ("TMB-4" or "2-PAM") before applying the copper choline test.

Davies et al. (1959) studied the efficacy of 2-hydroxyiminomethyl-N-methylpyridinium methane sulphate and atropine in the treatment of severs organophosphate (sarin or ethylpyrophosphate) poisoning and concluded that the effect of the size of the dose and the time of administration in relation to poisoning, oxime was very effective in conjuction with atropine when given either before or after poisoning at the dose rate of 30 mg/kg as optimum dose.

Karlog (1960) made experimental studies on the effect of P-2-AM in acute poisoning with alkyl phosphate and found that in both the paraoxon and parathion poisoning in rabbits,

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erythrocyte and plasma cholinesterase proceeded rapidly after I/P administration of P-2-AM (Pyridine-2-aldoxime-N-methiodide) (75 mg/kg body weight) .

The author further observed a marked reactivation of cholinesterase in whole blood by I/V injection of P-2-AM (50 mg/kg body weight) on 4 day old calves after 24 hours of dermal application of parathion (2 mg/kg body weight).

Svetlicic and Vondeker (1960) studied the therapeutic effect of Pyridine-2-aldoxime methiodide in mice, rats, rabbits, dogs and horses poisoned with parathion. Dog and horses were given PAM 50 mg/kg intraperitoneally and 20 mg/kg intravenously respectively after appearance of clinical symptoms. PAM reactivated the erythrocyte cholinesterase very markedly whereas its action on inhibited plasma cholinesterase was hardly noticeable. PAM showed an ability to counter act the nicotinic, muscurinic and central nervous system signs of anticholinesterase poisoning. They further observed that the conversion of parathion to paraoxon was slow, and as such a large and repeated doses of PAM was necessary for prolonged therapeutic effect.

Richter (1961) studied the toxicity of trichlorophon alone or in combination with pyridine aldoxime methiodie and atropine sulphate for ox, mouse and rats. The idea of combining above additives was to increase the tolerance and

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to reduce the acetylcholinesterase inhibiting action of the parasiticide. He reported that relatively low concentration of supplements appeared to increase the tolerance in mice and rats, whereas the cerebral acetylcholinesterase content in rats increased after doses of trichlorophon, alone or in combination with the stated additives. Erythrocyte cholinesterase level in rats and cattle were reduced.

Jung et al. (1963) studied the experimental toxicity of trichlorophon in mice, duck, pigs, sheep, cattle and horses They observed that LD50 of trichlorophon for mice by intraperitoneal injection was 450 and orally 660 mg/kg. They further observed that an intraperitoneal injection of atropine (10 mg/kg), atropine and PAM 10 mg and 50 mg/kg body weight, atropine and nikethamide (120), or atropine and leptaxol (30 mg/kg) increased the LD50 to 618, 1300, 1,312 and 1,217 mg/kg respectively. It was further observed that oral LD50 for duck was 44.9 mg/kg, oral toxic dose for pigs, sheep, cattle and horses were 65,200,100 and 50 mg/kg respectively.

Palmer et al.(1964) studied the toxicity of dimetilan in baby calves and observed that spray of 0.1 per cent (clinical signs appearing within 120 minutes) or oral dose of 0.5 mg/kg (clinical signs appearing within about 40 minutes)caused poisoning. The antidotal therapy with atropine sulphate, atropine sulphate either with PAM

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(P_2S, pyridine aldoxime methiodide) or DAM (diacetylmonoxime) was also given.

It was noticed that whole blood cholinesterase was hardly depressed except (by 57% from normal) in one calf which had been dosed orally with 0.5 mg/kg.

Palmer (1964) reported pyridinium oximes to be helpful as an antidote to anticholinesterase effect of organophosphorus, but tended to be specific for different species. Yearling cattle were given 37.5 mg/kg of coumaphos via dose syringe and treated after 6, 12 and 24 hours with one of 3 oximes (Diacetylmonoxime, protopam chloride and TMB-4). Atropine was also given to test animals and controls when signs of intoxication were observed. He opined that the poisonous effect was attributed to a metabolite of cumaphos and reached maximum after 72 hours. He further opined that the duration of poisoning was 4 to more than 10 days, and the effect of poison and of the antidotes differed among individuals. The combination of TMB-4 and atropine was the only treatment that raised the blood cholinesterase significantly, survival rates were as follows: (1) 1/4, (2) 5/6, (3) 4/4, controls 3/6.

Olekarlog (1964) studied the effect of pralidoxime treatment in 8 calves poisoned with parathion spray. After poisoning the calves showed respiratory difficulties and

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violent sweating and there was 20-60 per cent depression in cholinesterase activity of the blood. He concluded that treatment with 30-40 mg/kg pralidoxime I/m or I/v and washing the hair with 1 per cent solution resulted in full recovery without relapse.

Palmer (1965) studied about the therapeutic effect of three oximes in 4 yearling cattle poisoned with dioxathion. He used DAM (Diacetylmonoxime), 2-PAM (Protopam) and TMB-4 in doses of 10 or 20 mg/kg, 6, 24 and 48 hours after recognised toxic dose of 25 mg/kg dioxathion. He reported that only 4 of the 16 animals were more than mildly poisoned; 13 received atropine as required, 0.5 mg/kg initially and less subsequently He concluded that there was some return of the activity in 2-PAM group but a higher dose of 2-PAM or DAM might have had more efficacy ; the dose of 20 mg/kg TMB-4 appeared preferable. The mortality was as follows : controls, 2/4; 2-PAM 2/4; DAM 4/4 and TMB-4, 0/4 respectively.

Eidmann(1968) studied the antidotal treatment of organophosphorus poisoning by cholinesterase reactivators, pralidoxime or obidoxime (Toxogonin) and atropine.

Schmidt and Grutzmachur (1968) studied the late effect on respiration and circulation following the successful treatment of paraoxon poisoning in cats using esterase reactivators and atropine. They found that treatment by

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pralidoxime (130 mg/kg) or obidoxome (15 mg/kg) over came the symptoms of poisoning, but some times it was followed by respiratory alkaliosis, a fall in blood pressure and cardiae out put in anesthetized cats.

McCuruin (1969) reported that out of 24 dogs inadvertently treated with 15 per cent concentrated malathion dip solution on one to three occasions for eight days, 14 died, 2 dogs were killed and eight showed clinical signs of poisoning but recovered on treatment with pyridine-2-paldoxime and atropine sulphate by reactivating the inactivated cholinesterase and blocking the excessive cholinergic response.

Poloz and Lapushkov (1969) advocated the treatment of acute organophosphorus (methylmercaptophos) poisoning with tropacine and dipyroxime. The drugs were repeated as intramuscular inoculation of 4-10 mg/kg of tropacine with 12-15 mg/kg of dipyroxime in aquous solution 3-4 times at intervals of 4-5 hours. They claimed them to be the potent antidote leading to full recovery.

Norkowski and Kozsawski (1971) made studies on the investigation of the efficacy of 2-pyridine-aldoxime methiodide (PAM) and atropine sulphate in the treatment of phospho-choline intoxication in rabbits. Phospho-choline was injected intramuscularly at the dose rate of 400 mg/kg, atropine sulphate 0.5 mg/kg subcutaneously and PAM 15 mg/kg

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intramuscularly thus preventing the death of poisoned animals. The best result was obtained at a dose rate of 30 mg/kg of PAM and 0.5 mg/kg of atropine sulphate respectively.

Srivastava and Parasar (1971) studied the toxic effect of malathion in fowls. They observed that malathion dust 2.5 gm/kg and 50 ml of 1.25 per cent emulsion in 2.5 per cent sucrose solution was safe for external application whereas acute symptoms of poisoning were seen with 50 mg of 5 per cent emulsion of malathion in W.L.H. cockerels. The period of death was prolonged by treating the poisoned birds with atropine sulphate and pralidoxime.

Savateev et al. (1973) reported the analysis of mechanism of a therapeutic effect of oxygen under pressure in organophosphorus poisoning. They opined that after acute poisoning there was a fall in O_2 tension in muscle and venous blood and to a shift in acid-alkali balance in the detection of hypoxia without any normalization of disturbance of the acid-alkali balance when a pressure of 3 atmosphere was applied in rabbits. The survival period increased but the final out come remained unchanged.

Benes et al. (1976) studied the thereapy of an oral LD_{50} of EDMM /O-ethyl S-(2-dimethylaminoethyl) methyl phosphonothioate / at dosage of 0.209 mg/kg in four sheep

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of 30-50 kg body weight. They further observed that 120 minutes later when signs of acute poisoning appeared, 1/2 injection of TMB-4 (Trimedoxime bromide) at 10 mg/kg and atropine at 0.08 mg/kg produced complete recovery in 12 hours, two of four untreated sheep died.

Benes et al. (1976) studied the antidotal effect of TMB-4 comps (spofa) at the dose rate of 10 mg/kg body weight administered I/V in sheep 60 minutes after poisoning with EDMM gave an immediate clinical effect. They further concluded that the r.b.c. acetylcholinesterase was almost 100 per cent restored 15 minutes after the antidote was given whereas plasma cholinesterase restored 70-80 per cent only. It was further observed that the sheep returned to normalcy within 14 days.

Egyed et al. (1976) tried the comparative efficacy of pralidoxime iodide, obidoxime chloride and atropine in the treatment of oral diazinon poisoning in goslings. Pralidoxime iodide at the dose rate of 100 mg/kg was found to be an excellent antidote, whereas obidoxime chloride and atropine were proved to be unsatisfactory and inactive.

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MATERIALS AND METHODS

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MATERIALS AND METHODS

In the present study twenty three healthy, normal she-goats preferably of same age and weight were taken and maintained under the similar nutritional and environmental plane. They were randomly divided into four groups (group 1st, group 2nd, group 3rd and group 4th). A thorough clinical examination was made before carrying out the experiment.

Malathion (cythion, Cynamid India Ltd.) 50 per cent emulsifiable solution was orally given at the dose rate of 200 mg/kg body weight to produce acute poisoning in each group.

Groupwise study was carried out as follows:

Group - 1st (5 + 0 = 5 goats):

It consisted of five animals. Symptomatological, biochemical, haematological, post-mortem and histopathological changes in liver, kidneys, lungs, intestine, brain,heart were carried after dosing with malathion.

Group-2nd (5 + 1 = 6 goats):

This group was further divided into two subgroups (a) and (b).

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Subgroup (a) (5 + 0 = 5 goats) :- It consisted of five animals. The animals were soon subjected to treatment after the appearance of clinical symptoms of poisoning with the following drugs:

(1) Atropine sulphate (Bengal Immunity Ltd):- It was given half intravenously and half subcutaneously at the dose rate of 0.5 mg/kg body weight initially followed by 0.25 mg/kg by the above said routes subsequently if needed (Palmer,1964).

(2) DAM (Diacetylmonoxime, Loba Austranal Chemie) :- It was given intravenously at the dose rate of 30 mg/kg body weight as freshly prepared 10 per cent solution slowly and repeated if needed again whenever the clinical symptoms of poisoning reappeared,the sencond dose of drug (1) and (2) were given separately.

Subgroup (b) (1 + 0 = 1 goat) :- It consisted of only one goat. The animal was given same dose of malathion as in subgroup (a) and kept as control toadjudge the effect of the drug administered in subgroup (a).

Group - 3rd (5 + 1 = 6 goats):

It was also divided into two subgroups (a) and (b).

Subgroup (a) (5 + 0 = 5 goats):- It consisted of five animals. They were subjected to treatment after the appearance of clinical symptoms with the following drugs injected separately.

(1) Atropine sulphate :- Dose and routes of administration were same as indicated in subgroup (a) of group (2).

(2) DAM (Diacetylmonoxime):- Dose and route of administration were same as indicated in subgroup (a) of group (2).

(3) Coramine (Nikethamide,Ciba India Ltd.) :- It was administered half intravenously and half intramuscularly at the dose rate of 120 mg/kg body weight (Jung, 1963) and repeated thereafter when felt necessary.

Subgroup (b) (1 + 0 = 1 goat) :- It consisted of only one goat. Rest same as indicated in subgroup (b) of group (2).

Group-4th (5 + 1 = 6 goats):

It was further divided into two subgroups (a) and (b).

Subgroup (a) (5 + 0 = 5 goats) :- It consisted of five animals. They were treated soon after the appearance of clinical symptoms with the following drugs injected separately:

(1) Atropine sulphate :- Dose and routes of administration were same as indicated in subgroup (a) of group (2).

(2) DAM (Diacetylmonoxime):- Dose and route of administration were same as indicated in subgroup (a) of group (2).

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(3) Coramine :- Dose and route of administration were same as indicated in subgroup (a) of group (3).

(4) Magnesium sulphate :- 10 ml of 20 per cent solution of magnesium sulphate was injected subcutaneously.

Subgroup (b) (1 + 0 = 1 goat) :- It consisted of only one animal. Rest same as indicated in subgroup (b) of group (2).

All the twenty three goats of group (1st), group (2nd), group (3rd) and group (4th) were subject for detailed clinical examination, haematological, biochemical analysis and urine examination before and after the appearance of clinical symptoms and one hour after treatment and one week after treatment if survived.

In addition to above observations, time interval between administration of malathion and appearance of clinical symptoms, administration of malathion and death and survival period after the appearance of clinical symptoms were also recorded.

General clinical examinations:

Following observations were made :

(a) Pulse rate per minute
(b) Respiration rate per minute
(c) Rectal temperature (^{o}F).
(d) General condition.

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Haematological examinations.

It was done soon after the collection of blood from jugular vein in sterilized vials containing Heller and Paul (1934) anticoagulant solution in dry form obtained after keeping the vial in sterilizer at 60°C. The following haematological examinations were carried out :

(a) Haemoglobin (Hb%) :- It was determined by indirect method (acid haematin) using Sahli's haemoglobinometer (Oscar, W. Schalm, 1967).

(b) Packed cell volume (PCV%) :- It was determined by wintrobe haematocrit method (Oscar, W. Schaim, 1967).

(c) Total leucocyte count (TLC) :- It was determined by micropipette method (Boddie, 1962).

(d) Differential leucocyte count (DLC) :- For this Leishman's stain (supplied by Sarabhai Chemicals India Ltd.) was used. Method adopted was, as described by Boddie (1962).

Urine examination.

Urine samples were collected in a clean and dried test tube and was analysed as described by Ganti (1971) for the presence of sugar and albumin.

(a) Urine sugar :- Benedict's qualitative test was done to detect the presence of sugar in the collected urine

samples (Ganti, 1971).

(a) Urine albumin :- Robert's test was done to detect the presence of albumin in the collected urine sample (Ganti, 1971).

Biochemical estimation.

In this plasma ascorbic acid and blood glucose levels were estimated as follows :

(a) Blood glucose :- It was determined with the help of Klett-Colorimeter using folin-wu tube. For this blood glucose kit code L-7 supplied by Bharat Laboratory, Bombay was taken. Test was carried out according to method described in the leaflet supplied alongwith the kit.

(b) Plasma ascorbic acid :- It was done by titration method using dichlorophenol indophenol as described by King and Wootton (1964) in Micro-Analysis in Medical biochemistry.

Pathological examination.

Post-mortem examination of goats were conducted soon after death. The gross pathological lesions were noted.

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Histopathological examination.

Pieces of liver, kidneys, lungs, heart, intestine and brain were collected and fixed in 10 per cent formalin solution. Paraffin embedded tissue sections were cut at 4-5 mincrons by usual method. Sections were stained with haemdtoxylin and eosin and examined under microscope.

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RESULTS

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RESULTS

Poisoning.

A total number of 23 goats were taken and weighed to calculate the actual dose of malathion to produce acute poisoning. They were given malathion at the dose rate of 200 mg/kg body weight orally. The time interval between administration of malathion and appearance of symptoms is presented in Table 1.

Signs and symptoms.

The predominating symptoms in acute malathion poisoning in all the goats during the experiment were frothy salivation accompained by bleating at short intervals. As the time advanced bleating terminated into soft gnouring and salivation became profuse. Dyspnoea, Lacrimation, Frequent defecation, urination and hypermotility of the intestine visible through the abdominal wall were the obvious signs. Constriction of pupil (Pupilary miosis), muscle tremor commencing in head and neck, spreading over the body especially to the limb and hindquarter muscles causing the animal to walk with stiff, stilted movement were the additional signs. To and fro movement of head, streching of neck followed by prostration and clonic convulsions resulted finally to lateral recumbancy often complicated

by tympany. All the animals of group 1st died after exhibiting all the above symptoms (Photo A.B.C).

Clinical examinations.

An initial increase in pulse and respiration rates just after poisoning was recorded without any apparent change in body temperature, followed by a moderate fall after appearance of symptoms. Even after one hour of treatment the pulse, respiration and body temperature showed downard tendancy and same reached the normal level seven days after treatment. As shown in Table 2, 3, 4 and 17.

General condition of all the animals of the proposed research were thoroughly examined for general health and appearance, look of the animal, temperament, wound etc. on body and ectoparasites before administering the malathion to produce acute poisoning. On examination all the animals found to be in normal health, with good look and temperament with no wounds scratches over the body. The animals were also found free from ectoparasites.

Haematological examination.

Changes in total leucocytic count (TLC), differential leucocytic count (DLC), packed cell volume per cent (PCV) and haemoglobin concentration (Hb%) observed during the course of poisoning (Pre and Post), one hour post-treatment and 7 days post-treatment in all the four groups of goats are

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presented in Table 15, 5, 6, 7,9,10, 11, 8 and 17.

In all the four groups of goats poisoned with malathion the normal haematological picture is presented in Table 15. It was observed that, following poisoning there was very little increase in total leucocytic count on appearance of the symptoms that is from 10.3 ± 0.32 to 14.0 ± 0.37. As the symptoms progressed the total leucocytic count did not fluctuate much rather the level was maintained even after one hour of treatment that is 14.0 ± 0.37 to 15.16 ± 0.60 but the total leucocytic count tended to come to normal after seven days of treatment (Table 17).

Among the different leucocytic cells (DLC) during the course of poisoning there were changes mostly in neutrophils and lymphocytes. There was an increase of neutrophils from 37.45 ± 0.89 to 61.85 ± 1.89 at the peak of the symptoms (Table 6) with corrosponding decrease in lymphocytes from 54.4 ± 1.94 to 28.5 ± 0.74 (Table 7). The monocytes, eosinophils and basophils were found to be at almost normal level that is 1.9 ± 0.13, 5.8 ± 0.17 and zero in pre-poisoning (Table 15), 2.1 ± 0.95, 7.42 ± 0.13 and zero at the peak of symptoms (Table 9 and 8), 1.9±0.1, 8.06 ± 0.21 and zero at one hour after treatment (Table 9 and 8) and 2.0 ± 0.04, 4.15 ± 0.22 and zero 7 days post-treatment (Table 17).

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At the peak of symptoms the haemoglobin concentration and packed cell volume percentage in all the groups moderately increased from 9.11 ± 0.26 gm% and 26.7 ± 0.45 per cent to 9.85 ± 0.34 gm% and 30.05 ± 0.66 per cent respectively. One hour after treatment they were nearly same as at the peak of symptoms but 7 days after treatment the haemoglobin concentration and packed cell volume tended to come to normal (Table 17)

Even after one hour of treatment the PCV and haemoglobin concentration were at par with the peak of symptoms that is 30.05 ± 0.66, 9.85 ± 0.34 gm% and 31.5 ± 0.76 per cent, 9.6 ± 0.10 gm% respectively, however the above two blood values tended to come to normal 7 days after treatment (Table 17).

Biochemical estimations.

Blood glucose :- Result of analysis of blood glucose level in all the four groups of animals are presented in Table 12.

The normal blood glucose level of all the four groups of goats is presented in Table 15. It was observed that in all the four groups the blood glucose level markedly increased from 54.95 ± 2.40 mg% to 95.3 ± 3.92 mg% in group 1st; 64.28± 5.21 mg% to 125.3 ± 9.73 mg% in group 2nd; 69.24 ± 5.64 mg% to 128.8 ± 10.11 mg% in group 3rd and 76.72 ± 6.24 mg% to 142.9 ± 13.63 mg% in group 4th after poisoning with malathion. One hour after treatment the blood glucose level was further

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increased which is evident from Table 12. However, 7 days after treatment the blood glucose level tended to come to normal in all the survived goats (Table 17).

Plasma Ascorbic acid :- Results of the analysis of plasma ascorbic acid of the four groups of animals, following poisoning are presented in Table 13, whereas the normal value of plasma ascorbic acid level of the four groups of goats are presented in Table 15.

On analysis it was found that there was little variation in plasma ascorbic acid level between normal and the poisoned goats at the peak of symptoms (Table 13). One hour after treatment the increased level was maintained (Table 13), but 7 days after treatment plasma ascorbic acid almost reached the normal level (Table 17).

Urine Analysis.

Sugar and Albumen :- On qualitative test of the urine samples collected from all the four groups of poisoned goats were found to be negative for sugar and albumen.

Pathological changes.

Gross:

Liver :- The liver appeared to be dark brown in colour and few haemorrhagic spots were seen on its surface. Bloody fluid came out from the cut surface. The texture of the liver

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was found to be friable.

Kidneys :- The cortical portion of the kidney appeared haemorrhagic and was severly congested. No appreciable changes could be observed in the medullary region of the kidney.

Lungs :- The lungs were observed to be congested and haemorrhagic. Some pneumonic patches were also present. Lungs showed emphysema and appeared oedematous. The bronchi and trachea contained heavy froth.

Intestine :- The mucous membrane of the both large and small intestines were severly congested and haemorrhagic. The lumen contained large amount of mucous. The outer wall of the gut was also found to be congested.

Heart :- The heart musculature was congested. Few haemorrhagic spots were also seen on the heart.

Brain :-The cerebrum and cerebellum appeared to be congested and some haemorrhagic spots were present on the brain surface. The petechial haemorrhages on the duramater of meninges were also abserved.

Microscopic changes :- The hepatocytes around the central veins continued to contain vacuoles and vacuolation of the hepatocytes extended beyoned the midzonal region of the lobule. The liver cells around the periphery of the lobule were still

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disernible which did not contain any vacuole. In one case the vacuolar degeneration had subsided but cloudy swelling was persisting in the hepatocytes. There was also dilation of portal vein and oedema in the portal tract (Fig. 1).

The cut section of liver showed normal architectural pattern, which was not disturbed. The changes were mainly confined to the central vein. The liver cells around the central vein showed fatty changes and necrosis. The cytoplasm of some of the hepatocytes showed grannlarity and haziness of the cytoplasm . The nuclei of hepatocytes around the central vein were showing pyknosis, karryorrhexis and karyolysis. The vonkupffer cells showed hypertrophy and rounding of the cytoplasm. The blood vessels and sinusoids of the liver were packed with erythrocytes. There was infiltration of a few mononuclear cells in the portal tract (Fig. II). The bile duct did not show any significant changes.

The blood vessels of the myoeardium were severly congested and there was haemorrhage in the interfibrillar space. The muscle fibres at places were showing increased granularity and at some location there was almost complete lysis of the myocardial fib s. There was also mild infiltration of mononuclear cells (Fig. III, IV and V).

The blood vessels of the intertubular region and glomeruli were heavily packed with erythrocytes. There was

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haemorrhage in the intertubular and tubular space and also in the Bowman's space. The endothelial cells of the glomerular tuft were swollen and occupied almost complete space in the Bowman's capsule. The epithelial cells of the tubules were either disquamated or showing varying degrees of degenerative changes (Fig. VI).

In the lungs focal oedema and emphysema were very much pronounced. The bronchi and bronchioles showed least changes due to poisoning (Fig. VII).

The tips of intestinal villi were devoid of lining epithelial cells and there was a moderate amount of mononuclear cells infiltration in the lamina propria (Fig, VIII).

There was a mild congestion of blood vessels in the cerebrum but the most significant change was perineuronal oedema. However, satellitosis and neuronophagia were not pronounced in any part of the cerebrum (Fig. IX).

Time interval.

The time interval between administration of malathion and appearance of symptoms, administration and death of animal and survival period after appearance of clinical symptoms have been recorded in all the four groups of goats, which are presented in Table 1, 14, and 18. It was observed

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that the time interval in all the four groups of goats following poisoning and appearance of symptoms were 40.00 ± 3.53 minutes in group 1st, 40.00 ± 3.53 minutes in group 2nd, 38.00 ± 2.52 minutes in group 3rd and 41.00±1.98 minutes in group 4th respectively.

Chemotherapy.

The results of treatment of acute experimental malathion poisoning in goats with Atropine sulphate, DAM (Diacetylmonoxime) in group 2nd, Atropine sulphate, DAM and coramine (Nikethamide) in group 3rd and Atropine sulphate, DAM, coramine and 20 per cent magnesium sulphate in group 4th are presented in Table 16.

From Table 16 it is evident that after treatment with atropine sulphate at the dose rate of 0.5 mg/kg initially followed by 0.25 mg/kg half intravenously and half intramuscularly and DAM 30 mg/kg intravenously in the 2nd group of poisoned animals, none of the animal could survive. It was also observed that the goats died between 98.00 ± 7.36 minutes after the administration of malathion as shown in Table 14.

Goats of group 3rd treated with atropine sulphate and DAM at the same dose rate and route of administration as in group 2nd, alongwith coramine at the dose rate of 120 mg/kg body weight intramuscularly, only two goats out of

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five could survive (40%) Table 16. After 7 days of treatment the haematological and biochemical values of blood tended to come to normal Table 17.

The animals of group 4th treated with 20 per cent magnesium sulphate solution singly at the dose rate of 10.0 ml/animal subcutaneously alongwith the other combinations with same dose and route as in group 3rd only three goats out of five could survive (60%) as shown in Table 16, To over come the symptoms atropine sulphate, DAM and coramine were repeated frequently whenever the symptoms reappeared in all the three groups of animals.

In the above said three groups viz. group 2nd, 3rd and 4th one animal in each group was kept as control to judge the efficacy of the drugs administerd.

From the above findings it has been noticed that atropine sulphate, DAM, coramine and magnesium sulphate 20 per cent solution was found to be comparatively more efficacious as an antidote in acute malathion poisoning in goats than that of atropine sulphate, DAM, and atropine sulphate DAM and coramine.

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Table 1

Showing mean time period with S.E. between administration of malathion and appearance of symptoms in different stages.

Group	Post-poisoning mean ± S.E.	1 hour post-treatment mean ± S.E.
1st	40.00 ± 3.53	--
2nd	40.00 ± 3.53	40.00 ± 3.53
3rd	38.00 ± 2.55	38.00 ± 2.55
4th	41.00 ± 1.98	41.00 ± 1.98
	39.75 ± 2.89	39.66 ± 2.68

Table 1 (A)

Analysis of variance, table showing the effect on different groups and at different stages of treatment.

Sources of variation	Post-poisoning df.	Post-poisoning M.S.	1 hour post-treatment df.	1 hour post-treatment M.S.
Between groups	3	7.92 NS	2	11.67 NS
Within groups	16	53.13	12	50.00
Total	19		14	

Table 2

Showing mean pulse rate with S.E. after dosing with malathion in different stages of treatment.

Group	Post-poisoning mean ± S.E.	1 hour post-treatment mean ± S.E.
1st	109.6 ± 2.99	--
2nd	96.8 ± 3.74	75.2 ± 1.49
3rd	82.4 ± 3.49	96.0 ± 1.60
4th	73.6 ± 2.04	79.2 ± 3.20
	90. 6 ± 3.08	83.46 ± 2.09

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Table 2 (A)

Analysis of variance, table showing effect on different groupsand at different stages of treatment.

Sources of variation	Post-poisoning dff.	M.S.	1hr.post-treatment df.	M.S.
Between groups	3	1259.46++	2	609.07++
Within group	16	40.4	12	42.13
Total	19		14	

Table 3

Showing mean respiration rate with S.E. after dosing with malathion in different stages of treatment.

Group	Post-poisoning mean ± S.E.	1 hr post-treatment mean ± S.E.
1st	32.00 ± 1.79	--
2nd	32.00 ± 1.79	43.2 ± 1.96
3rd	24.80 ± 2.94	25.6 ± 0.88
4th	16.00 ± 1.26	16.8 ± 1.49
	26.2 ± 1.94	28.5 ± 1.44

Table 3 (B)

Analysis of variance, table showing effect on different groups and at different stages of treatment.

Sources of variation	Post-poisoning df.	M.S.	1 hr post-treatment df.	M.S.
Between groups	3	222.13++	2	903.47++
Within groups	16	33.30	12	19.73
Total	19		14	

N.S. = not significant; ++ = highly significant.

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Table 4

Showing mean temperature °F with S.E. after dosing with malathion in different stages of treatment.

Group	Post-poisoning mean ± S.E	1 hr post-treatment mean±SE
1st	102.5 ± 1.38	--
2nd	101.1 ± 1.59	99.2 ± 0.98
3rd	100.8 ± 2.34	100.8 ± 1.32
4th	100.9 ± 2.73	101.4 ± 1.63
	100.3 ± 2.01	100.4 ± 1.31

Table 4 (A)

Analysis of variance, table showing effect on different groups and different stages of treatment.

Sources of variation	Post-poisoning df.	M.S.	1 hr post-poisoning df.	M.S.
Between groups	3	413.76NS	2	356.21 NS
Within groups	16	787.32	12	378.83
Total	19		14	

Table 5

Showing mean total leucocytic count (TLC) with S.E. after dosing with malathion in different stages of treatment.

Group	Post-poisoning Mean ± S.E.	1 hr.post-treatment Mean ± S.E.
1st	15.2 ± 0.68	--
2nd	13.4 ± 0.32	15.6 ± 0.21
3rd	12.2 ± 0.12	13.9 ± 0.73
4th	15.2 ± 0.36	16.0 ± 0.87
	14.0 ± 0.37	15.16 ± 0.60

Table 5 (A)

Analysis of variance, table showing effect on different groupsand at different stages of treatment.

Source of variation	Post-poisoning df.	Post-poisoning M.S.	1 hr.post treatment df.	1 hr.post treatment M.S.
Between groups	3	97.62 NS	2	60.12 NS
Within groups	16	51.38	12	73.92
Total	19		14	

Table 6

Showing mean neutrophils with S.E. after dosing with malathion in different stages of treatment.

Group	Post-poisoning mean ± S.E.	1 hr post-treatment mean ± S.E.
1st	67.2 ± 1.38	--
2nd	63.0 ± 2.12	51.6 ± 1.98
3rd	52.4 ± 1.89	52.2 ± 0.89
4th	68.8 ± 2.17	62.8 ± 1.36
	61.85 ± 1.89	55.66± 1.41

Table 6 (A)

Analysis of variance, table showing effect on different groups and at different stages of treatment.

Source of variation	Post-poisoning df.	Post-poisoning M.S.	1 hr.post-treatment df.	1 hr.post-treatment M.S.
Between groups	3	18.2 NS	2	36.7 NS
Within groups	16	10.6	12	28.9
Total	19		14	

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Table 7

Showing mean lymphocytes with S.E. after dosing with malathion in different stages of treatment.

Group	Post-poisoning mean ± S.E.	1 hr.post-treatment mean±S.E.
1st	24.8 ± 0.63	--
2nd	28.2 ± 1.03	25.3 ± 1.08
3rd	39.10± 0.89	50.1 ± 2.31
4th	21.9 ± 0.41	26.80± 0.38
	28.50± 0.74	30.55± 1.25

Table 7 (A)

Analysis of variance, table showing effect on different groups and at different stages of treatment.

Source of variation	Post-poisoning df.	M.S.	1 hr post-treatment df.	M.S.
Between groups	3	60.97 ++	2	95.21++
Within groups	16	17.93	12	31.32
Total	19		14	

Table 8

Showing mean eosinophilic count with S.E. after dosing with malathion in different stages of treatment.

Group	Post-poisoning mean ± S.E	1 hr.post-treatment mean±S.E.
1st	5.5 ± 0.12	--
2nd	7.4 ± 0.09	1.6 ± 0.17
3rd	5.6 ± 0.11	5.4 ± 0.21
4th	11.2 ± 0.23	9.2 ± 0.26
	7.42 ± 0.13	8.06 ± 0.21

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Table 8 (A)

Analysis of variance, table showing effect on different groupsand at different stages of treatment.

Sources of variation	Post-poisoning df.	Post-poisoning M.S.	1 hr post-poisoning df.	1 hr post-poisoning M.S.
Between groups	3	12.32++	2	10.61++
Within groups	16	3.41	12	3.19
Total	19		14	

Table 9

Showing mean monocytic count with S.E.after dosing with malathion in different stage of treatment.

Group	Post-poisoning mean ± S.E.	1 hr.post-treatment mean±S.E.
1st	1.6 ± 0.07	--
2nd	2.6 ± 0.12	1.8 ± 0.18
3rd	2.0 ± 0.10	1.7 ± 0.19
4th	2.4 ± 0.09	2.2 ± 0.09
	2.15 ±0.09	1.90± 0.10

Table 9 (A)

Analysis of variance, table showing effect on different groups and at different stages of treatment.

Source of variation	Post-poisoning df.	Post-poisoning M.S.	1 hr. post-treatment df.	1 hr. post-treatment M.S.
Between groups	3	2.41 NS	2	1.97 NS
Within groups	16	1.63	12	1.38
Total	19		14	

N.S. = not significant.

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Table 10

Showing mean PCV % with S.E. after dosing with malathion in different stages of treatment.

Group	Post-poisoning mean ± S.E.	1 hr. post-treatment mean ± S.E.
1st	29.6 ± 0.32	--
2nd	32.8 ± 0.96	34.8 ± 0.96
3rd	29.6 ± 0.71	29.2 ± 0.37
4th	29.4 ± 0.67	30.6 ± 0.97
	30.05 ± 0.66	31.53 ± 0.76

Table 10 (A)

Analysis of variance, table showing effect on different groups and at different stages of treatment.

Source of variation	Post-poisoning df	Post-poisoning M.S.	1 hr. post-treatment df.	1 hr. post-treatment M.S.
Between groups	3	37.42 NS	2	18.49 NS
Within groups	16	29.73	12	20.12
Total	19		14	

Table 11

Showing mean haemoglobin concentration gm% with S.E. after dosing with malathion.

Group	Post-poisoning mean ± S.E.	1 hr. post-treatment mean ± S.E.
1st	9.4 ± 0.32	--
2nd	11.0 ± 0.48	10.7 ± 0.19
3rd	8.6 ± 0.17	8.0 ± 0.76
4th	10.4 ± 0.41	10.1 ± 0.41
	9.85 ± 0.34	9.6 ± 0.45

Table 11 (A)

Analysis of variance, table showing effect on different groups and at different stages of treatment.

Source of variation	Post-poisoning df.	Post-poisoning M.S.	1 hr. post-poisoning df.	1 hr. post-poisoning M.S.
Between groups	43	43.92+	2	12.59 NS
Within groups	16	20.13	12	8.36
Total	19		14	

Table 12

Showing mean blood sugar with S.E. after dosing with malathion in different stages of treatment.

Group	post-poisoning mean ± S.E	1 hr. post-treatment mean ± S.E.
1st	95.3 ± 3.92	--
2nd	125.3 ± 9.73	138.1 ± 14.73
3rd	128.8 ±10.11	152.5 ± 11.36
4th	142.9 ±13.63	160.2 ± 15.93
	123.07± 6.84	150.26 ± 14.00

Table 12 (A)

Analysis of variance, table showing effect on different groups and at different stages of treatment.

Source of variation	Post-poisoning df.	Post-poisoning M.S.	1 hr. post-treatment df.	1 hr. post-treatment M.S.
Between groups	3	125.97++	2	183.62++
Within groups	16	38.71	12	41.79
Total	19		14	

N.S. = not significant; + = significant; ++ = highly significant

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Table 13

Showing mean plasma ascorbicacid mg% with S.E. after dosing with malathion in different stages of treatment.

Group	Post-poisoning mean ± S.E.	1 hr.post-treatment mean ± S.E.
1st	0.97 ± 0.09	--
2nd	1.01 ± 0.07	1.07 ± 0.03
3rd	1.10 ± 0.05	1.11 ± 0.06
4th	1.00 ± 0.02	1.07 ± 0.04
	1.02 ± 0.05	1.08 ± 0.04

Table 13 (A)

Analysis of variance, table showing effect on different groups and at different stages of treatment.

Source of variation	Post-poisoning		1 hr.post-treatment	
	df.	M.S.	df.	M.S.
Between groups	3	3.61 NS	2	2.64 NS
Within groups	16	2.12	12	1.98
Total	19		14	

Table 14

Showing mean time interval between administration of malathion and death of the animal in different stage of treatment.

Group	Post-poisoning mean ± S.E.	1 hr.post-treatment mean ± S.E.
1st	166.0 ± 4.18	--
2nd	98.0 ± 7.36	--
3rd	160.0 ±10.19	218.31 ± 17.31
4th	347.0 ±18.32	380.00 ± 26.71
	167.7 ± 10.01	299.15 ± 14.67

-- = death of animals; N.S. = not significant.

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Table 15

Showing normal physiological, haematological and biochemical values of experimental goats.

Readings	Overall mean ± S.E.	Group 1st Mean±S.E.	Group 2nd Mean±S.E.	group 3rd Mean±S.E.	Group 4th Mean±S.E.
Pulse	100.3 ±2.58	112.2 ±2.34	104.6 ±2.80	96.0 ±3.20	88.4 ±2.01
Respiration	21.05 ±1.48	23.20 ±1.04	23.20 ±1.04	21.80 ±2.60	16.00 ±1.26
Temperature	102.6 ±1.56	103.2 ±1.02	102. 2 ±1.3 0	102.5 ±2.03	102.8 ±1.90
T.L.C.	10.30 ±0.32	10.70 ±0.59	8.80 ±0.29	9.10 ±0.16	12.60 ±0.24
Neutrophils	37.45 ±0.89	38.20 ±0.80	38.20 ±0.84	37.80 ±0.89	35.60 ±1.06
Lymphocytes	54.45 ±1.94	55.00 ±1.40	53.20 ±1.86	55.60 ±2.30	54.00 ±2.16
Eosinophils	5.80 ±0.17	5.20 ±0.10	5.20 ±0.08	4.40 ±0.14	8.40 ±0.36
Monocytes	1.90 ±0.13	1.60 ±0.26	1.80 ±0.12	2.20 ±0.06	2.00 ±0.09
Basophils	0	0	0	0	0
PCV%	26.70 ±0.45	26.60 ±0.40	28.20 ±0.64	25.60 ±0.40	26.40 ±0.36
Hb%	9.11 ±0.26	9.20 ±0.28	9.26 ±0.30	8.12 ±0.12	9.88 ±0.36
Blood glucose	66.29 ±4.87	54.95 ±2.40	64.28 ±5.21	69.24 ±5.64	76.72 ±6.24
Plasma Ascorbic Acid.	0.92 ±0.02	0.93 ±0.09	0.95 ±0.07	0.92 ± 0.05	0.91 ±0.08

Table 16

Showing the percentage survival of goats after different antidotal therapy in acute malathion poisoning.

Group	Treatment	No. of treated goats	No. of survived goats	% of survived goats.
1st	No treatment	--	--	--
2nd	(1)Atropine sulphate (2)DAM	5	nil	nil
3rd	(1)Atropine sulphate (2)DAM (3)Coramine	5	2	40
4th	(1)Atropine sulphate (2)DAM (3)Mag. sulphate (20%)	5	3	60

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Table 17

Showing Physiological, Haematological and Biochemical values 7 days after treatment.

Group	Physiological			Haematological								Biochemical	
	Pulse mean ±S.E.	Respi. mean ±S.E.	Temp. mean ±S.E.	TLC mean ±S.E.	Neutro mean ±S.E.	Lympho mean ±S.E.	Eosino mean ±S.E.	Mono mean ±S.E	Baso mean ±S.E.	PCV% Mean ±S.E.	Hb % mean ±S.E	Blood sugar mean ±S.E.	Plasma ascrobic acid mean±S.E.
1st	0	0	0	0	0	0	0	0	0	0	0	0	0
2nd	0	0	0	0	0	0	0	0	0	0	0	0	0
3rd	86.0 ±1.10	19.0 ±1.04	102.8 ±0.80	11.6 ±0.32	46.5 ±0.80	47.5 ±0.20	4.0 ±0.04	2.0 ± 0.80	0 0	27.5 ±0.64	9.3 ±0.28	65.0 ±3.40	1.01 ±0.04
4th	88.0 ±2.16	20.0 ± 1.10	102.8 ±1.12	12.3 ±0.28	35.0 ±0.64	58.6 ±0.48	4.3 ±0.24	2.0 ±0.04	0 0	22.6 ±0.96	9.2 ±0.36	81.1 ±4.28	0.90 ±0.02

0 = death of the animal.

Table 18

Showing mean survival period after appearance of symptoms in malathion poisoned goats.

Group	Post poisoning mean ± S.E.	
1st	26.0 ± 0.65	
2nd	58.0 ± 3.83	Time recorded in minutes.
3rd	122.0 ± 7.64	
4th	306.0 ±16.34	
	128.0 ± 7.11	

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FIGURE I.

GRAPH SHOWING THE EFFECT OF MALATHION ON BLOOD GLUCOSE LEVEL AT DIFFERENT STAGES OF POISONING IN DIFFERENT GROUPS.

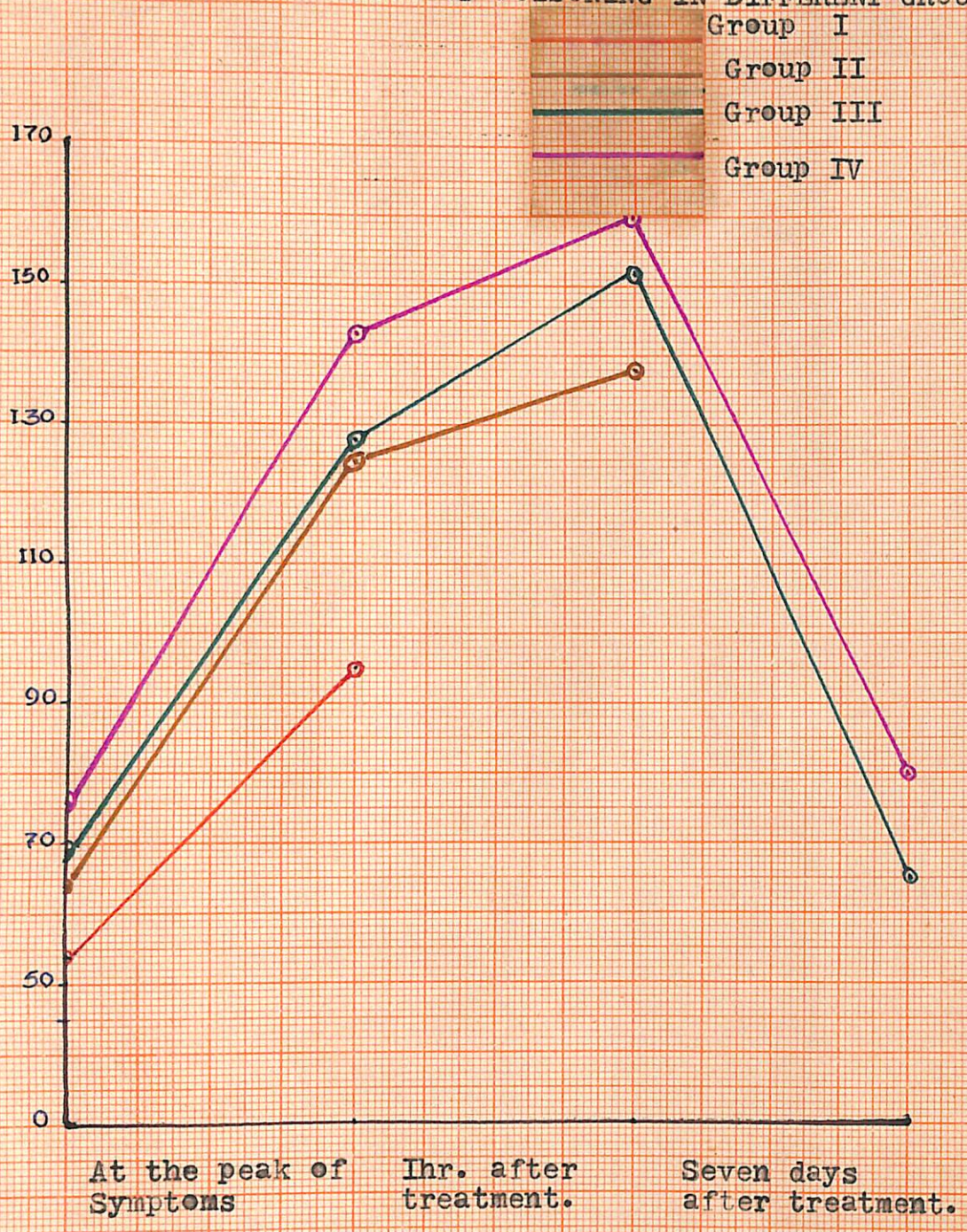

FIGURE II.

GRAPH SHOWING THE EFFECT OF MALATHION ON LYMPHOCYTIC COUNT AT DIFFERENT STAGE OF POISONING IN DIFFERENT GROUPS.

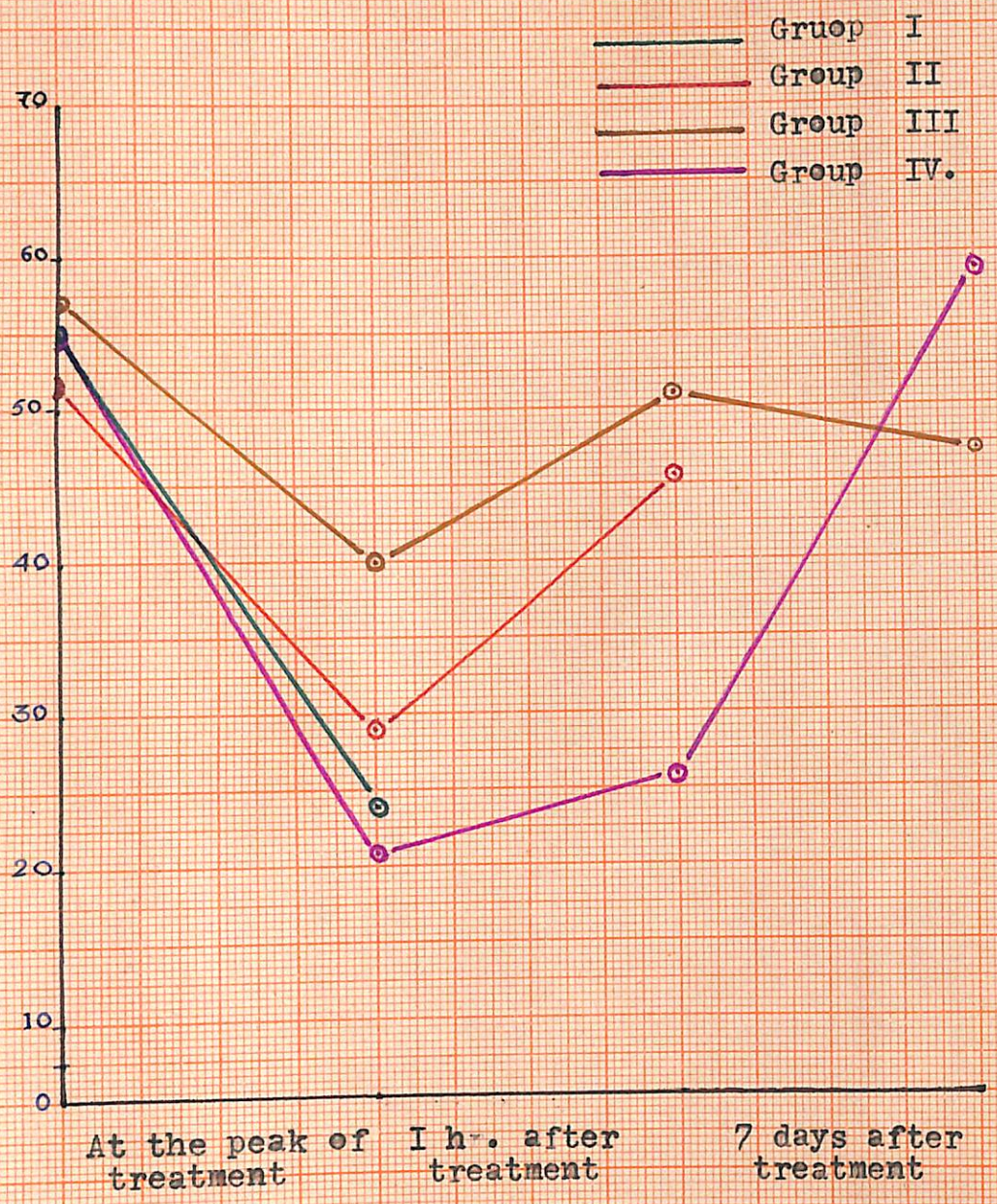

FIGURE III

GRAPH SHOWING THE EFFECT OF MALATHION ON THE PULSE RATE AT DIFFERENT STAGES OF POISONING IN DIFFERENT GROUPS.

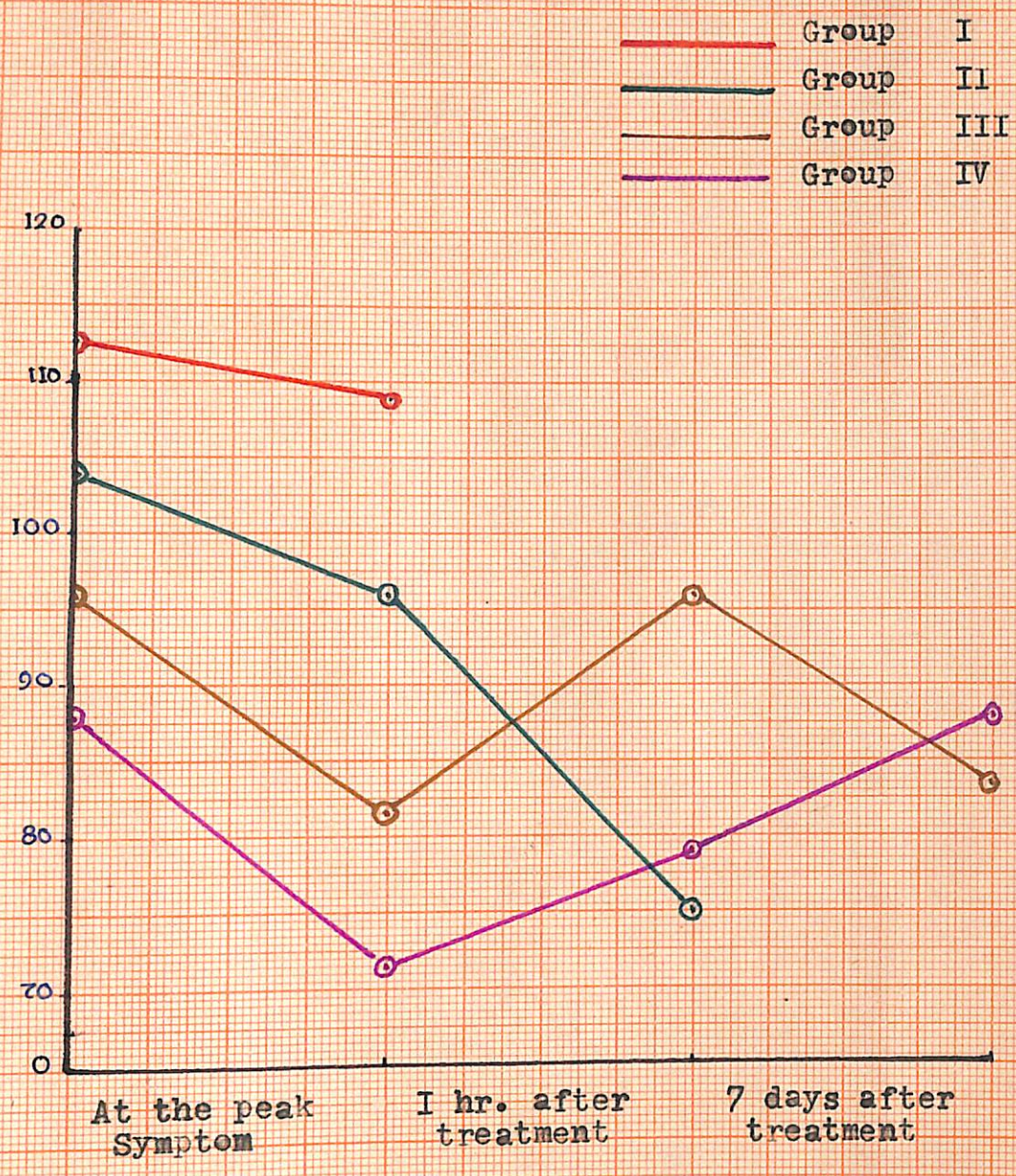

FIGURE IV

GRAPH SHOWING THE EFFECT OF MALATHION ON THE NEUTROPHILIC COUNT AT DIFFERENT STAGES OF POISONING IN DIFFERENT GROUPS.

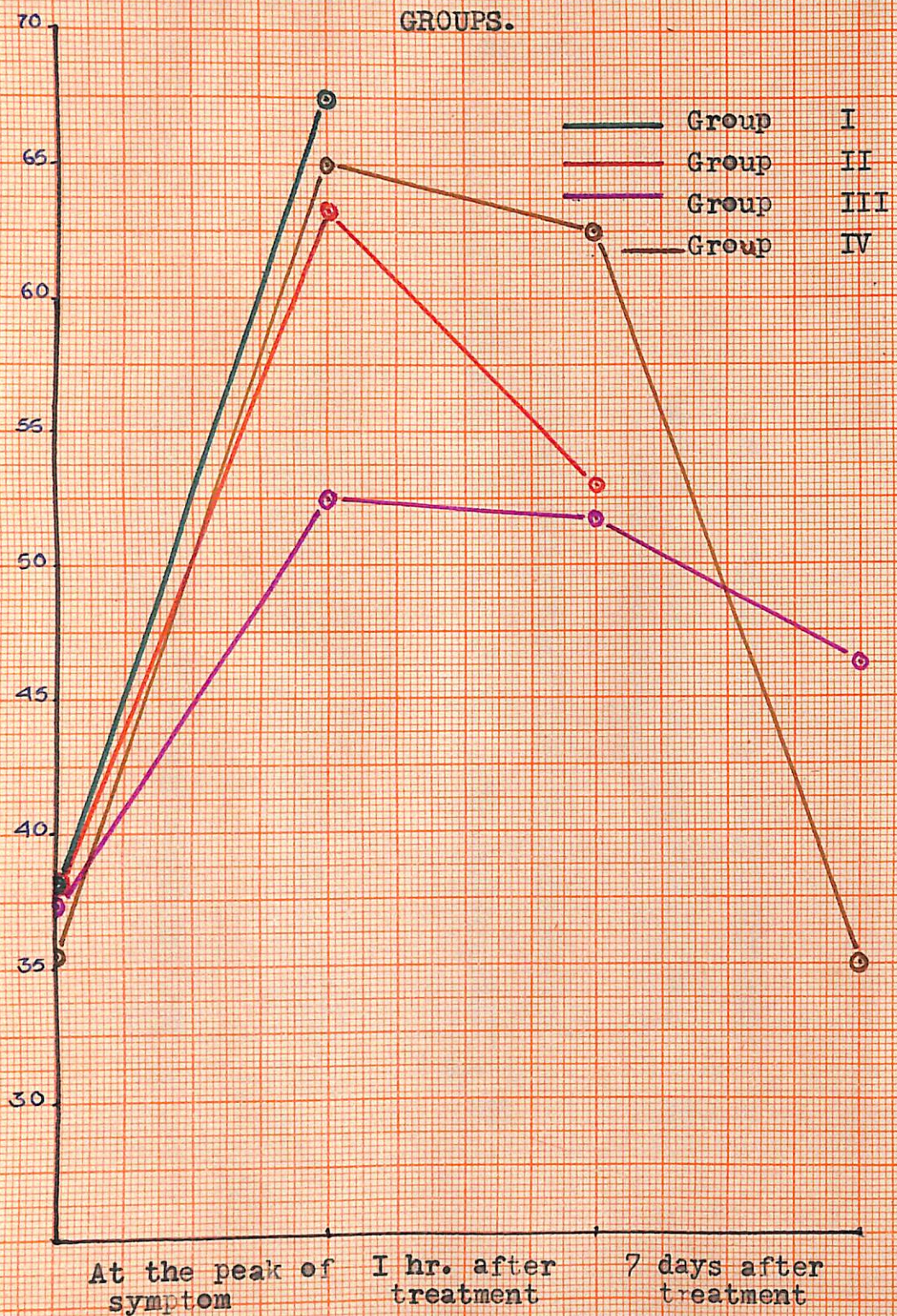

FIGURE V

HISTOGRAM SHOWING THE EFFECT OF MALATHION ON THE PLASMA ASCORBIC ACID AT DIFFERENT STAGES OF POISONING IN DIFFERENT GROUPS.

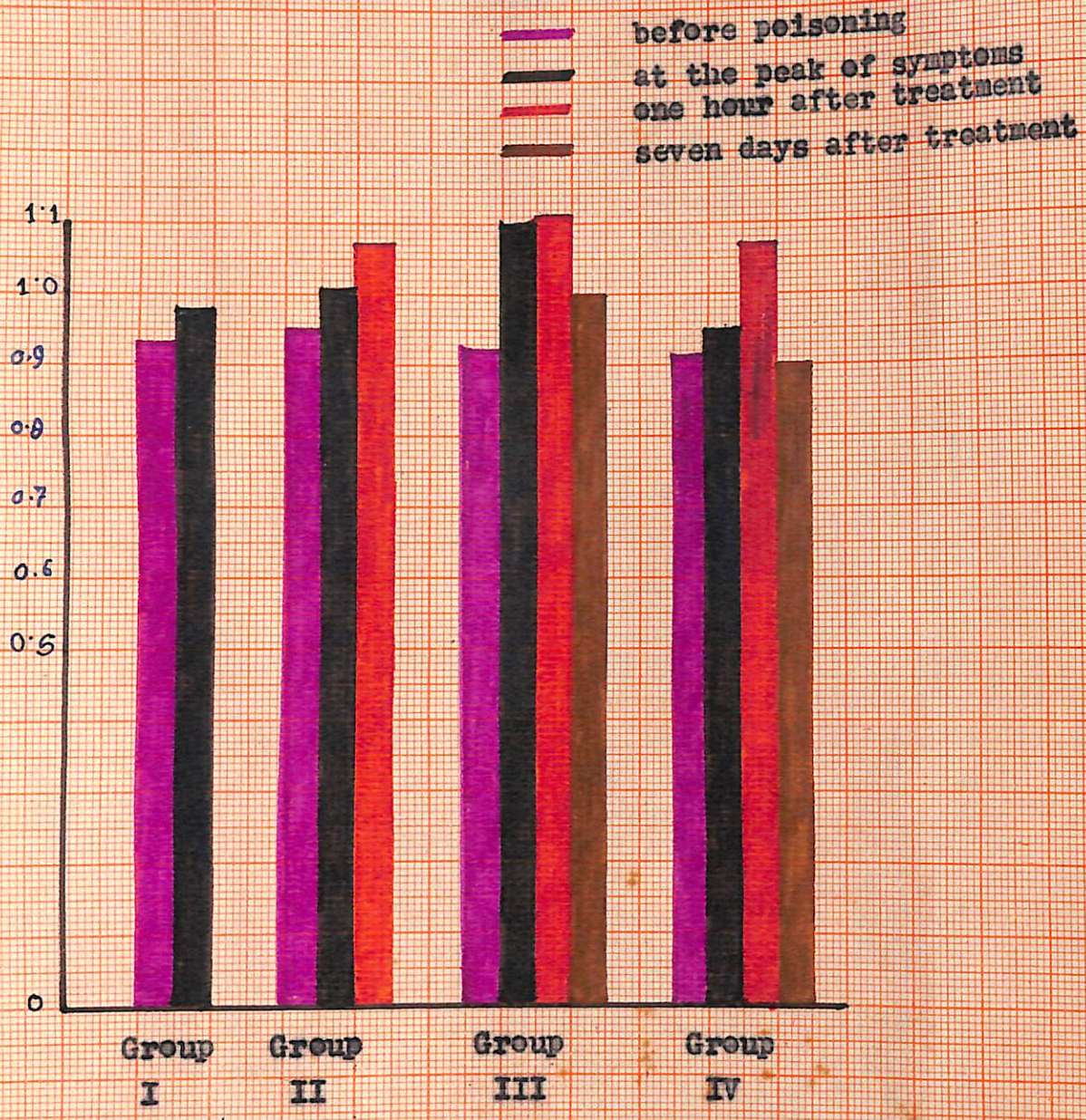

Fig. IV Section of myocardium showing infiltration of mononuclear Cells.

H & E x 400.

Fig. V Section of heart showing increased granularity and fragmentation of the myocardial fibres.

H & E x 400.

DISCUSSION

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DISCUSSION

An attempt was made to assess the toxic dose of malathion to produce acute toxicity in goats. By increasing the dose rate from 100 mg/kg body weight to 200 mg/kg body weight, it has been observed that upto 175 mg/kg body weight the animals poisoned did not reveal the signs of acute toxicity and as such dosage of 200 mg/kg body weight was found to be the optimum dose level to produce acute toxicity in the animals of all the groups. Dykstra (1955) observed the symptoms with 1 per cent suspension of malathion. Radeleff (1970) accordingly has recommended the minimum toxic dose approximately to be 100 mg/kg body weight for adult sheep and cattle, when applied topically as spray the minimum toxic concentration is 1 per cent for dairy calves under 2 weeks of age, more than 2 per cent for adult cattle and more than 1 per cent for sheep and goats. However, no specific toxic dose of malathion in goat has been mentioned.

Poisoning by organophosphates does not occur commonly but they are highly poisonous and heavy mortalities can occur in animals exposed to toxic amounts. Their toxic effects have been stated to be purely functional and probably no residual defects persist in recovered animals. The introduction of these compounds into animal therapeutics as treatment for nematodes, botfly, sheep nasal bot, warble fly and tick infestation has increased their importance of possible cause

of poisoning. Substances in organophosphate groups are included in the instrument of biological war fare as "nerve gasses".

In the present study, following poisoning at the dose rate of 200 mg/kg body weight in goats of all the groups the symptoms observed were frothy salivation accompained by bleating at short intervals. The other symptoms observed were dyspnoea, lacrimation, frequent defecation, urination, hypermotility of intestine and pupillary miosis. Muscle tremor, stilted movement, to and fro movemeant of head and neck followed by prostration and clonic convulsions and finally the lateral recumbency leading to death within 167.70 ± 10.01 minutes were the additional signs. All the five animals of group 1st (control) died following poisoning and showed the above predominating symptoms. It is generally agreed that asphyxiation is the ultimate cause of death fatally poisoned by an arganophosphate. Four different mechanisms appeared to be involved in fatal poisoning:

(1) Broncho secretion and Broncho constriction.

(2) Lowered blood pressure

(3) Neuromuscular block of the respiratory muscles, and

(4) Failure of the respiratory centers.

The respiratory failure might be due to result of extreme broncho secretion and broncho constriction which reduces the air way to the point where gasseous exhhange is inadequate. Paralysis of the muscles of respiration might

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also not mentioned about diarrhoea in cattle and sheep, but Blood and Henderson (1974), Hothi and Kwatra (1972), Vadalmudi and Paul (1974) and Golz (1957) have reported the presence of diarrhoea in addition to the above findings.

The signs of groaning shown by the poisoned animals are manifestations of severe pain in the abdomen or nervous derangement (Jackson et al., 1960, Smith and Jones, 1966).

Just after administration of malathion, the pulse and respiration rate and body temperature were recorded, and it was observed that there was an initial increase of pulse and respiration without any apparent change in body temperature. Whereas, the pulse and respiration rate and body temperature showed downward tendency one hour after treatment and thereafter. The initial increase of pulse and respiration rate may be only due to excitement being caused by handling of the goats while drenching malathion.

Mean time interval between administration of Malathion and appearance of symptoms:

The time period in the 4th group was maximum (41.00 minutes) and lowest in the 3rd group (38.00 minutes) whereas in one hour after treatment the same result was obtained, though there was variation between group as regard time period, no significant difference was recorded between group in both poisoning and one hour after treatment (Table 1).

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Pulse, Respiration and Temperature.

The maximum pulse rate was obtained in group 1st and lowest in group 2nd in the post-poisoned period, whereas in one hour after poisoning the maximum pulse rate was obtained in group 3rd and lowest being in group 2nd. Between groups the difference turned out to be highly significant in both post-poisoning and one hour after treatment (Table 2). In the post-poisoning period no significant difference was obtained in the pulse rate in group 3rd and 4th, whereas difference in other group combination revealed highly significant in one hour after poisoning, no significant difference was recorded between group 4th and 2nd, whereas pulse rate difference was obtained between group 2nd and 3rd and between group 3rd and 4th.

Highly significant respiration rate between groups was obtained in both post-poisoning and one hour after treatment. The maximum respiration rate was obtained in both the group 1st and 2nd, whereas group 4th recorded the lowest respiration rate (Table 3). In one hour after treatment the maximum mean respiration was exhibited by group 2nd and fell down sharply in group 4th which recorded the lowest value. Highly significant value were recorded in respiration difference between group 1st and 3rd, 1st and 4th and 3rd and 4th in post-poisoning, whereas in one hour after post-treatment all the groups from group 2nd to 4th revealed highly significant difference.

In post-poisoning period as well as in one hour after treatment temperature showed no significant difference (Table 4). In post-poisoning the maximum mean temperature was recorded in group 1st and lowest being in group 3rd, whereas in one hour after treatment the maximum temperature was recorded in group 4th and the lowest in group 2nd. Between the periods of poisoning no significant difference was found out (Table 4).

On perusal of literature it appears that no one has put much stress in the study of organophosphorus poisoning in animals in relation to pulse, respiration and temperature. However, in the present finding there has been decrease in pulse, respiration and body temperature, which seems to be attributed to hypotension, as affirmed by Goodman and Gilman (1971) who described that the blood vessels in general dilated, although the coronary and pulmonary circulation may show opposite response. They further postulated that the sum of the foregoing effects showed result in hypotension in organophosphorus poisoning. Thus, the present finding is in approximation with the finding of Goodman and Gilman (1971), but this also needs further investigation.

Haematology.

Total leucocytic count showed no significant difference between groups in stages of treatment in either post-poisoning

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or one hour after treatment periods. The mean maximum total leucocytic count (TLC) was observed in group 2nd and 4th in post-poisoning period whereas maximum value in one hour after treatment was observed in group 4th, lowest being recorded in group 3rd (Table 5).

In the present study the animals intoxicated with malathion showed a mild but progressive rise in total leucocytic count alongwith slight increase in PCV%, Hb%, eosinophilic and monocytic count. However, the lymphocytic count went down. From the available literature no relevant information could be had on the occurrence of leucocytosis due to insecticidal poisoning, however Wintrobe (1967) discussed leucocytosis in a variety of toxic conditions, he observed that leucocytosis might result from increase of lymphocytes. However, in present study the occurrence of neutrophillia was recorded. This rise of neutrophil might be the high level of organophosphorus administered. Hothi and Kwatra (1972) have reported the increase of lymphocytes resulting into leucocytosis. During the present study no relative increase of lymphocytes in circulation was detected. The repeated dosages of malathion might have resulted into lymphocytosis but due to the toxic dose of malathion in the present study, the lymphoid organ might not have got sufficient time for production of increased number of lymphocytes. The toxic drugs and chemicals, when administered in small repeated dosages stimulate the bone marrow to produce a large

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number of leucocytic cells (Florey, 1970). The increased neutrophil is attributed to the stimulation of the stress reaction which might have been caused due to malathion poisoning in the present study. Schalm (1967) has affirmed that neutrophilia is associated with variety of non-infection conditions which stimulates the stress reaction, but whether the insecticide can exert a similar effect on the lymphoid organs to produce more lymphocytes in the experimental animals needs further study.

In both post-poisoning and one hour after treatment between difference in eosinophilic count turned out to be highly significant. The maximum mean eosinophilic count was recorded in group 4th and lowest being in group 1st in post-poisoning period, whereas in one hour after treatment the maximum value in group 2nd and minimum in group 3rd. The group difference between group 1st and 4th, group 2nd and 4th and group 3rd and 4th turned out to be highly significant in post-poisoning whereas one hour after treatment highly significant difference was obtained between group 2nd and 3rd and 4th, excepting in group 2nd no significant difference were recorded between periods (Table 8).

Monocytic count did not reveal any significant difference between groups in both post-poisoning and one hour after treatment periods. The maximum monocytic count was recorded in group 2nd and minimum in group 1st in post-

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poisoning periods, whereas in one hour after treatment period the maximum was obtained in group 4th and minimun in group 3rd. Between periods no significant difference was obtained (Table 9).

PCV% was maximum in group 2nd in both post-poisoning and one hour after treatment periods, whereas lowest value was obtained in group 4th and group 3rd in post-poisoning and one hour after treatment periods respectively. No significant difference between group as well as between stage was recorded (Table 10).

Hb% in post-poisoning group revealed significant difference between groups. The lowest value being in group 3rd and highest in group 2nd, whereas in one hour after treatment no significant difference between groups could be obtained. The lowest value was recorded in group 3rd and highest in group 2nd. Between stages of poisoning no significant difference could be recorded (Table 11).

Dimitrieve (1975) observed disturbance of nervous system and haemopoietic organs by DDVP (Dichlorvos) poisoning in 30 hens. There were changes mostly in peripheral blood characterized by increase in leucocytes, erythrocytes and Hb% and appearance of large number of Pseudoeosinophils. In the present study also slight increase in leucocytes, Hb% and PCV has been recorded.

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The slight increase in PCV%, Hb%, eosinophilic and monocytic count and a decrease in lymphocytic count could not be substantiated due to lack of available literature, and as such it needs further study.

Biochemical changes.

As regard the blood sugar in the post-poisoning period there was sharp increase in its level from group 1st to 4th, group 1st showing minimum and group 4th maximum. Similar was the trend in one hour after treatment group 2nd showing lowest and group 4th highest values. Between periods no significant difference could be recorded (Table 12).

From perusal of the table 12 it seems that malathion poisoning in case of goat at the peak of poisoning symptoms caused highly significant increase of blood glucose level. Similar results have been observed by O'Brien (1957) who studied effect of malathion on carbohydrate metabolism in cases of mouse, cockroach and housefly and reported that malathion inhibits pyruvate oxidation perhaps by interference with citrate oxidising system. He further observed that glycolytic and tricarboxylic acid cycle in the mouse and cockroach were not substantially inhibited by this organophosphorus compounds in vitro. He concluded that this organophosphorus compound have similar inhibiting effect for insect than for mouse enzymes and so he considered this compound more

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toxic to insect than mammals.

However, Gupta (1974) studied the blood glucose and glycogen in rats after malathion poisoning and observed the increase in blood glucose level. He further reported that the glycogen content of liver, kidney, heart and spleen was higher upto 6 to 24 hours. The brain glycogen content did not show any change.

Madejski and Juszkiewiez (1966) studied the effect of thiometon poisoning in case of chicks and observed an increase in liver glycogen content.

There was also no significant difference between groups in both post-poisoning and one hour after treatment periods. Besides stages of poisoning also did not seem to affect the plasma ascorbic acid content significantly. The lowest value in plasma ascorbic acid was obtained in group 1st and the highest in group 3rd in post-poisoning periods whereas in one hour after treatment period group 3rd showed maximum the lowest being recorded in group 2nd and 4th (Table 13). The present finding is in agreement with that of Madejski and Juszkiewcz (1966) who had observed rise in blood ascorbic acid in chicks after Ekatin (Thiometon) poisoning.

Time interval between administration and death.

The mean time interval between administration and death of animals was minimum in group 1st and maximum was recorded in group 4th in post-poisoning period. In one hour after

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treatment the minimum was in group 3rd and maximum in group 4th

Pathological changes.

Macroscopic changes:

The gross changes at necropsy of the poisoned goats at the dose rate of 200 mg/kg body weight included congestion accompanied by haemorrhages of varying sizes on meninges of brain, heart, musculature, cortical region of kidneys, dorsal and ventral surface of liver, intestinal mucosae and in the lungs. The predominance of petechial were invariably seen on liver and kidneys. The present findings are in agreement with the findings of Fontanelli (1955), Radeleff et al. (1957), Yasnova (1969) and Hothi and Kwatra (1972) who could also see multiple haemorrhages in visceral organs. However, petechial haemorrhage in brain had not been recorded by Fontanelli (1955), Radeleff et al. (1957) and Yasnova (1969) which could be explain on the basis of high dose of malathion in the present study. This can further be substantiated on the plea that haemorrhages occur in association with convulsion and dyspnoeic anoxia (Smith and Jones, 1966), which were noticed in the present study on pathological examination of the poisoned goats. Similar findings have also been recorded by Yasnova (1969) and Dimitrieve(1975).

Microscopic changes:

Vacuolation of hepatocytes extended beyoned the midzonal

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region of the lobule. Liver cells around the periphery were disernible. Vacuolar degeneration and cloudy swelling and dilatation of portal vein was observed. Fatty changes and necrosis in the liver cells around the central vein were also observed in the present study. Hothi and Kwatra (1972) observed cloudy swelling and fatty changes in the cells of centrilobular and midzonal areas of hepatic lobules. Changes continued to progress as evidenced by the degenerative changes that involved in a greater area of the lobule. On the other hand Fontanelli (1955) and Gelati (1966) opined that degenerative changes in the liver were somewhat comparable with those noticed in the parathion poisoning in cattle. On the basis of microscopic examination of the liver it can be safely concluded that malathion is a potent hepatotoxin.

Haemorrhages in the inter fibrillar space of myocardium, increased granularity, complete lysis of myocardial fibres at some locations and infiltration of mononuclear cells were also observed. When the poison entered the systemic circulation then it was bound to cast its effect on the mycardium. The amount of damage would depend upon the dose of the poison and susceptibility of the myocardial tissues. The congestion of blood vessels and increased granularity of the muscle fibres indicated mild damage and focal lysis of muscle fibres pointed towards enhanced effect of the poison on the myocardium. However, the present findings did not indicate that malathion caused sever damage to the myocardium.

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Blood vessels of the intertubular region and glomeruli were heavily packed with erythrocytes, haemorrhages in intertubular and tubular space and also in Bowman's space. Swelling of endothelial cells of glomerular tuft and disquamation or varying degrees of degenerative changes in epithelial cells of tubules were observed. Thus, the present finding can be stated to be similar with Fontanelli (1966), Hothi and Kwatra (1972) and Dimitrieve(1975) who could also report that varying degrees of degenerative changes, uniformly distributed haemorrhages and the deposition of haemosidrinpigment. Thus, malathion also possessnephrotoxic effect ~~in to~~ being a potent hepatotoxic substance.

Focal oedema and emphysema were noticed in the lungs which is in agreement with the findings of Radeleff (1957), Yasnova (1969) and Hothi and Kwatra (1972). Oedema, emphysema and congestion of lung parenchyma was likely to cause respiratory trouble and finally might bring about the death of the animal by anoxia.

In the present study it was observed that tips of the intestinal villi were devoid of lining epithelial cells and a moderate number of mononuclear cell infiltration in the lamina propria. Yasnova (1969) also observed necrosis of intestinal mucous membrane. Fontanelli (1966) observed congestion of mucous membrane.

Mild congestion of blood vessels in the cerebrum and

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perineuronal oedema were observed in the present study. Satellitosis and neuronophagia were not observed. Hothi and Kwatra (1972) also reported satellitosis and neuronophagia at places in addition to perineuronal oedema. The present findings correlates well with that of Guarda (1959) who also observed lesion in the blood vessels of brain of fowls in acute and chronic poisoning with parathion. Examination of white matter of brain by routine staining techniques is not likely to reveal mild tissue alterations and in absence of special staining it was not possible to tell with certainty whether retrogressive changes like mild wallerian degeneration was caused by malathion or not. Endocrine glands were also not included in the present study and it is suggested that future study on this topic might be directed towards the detailed study of endocrine glands.

Therapeutics.

The organophosphorus compounds are highly toxic in nature and their accidental feeding many a times induces health hazard problems particularly for grazing ruminants. The various use of the compound such as agricultural pest control, treatment of nematodes, botfly, sheep nasal bot, warble fly and tick infestation have necessitated the treatment of such animals.

To combat the economic losses caused by organic-phosphate poisoning in animals in termsof heavy mortality

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and public health hazards a number of therapeutic and antidotal agents have been tried by various worker - (Hutter and Kostial (1954), Bergner (1959), Davies et al. (1959), Karlog (1960), Svetlicic and Vondekar (1960), Richter (1961), Jung et al. (1963), Olekarlog (1964), Palmer (1964), Palmer et al. (1964), Palmer (1965), Eidmann(1968), Schmidt and Grutzmachur (1968), McCurnin (1969), Polaz and Lapushkov (1969), Norkowski and Kozsawski (1971), Srivastava and Parasar (1971), Savateev et al. (1973), Benes et al. (1976), and Egyed et al. (1976).

In the present study four drugs in different combinations such as -

(1) Atropine sulphate and DAM (Diacetylmonoxime)

(2) As in (1) alongwith coramine (Nikethamide).

(3) As in (2) alongwith magnesium sulphate (20%)solution.

have been tried to treat the experimentally poisoned goats and also to evaluate the comparative efficacy of combinations

A combination of atropine sulphate at the dose rate of 0.5 mg/kg body weight initially followed by 0.25 mg/kg body weight half by intravenous and half by intramuscular route and DAM (Diacetylmonoxime) 30 mg/kg body weight by slow injection intravenously has been put to trial in animals of group 2nd. Atropine sulphate, a general pharmacological antidote counter acts the nicotinic,

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muscurinic and central nervous system action and DAM a cholinesterase reactivator compound reactivates the inhibited cholinesterase enzyme (Goodman and Gilman, 1970 and Jones, 1966) It was observed that none of the goats could survive even after completion of the treatment and all the goats died 98.0 ± 7.36 minutes after administration of malathion.

With the perusal of literature it appeared that the main cause of toxicity was the excessive accumulation of acetylcholine at the neuromuscular junction and thereby blocking the neuromuscular transmission due to the inhibitory action of organophosphorus compounds on cholinesterase enzyme, Garner (1957), Goodman and Gilman (1970) and Radeleff (1971). The present finding is in agreement with Palmer (1964) who also treated the yearling cattle poisoned with coumaphos with one of three oximes (DAM, PAM and TMB-4) alongwith atropine sulphate and found that the poisonous effect reached after 72 hours and duration of poisoning was 4 to more than 10 days and survival rates were (1) 1/4 (2) 5/6 (3) 4/4; control 3/6. Further Palmer (1965) studied the therapeutic effect of 3 oximes (DAM, PAM and TMB-4) at the dose rate of 10 or 20 mg/kg body weight in 4 yearling cattle poisoned with dioxathion. The mortality rates were reported to be controls 2/4; 2-PAM 2/4; DAM 4/4 and TMB-4 0/4 respectively. He concluded that there was some return of the activity in 2-PAM group but a higher dose of 2-PAM or DAM might have had more efficacy; the dose of 20 mg/kg TMB-4 appeared preferable

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However, the poisoned goats were found to be dead 98.0±7.36 minutes after administration of malathion following treatment in the present study but such period has not been recorded by Palmer (1964, 1965) where as the animals of untreated control group died only 66.o ± 4.18 minutes after administration but the mortality rate is almost similar with that of DAM and atropine sulphate as used by Palmer (1965).

The goats of group 3rd were treated with a combination of atropine sulphate and DAM (dose and route of administration as in group 2nd) alongwith coramine at the dose rate of 120 mg/kg body weight half intravenously and half intramuscularly. It was noted that two goats out of five could survive.

During the study it was interesting to note that when coramine was given in single dose (120 mg/kg) the symptoms of convulsions were stated and at the same time when coramine was given in divided doses on three or four occasions the convulsive symptoms were found to be absent. The present finding is in agreement with Jone (1969) who also stated that an excessive dose of nikethamide (coramine) stimulated the central cortex and the spinal cord, resulting in convulsion. Thus, it is apparent that the fractional doses of coramine might prove beneficial effect as compared to massive dose in treating organophosphorus poisoning cases. Jung et al. (1963) while studying the experimental toxicity of trichlorophon in mice, dog, pig, cat and horses reported

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that an intraperitoneal injection of atropine 10 mg/kg, atropine and PAM 10 ml and 15 ml/kg, atropine and nikethamide 120 or atropine and leptaxol 30 mg/kg increased the LD50 to 618, 1300, 1,312 and 1,217 mg/kg respectively.

The animals of group 4th when treated with a combination of magnesium sulphate (20%) solution at total dose of 10 ml subcutaneously, DAM, coramine and atropine sulphate (dose and route of administration as in group 2nd and 3rd), three goats out of five could survive. In this group magnesium sulphate helped in releasing the excessively accumulated acetylcholin from the neuromuscular junction and might have restored the neuromuscular transmission. The present finding is in similarity with Hutter and Kostial (1954) who observed the effect of calcium and magnesium ions on the release of acetycholin used in profused superior cervical ganglion from the preganglionic nerve endings and found that magnesium ions in concentration causing block of ganglionic transmission reduce the out put of acetylcholin whereas calcium ions releaved the block produced by magnesium ion and restored the out put of acetylcholine .

Urine analysis.

The sugar and albumen on qualitative test were found to be negative in urine samples collected from poisoned goats. The present finding however could not be substantiated

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for want of literature and needs further study.

Time interval.

The time interval between administration and appearance of clinical symptoms, administration and death of animal and survival time after appearance of clinical symptoms were found to be 39.75 ± 2.89, 167.70 ± 10.01 and 128.00 ± 7.11 minutes respectively. No one has so far especifically recorded the time interval and thus, it is left for further investigation.

From the above finding it can be concluded that the combination of atropine sulphate, DAM, coramine and magnesium sulphate (20%) solution has given the best effect , atropine sulphate, DAM and coramine were found to be intermediary in action whereas atropine and DAM has not been found effective.

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SUMMARY AND CONCLUSION

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SUMMARY AND CONCLUSION

Malathion at the dose rate of 200 mg/kg body weight was given to produce acute toxicity.

The predominating symptoms in all the poisoned goats were frothy salivation and bleating at short intervals.

As the disease caurse advanced the animals started grunting, salivation became profuse followed by frequent defecation and urination.

Muscular tremor in the head and neck region became apparent first, then spreading all over the body especially the limb and hind quarters.

To and fro movement of head and streching of neck were common accompaniments.

Prostration and convulsion finally resulted into lateral recumbency, often complicated by tympany.

A decrease in pulse, respiration rate and temperature was recorded after poisoning.

No marked changes in haematology was recorded although a moderate change in T.L.C., D.L.C. and PCV% was recorded.

A significant rise in blood glucose level was recorded whereas plasma ascorbic acid was slightly elevated.

The common gross lesions in vital organs recorded were haemorrhage and congestion. The liver texture was friable.

There were pneumonic patches and emphysema in lungs and lumen of intestine contained large amount of mucous.

The histopathological examination of the cut section of liver showed hepatocytes around the central veins and continued to contain vacuoles, and the liver cells around the periphery of the tubule were discernible. Fatty changes and necrosis were also near the central vein, blood vessels and sinusoids were packed with erythrocytes, infiltration of few mononuclear cells in the portal tract. Haemorrhage was seen in the interfibrillar space of myocardium. The fibres were showing increased granularity and complete lysis at some places and mild infiltration of mononuclear cells. The blood vessels of the intertubular region and glomeruli were heavily packed with erythrocytes. Endothelial cells of glomerular tuft were swollen and occupied almost complete space in Bowman's capsule. Focal oedema and emphysema were very much pronounced in the lungs.The tips of intestinal villi were devoid of lining epithelium. Perineuronal oedema was there in the cerebrum.

A combination of atropine sulphate, DAM, coramine and 20% magnesium sulphate solution gave the best result in malathion poisoning, atropine sulphate, DAM and coramine were found to be intermediary in action whereas atropine sulphate and DAM were found ineffective.

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