STUDIES ON THE PATHOLOGY OF EXPERIMENTAL SELENIUM TOXICITY IN GUINEA-PIGS

By

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CERTIFICATE I

This is to certify that this dissertation entitled: "Studies on the pathology of experimental selenium toxicity in guinea-pigs" submitted for the degree of Ph.D., in the subject of Veterinary Pathology, of the Haryana Agricultural University, is a bonafide research work carried out by Priya Mohan Das under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE II

This is to certify that this dissertation entitled "Studies on the pathology of experimental selenium toxicity in guinea-pigs" submitted by Priya Mohan Das to the Haryana Agricultural University in partial fulfilment of the requirements for the degree of Ph.D., in the subject of Veterinary Pathology, has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an External Examiner.

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IN

FOND MEMORY OF

MY FATHER

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(Priya Mohan Das)

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INTRODUCTION

The status of selenium in animal nutrition is paradoxical, because it has been well recognized as an essential micro-nutrient as well as a natural toxicant. At normal metabolic levels, the selenium possesses an antioxidant effect through glutathione peroxidase, a seleno-enzyme which protects the cells from degenerative and necrotic changes by destroying the oxidising agents such as hydrogen peroxide and lipid peroxides. In addition, the selenium has an effect on cyto-chrome P-450 and haem metabolism. A deficiency of selenium in the feed leads to the development of several disease conditions such as nutritional muscular dystrophy (white muscle disease), encephalomalacia, hepatosis dietetica, mulberry heart disease, exudative diathesis, liver necrosis, pancreatic degeneration, growth impairment, periodontal disease etc. in various species of animals including man. It has been observed that addition of selenium or selenium-vitamin E combinations to animal feed in selenium-deficient areas of the United States can prevent annual losses valued 545 million dollars from beef cattle and sheep, and 82 million dollars from poultry and swine (Shamberger, 1983).

Though selenium is considered as an essential mineral element in the diet since 1957 but interest in the biological significance of selenium was initially confined to its toxic

effects. The occurrence of selenium toxicity was first recorded in Western China as early as 1295 as reported by Rosenfeld and Beath (1964) from the writings of Marco Polo. These authors also referred to the report written in 1857 by T.C.Madison, an army surgeon, in which he described the sloughing of hooves, mane and tail among horses possibly due to selenium toxicity. This condition has often been referred in the literature as "alkali disease" or "blind staggers" and it was not until 1934 that selenium toxicity was identified as the cause of this disease.

Naturally occurring selenium toxicity has been reported in different species of animals from India and abroad. It is reported to cause serious economic losses as a result of mortality, decrease productivity, a failure of conception, abortion, teratogenesis and loss of functional activity of the livestock. The most common source of selenium toxicity is plants from seleniferous soils which may accumulate very high concentrations of selenium depending on several factors like plant species, stage of maturity, pasture management, climate and soil pH. The atmospheric and water pollution as a result of wastes from several industries such as electronics, glass, ceramics, paints, varnishes etc. are other possible sources of toxicity. Studies on the toxicological aspects of selenium got new dynamics in the recent days because of its wide application in the prevention of carcinogenesis in human and nutritional muscular dystrophy in animals (Ip,1985; Blood et al.,1983). It has also become important to recognize

the fundamental difference between the use of chemically defined selenium compounds and the undefined sources of organic selenium compounds normally found in the diets.

A perusal of the available literature indicates that there are number of reports describing the clinical signs, gross and histopathological changes in cases of selenium toxicosis, but the information regarding sequential pathology and biochemical alterations is rather sparse. According to Clark and Combs (1986), further animal studies are needed to identify the biochemical parameters associated with growth retardation, one of the earliest manifestations in animals fed on high selenium diets.

Taking into consideration the importance of problem and the limited informations available, the present studies were planned to investigate certain aspects of experimental selenium toxicity in guinea-pigs with the following objectives:

- To elucidate the clinical manifestations and pathology of experimental organic and inorganic forms of selenium toxicity in guinea-pigs.
- To examine some of the biochemical and haematological parameters with respect to above toxicities.
- To find out the protective effect of sodium arsenite, if any, in experimental inorganic selenium toxicity.

It is envisaged that the informations obtained from these studies may be of help in understanding the disease process in selenium toxicity.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Marco Polo was probably the first to encounter problems of selenium toxicity in "beast of burden" from Western China as early as 1295, as reported by Rosenfeld and Beath (1964). The same authors also referred to a report written in 1857 by T.C.Madison in which horses of South Dakota developed sloughing of hooves, mane and tail, possibly because of selenium toxicity. It was not until 1934 that selenium toxicity was identified as the cause of such a disease condition (Franke,1934).

The discovery of selenium as an essential element was based on the observations of Schwarz (1951) who noticed that rats fed on torula yeast diet developed a fatal liver necrosis which could be prevented by brewer's yeast, despite the absence of sufficient sulphur containing amino acid, cystine and vitamin E to account for its protective action. He designated this third anti-liver necrosis factor as "Factor-3". Later, Schwarz and Foltz (1957) recognised the selenium as an integral part of "Factor-3" which could prevent liver necrosis.

It is now well known that selenium is a component of the enzyme glutathione peroxidase (GSH-PX) which plays role in the protection of cells by destroying oxidising agents such as hydrogen peroxide and lipid peroxides (Rotruck et al., 1973). These oxidising agents, in the absence of GSH-PX, cause irreversible denaturation of essential cellular proteins which

lead to degeneration and necrosis. Besides, selenium also regulates the enzymes delta-amino levulinate synthase and haem oxygenase (Maines and Kappas,1976) which increase the haem synthetic activity of hepatocytes.

Burk and Masters (1975) studied the effects of selenium deficiency on hepatic microsomal cytochrome P-450 system in rats. They observed that selenium increased the activities of the hepatic microsomal cytochrome P-450 system. A number of studies have demonstrated the inhibitory effect of selenium on tumour formation (Shamberger and Rudolf,1966; Shamberger, 1969; Riley,1969). Recently, animal studies have shown protective effects of selenium supplementation during the initial and the promotion phases of carcinogenesis (Ip,1985).

Selenium is present atleast in trace amounts in all soils and in all natural feeds. Some soils contain an excess of selenium in forms which are available to plants and make the plants toxic to animals. Selenosis from seleniferous plants occurs in areas where the selenium content of the soil generally ranges from 1 to 6 ppm (Clarke et al.,1981). Some plants, known as the indicator plants, require selenium for their growth and may contain 1000 to 15000 ppm of the selenium. Selenosis in animals results from consumption of plants which contain more than 5 ppm of the selenium (Clarke et al.,1981).

The intake of seleniferous plants may lead to the development of either acute or chronic signs of toxicity in livestock depending upon the selenium content of the plant, period of consumption, species, sex, age of the animals, caloric intake etc. The clinical manifestations and lesions

observed in chronic selenosis also vary with the chemical characteristics of selenium. Rosenfeld and Beath (1964) have categorised chronic toxicity into three groups depending upon the clinical manifestations: (a) Alkali disease, characterized by loss of hair and deformation and sloughing of the hooves. The selenium compounds in the feed which led to the development of this type of syndrome were bound to the protein, such as, seleno-cystine, seleno-methionine etc. (b) Blind staggers, characterized by the signs of central nervous system involvement, develops following consumption of plants containing non-protein type seleno-aminoacids, such as, seleno-cystathionine, selenium-methylselenocysteine etc., and (c) Chronic selenosis produced experimentally by administration of selenate or selenite compounds to livestock.

Toxic Levels

Ammerman et al. (1978) reported that the requirement of selenium in diet was 0.1 ppm irrespective of monogastric or polygastric animal. The toxic levels of selenium for domestic animals were 5 ppm for dairy cattle, 3 to 20 ppm for sheep, 5 to 10 ppm for swine and 10 ppm for chicken of 0 to 8 weeks of age. Clarke et al. (1981) found that the levels in excess of 5 ppm of selenium were potentially toxic for grazing animals. Pathak and Datta (1983) observed that the daily oral administration of sodium selenite at the rate of 3 mg/kg body weight to goats did not produce toxicity but a concentration of 6 mg or more/kg body weight was toxic.

Among the common laboratory animals, the rat was most resistant to selenium toxicity and the cat least (Smith et al., 1937). A level of 2 or 3 ppm of sodium selenite or sodium selenate produced a slight decrease in weight in rats but a considerable mortality occurred when they were exposed to water containing 6 to 9 ppm selenium (Palmer and Olson, 1974). Hadjimarkos (1970) reported that hamsters were resistant to selenium toxicity as compared to other animals. Ray and Ray (1982) induced selenium toxicity in guinea-pigs by intra-peritoneal injections of sodium selenite at the rate of 0.5 mg/kg body weight.

Natural Selenium Toxicity

Gabbedy and Dickson (1969) reported an outbreak of selenium toxicity from Australia wherein 180 (out of 190) six-weeks old lambs died. Hosseinion et al. (1972) recorded natural selenium toxicity in a mixed flock of 1500 sheep and goats from Iran. The animals were grazed on a low rainfall pasture where seleniferous weeds were widely distributed and in less than 15 days period, 52 ewes, five goats and one ram died due to selenium toxicity.

Among the 1400 sheep which grazed on a field in southern Utah, 200 died suddenly and 25 more succumbed within 30 days after the flock had been moved to another area (James et al., 1982). The mortality was attributed to selenium toxicity and the plants in that field contained 21.44 to 80.00 ppm of selenium. Gupta et al. (1982) described natural cases of toxicity in buffaloes, cattle and goats from Hoshiarpur

district of Punjab. Hoof and horn deformities were noted when the animals were reared at a particular area for more than 6 months. The selenium content of fodder samples ranged from 3.27 to 5.39 (average 4.33) ppm in wheat straw and 4.74 to 5.41 (average 5.07) ppm in berseem. The level of selenium in hair varied from 7.50 to 16.32 ppm. Harrison et al. (1983) observed a paralytic disease of swine at 3 farms of Georgia and diagnosed it as selenium toxicity. They estimated the selenium content of feed which ranged from 19.0 to 24.0 ppm. A report of lameness in exotic Jersey cattle at the Model Exotic Farm, Bhiwani (India) was investigated and attributed to selenium toxicity (Anon.,1984). Casteel et al. (1985) reported selenium toxicosis in a group of feeder pigs. Lumbar poliomyelomalacia and coronary band necrosis were the principal lesions. The selenium concentrations in liver and kidney were 1.5 to 4.5 and 8.0 µg/g, respectively whereas in feed samples, it ranged from 9.7 to 27.0 µg/g.

Experimental Studies

A perusal of the available literature shows that experimental studies on various aspects of selenosis have been carried out in laboratory animals like rats (Smith et al., 1937; Lewis et al.,1940; Fitzhugh et al.,1944; Rosenfeld and Beath,1954; Halverson et al., 1970; Schroeder and Mitchener, 1971; Palmer and Olson,1974; Pierce and Tappel,1977; Pierce and Tappel,1978; Hatch et al.,1979; Behne and Wolters,1983; Levander et al.,1983), mice (Hurlbut and Martin,1972), rabbits (Berschneider et al.,1977a,b) and hamsters (Julius et al.,

1983; Birt et al.,1983; Birt et al.,1986). Recently, some experimental studies have also been carried out in domestic animals like sheep (Madan Mohan et al.,1976), buffaloes (Bakshi et al.,1984; Prasad and Arora,1984a,b; Jakhar,1984) and goats (Pathak and Datta,1984). The available literature has been reviewed under following subheads.

Clinical Signs

The selenium toxicity in laboratory animals has been divided into acute, subacute and chronic types depending on the dose and duration of selenium administration (Rosenfeld and Beath,1964).

Czapek and Weil (1893) described acute selenium poisoning in rabbits, cats and dogs due to sodium selenite or selenious acid. The clinical signs appeared within 5 minutes following subcutaneous injection or 15 minutes after oral administration of selenite. Garlicky odour of breath, nervousness and fear, loud crying etc. were the primary signs. Excitement was more pronounced in rabbits than dogs but vomition and diarrhoea were more in dogs. These primary signs were followed by quiteness, somnolence, difficult respiration and decreased reflexes. Laboured breathing was followed by opisthotonos, tetanic spasms of the extremities, clonic spasm and death. Smith et al.(1937) observed similar symptoms in rats. McConnell and Portman(1952) noticed garlicky odour of breath and convulsions in mice but not in rats; the death occurred within a few to 36 hrs following subcutaneous injection of MLD_{50} of dimethyl selenite.

Ray and Ray (1982) injected sodium selenite at the rate of 0.5 mg/kg body weight (single dose) intraperitoneally in male guinea-pigs. The animals became dull and depressed and the food consumption and body weight, in comparison to paired control animals, were decreased within 48 hrs and became normal in 6 day of the experiment.

Marked loss in body weight, anorexia followed by cachexia, ascites and oedema were observed in rats in subacute and chronic selenosis (Smith et al.,1937; Smith,1941; Rosenfeld and Beath,1947). The emaciated animals assumed a hunched posture and the fur was coarse and ruffled. Anaemia was noticed in chronic selenosis in rats (Franke and Potter,1934; Fitzhugh et al.,1944; Mahalanobis and Ray,1954). Franke (1934) observed paralysis of hind legs and dark yellow stain around the genitalia in few rats. According to Rosenfeld and Beath (1954), 1.5 and 2.5 ppm of selenium as potassium selenite in drinking water for two generations had no effect on reproduction in rats. However, 7.5 ppm selenium prevented reproduction in the females but fertility of the males was not affected. Palmer and Olson (1974) reported that 2 or 3 ppm of sodium selenite or sodium selenate in drinking water caused decrease in weight of rats in four to six weeks whereas 6 or 9 ppm of selenium resulted in considerable mortality.

Berschneider et al.(1977b) observed that rabbits fed 9.83 or 19.5 mg selenium/kg feed developed signs of selenosis after 2 weeks. These were characterized by inappetance, apathy, increased pulse and respiration rates, ataxia, paresis in some cases, loss of hair, at the late stages icterus and in some

cases haemoglobinuria.

Decreased feed consumption and depressed growth rate were also observed in Syrian Hamsters which were given diets containing 5 ppm or more of sodium selenite (Julius et al., 1983; Birt et al.,1986).

Pathak and Datta (1983) recorded the clinical signs in goats after oral administration of different doses of sodium selenite. Loss of appetite and body weight, depression, constipation followed by diarrhoea mixed with blood or mucus, polydipsia, polyuria, foamy salivation and recumbency were the signs. In some of the long standing cases, impairment of vision and alopecia were also observed.

Jakhar (1984) studied the clinico-pathological aspects of experimental selenium toxicity in buffalo calves by feeding selenium-enriched (4.2 ppm selenium) rice straw in one group and by oral administration of sodium selenite @ 1.5 mg/kg body weight for first 20 days followed by 2.0 mg/kg body weight for the remaining 22 days in another group of animals. He noticed anorexia, gradual weight loss, weakness, emaciation, subnormal temperature, increased respiration and pulse rates, lacrimation and salivation, alopecia, depression, disinclination to move and stiffness of the joints in animals suffering from organic and inorganic form of selenium toxicity. The loss of body weight was comparatively less marked in inorganic selenosis than the organic one.

Haematological Studies

In mammals, progressive anaemia with continuous decrease of haemoglobin has been reported by several investigators (Franke and Potter,1934; Smith et al.,1937; Rhian and Moxon, 1943; Mahalanobis and Ray,1954). Anderson and Moxon (1942) carried out haematological investigations in dogs following subcutaneous injections of sodium selenite under barbital depression and recorded a marked increase in haemoglobin and packed cell volume values, as much as 62 per cent. Smith et al. (1937) and Moxon and Rhian (1943), on the basis of their observations, reported that anaemia was a common manifestation of selenosis in all species, but in rats and dogs it was of progressive severity and microcytic and hypochromic in nature. The animals which died had haemoglobin values as low as 2 g/ 100 ml. While studying anaemia of selenite toxicity in rats with ^{59}Fe, Halverson et al.(1970) observed that the anaemia was caused by haemolysis rather than by a defect in red blood cell synthesis. A reduction in haemoglobin, packed cell volume and total red blood cell counts due to selenium toxicity has been reported in Syrian Hamsters (Birt et al.,1983,1986).

Jakhar (1984) recorded significant decrease in haemoglobin and packed cell volume in both organic and inorganic selenium toxicity in buffalo calves from third week onwards. The values were comparatively lower in inorganic than the organic toxicity. No significant difference between groups was noticed in respect of MCHC. Regarding leucocytes, no significant difference was noticed in TLC, DLC and absolute lymphocyte counts in the

two groups at various intervals of feeding. Pathak (1984) found decreased haemoglobin, packed cell volume, total erythrocyte count, total leucocyte count and lymphocyte percentage in experimentally induced selenosis in goats.

Hogan (1986) injected sodium selenite intraperitoneally in different groups of male mice at the rate of 2.0 mg/kg/day for various periods and examined the peripheral blood leucocytes. He reported that sodium selenite was capable of causing a dramatic decline in the number of circulating leucocytes within 8 to 16 days of selenite treatment and the decline was due to decrease in neutrophilic granulocytes.

Biochemical Studies

Blood glucose There appears to be no agreement regarding the effect of selenium toxicity on carbohydrate metabolism. In chronic selenosis, the blood glucose and liver glycogen levels were found to increase or decrease depending upon the nutritional status of the animal (Jones, 1909; Levine and Flaherty, 1926; Potter et al., 1939; Wright, 1941). According to Potter et al. (1939), the variations in liver glycogen and blood glucose values in chronic selenosis might be secondary to the decreased food intake. Schroeder and Mitchener (1971) administered selenium @ 2 ppm of drinking water to the weaning rats for one year and then increased the selenium concentration to 3 ppm for the remaining experimental period i.e. 20 to 23 months. They observed higher values of serum glucose in selenite fed male rats than the controls.

Serum proteins Rosenfeld and Beath (1946) reported a decrease in blood proteins and an increase in non-protein nitrogen content in sheep. Discontinuation of selenium feeding resulted in slow but gradual increase in blood protein values and a decrease in non-protein nitrogen. A gradual decrease of protein level was also noticed in liver.

A significant decrease in total serum proteins in the organic and inorganic selenium toxicoses has been reported by Jakhar (1984) in buffalo-calves. The values decreased gradually during the entire course of the experiment (42 days).

Serum glutamic oxalacetic transaminase (SGOT) and Serum glutamic pyruvic transaminase (SGPT) Buck et al.(1961) reported elevation of SGOT values in sheep fed Astragalus pubentissimes. No appreciable elevation of SGPT values was noticed in any of the animals. Diehl and Mahan (1973) recorded an initial increase in SGOT activity upto 24 hrs followed by a decrease to almost normal in pigs injected with sodium selenite. Madan Mohan et al.(1976) observed that feeding of selenium in the form of selenium dioxide to Magra sheep produced lowering of SGOT and SGPT when selenium content in feed was 30 and 40 ppm but not at 10 and 20 ppm. Increased SGOT activity following ingestion of loco weed (Astragalus) by livestock was recorded by James and Kampen (1974). However, Goehring et al.(1984) did not observe any significant difference in SGOT and SGPT activities in swine fed on subtoxic to toxic levels of organic and inorganic selenium.

In goats, Pathak and Datta (1984) recorded marked increase in SGOT and slight increase in SGPT activity following oral administration of subtoxic to toxic doses of sodium selenite daily for 4 to 130 days.

Jakhar (1984) observed significantly lowered SGOT activity both in the organic and inorganic selenium toxicity in buffalo-calves as compared to controls. Although there was no regular pattern of SGOT activity during the course of 42 days but SGPT exhibited increased activity in the first week followed by a gradual decrease. However, the values in organic toxicity did not differ significantly from that in inorganic toxicity.

Erythrocytic glutathione peroxidase (GSH-PX) Rotruck et al. (1973) demonstrated that the selenoenzyme, GSH-PX, contained atleast 2 gram atom of selenium per mole of the enzyme. They also reported that the vitamin E prevented fatty acid hydroperoxidase formation, whereas, the sulphur amino acids and selenium were involved in peroxides destruction, thus all of them prevented the oxidative damage to the cells.

Oh et al. (1974a), by purifying the ovine erythrocytic GSH-PX, demonstrated the presence of 4 gram atoms of selenium per mole of enzyme. Subsequently, they (Oh et al., 1974b) carried out experimental studies in new-born lambs and observed that the dietary selenium level had a marked effect on the selenium concentration and GSH-PX activity in different body tissues. The GSH-PX reached a plateau at 0.10 ppm selenium in all tissues analysed except pancreas and erythrocytes, but the selenium content of all the tissues increased with each increment of dietary selenium. Thompson et al. (1976)

analysed the levels of blood selenium and GSH-PX in sheep, cows and pigs under farm conditions and reported a significant positive correlation between the two. Pierce and Tappel(1977) reported an increase in GSH-PX activity within 48 hrs after a single dose of 300 µg selenium administration (in the form of selenite and selenomethionine) by stomach tube in rats. The effects of selenite (inorganic) and selenomethionine (organic) on GSH-PX activity were found to be similar. Hoffman et al. (1978) in one of their experimental studies injected (single dose) 5 mg sodium selenite and 68 I.U. vitamin E per 60 kg body-weight intramuscularly to heifers and determined the erythrocytic GSH-PX activity from blood collected at 2 week-intervals. They observed 16 per cent increase in the activity of this enzyme at 4 weeks post-treatment. In another experiment, they injected only sodium selenite (5 mg, I/M) to heifers and recorded 67 per cent increase (24.4 ± 3.0 EU/mg Hb) in enzyme activity from the initial level (14.6 ± 2.3 EU/mg Hb) at 30 days post-treatment. Little et al.(1979) reported that sub-cutaneous injection of sodium selenite, at different doses (0.05 to 0.15 mg Se/kg body-weight), in dairy cows caused significant increase of GSH-PX activity in blood which remained higher for 182 days. Thompson et al.(1980) found slow rise in GSH-PX activity in erythrocytes of calves and the levels remained elevated several months after the serum selenium concentration became marginal or deficient.The rise or fall of GSH-PX activity and the selenium levels were more rapid in serum than that of erythrocytes.

The presence of GSH-PX in red blood cells of guinea-pigs and its decrease in selenium deficiency was demonstrated by Burk et al. (1981). Birt et al. (1983 and 1986) and Julius et al. (1983) observed increased erythrocytic GSH-PX activity in Syrian hamsters when fed diets containing excess of selenium. Behne and Wolters (1983) noted that the main pool for GSH-PX in rat was the erythrocytes and the liver. Prasad and Arora (1984a) found that the erythrocytic GSH-PX activity remained consistently higher in buffalo-calves fed on selenium-rich rice straw. Moreover, the erythrocytic GSH-PX activity was higher in buffalo-calves given I/M injections of seleno-methionine (organic) than sodium selenite (Prasad and Arora, 1984b). However, Jakhar (1984) recorded slightly more erythro-cytic GSH-PX activity in the inorganic than the organic selenosis in buffalo-calves and the values were significantly higher than that in control group. Increased blood GSH-PX has also been observed in swine when fed selenium-rich oats or sodium selenite in feed (Goehring et al.,1984). Thomson et al. (1985) found that erythrocytic GSH-PX activity increased in human when supplemented with high selenium wheat bread. Recently, McClure et al. (1986) observed increased erythrocytic GSH-PX activities in cows following treatment with oral selenium pellets.

Selenium in Farm Crops

Williams et al. (1941) estimated the selenium content of wheat and wheat products, barley, corn, oats and rye from seven

Western States of the United States and found that the level ranged from 0.1 to 30 ppm. The selenium content of barley grain reached 20 ppm when it was grown in an area where the Astragalus species grew (Beath, 1937). Moxon et al. (1950) noted that the selenium content of barley remained fairly constant at all stages of the growth. Ganje (1966) found that the corn grown in cultural solutions containing 5 ppm of selenite or organic selenium, accumulated 200 and 1,000 ppm of selenium, respectively and when the selenium content was increased to 10 ppm, the corn accumulated 300 ppm from the selenite and more than 1,500 ppm from the organic form of selenium. Ammerman et al. (1978) reported that the selenium concentration of forages was dependent on soil factors, plant species, stage of maturity, yield, pasture management, climate and soil pH. Prasad and Arora (1980) found that the rice straw and grains accumulated as high as 12.80 ppm selenium from the soil which contained 1.588 ppm.

Selenium Levels in Tissues

According to Dudley (1936), the selenium concentration in various tissues of hogs and horses suffering from "blind stagger" ranged from 9 to 25 ppm in liver, 1 to 6 ppm in spleen and 8 to 20 ppm in hooves, while in "alkali disease" the selenium content of hooves was 8 and 5 ppm in horses and cattle, respectively. Rosenfeld and Beath (1945) reported that the selenium after ingestion accumulated in various tissues with the highest concentration in liver and kidneys. Under toxic conditions, the selenium level of liver and kidneys reached

20 to 30 ppm but in most of the other tissues, the level ranged between 4 and 7 ppm. Later on, they (Rosenfeld and Beath, 1964) observed that the distribution and retention of selenium in chronic and subacute selenosis depended on the daily dose, route of administration, experimental animal used and the form of selenium administered to produce the toxicity. They noted that the organic selenium accumulated in higher quantities and persisted for longer period in tissues as compared to inorganic selenium. As reported by Glenn et al. (1964), Gardiner (1966) and Morrow (1968), the selenium concentration was highest in the liver, followed by (in descending order) the kidneys, lungs, spleen, myocardium, wool and virtually not at all in skeletal muscle and brain in sodium selenite or selenate induced selenosis in sheep.

Gabbedy and Dickson (1969) reported an outbreak of sodium selenite toxicity in lambs. The liver concentration at the time of poisoning averaged 64 ppm but 15 days later, liver and kidney concentrations of selenium averaged 26 and 7.4 ppm, respectively. Schroeder and Mitchener (1971) observed the accumulation of selenium in different organs of the rats even though the diet contained only 0.05 /ug/g wet-weight.

Hurlbut and Martin (1972) observed that the selenium levels in liver, kidney, pancreas and hair were identical when the mice were fed same dose of either sodium selenite or selenium-methyl selenocystein. The selenium content returned to normal level after one week except in hair. Further, it was noticed that the selenium content of tissues was two to eight

times higher in mice fed selenomethionine than those fed sodium selenite or selenium methyl selenocystein. Herigstad et al. (1973) reported that the pigs with toxicosis had greater concentrations of selenium in liver than in kidney, but those animals which did not develop toxicosis had greater concentration in kidney than the liver.

The increase in dietary selenium has been reported to significantly increase the selenium level of plasma, muscle and liver in guinea-pigs (Stosic, 1974) and calves (Thompson et al., 1980). Bhatia et al. (1982) during their studies on chronic selenium toxicity in buffalo-calves estimated the selenium concentration in different tissues and found that the selenium content of liver, skin, kidney and spleen was 49.15, 5.50, 5.10 and 1.5 ppm, respectively. As reported by Gupta et al. (1982), the selenium content of hair in natural chronic selenosis in buffaloes, cattle and goats ranged from 7.5 to 16.32 ppm.

Birt et al. (1983) estimated the selenium contents of liver, kidney and pancreas in Syrian hamsters after feeding of different doses (0.25 to 10.00 ppm) of sodium selenite. The selenium content was found to range from 30 to 114 ppm in liver, 48 to 125 ppm in kidney and 12 to 27 ppm in pancreas. Jakhar (1984) observed that the mean selenium level in different tissues (liver, kidney, spleen and skin) was significantly higher in inorganic than organic selenium toxicosis in buffalo calves, in comparison to the controls. A linear type correlation between the ingestion of seleniferous

grains or inorganic selenium and the concentration of selenium in blood, hair, liver, kidney, heart and spleen of rats and swine has been reported by Goehring et al.(1984). Similar observations have been made by Mallinson et al.(1985) in heifers, Anderson et al.(1985) and Hopper et al.(1985) in lambs, Ringdal et al.(1985) in rats and Jenkins and Hidiroglou (1986) in calves.

Protective Effect of Arsenite

The protective action of sodium arsenite was first reported by Moxon (1938) who observed that the administration of sodium arsenite at the rate of 5 ppm in drinking water gave full protection against liver damage in rats fed on diets containing 15 ppm of selenium as seleniferous wheat or as sodium selenite. In his further study (Moxon,1941), he observed that the selenium deposition in liver, kidney, hair and muscle was reduced about 30 per cent in pigs which were fed on selenium rich diet (9 ppm) supplemented with 5 ppm of sodium arsenite, in comparison to those receiving only selenium. The lungs of the selenized animals contained 9.2 ppm of selenium compared to 1.2 ppm in arsenic treated animals.

According to Hendrick et al.(1953), the organic arsenicals provided partial protection against selenium toxicity in rats. Sodium arsenite or arsenilic acid were partially effective in counteracting the toxic effects of sodium selenite in poultry (Carlson et al.,1954). Addition of 0.01 per cent arsenilic acid to a ration containing 12 ppm selenium slightly reduced the symptoms of selenium toxicity in beef cattle (Minyard et al.,

1960). Halverson et al. (1970) reported that addition of arsenite to a selenite treated diet caused reduction in haemolysis and selenium accumulation in the liver and kidney. Levander (1972) reviewed the literature on metabolic interrelationship and adoptation in selenium toxicity and reported that arsenic diminished the selenium toxicity by stimulating its excretion into the bile. Hill (1975) also reported that arsenicals increased the selenium excretion through bile.

Pathology

The gross pathological changes in subacute selenosis have been studied in laboratory animals (Franke, 1934; Munsell et al., 1936; Smith et al., 1937; Fitzhugh et al., 1944). These changes, in general, include dilatation of veins in visceral organs, enlargement of vena cava and right auricle, congestion of lungs and liver, degeneration in thymus, hypoplasia and degeneration of reproductive organs, haemorrhages in stomach and intestine and distension of urinary bladder. In chronic selenosis, the outstanding pathological changes occurred in liver which became hard, nodular and shrunken in size (nodular cirrhosis). Thymus and gonads were atrophied, heart and spleen enlarged, and the lymph nodes were enlarged and congested.

Harr et al. (1967) studied the pathological changes in rats after feeding them on semipurified diet containing upto 16 ppm of added selenium in the form of selenite or selenate. The changes observed were toxic hepatitis, myocarditis, nephritis and pancreatitis. Berschneider et al. (1977b) reported

congestion, haemorrhage, liver dystrophy or cirrhosis,nephrosis or cholaemic nephrosis in rabbits fed on diets containing 9.83 and 19.5 mg Se/kg body weight.

Gross changes in the experimentally induced selenosis in goats included erosion in gastro-intestinal tract with or without mucosal ulceration; reddish to greenish and sometimes blackish areas of discolouration in rumen and reticulum; proctitis; congestion and haemorrhage in heart with hydro-pericardium; congestion and focal to diffuse haemorrhages with emphysema in the lungs; congestion and focal necrosis in liver and kidney; thickening of the urinary bladder; congestion of spleen; and congestion followed by petechial or ecchymotic haemorrhages in the brain (Pathak and Datta,1983).

Jakhar (1984) observed gelatinization of the body fat, vascular congestion, oedema, petechial haemorrhages in some visceral organs, and fibrosis in the lungs and liver during his studies on experimental selenium toxicity in buffalo calves.

Microscopic pathology Duhamel (1913) described the pathological changes produced in rabbits by the administration of colloidal selenium and selenius acid. These included toxic degenerative changes in the liver; subacute glomerulitis with tubular degeneration and casts in the kidney; dilated capillaries; haemorrhagic exudate in the alveoli and bronchi of the lungs. According to Lillie and Smith (1940), the destructive process in liver was continuous in selenosis and passed through necrosis, haemorrhage and fibrosis to periportal

cirrhosis. Fitzhugh et al. (1944) noticed liver hyperplasia, increased cystic sinusoids, and focal myelosis in subacute selenium toxicity in rats. After a few months, there was focal fibrosis, distortion of the normal hepatic architecture, hepatic cell atrophy and Kupffer cell hyperplasia. At this stage, the liver damage was without intrahepatic haemorrhage and necrosis and the cystic dilation of sinusoids and myeloid foci were also less. Rosenfeld and Beath (1948) reported telangiectasis with focal necrosis in some stages of liver damage in rats.

As reported by Smith et al. (1937), the changes in heart included varying degrees of degeneration of muscle fibres, necrosis, lymphocytic infiltration, fibrosis and scarring. The stomach in early stages showed lymphocytic infiltration of the mucosa and submucosa which was followed by atrophy of the mucosa, necrosis and ulcers in later stages. Mild tubular degeneration with acute glomerular injury was seen in kidney. They also observed foci of myeloid metaplasia in the liver and spleen, and hypoplasia of bone marrow. Klug and Hendrick (1954) observed nodular fibrosis without any sign of malignancy in the lungs of rats which were fed on naturally occurring selenium for 16 months. Keratinization in the deeper epidermal layers with the disappearance of the basal cells was observed by Butcher (1957) when sodium selenite was applied in 5 per cent sulphonate on the epidermis of rats.

Pathak and Datta (1983) recorded the microscopic changes in different organs of goats following experimental induction

of selenium toxicity with sodium selenite. There was congestion, haemorrhage, oedema, leucocytic infiltration, degeneration and necrosis in different layers of gastrointestinal tract. The left ventricle of the heart was severely affected wherein the myocardium showed fatty degeneration, oedema, necrosis, moderate to severe haemorrhage, leucocytic infiltration and fibroblastic proliferation. Changes in the lungs included congestion, haemorrhage, alveolar oedema and thickening of interalveolar septae by infiltrating leucocytes. Hyperplasia of the broncheolar epithelium was also seen in animals given higher doses of sodium selenite. The microscopic changes observed in liver were congestion of the sinusoids and central veins, parenchymatous degeneration, fatty changes, coagulative necrosis in centrilobular areas, fibrosis of the hepatic parenchyma and epithelial hyperplasia of the bile ducts. Haemorrhages in the glomeruli and intertubular spaces were observed in the kidney. Tubules of the kidney showed degenerative changes and coagulative necrosis. The changes in the urinary bladder were congestion, haemorrhage and oedema with leucocytic infiltration in the lamina propria and muscularis mucosae. Hyperplasia of the bladder epithelium was also noted. In the spleen and lymph nodes, atrophy of the germinal centres and depletion of lymphoid cells in the Malpighian corpuscles alongwith haemosiderosis were the characteristic changes. Congestion and nuclear degeneration were seen in the gray matter of brain. In some cases, change like degeneration and necrosis were observed in the skeletal

muscles. Almost similar changes have been observed in experimental selenium toxicity in buffalo calves by Jakhar (1984). In addition, he noticed congestion in the cortical region of adrenal gland; congestion of the meningeal blood vessels, mild lymphocytic infiltration in Virchow-Robin space alongwith liquefaction and mild microglial cell proliferation in the brain. The vascular changes in different organs, according to him, were of greater intensity in the inorganic than the organic selenium toxicity.

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The present studies were undertaken to experimentally induce the organic and inorganic selenium toxicity in recently weaned guinea-pigs. For the production of organic selenium toxicity, selenium-enriched barley was fed to these animals, whereas inorganic toxicity was induced by feeding sodium selenite mixed with the ordinary barley. Attempts were also made to examine the protective effect of sodium arsenite in cases of inorganic selenium toxicity.

Experimental Animals

These studies were conducted on 131 albino English breed guinea-pigs procured from the Disease Free Small Animal House of the Haryana Agricultural University, Hisar. The animals were between 21 to 25 days of age with an average body weight of 153.27 g. On their arrival in the Department, they were kept in cages under strict hygienic conditions. The space in the cages was sufficient to allow free movement of the animals. These guinea-pigs were maintained on 48 hrs water-soaked ordinary barley to which vitamin C (0.2 % of feed) was added. The feed and clean water were given to these animals *ad libitum*.

Feed

Two types of feed were used for the production of selenium toxicity in guinea-pigs.

i) Selenium-enriched barley

ii) Ordinary barley mixed with sodium selenite

Selenium-enriched barley The selenium-enriched barley was obtained from crop grown on selenium-rich soil prepared earlier for this purpose. Joya variety of paddy was transplanted in about 0.2 acre of land. Ten days after paddy transplantation, 20 kg urea was added to the field followed by second treatment with 32 kg urea after a week. Both these applications together provided the recommended dose of nitrogen. Five days after second urea treatment, 0.5 kg sodium selenite (Arnold Otto Meyer) was thoroughly mixed with 10-15 kg of soil and applied uniformly along the plant line. One week later, 20 kg Diammonium phosphate (DAP) was also applied to the field. After harvesting the paddy crop, barley was sown in this field. This barley crop was utilized for production of organic selenium toxicity in guinea-pigs and it was found to contain 5.10 ppm of the selenium.

Ordinary barley Ordinary barley was procured from the Haryana Agricultural University Farm. This was found to contain only 0.86 ppm of selenium. This was used for feeding of control guinea-pigs as well as for formulation of feed for the inorganic selenium toxicity. Sodium selenite was thoroughly mixed with water soaked barley at the rate of 30 ppm and 15 ppm on dry weight basis. This sodium selenite added

barley was used for the production of inorganic selenium toxicity.

Experimental Design

After five days of initial feeding on ordinary barley, three guinea-pigs were selected at random. The rectal temperature was recorded and the blood samples were obtained from them for haematological and biochemical studies. Thereafter, these were killed and different tissues were collected for histopathological studies and selenium estimation.

The remaining 128 guinea-pigs were divided randomly into four groups. Animals in group A (40) were given water-soaked selenium-enriched barley (containing 5.10 ppm selenium) with vitamin C (0.2 % of feed) throughout the course of the experiment. Group B animals (40) were fed on water-soaked ordinary barley (containing 0.86 ppm selenium) to which sodium selenite (@ 30 ppm of the dry feed) and vitamin C (0.2 % of dry feed) was added. After 23 days, the selenium content of the feed was reduced to 15 ppm and continued till the end of the experiment (60 d). The guinea-pigs of group C (24) were kept on water-soaked ordinary barley (containing 0.86 ppm selenium) with vitamin C and served as control. Group D animals (24) were fed on water-soaked ordinary barley mixed with sodium selenite (30 ppm), vitamin C (0.2 %) and sodium arsenite (10 ppm) throughout the experimental period. This constituted the treated group.

The animals in different groups were closely observed daily for clinical signs and weighed at 3 days interval upto

the end of the experiment (60 d). Blood samples were collected from three randomly selected guinea-pigs in each group from the heart at 10 days interval and placed separately in vials containing EDTA (10 mg/ml), heparin (10 I.U./ml) and in sterilized tubes for serum separation. The blood and serum samples were subjected to haematological examinations and biochemical estimations (as mentioned below). For differential leucocyte counts (DLC), thin blood smears were prepared from fresh blood and stained with Giemsa's stain. Before collection of blood, rectal temperature of these animals was noted. The animals were then killed and subjected to detailed necropsy examination. Tissues were collected for histopathology and selenium estimation.

Clinical Examination

Guinea-pigs in different groups were examined daily for the clinical signs of toxicity, if any. The feed consumed by the guinea-pigs in different groups was recorded daily (group-wise) by substracting the feed leftover from the feed supplied. The spilled feed, if any, was picked up carefully from the faeces and washed. After washing it was mixed with the remaining feed of the pots and dried properly under direct sunlight. This feed was weighed and taken as the leftover feed. The data was analysed to calculate the average feed consumed by an individual guinea-pig.

Haematological Studies

These included total leucocyte and erythrocyte counts

(TLC and TEC), packed cell volume (PCV), haemoglobin (Hb) estimation and differential leucocyte count (DLC).

The TLC and TEC were determined by the improved Neubaur haemocytometer and PCV by the microhaematocrit method (Schalm et al.,1975). The Hb levels were determined by cyanmethaemoglobin method (Makarem,1974) using Spectronic-20 at 540 nm against haemoglobin reagent blank. The following formula was applied for determining the Hb values:

$$g\ Hb/100\ ml = A_{540} \times 36.8$$

For DLC, different cells in Giemsa's stained blood smears were counted by zigzag method in the uniformly cell distributed areas. Based upon TLC and DLC values, absolute leucocytic counts for lymphocytes and neutrophils were determined. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were derived from the values of PCV, Hb concentration and TEC.

Biochemical Studies

These included the estimation of blood glucose, total serum proteins, serum glutamic oxalacetic transaminase, serum glutamic pyruvic transaminase and erythrocytic glutathione peroxidase activity at different intervals.

Blood glucose Blood glucose was estimated within 4 hrs of the sample collection following the procedure described by King (1951). The technique was first standardized and the calibration curve prepared (Fig.1) for determining the values in unknown samples.

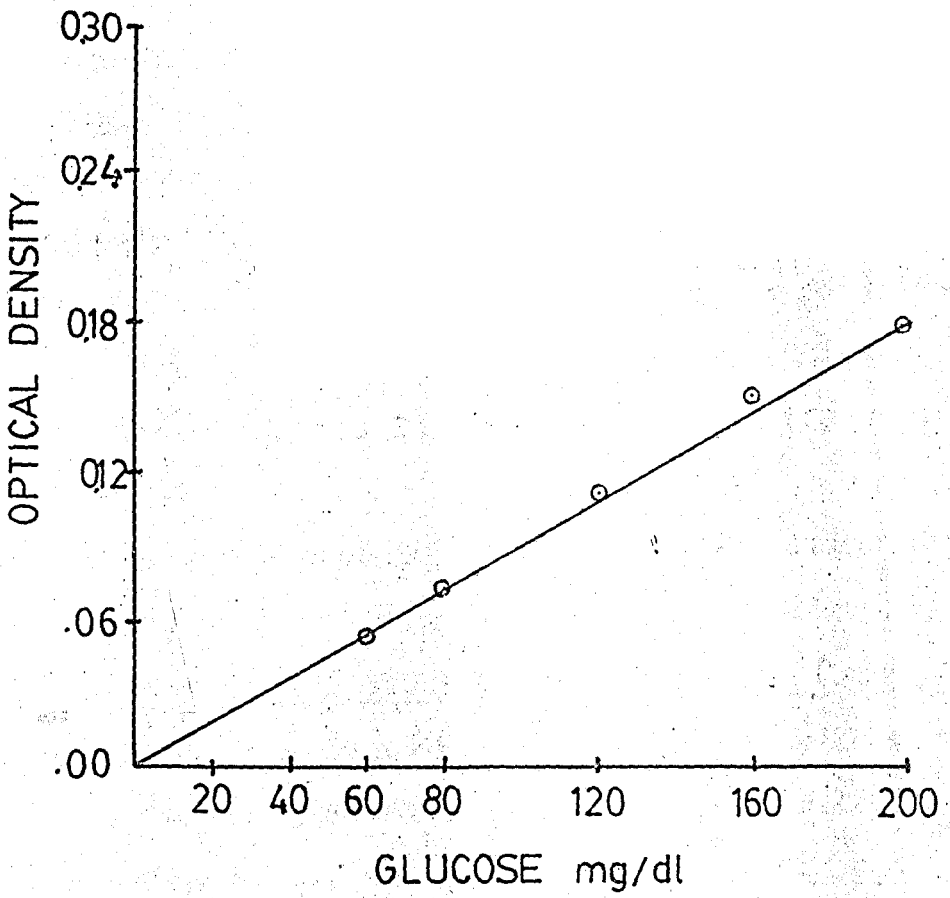


FIG. 1. CALIBRATION CURVE FOR BLOOD GLUCOSE DETERMINATION

Total serum proteins Total serum protein concentration was determined by the modified Biuret method of Wootton (1974).

Serum glutamic oxalacetic transaminase (SGOT) and Serum glutamic pyruvic transaminase (SGPT) These enzymes were estimated as per the procedures described by Coles (1967). The calibration curves (Fig.2) were prepared earlier on the basis of which the values of unknown samples were determined. The Karman (Sigma Frankel) units per ml of SGOT and SGPT activities were converted into I.U. by multiplying the value with 0.48 (Doxey,1971).

The serum proteins, SGOT and SGPT estimations were completed within 4 days of the sample collection.

Erythrocytic glutathione peroxidase (GSH-PX) activity The glutathione peroxidase activity in erythrocytic haemolysate of the heparinized blood was determined within 48 hrs of collection as per the method of Hafeman et al.(1974). The calibration curve prepared earlier (Fig.3) was used to calculate the GSH-PX values. Later, the haemoglobin content in the haemolysates was determined following the method of Lowry et al.(1951) and calculated from the standard curve (Fig.4). The values of GSH-PX activities were expressed as E.U./mg Hb.

Estimation of Selenium

The selenium content of barley and different tissues like liver, kidney, spleen and hair from killed guinea-pigs was determined at 20 days interval following the method of Cummins et al.(1965) and subsequently modified by Levesque

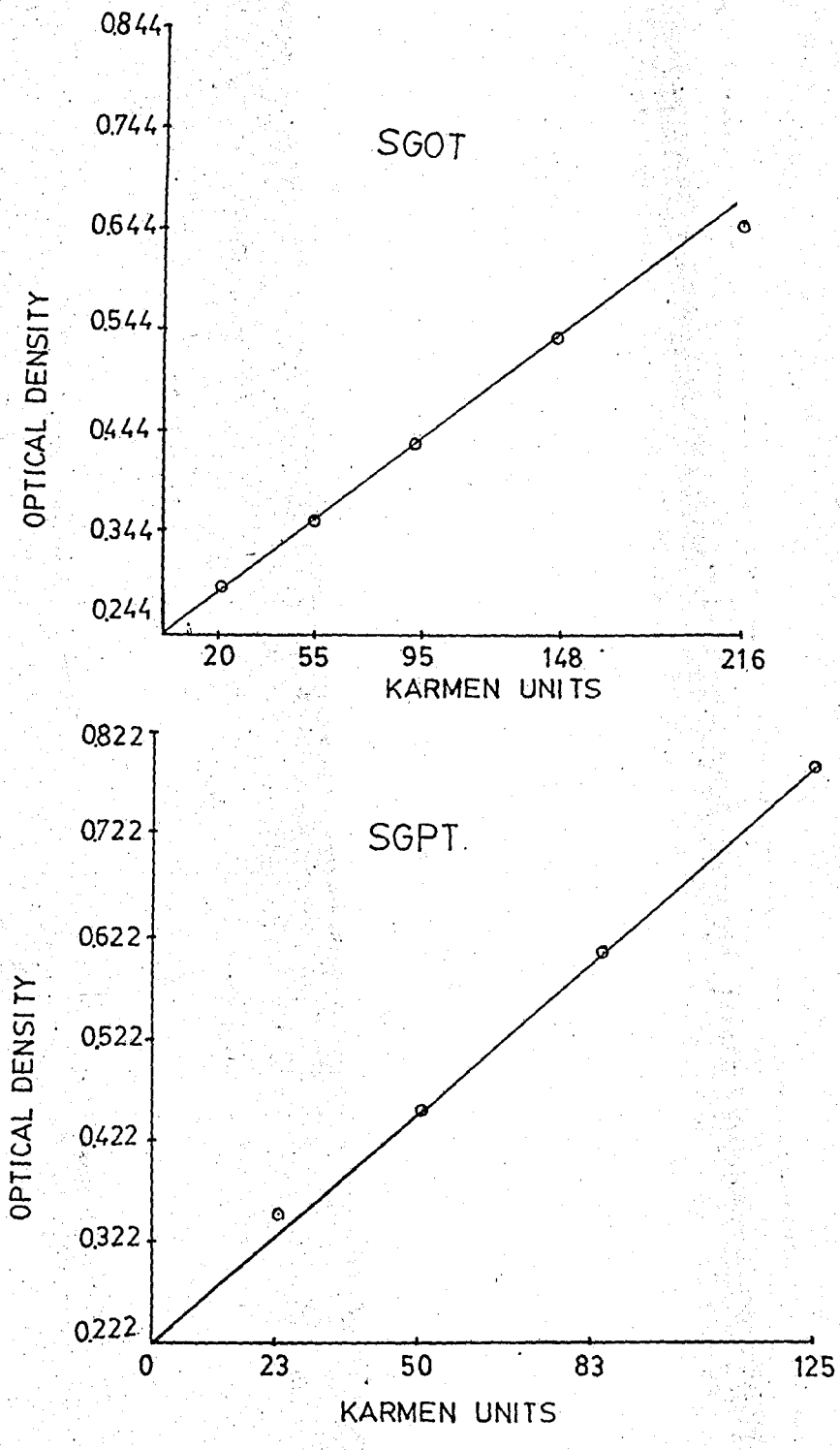


FIG. 2. CALIBRATION CURVES FOR SGOT & SGPT

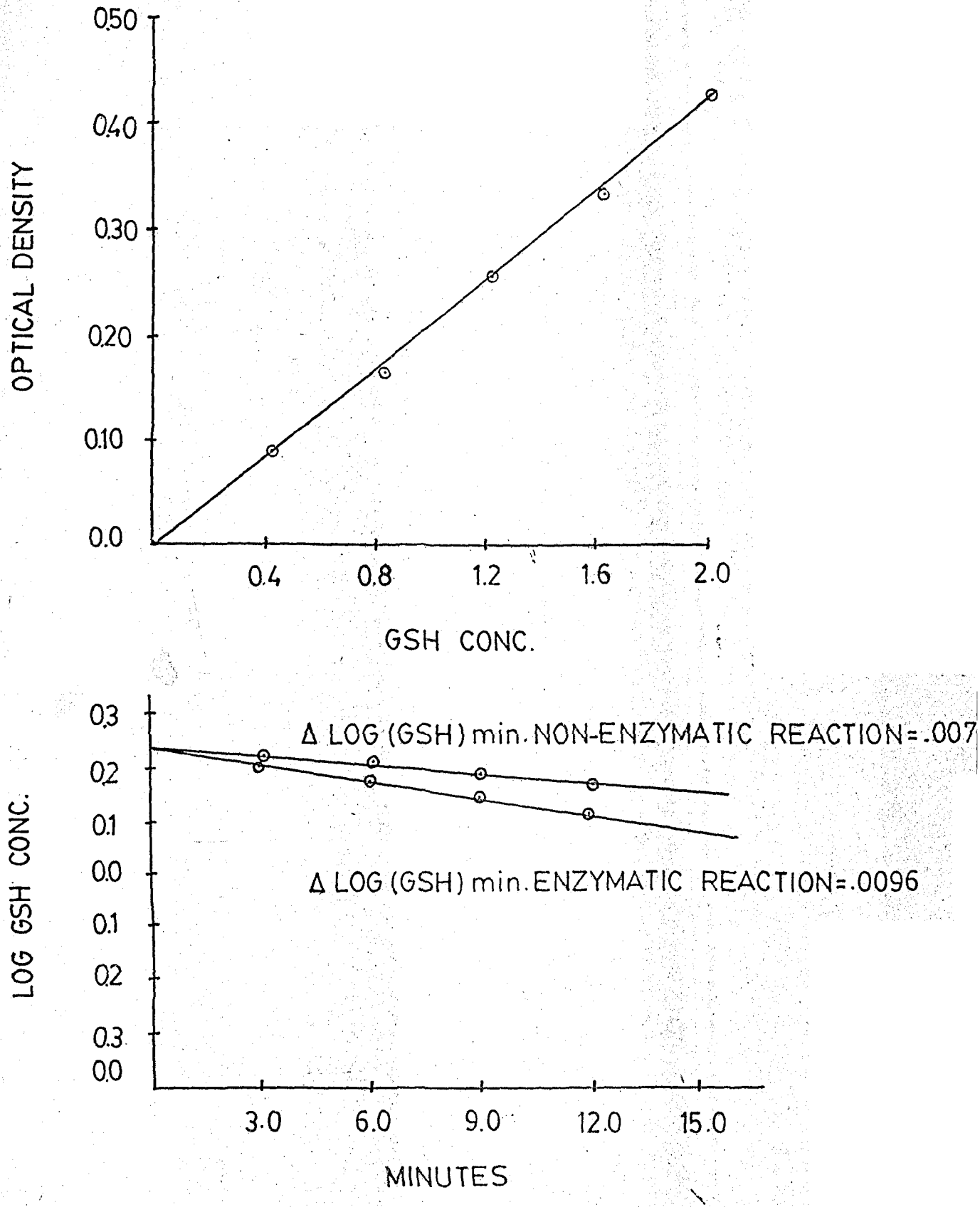


FIG. 3. CALIBRATION CURVES FOR GLUTATHIONE - PEROXIDASE

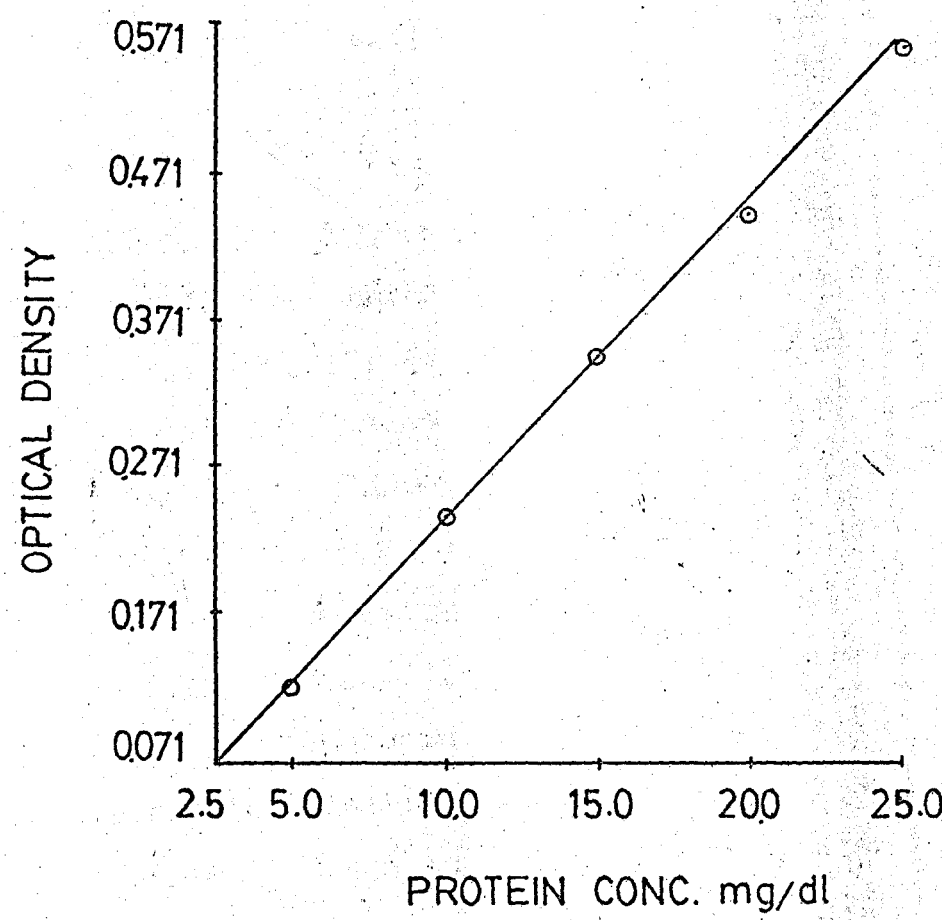


FIG. 4. CALIBRATION CURVE FOR PROTEIN DETERMINATION

and Vendette (1971). The calibration curves for different concentrations of selenium (Fig.5) were used to determine the values in unknown samples.

Pathological Studies

All those guinea-pigs which died during the course of the experiment or killed at different intervals were subjected to detailed necropsy examination. Gross lesions, if any, in different organs/tissues were recorded. Representative tissues from lung, heart, liver, kidney, spleen, stomach, intestine, muscle (sternal area), skin, thymus, lymph node, adrenal and testis were collected in 10 per cent formol saline for histopathological studies. The fixed tissues were processed for paraffin embedding using ethanol as dehydrating agent and chloroform for clearing. Sections were cut at 4-5 μ thickness and stained by haematoxylin and eosin method. Gomori's method was used for the demonstration of haemosiderin in tissue sections (Luna, 1968).

Statistical Analysis

The data generated by these observations were analysed by the analysis of variance (Alder and Roessler, 1964).

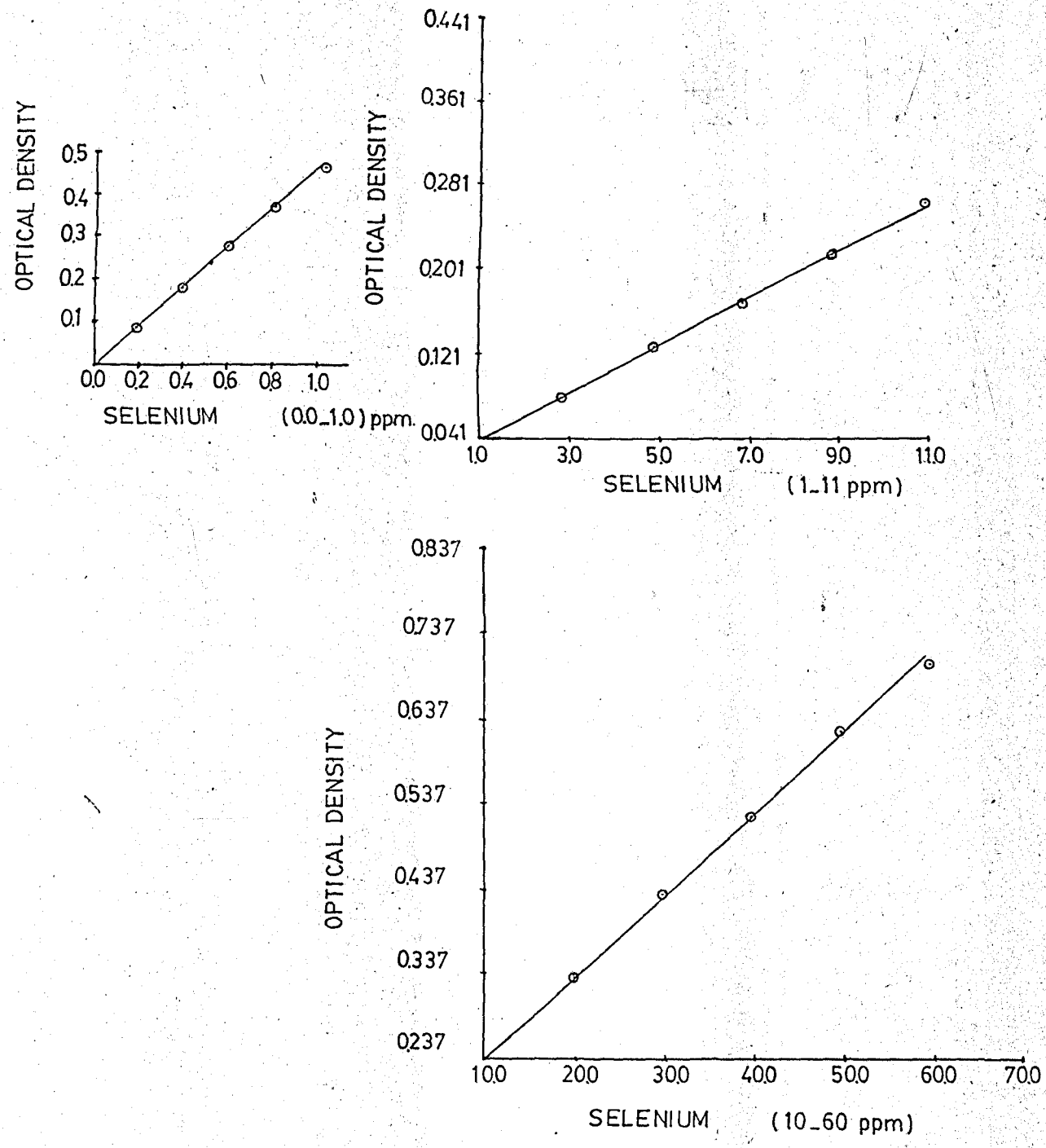


FIG.5. CALIBRATION CURVES FOR SELENIUM DETERMINATION

RESULTS

CHAPTER IV

RESULTS

The observations with respect to the clinical signs, mortality pattern, haematological and biochemical investigations, pathological changes and selenium estimation during the course of present experimental studies are presented as under:

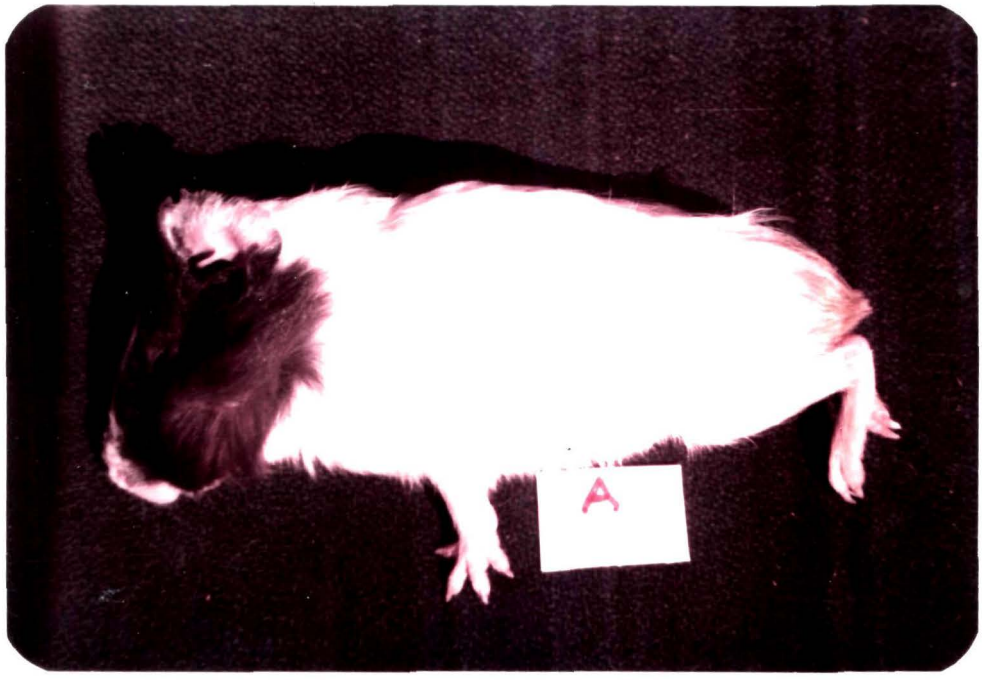
Clinical Signs

Group A (Organic selenium toxicity) Abnormalities in the general behaviour of this group of animals were first recorded on 11th day of the feeding of selenium-enriched barley. These included loud crying, slight lacrimation and/or salivation in some animals, dullness, depression, progressing weakness, retarded movement of the animals towards feed and water, and slight nervousness. With the advancement of experimental period, the guinea-pigs showed signs of nervous involvement characterized by restlessness and pressing of head against the cage-wall, especially when there was sharp sound or stimuli; convulsions started in some cases suddenly which persisted for about one minute and then the animal became normal. The fur was ruffled and a patchy alopecia was first noticed at the base of the ear (Fig.6) or at the forehead and thereafter at other parts of the body. The affected animals were very much emaciated and dejected with oedematous

Fig.6 Patchy alopecia at the base of ear in a guinea-pig (Group A, 32 DPE).

Fig.7 Emaciation and paralysis of hind leg in a guinea-pig (Group A, 28 DPE).


ORGANIC
SELENIUM


A

swelling of the head region. The visible mucous membrane was pale and the respiration was laboured. With the progression of disease, paralysis developed in the hind legs (Fig.7) and the animals died within 48 hrs of the manifestation of paralysis. Prior to death, the animals lost tonacity of the muscles and the body was stretched at its back as in case of tetanus.

Group B (Inorganic selenium toxicity) The clinical signs observed in this group of animals were more or less similar but comparatively more severe than that in group A. The signs appeared first on the 8th Day Post-experimentation (DPE) in this group. Oedema of the head and alopecia were comparatively more marked (Fig.8) and the nervousness and convulsions exhibited by this group of animals were relatively more pronounced. Although paralysis involved mostly the hind legs (Fig.9), but in some cases the forelegs were also affected. The emaciation and cachexia were more marked.

Group C (Control) None of the animals in this group exhibited any abnormality during the 60 days of experimentation (Fig.10).

Group D (Treated) The animals of this group exhibited abnormalities in their behaviour first on 16 DPE and the clinical signs were similar to that in group B. Though alopecia was not recorded but the hair distribution in some of the animals was scanty. Oedema of the face region was not marked and the paralysis of hind legs was observed in few cases (Fig.11).

Fig.8 A dull and depressed guinea-pig from group B (2º DPE). Patchy alopecia at the flank area and oedema of head region is also visible.

Fig.9 Oedematous swelling of the head region and paralysis of the hind leg in a guinea-pig (Group B, 41 DPE).


INORGANIC SEL

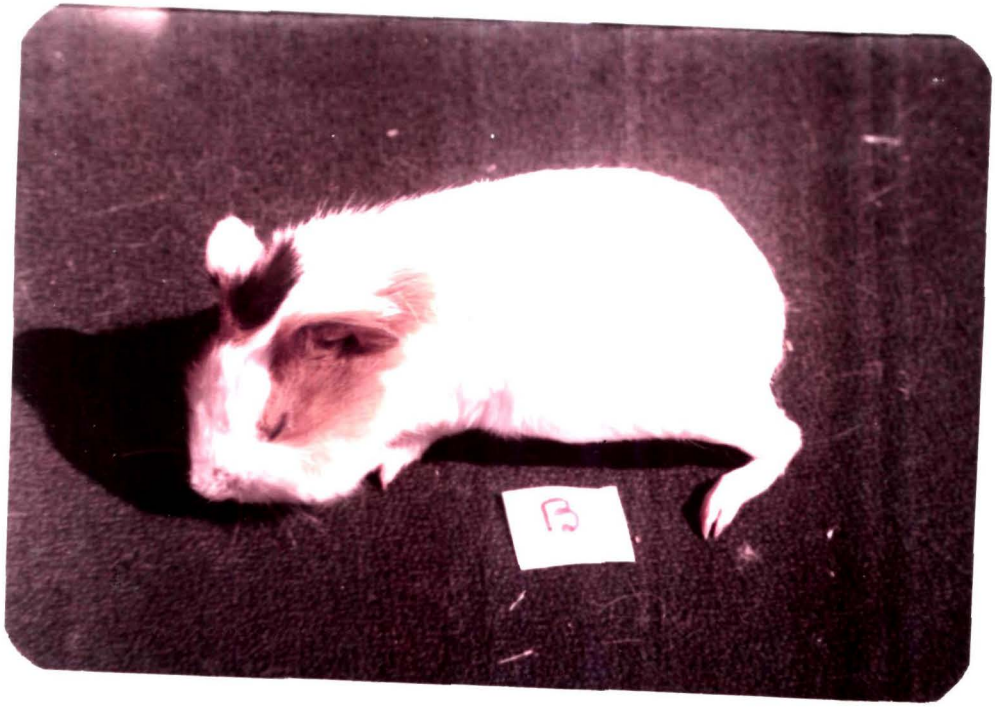
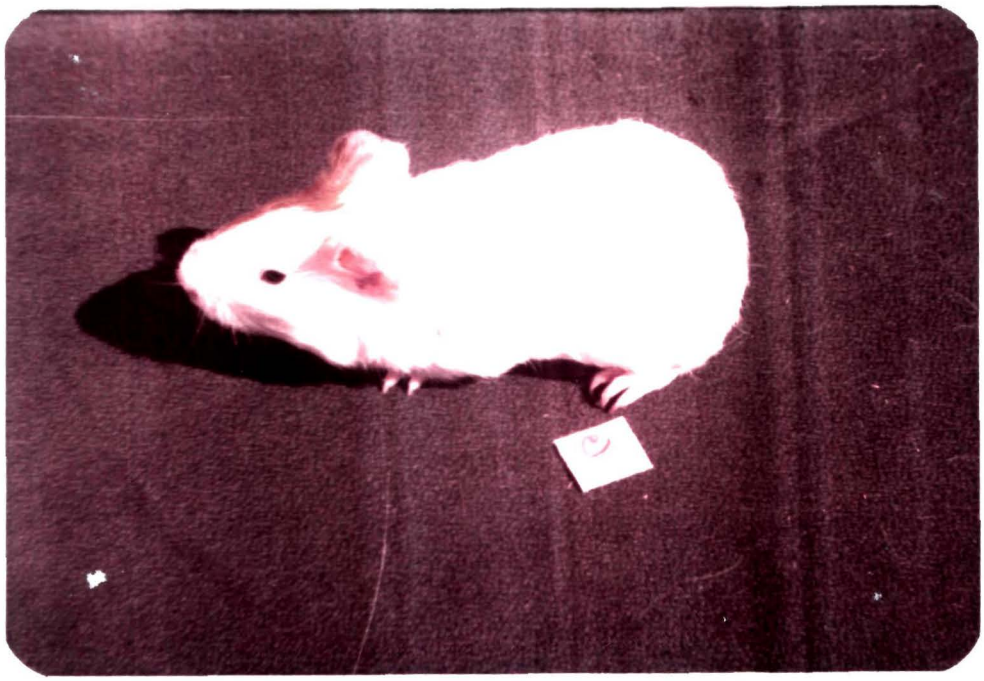

B

Fig.10 An alart and active guinea-pog from the control group (Group C, 27 DPE).

Fig.11 A guinea-pig from treated group (Group D, 36 DPE) showing ruffled and scanty distribution of fur with paralysis of hind leg.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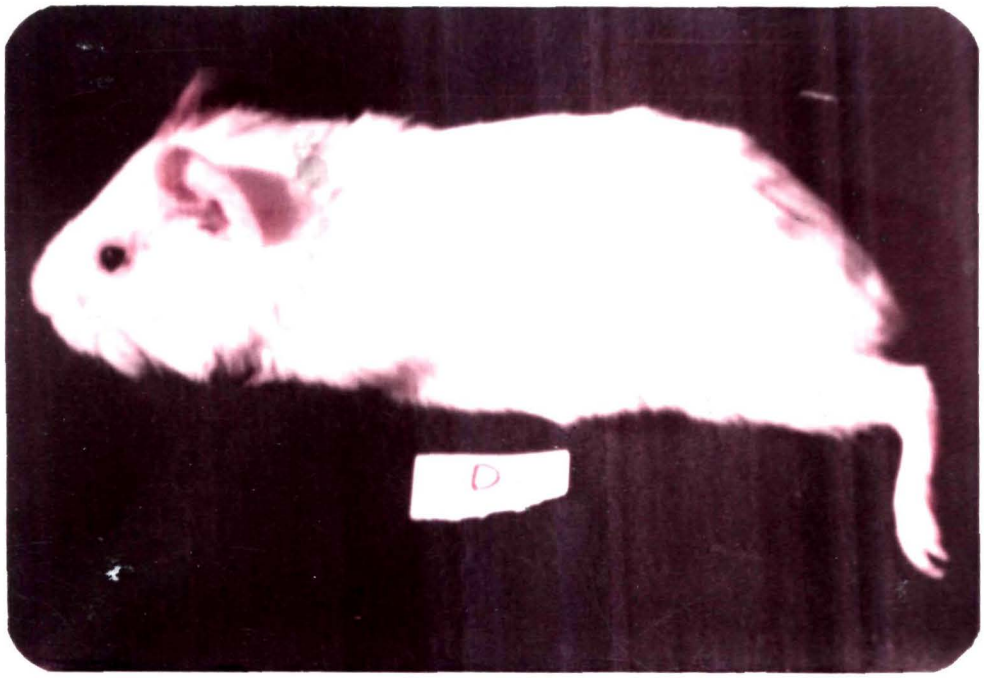
D

Mortality Pattern

The occurrence of mortality was first recorded on 11th day post-experimentation in B group, followed by 14th day in A and 17th day in group D (Table 1). The overall mortality was 55.0, 55.0 and 37.5 per cent in group A, B and D, respectively. Although there was no definite pattern of mortality in various groups but the highest mortality was observed in group A and B between 40 to 50 days of experimentation followed by that in 10 to 20 DPE whereas, the mortality increased continuously after 17th day to 40 DPE in animals of group D. No mortality was, however, observed in any group before 10 days and after 50 days of experimental feeding.

Feed Consumption

The average feed consumed by guinea-pigs of different groups at various intervals is given in Table 2 and presented graphically in Fig.12. From the table it is apparent that the average feed consumed by the guinea-pigs was significantly ($P < 0.05$) reduced in group A and B in comparison to controls (group C). However, no significant difference in feed consumption was noticed between animals of group C and D.

Body Weight

The average weight gain/loss in guinea-pigs at various intervals of feeding are presented in Table 3. A reference to the table indicates that guinea-pigs fed on selenium-enriched barley (group A) or barley mixed with sodium selenite (group B)

TABLE 1

Mortality pattern in different experimental groups of guinea-pigs

Group	Number died at various intervals (days)						Overall percent mortality
	10	20	30	40	50	60	
A (Organic Se toxicity)	0/40	9/37	6/25	0/16	7/13	0/3	55.0
B (Inorganic Se toxicity)	0/40	10/37	6/24	2/15	4/10	0/3	55.0
C (Control)	0/24	0/21	0/18	0/15	0/12	0/9	0.0
D (Treated)	0/24	3/21	3/15	3/9	0/3	NA	37.5

NA = None available

The denominator indicates the number of guinea-pigs at each interval while the numerator the number died

TABLE 2

Average feed consumed (Mean ± SE) by guinea-pigs in different groups at various intervals

Group	Feed consumption (g) at different intervals (days)						Overall mean
	10	20	30	40	50	60	
A (Organic Se toxicity)	11.59 ±0.30 (40)	12.01 ±0.35 (28)	16.73 ±0.38 (19)	20.50 ±0.25 (16)	19.45 ±1.11 (6)	11.20 ±0.24 (3)	15.25[b]
B (Inorganic Se toxicity)	11.05 ±0.18 (40)	11.07 ±0.42 (27)	16.95 ±0.21 (18)	18.31 ±0.36 (13)	19.79 ±0.51 (6)	17.30 ±0.49 (3)	15.75[b]
C (Control)	13.53 ±0.12 (24)	16.61 ±0.35 (21)	19.57 ±0.17 (18)	20.52 ±0.06 (15)	22.76 ±0.33 (12)	23.17 ±0.26 (9)	19.36[a]
D (Treated)	11.86 ±0.27 (24)	14.23 ±0.18 (18)	20.20 ±0.44 (12)	21.43 ±0.49 (6)	18.09 ±0.65 (3)	NA	17.16[ab]

NA = None available

Figures in parentheses indicate the number of guinea-pigs

Figures having superscripts in common (within a column) do not differ significantly at 5 per cent level of probability ($P < 0.05$)

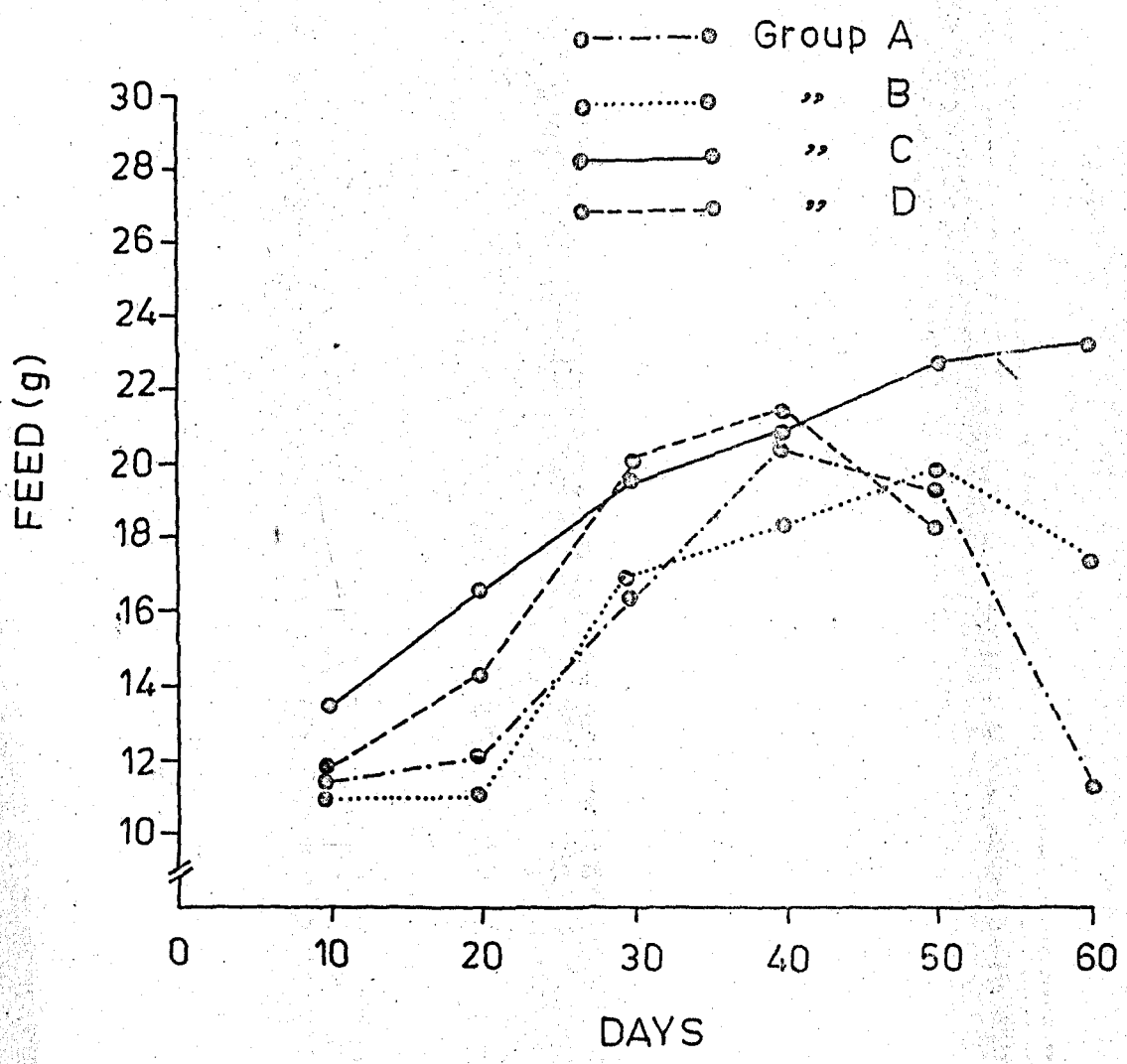

FIG. 12. MEAN FEED CONSUMED (g) BY A GUINEA-PIG IN DIFFERENT GROUPS AT VARIOUS INTERVALS

TABLE 3

Average body weight gain/loss (Mean ± SE) in guinea-pigs of different experimental groups

Group	Body weight gain or loss (g) at different intervals (days)						Overall mean
	9	21	33	45	57	60	
A (Organic Se toxicity)	0.94 ±0.70	6.73 ±1.22	0.36 ±0.17	-0.28 ±0.84	-7.26 ±4.19	-12.38	-1.98[b]
B (Inorganic Se toxicity)	0.25 ±0.12	6.34 ±2.25	6.02 ±3.51	7.28 ±1.34	-12.33 ±2.45	-16.50	-1.49[b]
C (Control)	0.44 ±0.24	7.72 ±2.36	19.45 ±3.90	32.36 ±0.09	52.76 ±5.83	63.22	29.37[a]
D (Treated)	0.88 ±0.56	11.10 ±2.94	12.44 ±4.75	25.11 ±3.39	NA	NA	12.38[a]

NA = None available

Figures having superscripts in common in a column do not differ significantly at 5 per cent level of probability ($P < 0.05$)

experienced a significant ($P < 0.05$) retardation in body-weight gain in comparison to control. The loss of body-weight or failure to weight gain was maximum in animals of group A. No significant difference was noticed in the treated group as compared to control.

Body Temperature

The average rectal temperature recorded in animals of different groups (Table 4) was within normal range and no significant difference was observed in any of the experimental groups in comparison to control.

Haematological Studies

The results of haematological studies like packed cell volume, haemoglobin level, total erythrocyte count, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, total and differential leucocyte counts and absolute leucocytic values at various intervals of the experimental feeding are given below:

Packed cell volume (PCV) Mean values of PCV in different groups are given in Table 5 and Fig.13. A reference to this table indicates a significant ($P < 0.01$) difference in the PCV value between the groups. The animals of group A and B showed a significantly lower values than that in group C. However, the value in group A did not differ significantly from that in group B. Though the difference in mean PCV between group C and D was not significant but the value was

TABLE 4

Average rectal temperature (^{o}F) of guinea-pigs in different experimental groups

Group	Temperature (Mean ± SE) at various intervals (days)							Overall mean
	0	10	20	30	40	50	60	
A (Organic Se toxicity)	101.63 ± 0.12	100.73 ± 0.30	101.80 ± 0.28	99.40 ± 1.23	99.77 ± 0.23	101.65 ± 0.46	100.20 ± 0.14	100.74
B (Inorganic Se toxicity)	101.63 ± 0.12	99.77 ± 0.41	100.40 ± 0.68	99.10 ± 0.45	98.83 ± 0.26	100.60 ± 0.28	100.90 ± 0.07	100.18
C (Control)	101.63 ± 0.12	100.20 ± 0.09	100.80 ± 0.66	100.40 ± 0.57	99.83 ± 0.26	100.10 ± 0.05	101.60 ± 0.33	100.65
D (Treated)	101.63 ± 0.12	102.13 ± 0.05	99.07 ± 0.11	100.67 ± 0.63	99.20 ± 0.64	100.65 ± 0.25	NA	100.56

NA = None available

TABLE 5

Mean packed cell volume (per cent) in guinea-pigs of different experimental groups

Group	PCV (Mean ± SE) at different intervals (days)							Overall mean
	0	10	20	30	40	50	60	
A (Organic Se toxicity)	38.33 ±0.72	36.33 ±0.72	32.67 ±2.60	32.67 ±1.44	32.33 ±0.72	31.50 ±1.06	32.50 ±1.77	33.76[b]
B (Inorganic Se toxicity)	38.33 ±0.72	33.67 ±0.72	32.33 ±1.44	31.33 ±0.72	32.67 ±0.54	32.50 ±1.06	33.00 ±2.12	33.40[b]
C (Control)	38.33 ±0.72	37.67 ±0.54	39.67 ±0.72	38.67 ±1.66	40.00 ±1.25	36.67 ±0.27	36.67 ±0.98	38.24[a]
D (Treated)	38.33 ±0.72	35.67 ±1.66	37.33 ±1.66	38.00 ±0.94	34.50 ±3.18	36.00 ±1.41	NA	36.64[a]

NA = None available

Figures having superscripts in common in a column do not differ significantly at 1 per cent level of probability ($P < 0.01$)

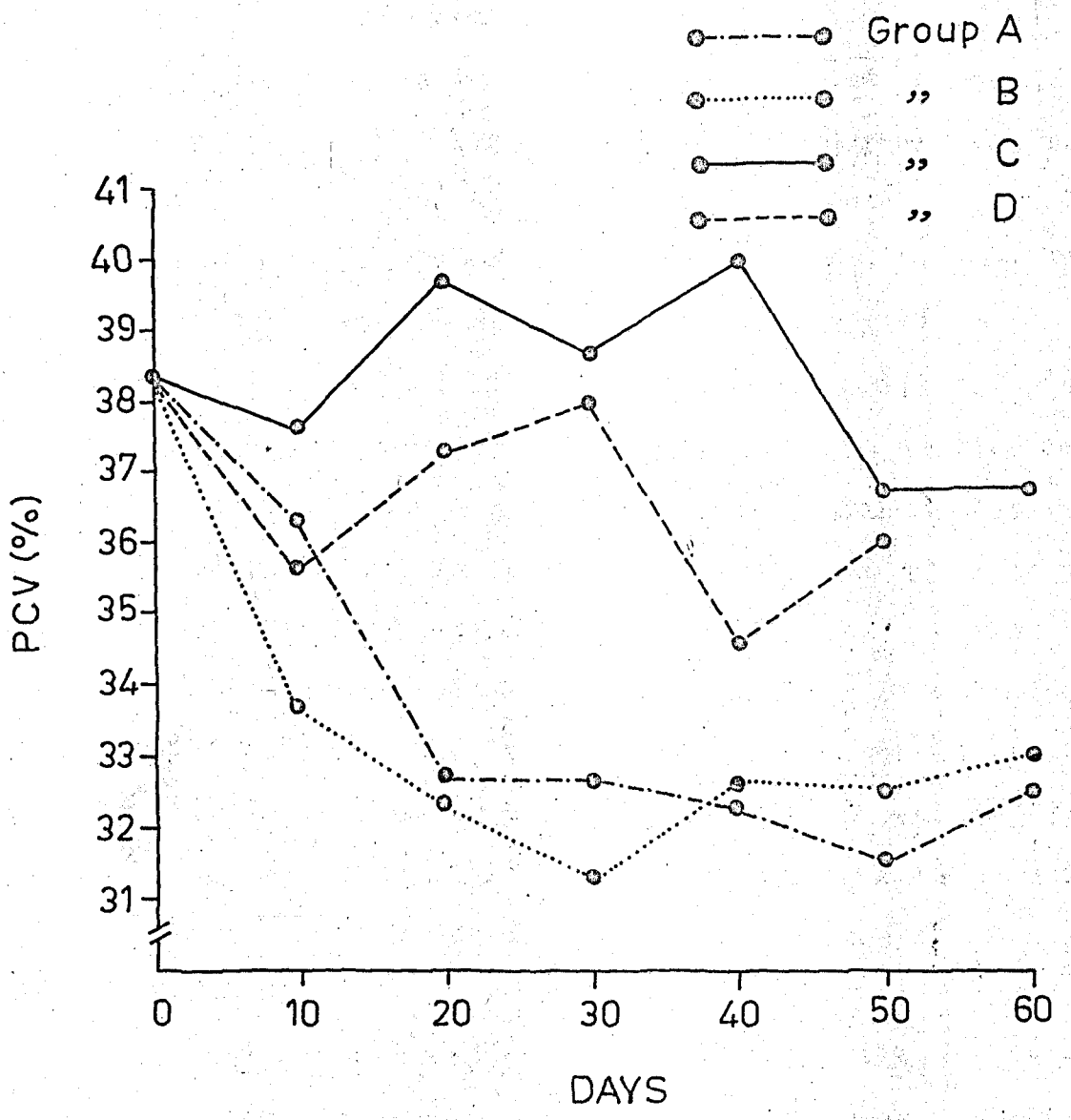

FIG. 13. MEAN PACKED CELL VOLUME (PER CENT) OF GUINEA-PIGS IN DIFFERENT GROUPS AT VARIOUS INTERVALS

comparatively higher in group C (38.24 per cent) than the group D (36.64 per cent). The drop in PCV values was noticed from 20 day onwards in both the organic and inorganic selenium toxicity but in the treated group, the decrease was seen after 40 days.

Haemoglobin (Hb) concentration The average Hb concentration observed in guinea-pigs of different experimental groups is given in Table 6 and presented graphically in Fig.14. A highly significant ($P < 0.01$) decrease in Hb concentration was seen in all the 3 groups (A,B,D) of guinea-pigs fed on high selenium diet in comparison to control group (C). Although no significant difference was found to exist between group B and D but the mean values of these groups differed significantly from that in group A. The overall mean values in the animals of group A, B, C and D were 9.94, 10.49, 11.64 and 10.80 g/dl, respectively. The earliest decrease (10 days onward) was noticed in group B followed by that in group A (30 days onwards) and D (40 days).

Total erythrocyte count (TEC) The result in respect of total erythrocyte count in different groups of guinea-pigs at various intervals are presented in Table 7 with graphical representation in Fig.15. From the table it is apparent that there is no significant difference in TEC values amongst different experimental groups.

Mean corpuscular volume (MCV) The MCV calculated in animals of different groups at various intervals are given in Table 8 and Fig.16. Significantly ($P < 0.01$) lower values

TABLE 6

Mean haemoglobin concentration (g/dl) in guinea-pigs of different experimental groups

Group	Hb value (Mean ± SE) at various intervals (days)							Overall mean
	0	10	20	30	40	50	60	
A (Organic Se toxicity)	11.63 ±0.18	10.16 ±0.14	10.06 ±0.08	9.76 ±0.29	9.38 ±0.28	9.15 ±0.51	9.44 ±0.51	9.94[c]
B (Inorganic Se toxicity)	11.63 ±0.18	10.45 ±0.14	10.29 ±0.65	9.76 ±0.29	10.67 ±0.31	10.16 ±0.21	10.49 ±0.65	10.49[b]
C (Control)	11.63 ±0.18	11.74 ±0.16	11.96 ±0.09	11.19 ±0.24	11.66 ±0.61	11.42 ±0.55	11.85 ±0.24	11.64[a]
D (Treated)	11.63 ±0.18	10.50 ±0.29	10.98 ±0.23	10.87 ±0.23	10.49 ±0.65	10.31 ±0.10	NA	10.80[b]

NA = None available

Figures having superscripts in common in a column do not differ significantly at 1 per cent level of probability ($P < 0.01$)

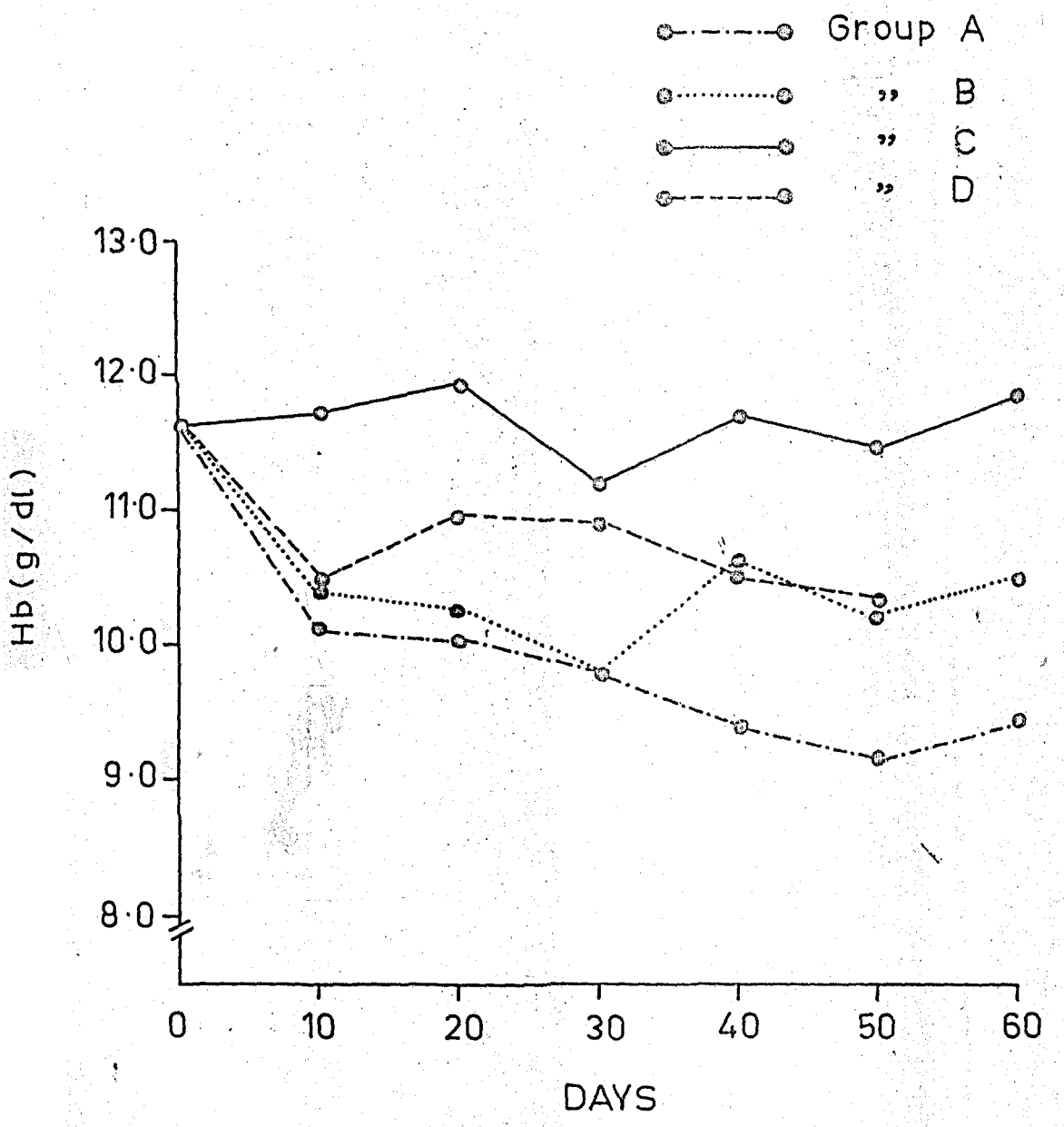

FIG. 14. MEAN HAEMOGLOBIN VALUE (g/dl) OF GUINEA-PIGS IN DIFFERENT GROUPS AT VARIOUS INTERVALS

TABLE 7

Average (Mean ± SE) total erythrocyte count in guinea-pigs of different experimental groups

Group	TEC (millions/µl) at various intervals (days)							Overall mean
	0	10	20	30	40	50	60	
A (Organic Se toxicity)	4.76 ±0.05	4.86 ±0.11	4.81 ±0.10	5.82 ±0.35	6.15 ±0.11	6.80 ±0.65	5.86 ±0.10	5.58
B (Inorganic Se toxicity)	4.76 ±0.05	4.55 ±0.10	4.78 ±0.08	4.98 ±0.43	5.97 ±0.13	6.58 ±0.15	5.87 ±0.06	5.36
C (Control)	4.76 ±0.05	4.91 ±0.05	4.97 ±0.05	5.40 ±0.13	5.65 ±0.21	5.74 ±0.18	5.24 ±0.31	5.24
D (Treated)	4.76 ±0.05	4.63 ±0.17	4.85 ±0.15	5.55 ±0.50	5.73 ±0.14	6.60 ±0.51	NA	5.35

NA = None available

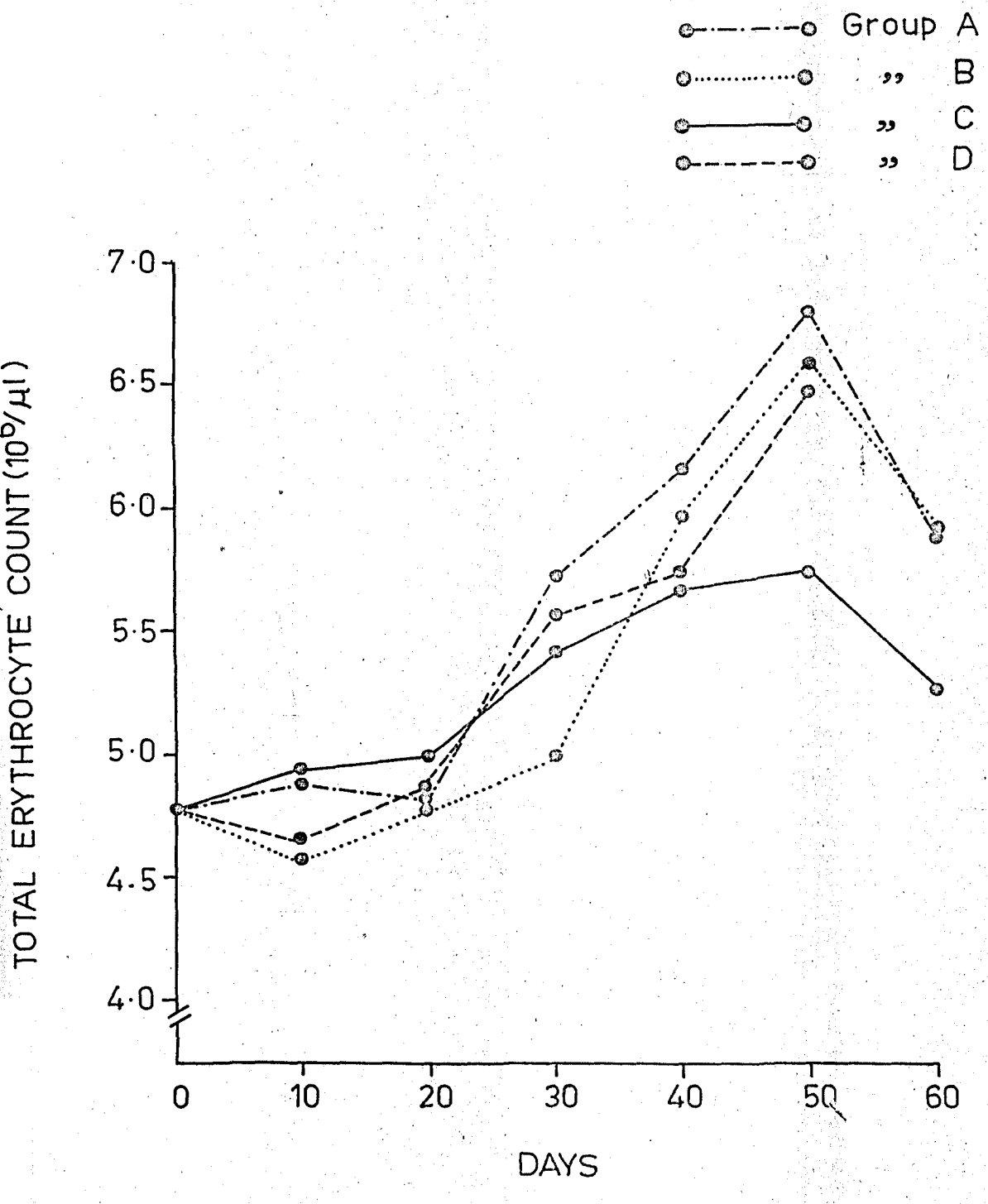

FIG. 15. MEAN TOTAL ERYTHROCYTE COUNT ($10^6/\mu l$) OF GUINEA-PIGS IN DIFFERENT GROUPS AT VARIOUS INTERVALS

TABLE 8

Mean corpuscular volume in guinea-pigs of different experimental groups

Group	MCV (cubic micron) at different intervals (days)							Overall mean
	0	10	20	30	40	50	60	
A (Organic Se toxicity)	80.58 ±0.92	74.93 ±2.23	67.70 ±4.08	56.41 ±2.09	52.57 ±0.52	46.91 ±2.91	55.60 ±3.96	62.10[b]
B (Inorganic Se toxicity)	80.58 ±0.92	74.06 ±0.36	67.56 ±2.17	64.45 ±5.98	54.86 ±1.87	49.55 ±2.71	56.30 ±4.16	63.91[b]
C (Control)	80.58 ±0.92	76.87 ±1.93	79.79 ±0.68	71.84 ±4.30	71.19 ±3.69	64.10 ±2.37	70.84 ±4.91	73.60[a]
D (Treated)	80.58 ±0.92	77.02 ±2.07	76.83 ±1.09	68.57 ±2.04	60.69 ±7.32	54.92 ±2.12	NA	69.77[a]

NA = None available

Figures having superscripts in common in a column do not differ significantly at 1 per cent level of probability ($P < 0.01$)

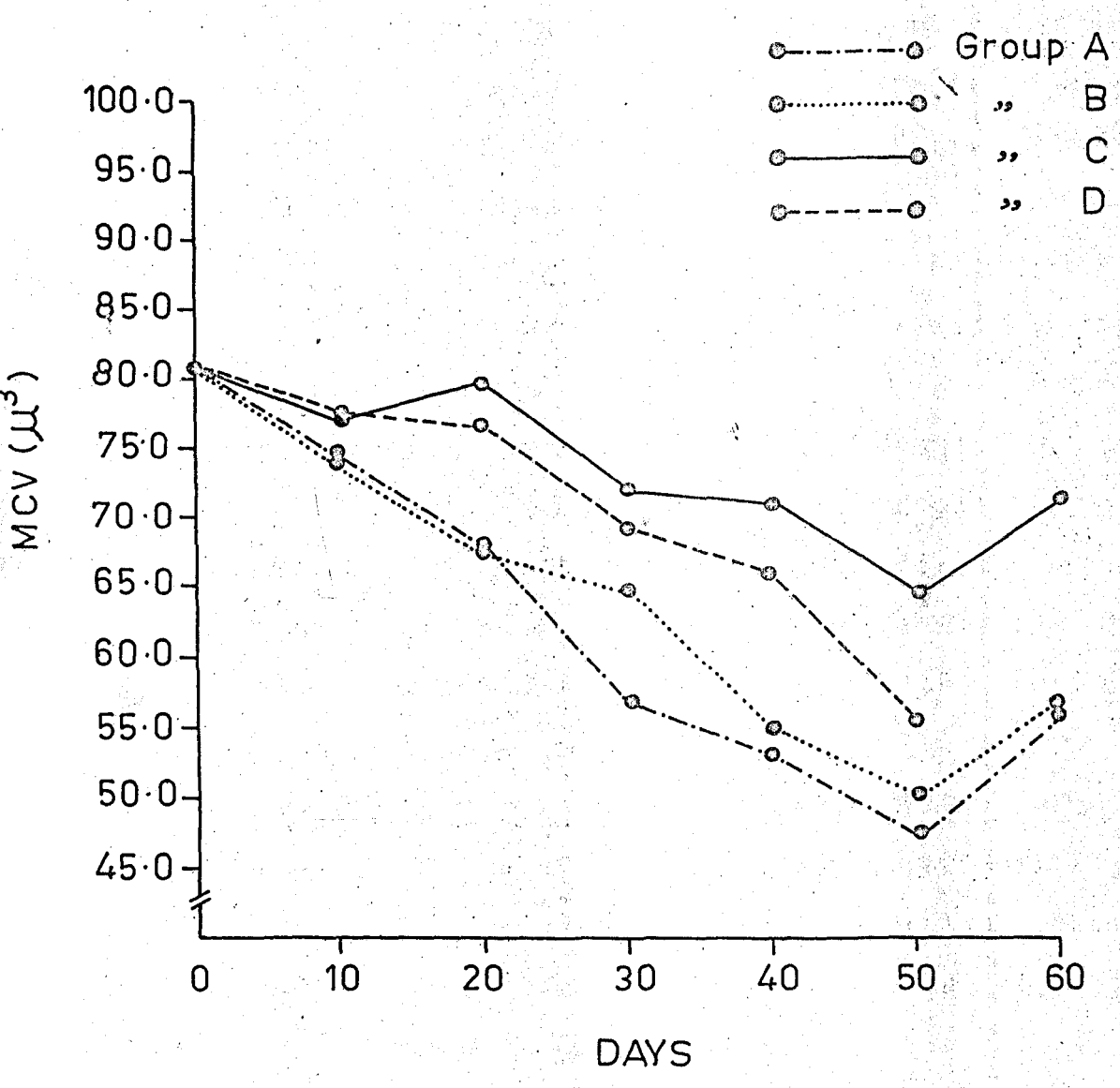

FIG. 16. MEAN CORPUSCULAR VOLUME (μ^3) OF GUINEA-PIGS IN DIFFERENT GROUPS AT VARIOUS INTERVALS

were observed in animals of group A and B in comparison to group C (control group). However, no significant difference in this respect was noticed between the control (group C) and the treated (group D) groups. Though a slight decrease in MCV values was observed at various stages of the experimental period but significant decrease was noticed from 30 day onwards in group A, B and D.

Mean corpuscular haemoglobin (MCH) Mean values of MCH in different groups are depicted in Table 9 and Fig.17. It is apparent that the MCH values in group A, B and D were significantly lower in comparison to the control (group C). The overall reduction in MCH was highest in group A followed by that in group B and D. However, the difference between group B and D was not significant. Though MCH values declined progressively in all groups of animals but the rapid decrease was observed on 30 day onwards.

Mean corpuscular haemoglobin concentration (MCHC) The MCHC values in guinea-pigs of different experimental groups (Table 10) did not differ significantly from one another.

A comparison of the erythrocytic indices in all the four experimental groups is given in Table 11.

Total leucocyte count (TLC) The mean values of TLC recorded in the four groups at different days of the experiment are presented in Table 12 and Fig.18. A significant drop in TLC was observed in both group A and B in comparison to control (group C). Although the mean TLC in group D (treated) was slightly lower than that of control but the difference was not significant.

TABLE 9

Mean corpuscular haemoglobin in guinea-pigs of different experimental groups

Group	MCH ($\mu\mu$g)* at various intervals (days)							Overall mean
	0	10	20	30	40	50	60	
A (Organic Se toxicity)	24.46 ±0.57	20.96 ±0.71	20.94 ±0.45	17.07 ±1.64	15.26 ±0.44	13.57 ±0.55	16.15 ±1.14	18.34^{c}
B (Inorganic Se toxicity)	24.46 ±0.57	23.01 ±0.22	21.53 ±1.30	16.64 ±1.59	17.93 ±0.77	15.48 ±0.66	17.90 ±1.28	19.56^{bc}
C (Control)	24.46 ±0.57	23.95 ±0.46	24.08 ±0.38	20.74 ±0.49	20.84 ±1.86	19.97 ±1.17	22.94 ±1.73	22.43^{a}
D (Treated)	24.46 ±0.57	22.71 ±0.26	22.66 ±0.49	19.61 ±0.47	18.29 ±0.60	15.80 ±1.07	NA	20.59^{b}

*Mean ± SE

NA = None available

Figures having superscript in common in a column do not differ significantly at 1 per cent level of probability ($P < 0.01$)

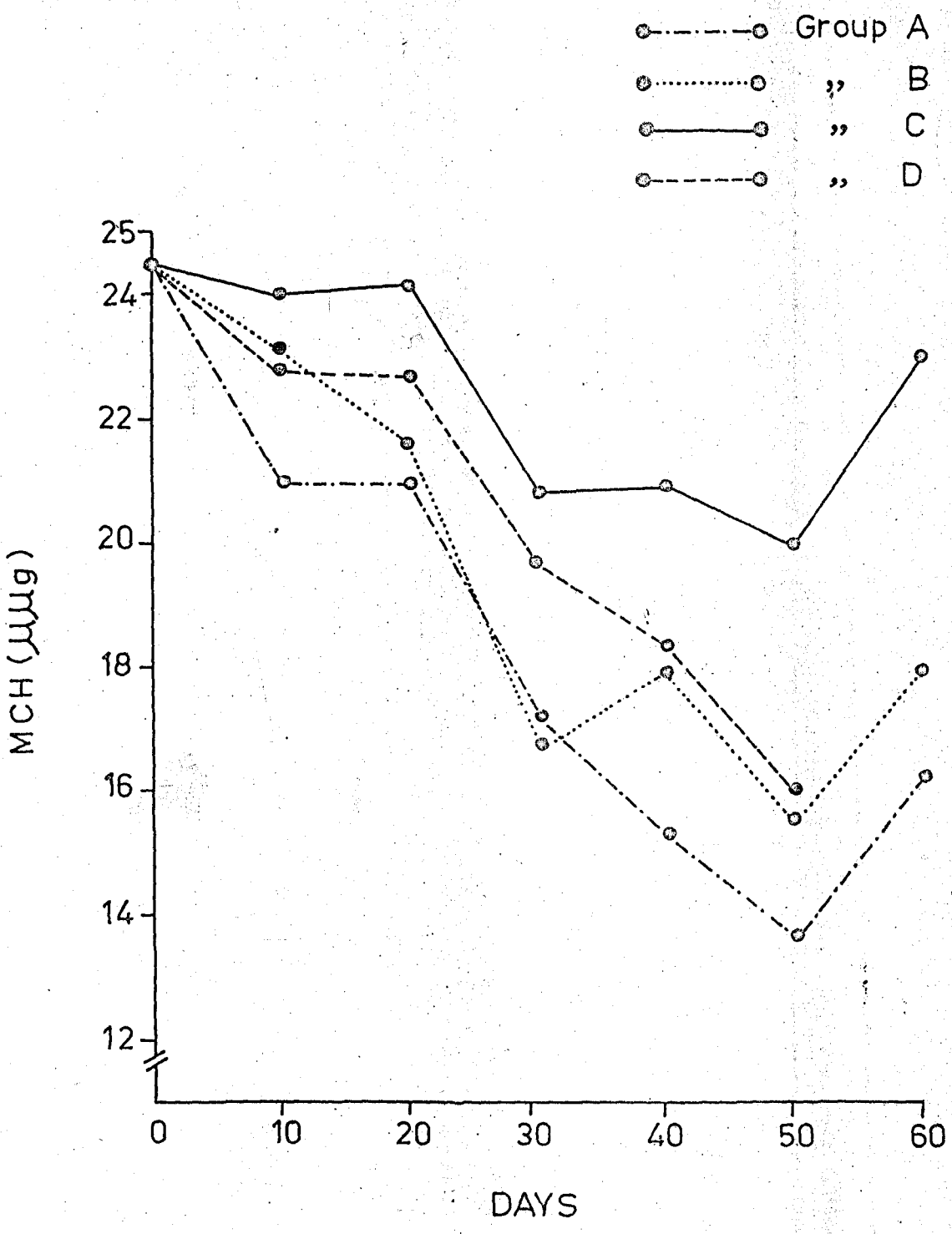

FIG. 17. MEAN CORPUSCULAR HAEMOGLOBIN (μμg) OF GUINEA-PIGS IN DIFFERENT GROUPS AT VARIOUS INTERVALS

TABLE 10

Mean corpuscular haemoglobin concentration in guinea-pigs of different experimental groups

Group	MCHC (%) at various intervals (days)							Overall mean
	0	10	20	30	40	50	60	
A (Organic Se toxicity)	30.38 ±0.78	27.97 ±0.43	31.32 ±2.21	30.16 ±2.14	29.07 ±1.14	28.99 ±0.63	29.05 ±0.01	29.56
B (Inorganic Se toxicity)	30.38 ±0.78	31.07 ±0.28	31.93 ±1.91	26.84 ±4.36	30.66 ±0.43	31.28 ±0.38	31.80 ±0.07	30.57
C (Control)	30.38 ±0.78	31.17 ±0.23	30.18 ±0.64	29.18 ±1.90	29.25 ±1.91	31.14 ±1.39	32.39 ±1.03	30.53
D (Treated)	30.38 ±0.78	29.56 ±1.01	29.53 ±1.03	28.62 ±0.18	31.29 ±4.77	28.69 ±0.84	NA	29.68

NA = None available

TABLE 11

Erythrocytic indices* of guinea-pigs in different experimental groups

Group	Group mean					
	PCV (%)	Hb (g/dl)	TEC (10^6/µl)	MCV (μ^3)	MCH (µµg)	MCHC (%)
A (Organic Se toxicity)	33.76	9.94	5.58	62.10	18.34	29.56
B (Inorganic Se toxicity)	33.40	10.49	5.36	63.91	19.56	30.57
C (Control)	38.24	11.64	5.24	73.60	22.43	30.53
D (Treated)	36.64	10.80	5.35	69.77	20.59	29.68

*Average of the values observed at various intervals

TABLE 12

Total leucocyte count ($10^3/\mu l$) in guinea-pigs of different experimental groups

Group	TLC (Mean ± SE) at various intervals (days)							Overall mean
	0	10	20	30	40	50	60	
A (Organic Se toxicity)	4.13 ±0.07	3.82 ±0.04	3.52 ±0.04	3.40 ±0.17	3.22 ±0.13	2.75 ±0.07	2.75 ±0.21	3.37[b]
B (Inorganic Se toxicity)	4.13 ±0.07	3.65 ±0.11	3.47 ±0.05	3.02 ±0.10	2.90 ±0.14	3.01 ±0.08	2.53 ±0.12	3.24[b]
C (Control)	4.13 ±0.07	4.00 ±0.02	3.88 ±0.08	3.57 ±0.10	3.33 ±0.05	3.38 ±0.09	3.17 ±0.10	3.64[a]
D (Treated)	4.13 ±0.07	3.77 ±0.11	3.90 ±0.05	3.67 ±0.18	3.03 ±0.09	3.13 ±0.02	NA	3.61[a]

NA = None available

Figures having superscript in common in a column do not differ significantly at 1 per cent level of probability ($P < 0.01$)

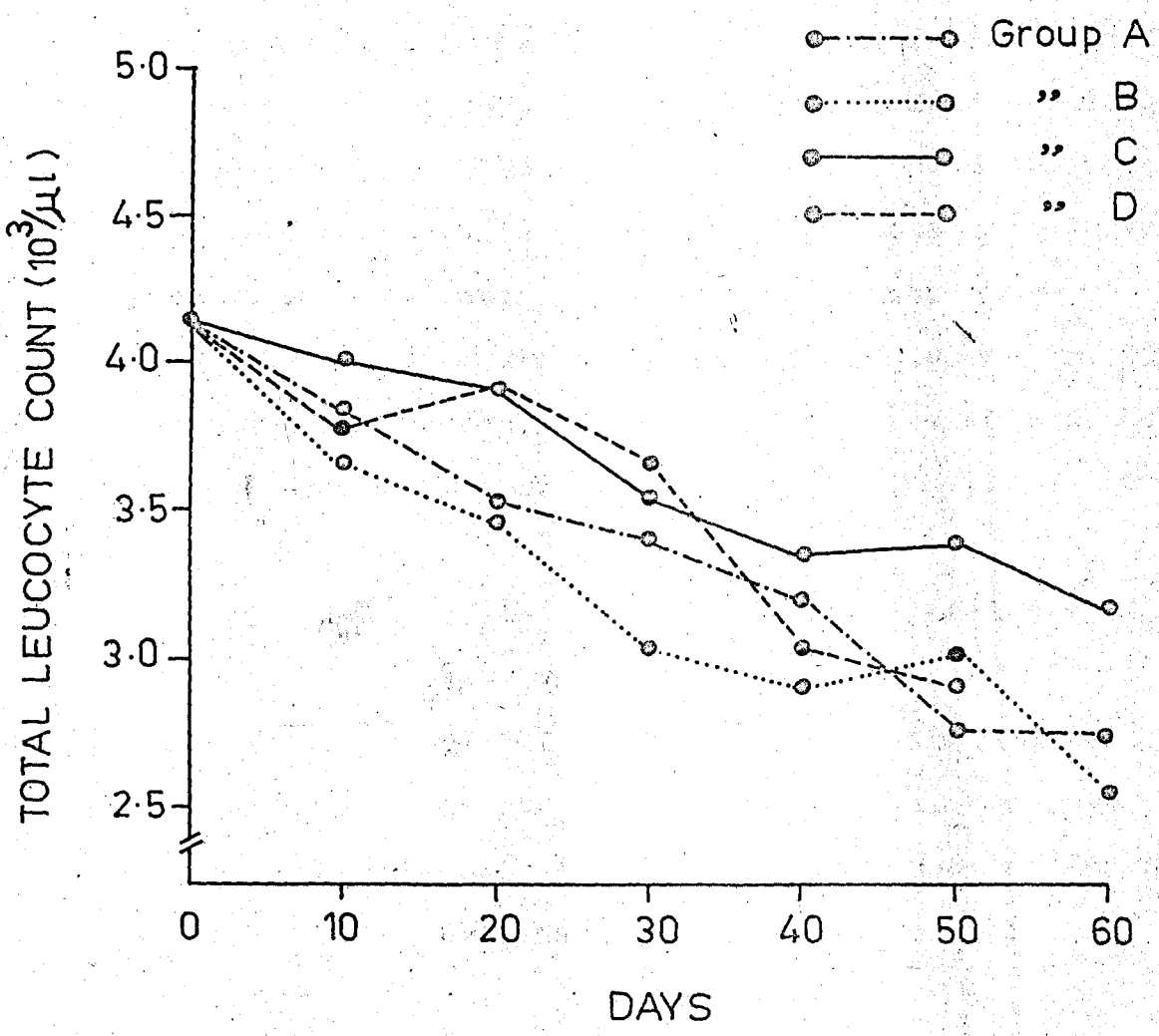

FIG. 18. MEAN TOTAL LEUCOCYTE COUNT ($10^3/\mu l$) OF GUINEA-PIGS IN DIFFERENT GROUPS AT VARIOUS INTERVALS

Differential leucocyte count (DLC) The mean per cent values of different leucocytes in the blood of guinea-pigs in different experimental groups at various intervals are presented in Table 13. A comparison of these values in different groups revealed a significant increase ($P < 0.01$) in the percentage of neutrophils with a corresponding decrease in lymphocytes in group A. A similar increase in the percentage of neutrophils was also noticed in group B and D but it was not associated with significant decrease in lymphocytes in comparison to control (group C). The percentage of eosinophils was also higher in group A in comparison to the control group. However, no significant difference was observed among the groups in regard to per cent monocyte and basophil counts.

Absolute leucocyte values The estimation of absolute neutrophil and lymphocyte counts (Tables 14 and 15, Fig.19) derived from TLC and DLC values in different experimental groups revealed that the leucopenia in group A was the result of lymphopenia whereas, in group B it was due to decrease in the number of lymphocytes as well as neutrophils.

Biochemical Studies

Blood glucose The average blood glucose concentration observed in different experimental groups at various intervals is given in Table 16 and Fig.20. A significant ($P < 0.01$) drop in blood glucose level, in comparison to controls, was observed in group A and B which continued upto the end of the experiment (60 days). Although a significant drop was noticed

TABLE 13

Differential leucocyte count (per cent) in guinea-pigs of different experimental groups

Group		DLC (Mean ± SE) at various intervals (days)							Overall mean
		0	10	20	30	40	50	60	
1		2	3	4	5	6	7	8	9
	N	41.67 ±0.98	47.67 ±2.60	43.67 ±2.60	49.33 ±0.27	58.67 ±4.84	55.00 ±0.00	53.50 ±3.18	49.93[a]
	L	49.00 ±0.94	40.00 ±3.77	44.00 ±1.89	41.00 ±0.94	32.33 ±4.53	34.00 ±1.41	37.00 ±2.12	39.62[q]
A (Organic Se toxicity)	M	6.00 ±0.47	5.00 ±0.47	8.33 ±1.66	5.33 ±0.27	4.67 ±0.27	6.50 ±0.35	6.50 ±0.35	6.05
	E	3.00 ±0.47	7.00 ±0.82	4.00 ±0.94	4.33 ±0.72	4.33 ±0.27	4.50 ±1.06	3.00 ±0.71	4.31[x]
	B	0.33 ±0.27	0.33 ±0.27	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.09
	N	41.67 ±0.98	34.67 ±4.65	38.67 ±0.54	39.67 ±5.17	48.33 ±3.07	50.00 ±0.71	51.00 ±0.00	43.43[b]
	L	49.00 ±0.94	54.00 ±4.71	55.00 ±1.41	53.00 ±5.35	43.00 ±1.89	39.50 ±1.06	42.50 ±0.35	48.00[p]
B (Inorganic Se toxicity)	M	6.00 ±0.47	6.00 ±1.41	4.00 ±1.63	4.67 ±0.72	5.00 ±0.94	4.50 ±1.06	4.00 ±0.00	4.88
	E	3.00 ±0.47	5.00 ±0.47	2.33 ±0.27	2.67 ±0.27	3.67 ±0.27	6.00 ±0.71	2.50 ±0.35	3.60[y]
	B	0.33 ±0.27	0.33 ±0.27	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.09

Contd.....

1		2	3	4	5	6	7	8	9
C (Control)	N	41.67 ±0.98	39.33 ±1.09	43.67 ±2.13	41.00 ±3.68	42.33 ±3.93	39.67 ±0.72	37.33 ±1.44	40.71^{c}
	L	49.00 ±0.94	50.00 ±1.25	49.00 ±2.49	51.67 ±3.54	48.00 ±3.27	52.67 ±0.54	54.33 ±2.13	50.67^{p}
	M	6.00 ±0.47	6.67 ±0.27	4.33 ±0.98	4.00 ±0.94	5.33 ±0.54	4.67 ±0.54	6.00 ±0.47	5.29
	E	3.00 ±0.47	3.67 ±0.72	2.67 ±0.27	3.37 ±0.72	4.33 ±0.54	3.00 ±0.47	2.33 ±1.73	3.19^{y}
	B	0.33 ±0.27	0.33 ±0.27	0.33 ±0.27	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.14
D (Treated)	N	41.67 ±0.98	37.00 ±3.68	40.00 ±0.94	45.33 ±7.46	48.00 ±3.54	50.00 ±5.66	NA	43.67^{b}
	L	49.00 ±0.94	51.33 ±2.37	52.00 ±1.70	47.00 ±7.59	46.50 ±3.18	42.50 ±6.01	NA	48.06^{p}
	M	6.00 ±0.47	6.00 ±0.94	5.00 ±0.47	3.67 ±0.27	3.50 ±0.35	4.00 ±0.71	NA	4.70
	E	3.00 ±0.47	5.67 ±0.98	3.00 ±0.47	4.00 ±0.94	2.00 ±0.00	3.00 ±0.71	NA	3.45^{y}
	B	0.33 ±0.27	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.33 ±0.27	NA	0.09

NA = None available

Figures with superscript in common in a column do not differ significantly at 1 per cent level of probability ($P < 0.01$)

TABLE 14

Absolute neutrophil counts in guinea-pigs at various intervals of the experiment

Group	Neutrophil count (Mean ± SE) per µl of blood at various intervals (days)							Overall mean
	0	10	20	30	40	50	60	
A (Organic Se toxicity)	1718.10 ± 30.58	1820.33 ±105.60	1534.83 ± 87.31	1391.67 ± 57.63	1367.67 ± 72.33	1512.25 ± 39.07	1484.75 ±201.00	1618.51
B (Inorganic Se toxicity)	1718.10 ± 30.58	1280.00 ±211.55	1340.00 ± 19.69	1205.83 ±187.62	1394.67 ± 76.33	1506.10 ± 60.18	1287.75 ± 63.11	1390.28
C (Control)	1718.10 ± 30.58	1573.33 ± 44.46	1693.17 ± 73.90	1464.50 ±143.49	1667.00 ± 91.37	1342.67 ± 44.02	1184.00 ± 68.06	1520.40
D (Treated)	1718.10 ± 30.58	1401.33 ±170.66	1560.67 ± 49.53	1671.17 ±311.39	1445.75 ± 64.52	1560.50 ±167.94	NA	1559.59

NA = None available

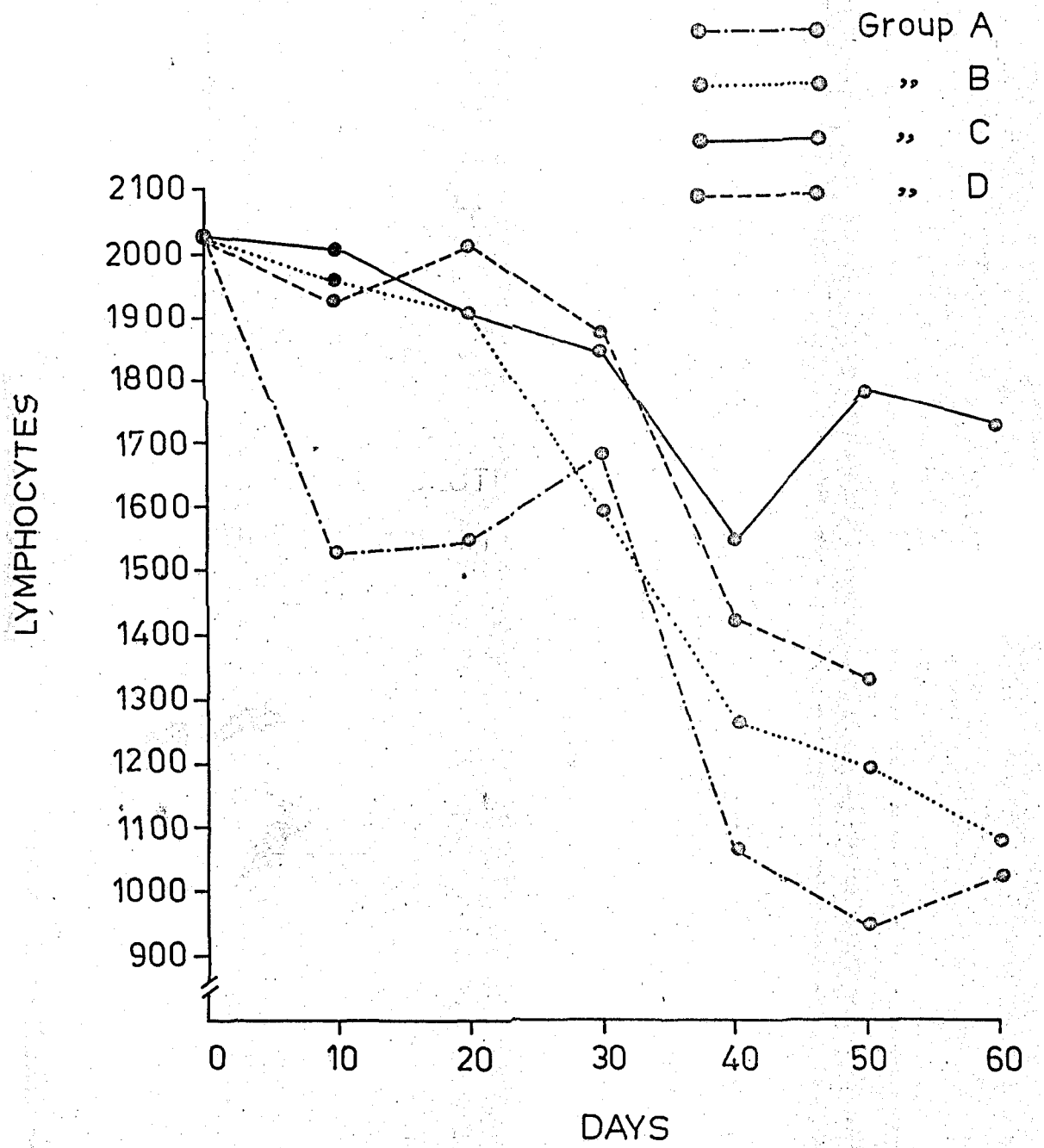

FIG. 19. MEAN ABSOLUTE LYMPHOCYTE COUNTS OF GUINEA-PIGS IN DIFFERENT GROUPS AT VARIOUS INTERVALS

TABLE 15

Absolute lymphocyte counts in guinea-pigs at various intervals of the experiment

Group	Lymphocyte count (Mean ± SE)/µl of blood at different intervals (days)							Overall mean
	0	10	20	30	40	50	60	
A (Organic Se toxicity)	2023.73 ± 71.11	1525.33 ±138.37	1546.00 ± 57.71	1678.50 ± 89.83	1058.17 ±184.86	933.00 ± 14.85	1008.50 ± 20.15	1396.18[c]
B (Inorganic Se toxicity)	2023.73 ± 71.11	1956.00 ±116.07	1904.67 ± 27.49	1588.00 ±137.49	1251.00 ± 96.80	1187.30 ± 12.02	1072.25 ± 43.66	1568.99[b]
C (Control)	2023.73 ± 71.11	2000.67 ± 59.65	1903.50 ±110.86	1841.33 ±130.87	1538.33 ±102.97	1781.33 ± 42.90	1718.67 ± 70.59	1801.08[a]
D (Treated)	2023.73 ± 71.11	1926.33 ± 45.72	2026.33 ± 53.27	1868.33 ±350.84	1412.25 ±998.62	1330.25 ±195.34	NA	1764.54[a]

NA = None available

Figures having superscript in common do not differ significantly at 1 per cent level of probability ($P < 0.01$)

TABLE 16

Mean blood glucose concentration (mg/dl) in guinea-pigs at various intervals of experimental feeding

Group	Blood glucose level (Mean ± SE) at different intervals (days)							Overall mean
	0	10	20	30	40	50	60	
A (Organic Se toxicity)	95.33 ±1.09	81.33 ±8.27	60.67 ±2.37	61.67 ±4.75	59.33 ±2.72	59.50 ±2.48	57.00 ±2.12	67.83[c]
B (Inorganic Se toxicity)	95.33 ±1.09	84.00 ±3.27	61.67 ±2.42	56.00 ±2.94	55.00 ±2.16	57.00 ±2.12	61.00 ±3.54	67.14[c]
C (Control)	95.33 ±1.09	93.33 ±2.37	79.00 ±3.56	77.33 ±1.09	75.67 ±2.13	80.00 ±5.74	74.00 ±2.45	82.09[a]
D (Treated)	95.33 ±1.09	81.67 ±5.46	66.67 ±1.91	65.33 ±6.06	71.00 ±3.54	67.00 ±2.83	NA	74.50[b]

NA = None available

Figures having superscript in common in a column do not differ significantly at 1 per cent level of probability ($P < 0.01$)

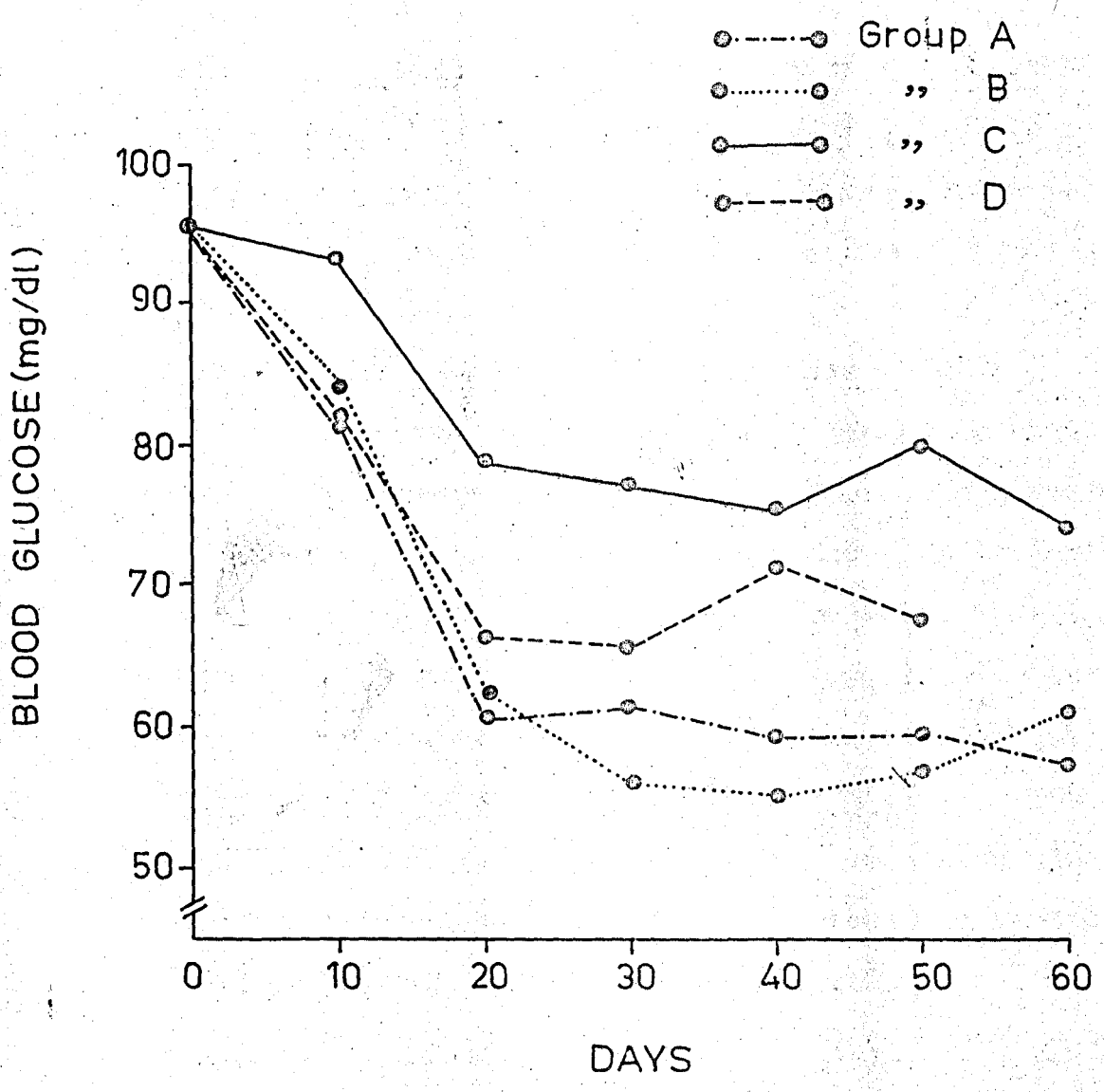

FIG. 20. MEAN BLOOD GLUCOSE (mg/dl) OF GUINEA-PIGS IN DIFFERENT GROUPS AT VARIOUS INTERVALS

in the blood glucose level of group D animals, in comparison to controls, but the decrease was significantly lower than that observed in group A and B.

Total serum proteins Estimation of total serum proteins at different intervals (Table 17 and Fig.21) revealed a significant drop in all the three experimental groups as compared to the control; the value beings lowest in group B followed by A and D.

Serum glutamic oxalacetic transaminase (SGOT) The estimation of SGOT activity in different groups at various intervals of the experiment (Table 18 and Fig.22) revealed a significant decrease in SGOT in group A and B in comparison to group C (control) animals; the decrease being slightly more in inorganic selenium toxicity. The decrease in SGOT activity observed in the treated (D) group was, however, insignificant.

Serum glutamic pyruvic transaminase (SGPT) The mean SGPT activity in all the three groups fed on barley containing high selenium content was slightly lower than the controls (Table 19) but the difference was not significant.

Erythrocytic glutathione peroxidase activity (GSH-PX) The mean value of erythrocytic glutathione peroxidase (GSH-PX) activity observed in different groups at various intervals is given in Table 20 and Fig.23. A reference to this table shows that there was a highly significant ($P < 0.01$) increase in GSH-PX activity in guinea-pigs fed on selenium-enriched barley (group A) as well as barley mixed with sodium selenite (group B), as compared to controls (group C). A slight but insignificant increase in GSH-PX activity was also noticed in

TABLE 17

Total serum proteins (g/dl) in guinea-pigs at various intervals of experiment

Group	Protein concentration (Mean±SE) at different intervals (days)							Overall mean
	0	10	20	30	40	50	60	
A (Organic Se toxicity)	6.32 ±0.05	6.20 ±0.19	5.04 ±0.12	5.00 ±0.70	4.31 ±0.17	5.54 ±0.08	5.14 ±0.17	5.36^{bc}
B (Inorganic Se toxicity)	6.32 ±0.05	6.13 ±0.18	4.60 ±0.16	4.70 ±0.13	4.65 ±0.10	4.72 ±0.22	4.99 ±0.27	5.16^{c}
C (Control)	6.32 ±0.05	6.45 ±0.10	6.66 ±0.10	6.19 ±0.39	5.94 ±0.10	6.55 ±0.11	6.04 ±0.36	6.31^{a}
D (Treated)	6.32 ±0.05	6.25 ±0.09	5.22 ±0.17	6.18 ±0.20	4.79 ±0.13	5.20 ±0.15	NA	5.66^{b}

NA = None available

Figures having superscripts in common do not differ significantly at 1 per cent level of probability ($P < 0.01$)

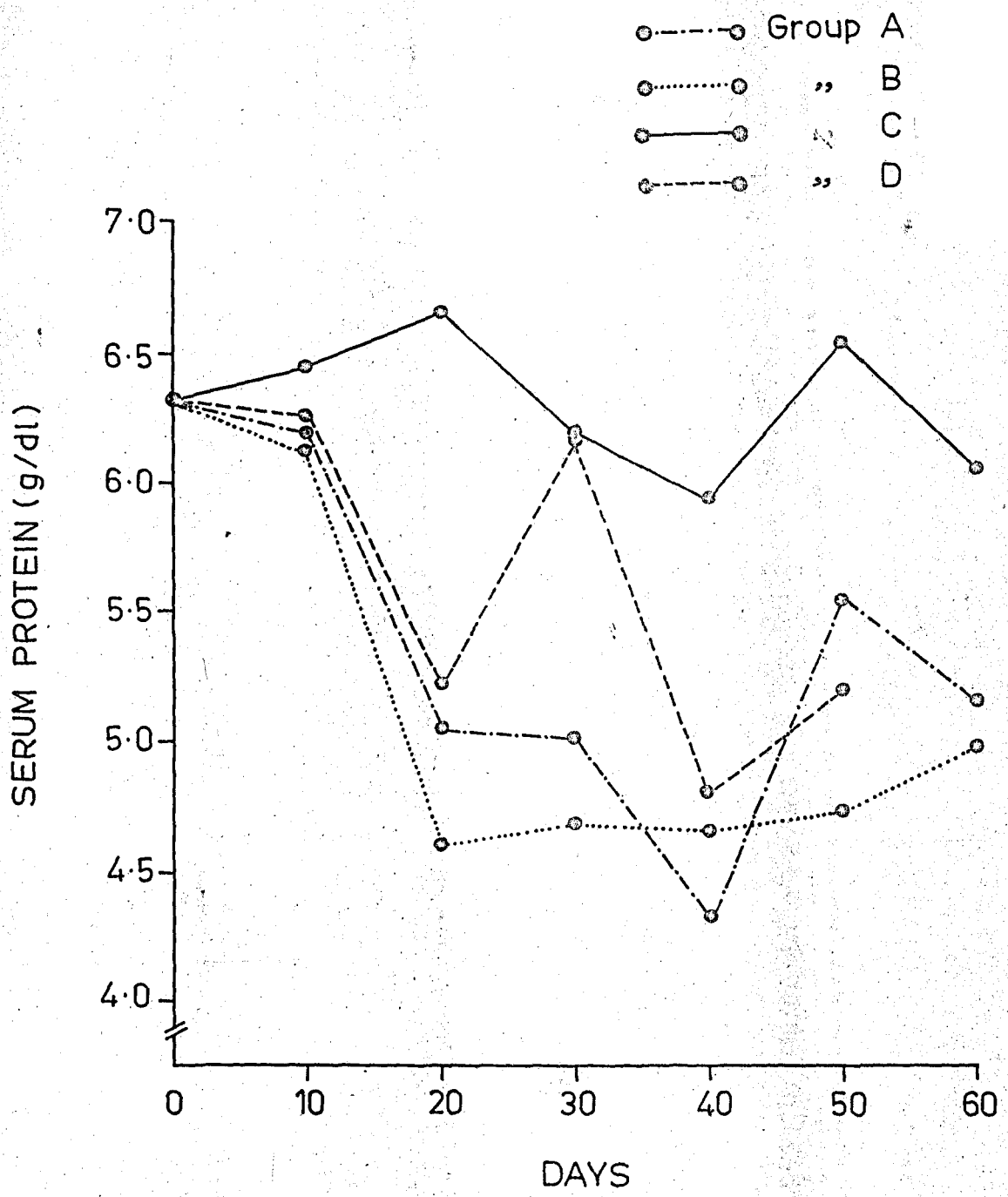

FIG. 21. MEAN TOTAL SERUM PROTEIN (g/dl) OF GUINEA-PIGS IN DIFFERENT GROUPS AT VARIOUS INTERVALS

TABLE 18

Serum glutamic oxalacetic transaminase activity (IU/ml) in guinea-pigs at various intervals of experiment

Group	SGOT activity (Mean ± SE) at different intervals (days)							Overall mean
	0	10	20	30	40	50	60	
A (Organic Se toxicity)	33.92 ±1.83	33.92 ±2.65	42.24 ±2.07	44.16 ±3.14	45.20 ±1.36	48.96 ±2.04	53.28 ±3.06	43.10[b]
B (Inorganic Se toxicity)	33.92 ±1.83	29.12 ±2.32	41.60 ±10.96	56.32 ±4.88	40.32 ±1.36	40.32 ±2.04	40.80 ±7.81	40.34[b]
C (Control)	33.92 ±1.83	38.40 ±0.78	49.89 ±6.10	51.20 ±1.83	53.76 ±7.28	61.12 ±2.87	56.17 ±3.72	49.21[a]
D (Treated)	33.92 ±1.83	33.60 ±4.36	48.00 ±6.70	51.84 ±7.41	48.96 ±2.04	52.66 ±8.94	NA	44.83[ab]

NA = None available

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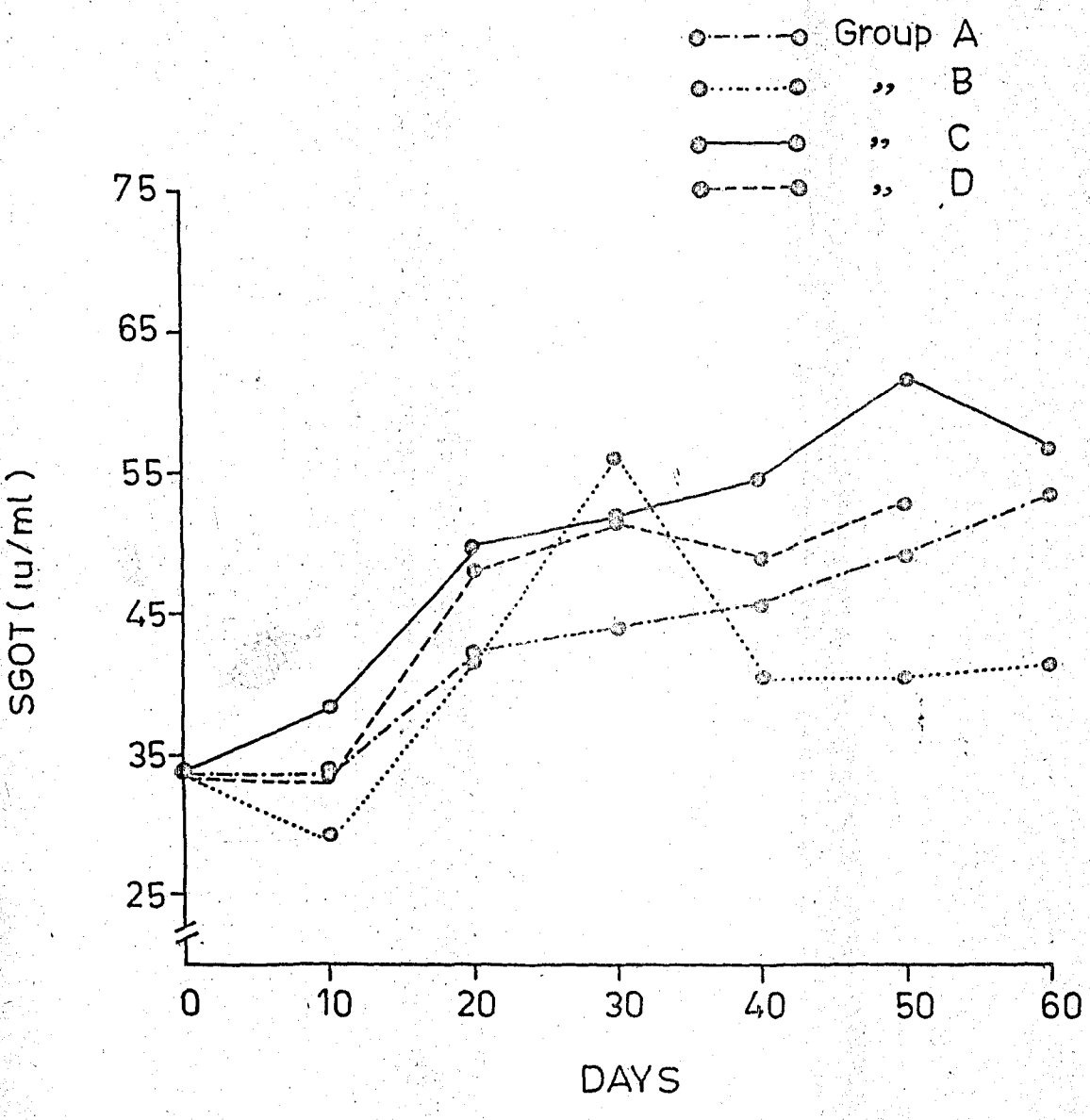


FIG. 22. MEAN SERUM GLUTAMIC OXALACETIC TRANSAMINASE (iu/ml) OF GUINEA-PIGS IN DIFFERENT GROUPS AT VARIOUS INTERVALS

TABLE 19

Serum glutamic pyruvic transaminase activity (IU/ml) in guinea-pigs at various intervals of the experiment

Group	SGPT (Mean ± SE) at different intervals (days)							Overall mean
	0	10	20	30	40	50	60	
A (Organic Se toxicity)	18.72 ±0.78	19.52 ±0.57	29.12 ±1.95	30.88 ±5.22	24.64 ±1.52	21.12 ±5.09	20.40 ±0.51	23.49
B (Inorganic Se toxicity)	18.72 ±0.78	15.68 ±2.10	22.56 ±9.86	31.20 ±3.06	25.76 ±0.52	26.40 ±3.73	25.68 ±0.85	23.71
C (Control)	18.72 ±0.78	20.32 ±0.91	26.56 ±3.28	37.12 ±2.49	27.04 ±0.69	30.40 ±2.10	27.52 ±1.76	26.81
D (Treated)	18.72 ±0.78	16.16 ±1.59	15.52 ±4.49	36.00 ±3.33	22.56 ±1.36	35.52 ±1.36	NA	24.08

NA = None available

TABLE 20

Erythrocytic glutathione peroxidase activity (EU/mg Hb) in guinea-pigs of different experimental groups

Group	EGSH-PX activity (Mean ± SE) at different intervals (days)							Overall mean
	0	10	20	30	40	50	60	
A (Organic Se toxicity)	1.91 ±0.50	3.70 ±0.67	12.42 ±5.82	17.69 ±4.16	20.97 ±3.11	19.61 ±1.22	18.36 ±7.03	13.52[b]
B (Inorganic Se toxicity)	1.91 ±0.50	6.20 ±1.41	28.50 ±4.88	33.25 ±0.73	22.24 ±7.39	20.67 ±0.47	20.34 ±0.04	19.02[a]
C (Control)	1.91 ±0.50	2.25 ±0.22	8.63 ±2.90	7.38 ±2.56	10.79 ±5.57	7.90 ±1.92	5.38 ±0.43	6.32[c]
D (Treated)	1.91 ±0.50	3.15 ±1.44	5.96 ±0.53	14.26 ±5.55	15.49 ±6.23	8.82 ±4.14	NA	8.27[c]

NA = None available

Figures having superscripts in common in a column do not differ significantly at 1 per cent level of probability ($P < 0.01$)

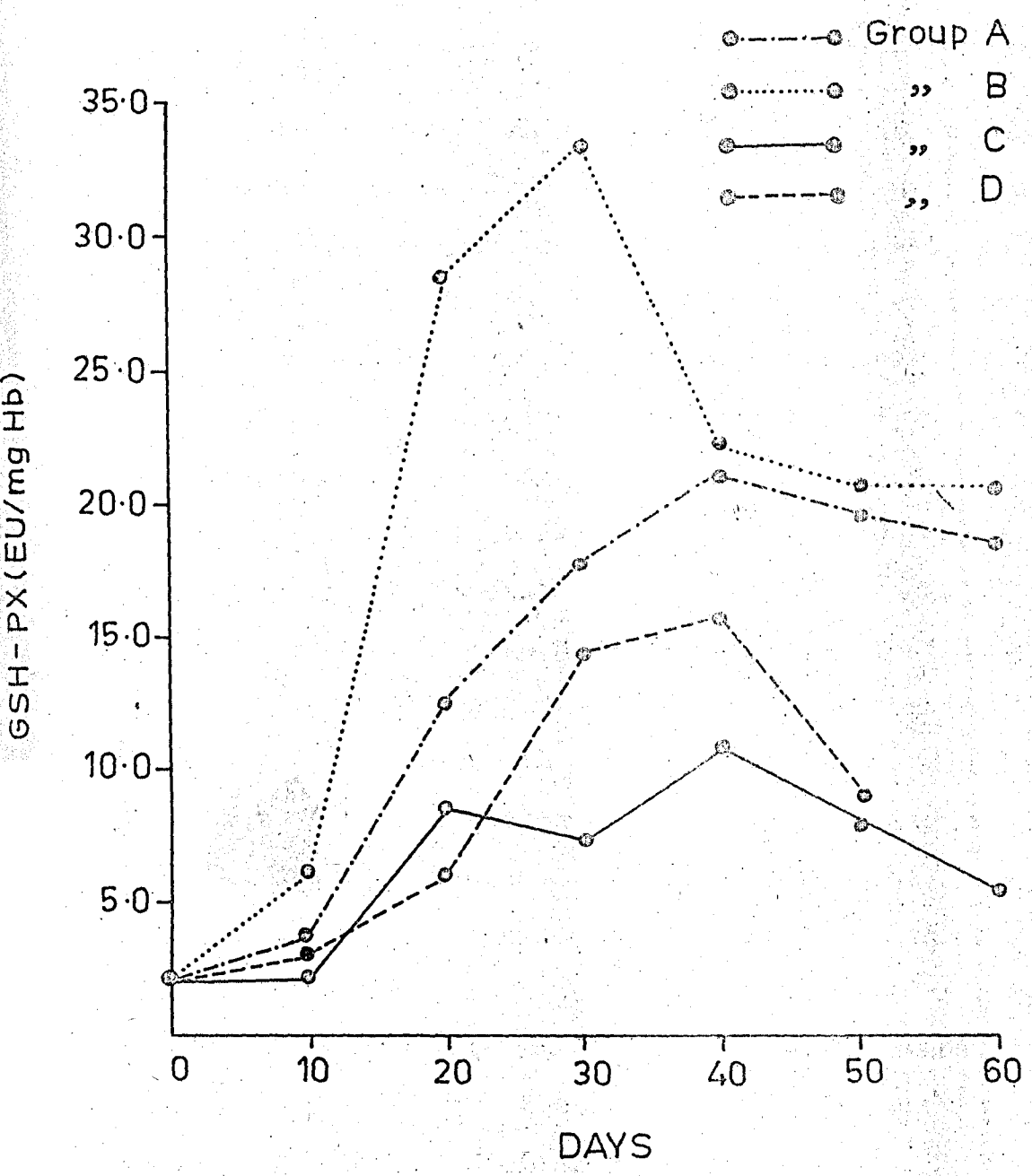


FIG. 23. MEAN ERYTHROCYTIC GLUTATHIONE PEROXIDASE ACTIVITY (EU/mg Hb) OF GUINEA-PIGS IN DIFFERENT GROUPS AT VARIOUS INTERVALS

the treated group (group D).

Estimation of Selenium

The results of selenium estimations in various tissues like liver, kidney, spleen and hair from animals in different groups at various intervals of the experiment are presented in Table 21 and Fig.24. A highly marked elevation in the selenium concentration, in comparison to controls (group C), was noticed in all the four tissues tested; the values being highest in group B followed by that in group A and D. Among the different tissues examined, the maximum concentration was detected in hair wherein a continuous increase was noticed throughout the experiment. The selenium concentration of hair was highest in group B followed by that in group A and D as compared to group C. In liver, the selenium concentration was found to increase continuously in group A and D, whereas, a slight decrease was noticed after an initial increase at 20 DPE in group B. The selenium concentration in kidney was maximum at 40 DPE in group A and 20 DPE in group B. Thereafter, it had a decreasing trend. The selenium content of kidney was always found higher than that of liver in both organic and inorganic forms of selenium toxicity.

Pathology

Gross lesions

None of the guinea-pigs killed from the control group (group C) revealed any gross lesion at any interval/stage of the experiment. In the remaining groups, slight variations

TABLE 21

Average selenium content in various tissues of guinea-pigs in different experimental groups

Group		Selenium concentration (ppm) at different intervals (days)				Overall mean
		0 day	20 day	40 day	60 day	
A (Organic Se toxicity)	Liver	3.00	19.40	23.00	37.00	20.60
	Kidney	3.55	40.00	59.60	47.40	37.64
	Spleen	0.45	13.40	13.80	16.60	11.06
	Hair	0.45	9.20	40.00	60.60	27.56
B (Inorganic Se toxicity)	Liver	3.00	50.40	47.40	44.60	36.35
	Kidney	3.55	73.60	69.00	50.40	49.14
	Spleen	0.45	21.60	15.60	19.80	14.36
	Hair	0.45	19.40	73.60	83.04	44.12
C (Control)	Liver	3.00	7.44	7.92	5.76	6.03
	Kidney	3.55	7.76	7.76	6.96	6.51
	Spleen	0.45	4.40	3.84	3.68	3.09
	Hair	0.45	2.48	7.72	7.92	4.64
D (Treated)	Liver	3.00	15.00	40.00	NA	19.33
	Kidney	3.55	14.50	50.40		22.82
	Spleen	0.45	5.00	14.40		6.62
	Hair	0.45	3.00	44.60		16.02

NA = None available

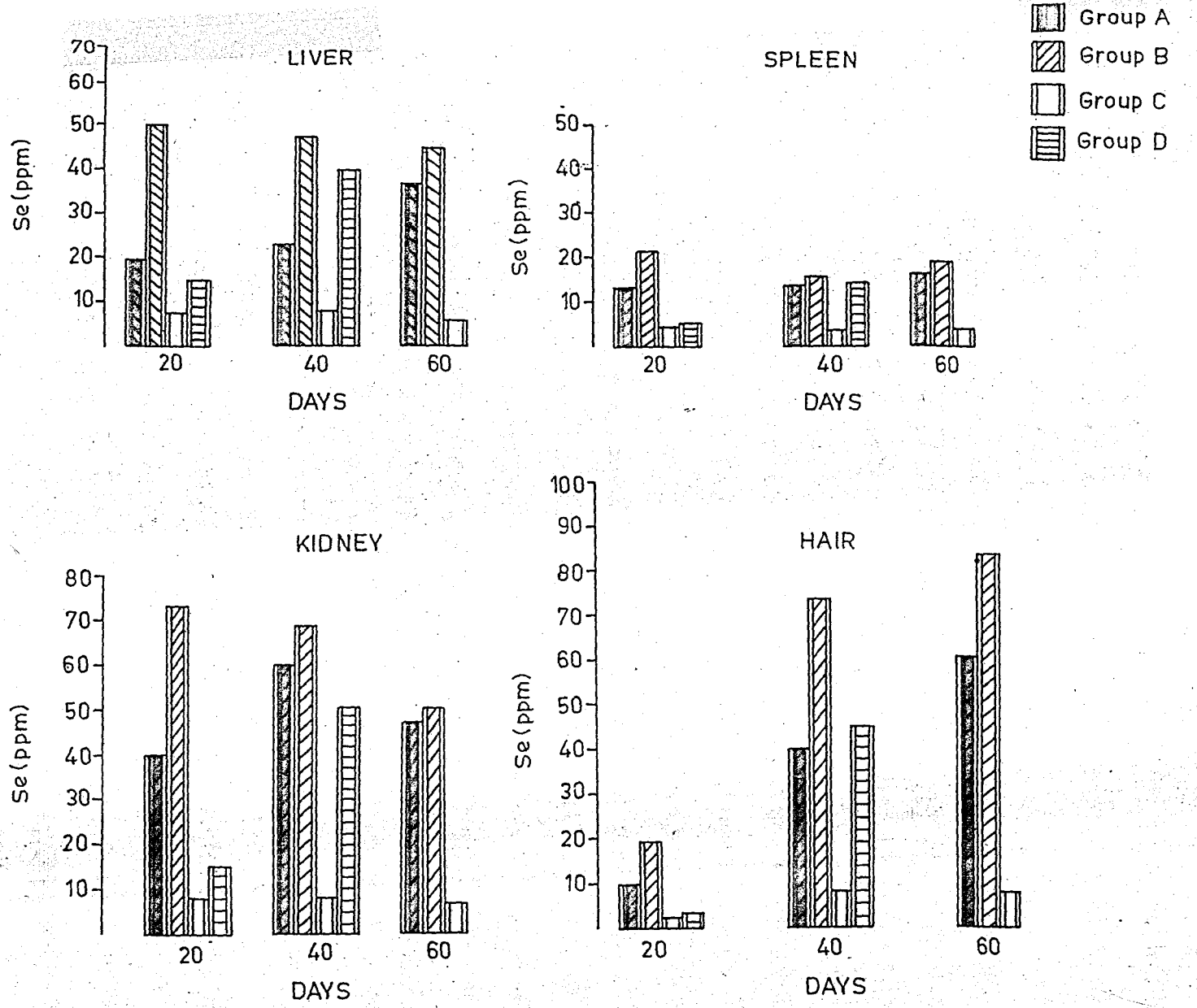


FIG. 24. SELENIUM CONTENT (ppm) IN DIFFERENT TISSUES OF GUINEA-PIGS IN DIFFERENT GROUPS AT VARIOUS INTERVALS

were observed in the gross lesions between animals of different experimental groups as well as between the animals which died during the course of experiment and those which were killed.

Naturally dead animals

Group A A total of 22 guinea-pigs died during the course of 60 days of feeding on selenium-enriched barley. The mortality started from 14 DPE and continued upto 50 DPE. The carcases of majority of these animals were anaemic and emaciated. The gross changes (Fig.25) included congestion, emphysema and focal haemorrhages in the lungs; swollen and congested liver with diffuse whitish necrotic foci; congestion, oedema and haemorrhages in the stomach and small intestines; dark tan coloured spleen which was somewhat swollen in the early stage; congestion of heart and kidneys coupled with petechial haemorrhages in a few cases. The gall bladder and the urinary bladder were distended in almost all cases and petechial haemorrhages were observed in the mucosa of urinary bladder. A mild to moderate degree of ascites was also noted in some cases.

With the advancement of feeding period i.e. 30 days onwards, the lungs became grayish-red and leathery in consistency. The colour of the liver changed to dark brown with small shallow pits on the parietal surface in some cases. Haemorrhage and congestion in the stomach and small intestines were less marked. The spleen was atrophied with a thick and wrinkled capsule. The heart was soft and flabby with petechial haemorrhages in the epicardium in few cases. Petechial to ecchymotic haemorrhages were seen in the cortical area of the

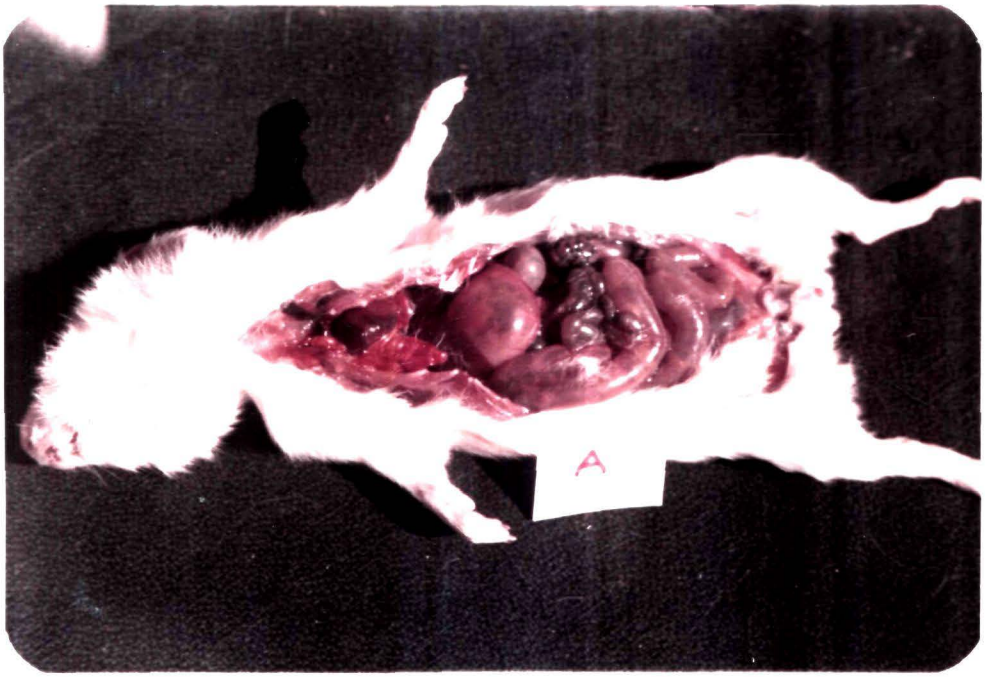
kidney. The adrenal glands were slightly enlarged reddish in colour and studded with few haemorrhages. The thymus was atrophied and the testes underdeveloped. No gross changes were observed in the skeletal muscles and skin. The meningeal and cerebral blood vessels were congested.

Group B In this group also, 22 animals died within 11 to 48 DPE. The gross changes, in general, were similar to that in group A, but the initiation was earlier and the vascular changes in the visceral organs were more prominent. The lungs showed severe congestion (Fig.26) and haemorrhages in animals which died before 30 DPE. Besides severe congestion and haemorrhages, erosion of the mucosa was observed in stomach and small intestines. Mild vascular changes were also noticed in large intestines in a few cases. Proctitis and protrusion of the rectum was observed in two cases. Haemorrhage and congestion in both the cortical and medullary portions of kidneys were common. Ascites was seen in more number of cases as compared to group A. Ecchymotic haemorrhages were noticed in the thigh muscles in three cases. The gross changes in animals which died after 30 days of experimental feeding were more or less similar to that in group A.

Group D Nine animals of this group died between 17 and 40 days of experimental feeding. The gross lesions observed were almost similar to that in group B, but the initiation of the lesions was late and the changes were comparatively less severe.

Fig.25 A naturally dead guinea-pig from group A(25 DPE) showing congested lungs, petechiae on heart,distended gall bladder and urinary bladder.

Fig.26 A naturally dead guinea-pig from group B(30 DPE) showing congested lungs and distended urinary bladder.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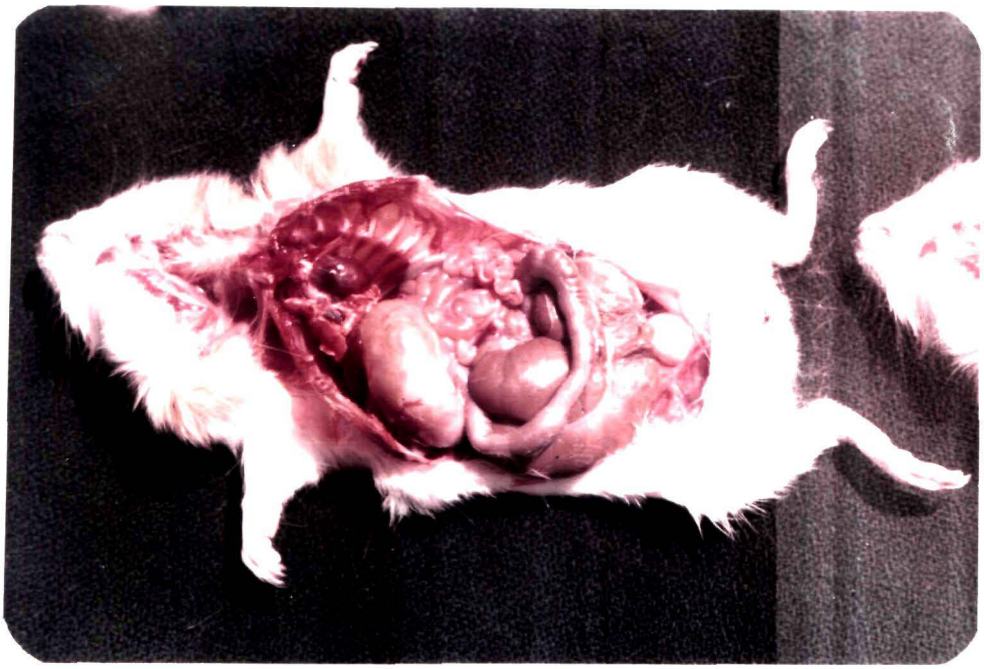


Killed Animals

The distribution and type of gross lesions observed in the killed animals of group A, B and D were, in general, similar with slight variation in severity at the early phase. The lesions as observed at different intervals are as follow:

0 DPE No change was detected in any organ of the animals killed at this stage.

10 DPE In group A, varying degree of congestion was observed in lungs, liver, stomach and intestines. A severe congestion coupled with haemorrhages was noticed in these organs in group B, whereas no significant alterations were recorded in animals of group D.

20 DPE The general condition of the animals was poor and anaemic at this stage and the lesions in the lungs, liver and gastro-intestinal tract were more severe than observed earlier. Enlargement of spleen, distension of gall and urinary bladder and congestion of kidneys were seen in some cases of groups A and B. However, the changes in the lungs, liver and gastro-intestinal tract in animals of group D resembled those of group B at 10 DPE.

30 DPE The animals killed on 30 DPE were much more anaemic and emaciated. Lungs were reddish-brown in colour and the liver was swollen, pale and studded with necrotic foci. The changes in the gastro-intestinal tract were, however, less severe. The spleen was atrophied, dark tan in colour with a thick and wrinkled capsule. The thymus and testis were smaller in size in comparison to controls. Haemorrhages

(petechial to ecchymotic) were observed in the kidneys. Animals of treated group (D) exhibited less severe lesions as compared to the toxicoses groups (A and B).

40, 50 and 60 DPE The animals killed at these intervals were in very poor condition and a straw-coloured oedematous fluid was seen in the peritoneal cavity of some of these cases. Hydropericardium was also observed in a few cases but petechial haemorrhages in the epicardium were seen in most of the animals. The heart was soft and flabby. A frothy exudate was observed in the bronchi and bronchioles. The brownish-coloured lungs were somewhat leathery in consistency and the pleura was slightly thickened in some of these cases. The wall of the urinary bladder was somewhat thickened and both the gall bladder and urinary bladder were always found distended. Thymus and spleen were atrophied and the gonads (testes) appeared underdeveloped in comparison to controls. Comparatively less vascular changes were observed in the stomach and intestines. Haemorrhages in the kidney were present at this stage also. The adrenal gland was enlarged in few cases but atrophied in others. Overall vascular changes at this stage were less marked in comparison to early stages.

Microscopic lesions

Naturally dead animals

Group A The microscopic changes in the lungs of animals which died on or before 30 DPE included acute dilatation of pulmonary capillaries and other blood vessels, serous exudation in the alveoli and intra-alveolar

haemorrhages (Figs. 27 and 28). The inter-alveolar septa were thickened due to capillary congestion and infiltration of mononuclear cells. A few of the mononuclear cells were also seen in the alveolar spaces. Some of the mononuclear cells (macrophages) contained haemosiderin which was confirmed by prussian blue reaction. There was hyperplasia of bronchial epithelium (Fig.29) and slight lymphocytic infiltration around the bronchioles. Focal aggregation of mononuclear cells was also noticed around the blood vessels and, in some cases, serous exudate was seen around the arterioles. As the condition progressed, the vascular changes like congestion and haemorrhages were reduced in intensity.

The liver showed dilation of the sinusoids and central veins associated with degenerative changes in the hepatocytes. Multiple foci of coagulative necrosis were seen in the liver parenchyma (Fig.30) which at places were infiltrated with polymorphonuclear cells. There was hyperplasia of bile duct epithelium and some of these cells were transformed into goblet type cells (Fig.31). With the advancement of condition, there was hyperplasia of Kupffer cells, alongwith mononuclear cell infiltration and increased amount of fibrous tissue in the portal area (Fig.32).

Microscopic changes in the heart included congestion of the epicardial and subepicardial tissues, congestion, inter-muscular haemorrhage and focal areas of hyaline degeneration in the myocardium. A mild mononuclear infiltration was also noticed in between the muscle fibres. In advanced

Fig.27 Severe congestion, alveolar oedema and mild mononuclear infiltration in the lung of a naturally dead guinea-pig (Group A, 23 DPE).

H & E x100

Fig.28 Severe congestion and intra-alveolar haemorrhage in the lung from a naturally dead guinea-pig (Group A, 28 DPE).

H & E x200

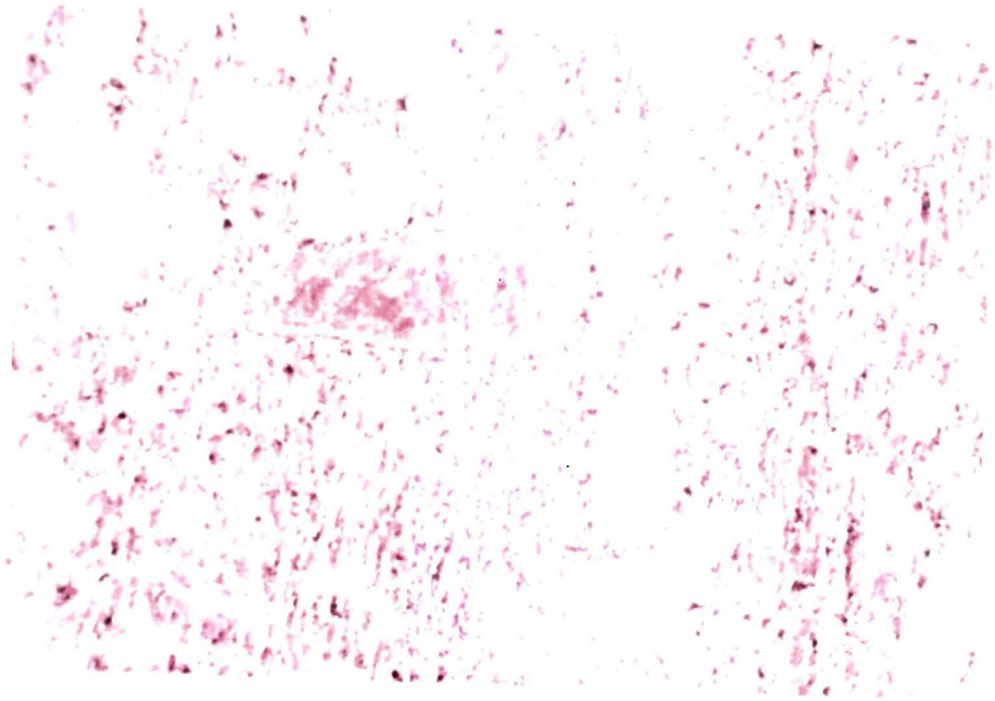


Fig.29 Congestion and hyperplasia of bronchial epithelium in a naturally dead guinea-pig from group A (46 DPE).

H & E x200

Fig.30 Congestion and focal areas of necroses in the liver of a naturally dead guinea-pig from group A(14 DPE).

H & E x100

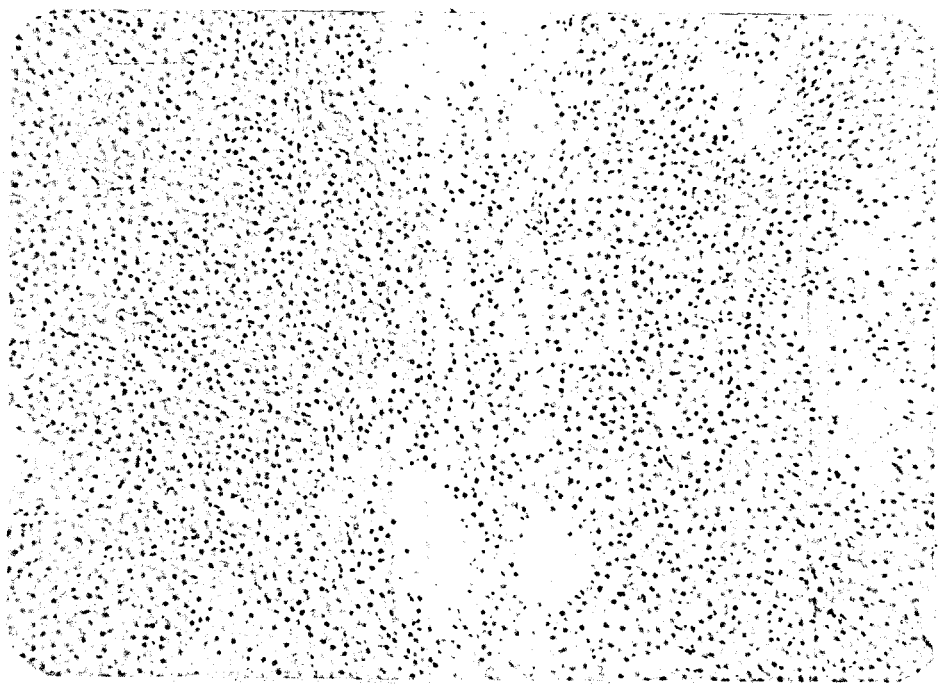
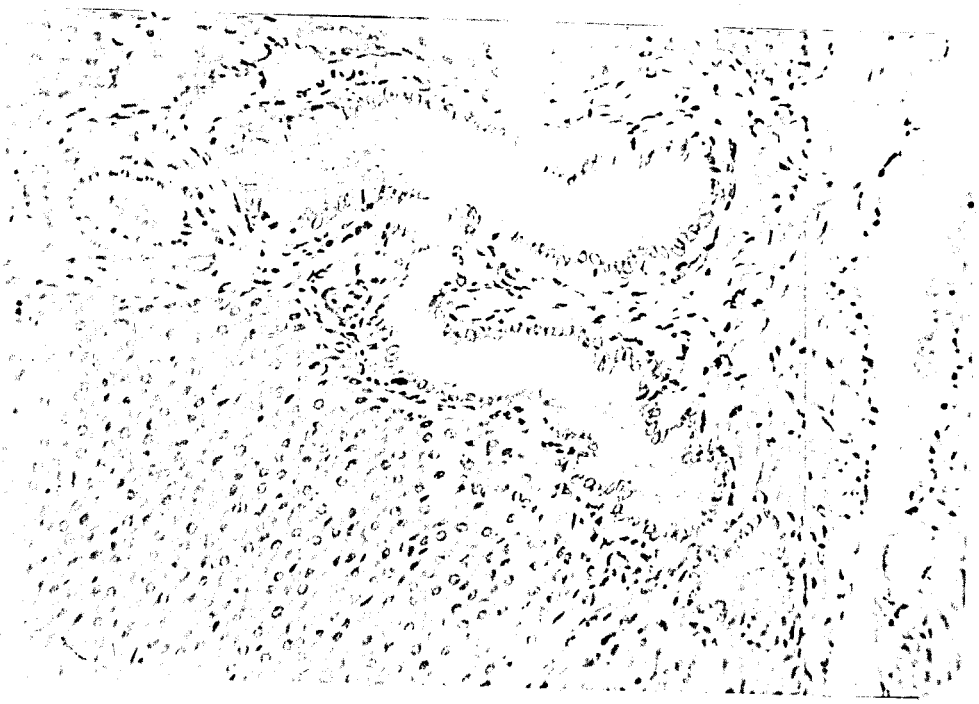


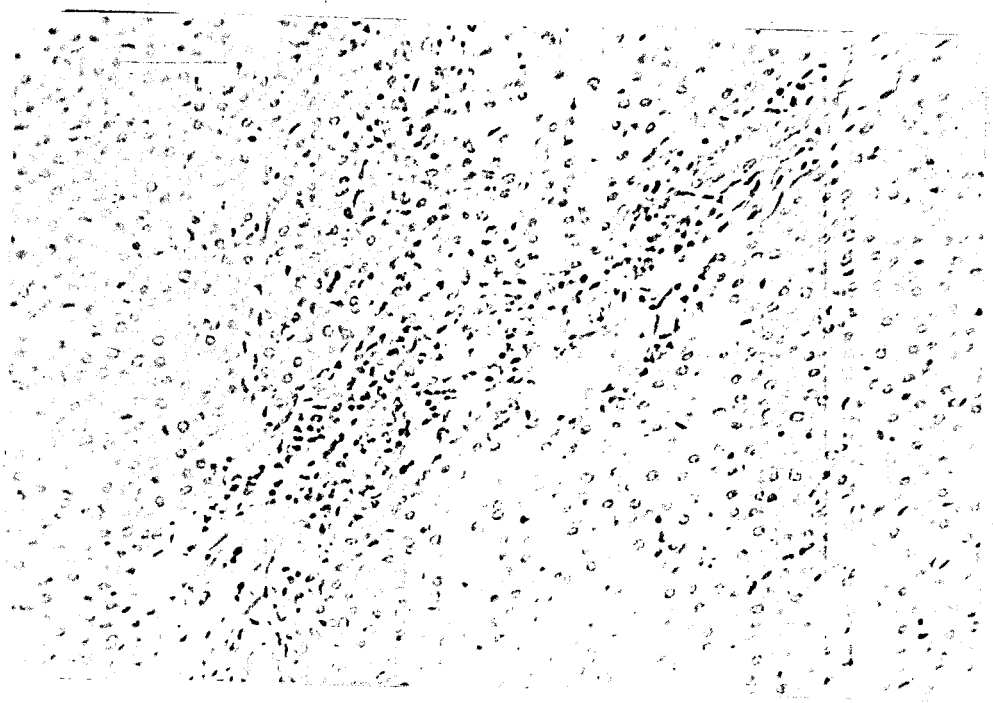
Fig.31 Bile duct hyperplasia with many goblet cells in the liver of a naturally dead guinea-pig from group A (22 DPE).

H & E x200

Fig.32 Mononuclear cell infiltration and fibroblastic proliferation in the portal area of liver from a naturally dead guinea-pig of group A (30 DPE).

H & E x200





cases, the necrotic areas were replaced by proliferating fibroblastic tissue (Fig.33).

The vascular changes in the kidney were not so prominent in early stages of the toxicity but later on congestion and varying degree of haemorrhages were observed in the inter-tubular tissue. These were accompanied by mild parenchymatous or hydropic changes in the tubular epithelium. With the advancement of toxicity, the haemorrhage and congestion became more severe (Fig.34) and involved both the cortex and medulla. The tubular epithelium, in some cases was necrotic. Proteinacious or cellular casts were noticed in some of the tubules. In some cases, focal aggregation of mononuclear cells was observed in the interstitial connective tissue.

The spleen revealed severe congestion and haemorrhage in early stages which was followed by depletion of lymphoid cells in the Malpighian corpuscles (Fig.35) and hyperplasia of reticulo-endothelial cells in later stages of toxicity. The fibromuscular capsule was thickened due to proliferation of fibroblasts and there was increase in the number and size of trabeculae (Fig.36). Haemosiderosis was a constant feature in the spleen of all these cases. Slight depletion of lymphoid tissue was also seen in the cortical portion of lymph nodes but medulla was congested and haemorrhagic.

Sections of the brain revealed congestion in the meningeal blood vessels and capillaries of the brain tissue in this group of animals.

Marked congestion was noticed in the capsular, sub-capsular, cortical and medullary vessels of the adrenal gland

Fig.33 Myocardial degeneration and fibrosis in the heart of a naturally dead guinea-pig from group A (48 DPE).

H & E x200

Fig.34 Congestion and intertubular haemorrhage in the kidney of a naturally dead guinea-pig from group A (48 DPE).

H & E x100

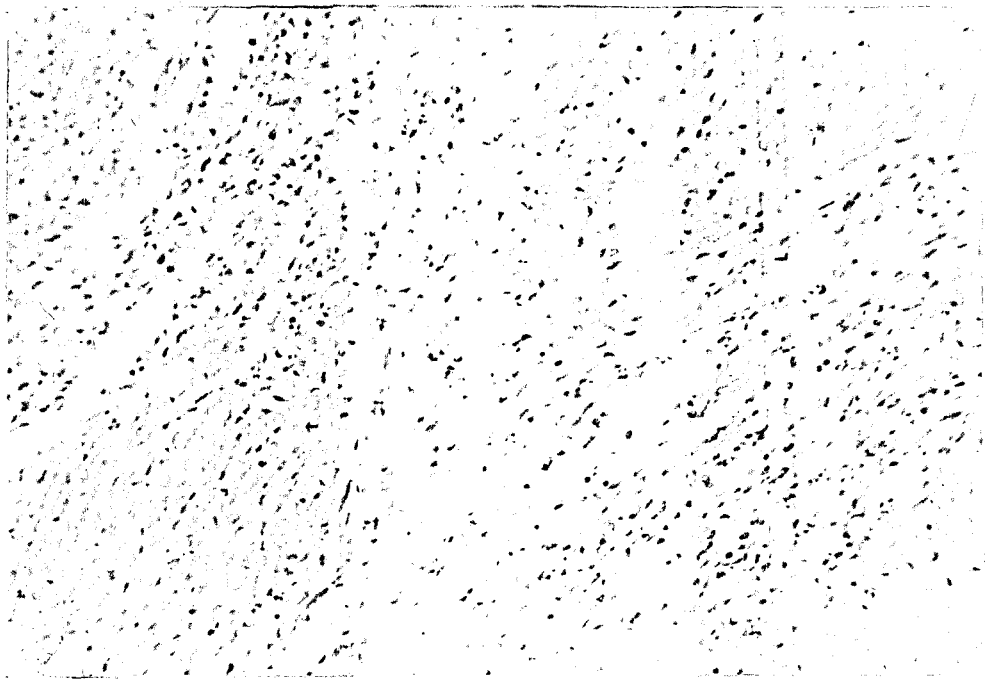
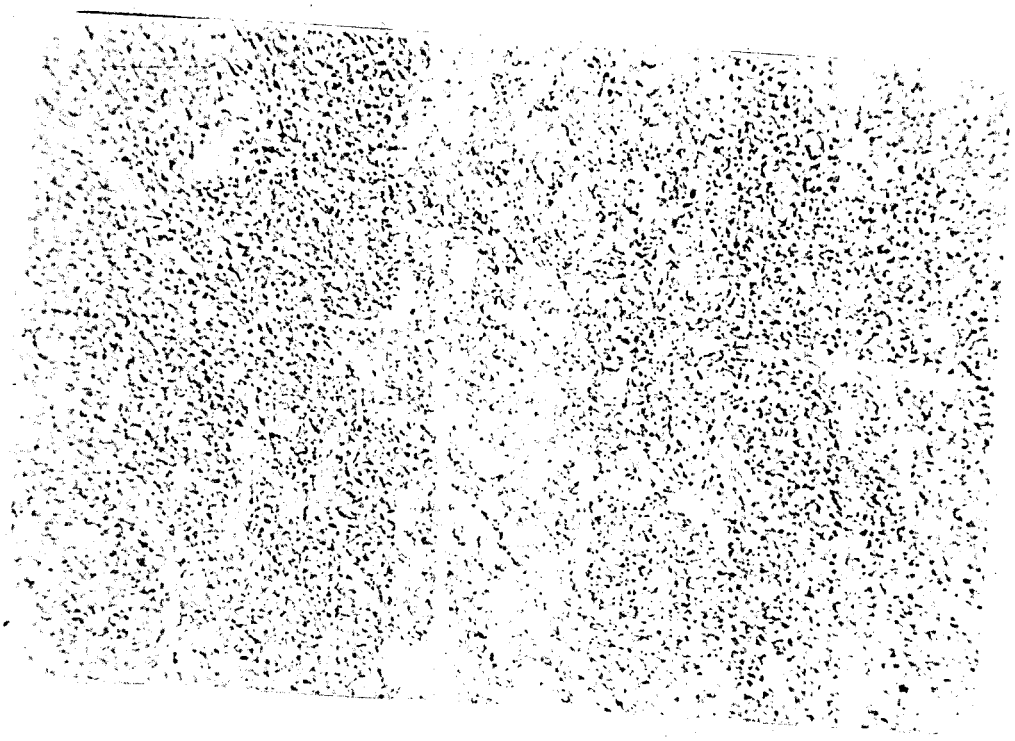


Fig.35 Depletion of lymphoid elements in the spleen of a naturally dead guinea-pig from group A (20 DPE).
H & E x100

Fig.36 Increase in number and size of fibro-muscular trabeculae of spleen in a naturally dead guinea-pig of group A (48 DPE).
H & E x100



(Fig.37). The cells of zona glomerulosa showed hydrophic changes followed by atrophy in later stage. The vascular changes like congestion and haemarrhages were so extensive in zona fasciculata and reticularis that these led to degeneration and disruption of the cells. Severe congestion and haemorrhage were also noticed in the medullary region.

The microscopic changes in the stomach and small intestines included congestion, haemorrhage and oedema with mild infiltration of mononuclear cells in the submucosal tissues. The lining epithelium, as places, was desquamated.

The skeletal muscles in some cases showed serofibrinous exudate, intermuscular haemorrhage and fragmentation of muscles with focal infiltration of mononuclear cell.

The histological changes in the testis included congestion of the intertubular tissue and absence of spermatogenesis. The seminiferous tubules were lined by a single layer spermatogonia and there was no sign of hyperplasia. The presence of cytoplasmic fragments was noticed in some of the tubules. The interstitial cells were undifferentiated and mostly fibroblast type.

Severe capillary congestion, haemorrhage and focal areas of epithelial hyperplasia were the salient changes in urinary bladder. The superfacial cells were enlarged with vacuolated cytoplasms and desquamated. The muscular tissue was widely separated because of oedematous fluid.

Group B The general histopathological changes observed in this group of animals resembled to those of group A except that the initiation of the changes was earlier and

the vascular changes were of greater intensity.

Severe congestion and haemorrhage (Fig.38) with focal mononuclear infiltration was observed in the lungs in early stages. The cellular infiltration was conspicuous around the bronchioles and the blood vessels (Fig.39). Thickening of the inter-alveolar septa because of severe vascular changes and infiltration of macrophages was more marked. Many of the macrophages were laden with haemosiderin pigment (Fig.40).The emphysema involving a group of alveoli was more marked but the hyperplasia of the bronchiolar epithelium was comparatively less. There was thickening of the arteriolar walls due to hypertrophy of muscular tissue and the serofibrinous exudation around the pulmonary vessels (Fig.41) was comparatively more in these animals.

The vascular changes in liver were comparatively more marked in this group. Focal necrosis and mononuclear cell infiltration in the hepatic parenchyma were observed in some cases (Fig.42). Though a mild degree of epithelial hyperplasia was observed in the intrahepatic bile ducts but there was no goblet cell metaplasia. Congestion and intermuscular haemorrhages (Fig.43) coupled with hyalinization of muscle fibres were more prominent in myocardium but there was no fibroblastic proliferation as observed in group A.

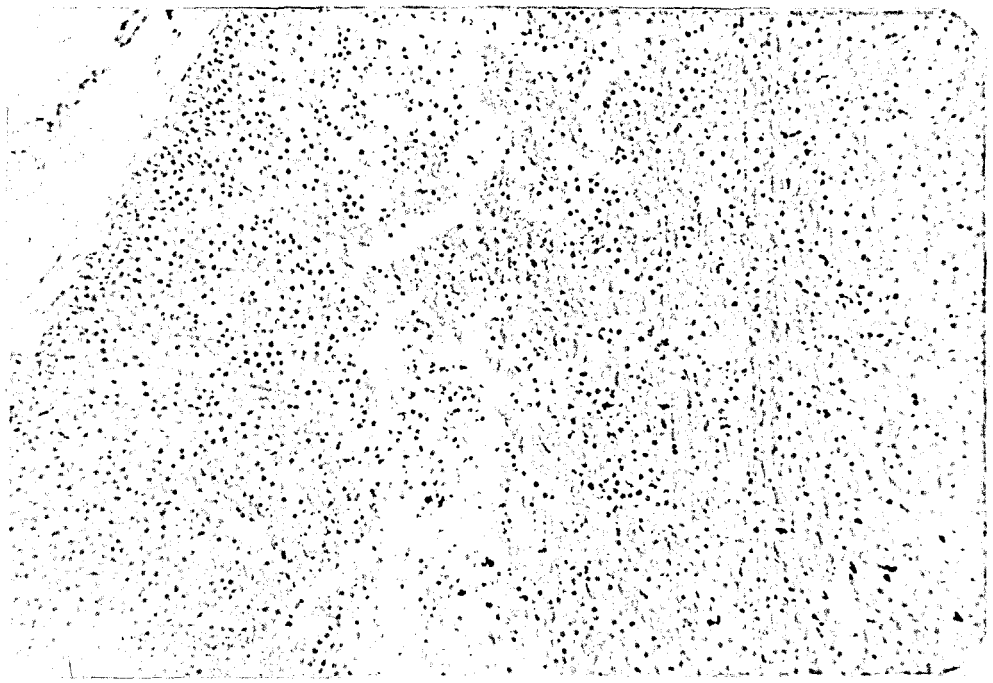
In kidneys, capillary congestion and haemorrhages were more frequent in glomeruli (Fig.44) and intertubular tissue. The degeneration and necrosis of tubular epithelium was more marked. At places, there was slight fibroblastic proliferation in the intertubular areas.

Fig.37 Adrenal gland of a naturally dead guinea-pig from group A (46 DPE) to show severe congestion and haemorrhage in cortex and medulla.

H & E x100

Fig.38 Congestion, haemorrhage and perivascular mononuclear cell infiltration in the lung of a naturally dead guinea-pig from group B (11 DPE).

H & E x100



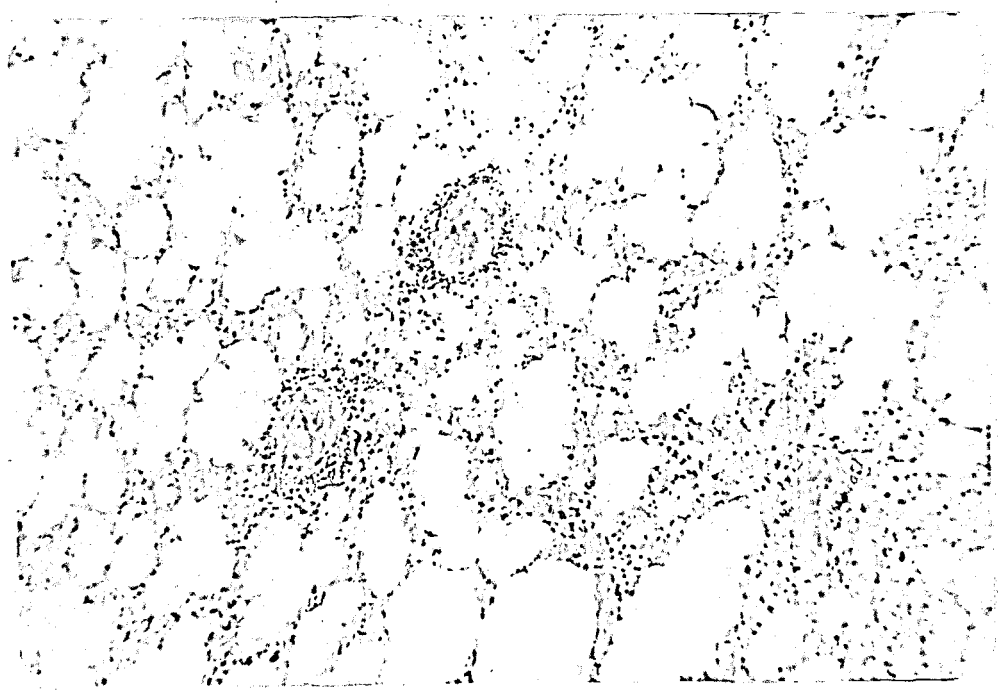
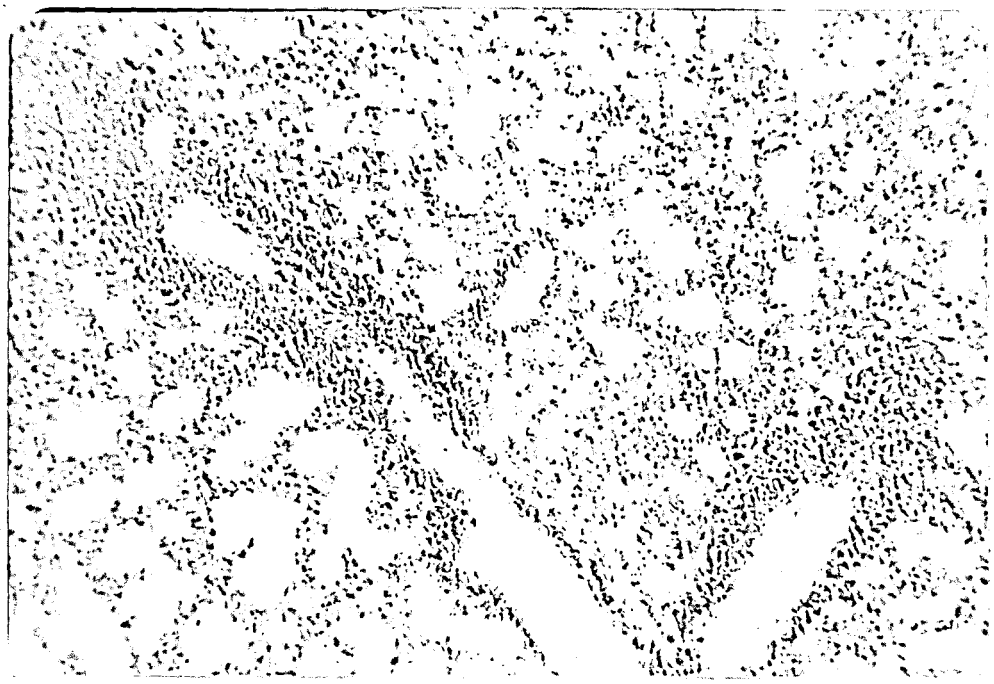


Fig.39 Marked infiltration of mononuclear cells around large blood vessels in the lung of a naturally dead guinea-pig from group B (18 DPE).

H & E x100

Fig.40 Prussian blue reaction in the section of lung from a naturally dead guinea-pig of group B (18 DPE).

Gomori's x200



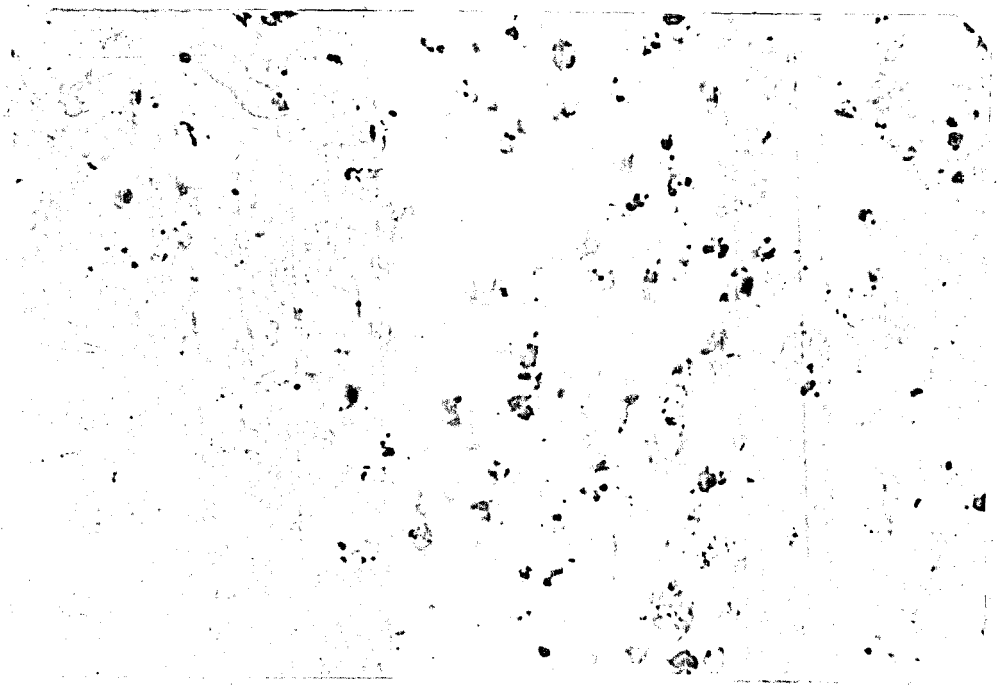
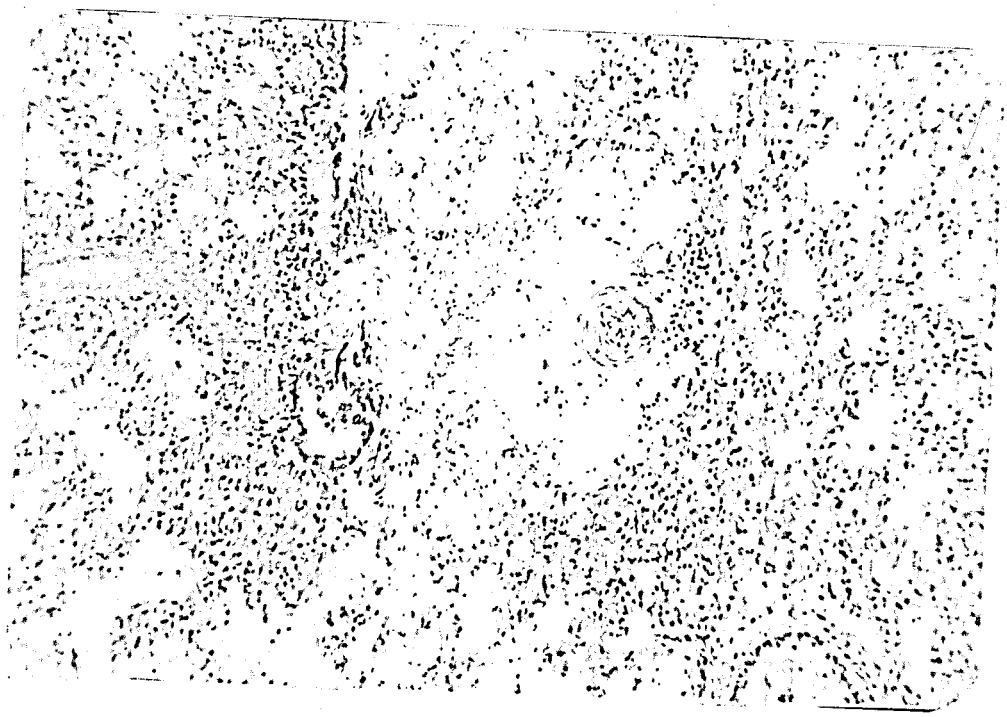


Fig.41 Congestion, haemorrhage and marked perivascular oedema in the section of lung from a naturally dead guinea-pig of group B (23 DPE).

H & E x100

Fig.42 Focal mononuclear cell infiltration in the liver of a naturally dead guinea-pig from group B (15 DPE).

H & E x200



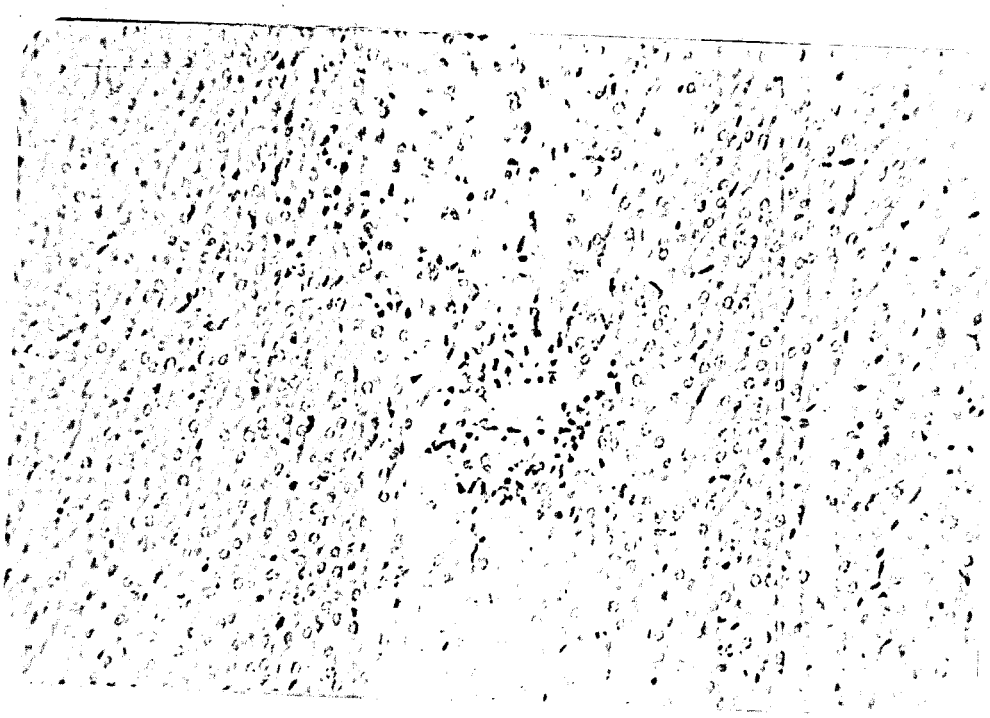
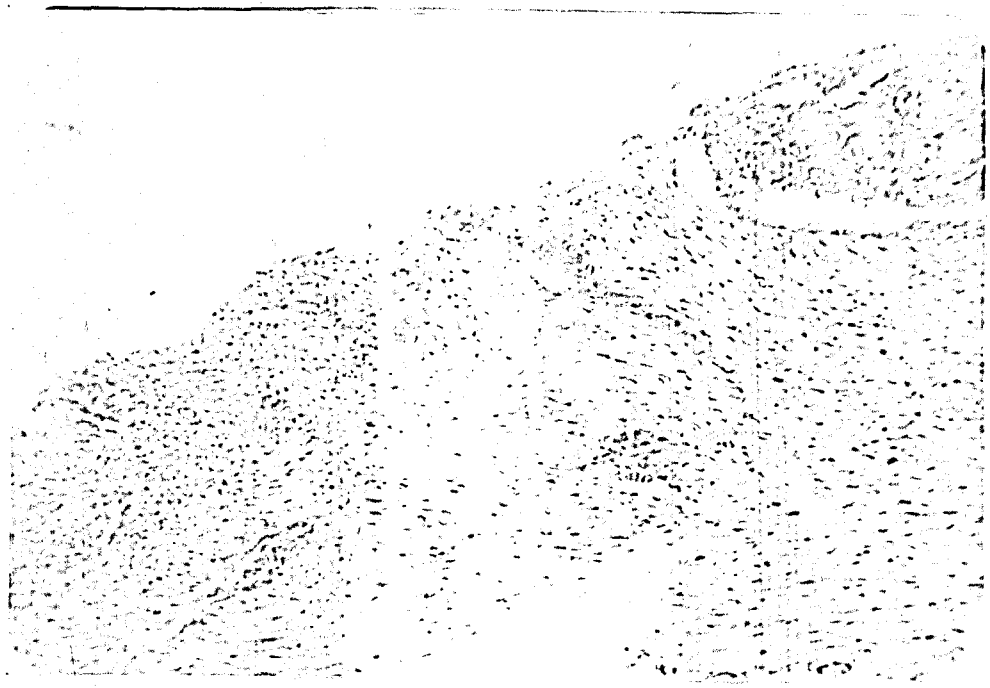
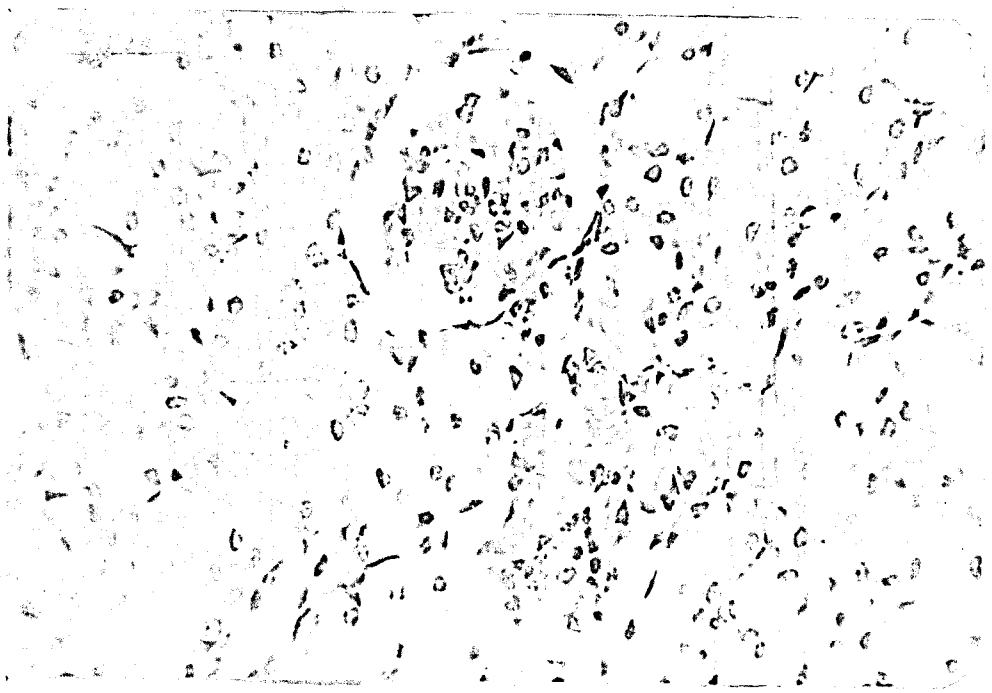


Fig.43 Congestion and haemorrhage in the heart of a naturally dead animal from group B (29 DPE).
H & E x100

Fig.44 Marked congestion and slight glomerular haemorrhages in the section of kidney from a naturally dead guinea-pig of group B (11 DPE).
H & E x400





The changes in the spleen were similar to that in group A and haemosiderosis was also prominent feature (Fig.45) in these cases.

Besides congestion of meningeal and cerebral blood vessels, focal areas of haemorrhages were also seen in the cerebrum (Fig.46).

The changes in the adrenal gland was similar to that in group A but these were comparatively more marked. The vascular changes in the cortex were so marked that it not only caused degeneration but individualization of the cells of zona fasciculata. The medulla was atrophic and almost replaced by extravascular red blood cells (Fig.47).

The vascular changes in the stomach and small intestine were more marked (Fig.48). In addition, there was slight necrosis of lymphoid tissue in the payer's patches of intestine (Fig.49).

The microscopic changes in the skeletal muscules, testes and urinary bladder were similar to that in group A but the capillary congestion, oedema and hyperplasia of epithelium in urinary bladder (Fig.50) was more marked. A mild degree of hyperkeratosis of the skin was observed in a few cases.

Group D The histopathological changes observed in the naturally dead animals of group D were almost similar to that of group B with variation in initiation and severity only.

Although pulmonary congestion was noticed in animals of this group but the haemorrhage was very mild. Emphysema, diffuse or focal mononuclear infiltration, epithelial

Fig.45 Haemosiderin deposition in the white pulp of spleen in a naturally dead animal of group B (24 DPE).
Gomori's x200

Fig.46 Congestion and focal haemorrhage in the brain from a naturally dead animal of group B(47 DPE).
H & E x 100

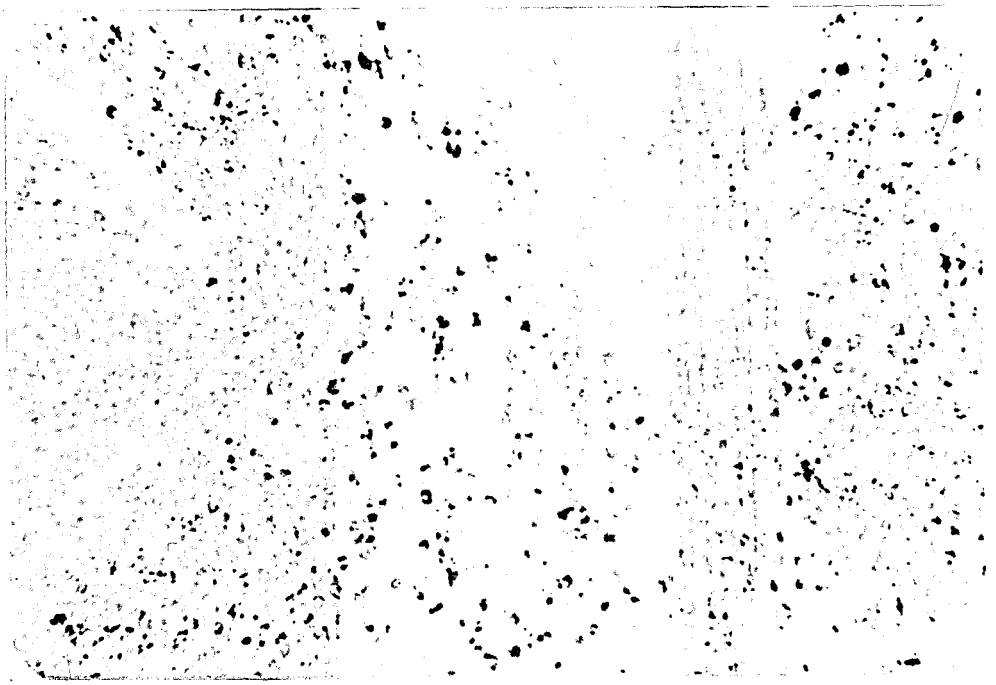
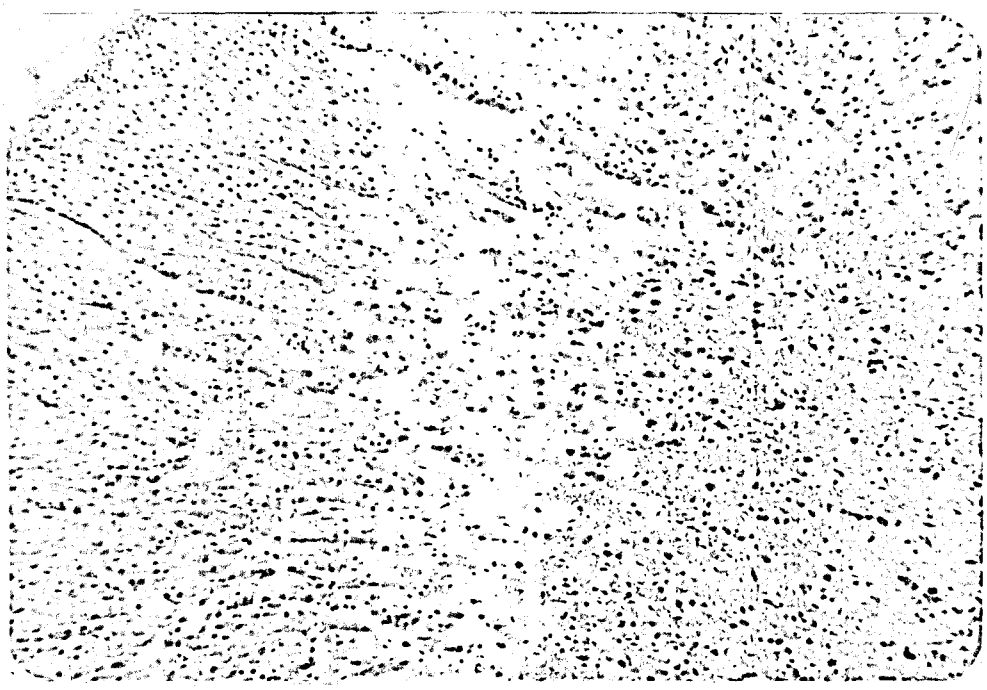


Fig.47 Degenerative changes with individualization of the cells of zona fasciculata in the adrenal gland of a naturally dead guinea-pig from group B (48 DPE). The medulla is almost replaced by extra vassated blood.

H & E x100

Fig.48 Severe congestion, haemorrhage and desquamation of linning epithelium in the section of intestine from a naturally dead guinea-pig of group B (21 DPE).

H & E x100



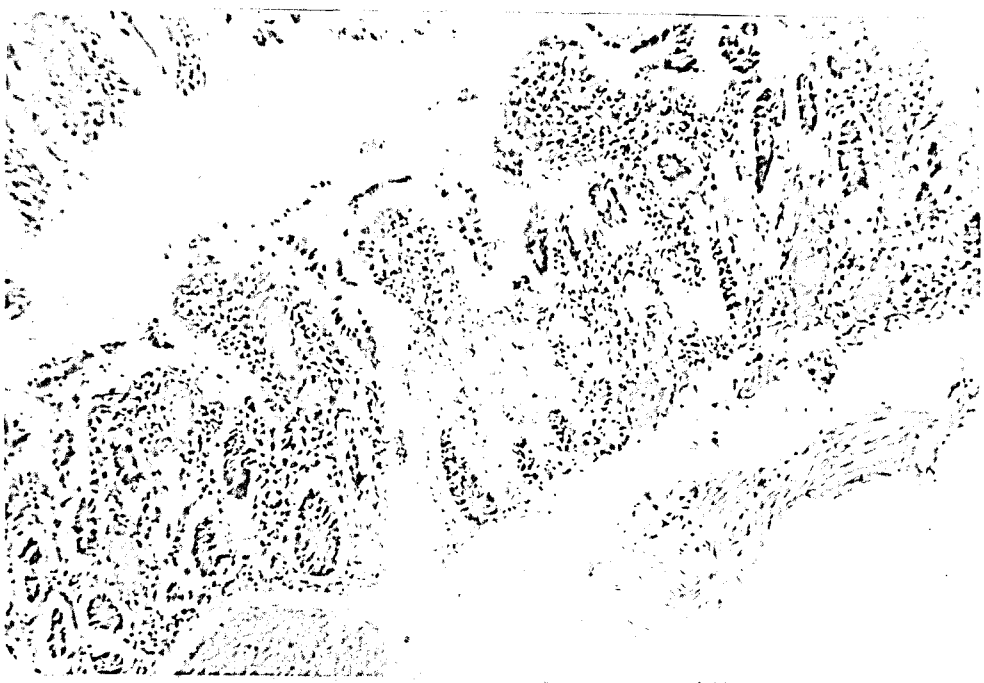
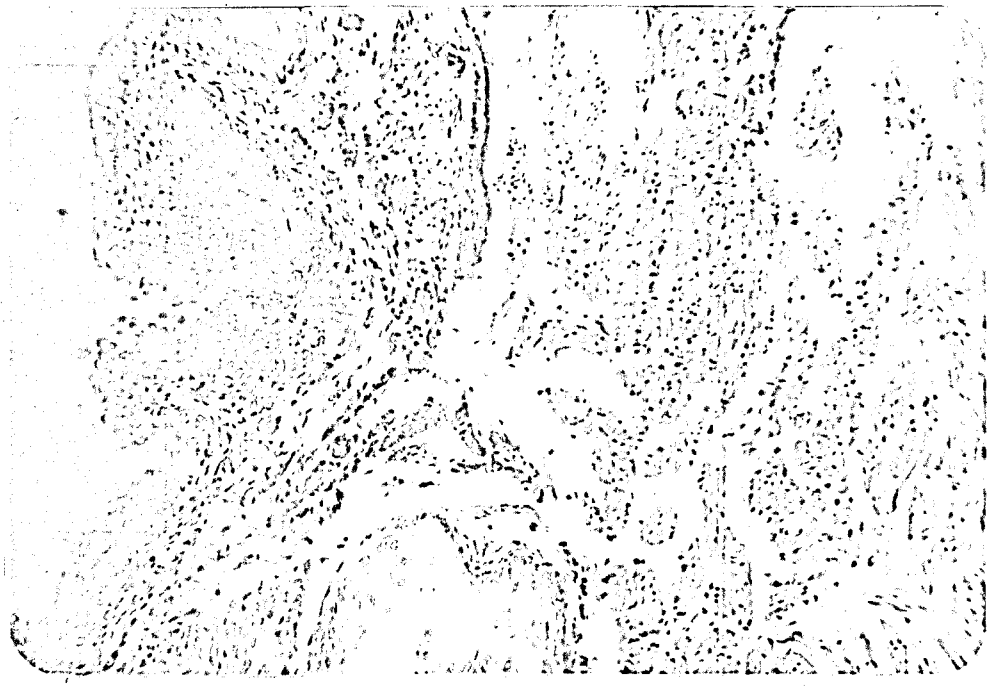


Fig.49 Congestion and depletion of lymphoid cells in the payer's patches of a naturally dead animal from group B (30 DPE).

H & E x100

Fig.50 Congestion, haemorrhage and intermuscular oedema in the wall of the urinary bladder of a naturally dead guinea-pig from group B (42 DPE). The linning epithelium is hyperplastic and desquamated.

H & E x100



hyperplasia in bronchioles and slight thickening of the arteriolar walls were the other changes in the lungs. The changes in the liver were similar but less severe in comparison to that in group B.

The microscopic changes of the heart included congestion, haemorrhage, degenerative changes in the muscle fibres but there was no proliferation of fibrous tissue.

Mild congestion, haemorrhage and tubular degeneration with the formation of hyaline casts in the lumen of renal tubules were noticed in the kidney. Congestion, haemosiderosis and depletion of lymphid cells was seen in spleen but there was no increase in the number or size of the trabeculae. The changes in skeletal muscle included intermuscular haemorrhage and hyalinization of some muscle fibres (Fig.51).

Though the change in brain, adrenal gland, lymph node, testis, stomach, intestine and urinary bladder were similar to that in group B but these, in general, were less severe.

Killed animals

The microscopic examination of sections from the control group (group C) did not reveal any appreciable change. In the remaining three experimental groups (A, B and D), the changes, in general, were more or less similar with slight variation in severity as well as distribution, which are mentioned below:

0 DPE No appreciable microscopic change was observed in any organ/tissue of the animals killed at this stage.

10 DPE Pulmonary congestion was observed in a few animals but in addition to congestion, slight inter-alveolar

haemorrhage was also noted in the animals of group B.

Mild congestion of sinusoids and central vein was noticed in the liver of some animals in groups A and B, but no changes were observed in group D. Focal necrosis was observed in one animal of group B. Congestion in the stomach and intestine was seen in groups A and B with slight haemorrhage in the submucosa in one animal of group B. No other changes were observed in any organ of these animals.

20 DPE The changes in the lungs included diffuse mononuclear infiltration in group A, but focal infiltration in group B. This was in addition to the pulmonary congestion and haemorrhages observed in both groups.

Dilation of the central veins, focal areas of haemorrhage, mild mononuclear infiltration and degeneration followed by necrosis of hepatic cells were the pathological changes observed in liver.

The changes observed in the stomach and intestine were haemorrhage in the mucosa, and congestion, haemorrhage and slight mononuclear cell infiltration in submucosal tissue.

The kidneys showed congestion and haemorrhage in the cortical area. Slight hydropic degeneration was seen in the tubular epithelium. Haemorrhages were observed in the spleen. The urinary bladder showed congestion and slight haemorrhage in the submucous area. Mild to marked congestion was noticed in the meningeal vessels in a few cases. No appreciable microscopic changes were observed in different tissues of the animals from group D except for the mild congestion and haemorrhage in the liver and lungs.

30 DPE Haemorrhage in the lungs was not a constant feature at this stage. Thickening and mononuclear cells (macrophages) infiltration in the inter-alveolar septa, and hyperplasia of bronchial epithelium were seen in some of the lung sections.

Congestion, degenerative changes in the hepatic cells, Kupffer cell hyperplasia and mononuclear cell infiltration was observed in the liver. Intestines revealed congestion and desquamation of the epithelial cells in some cases. Congestion, haemorrhage and tubular degeneration were seen in the sections of kidney. In the testis, the tubules were lined by a single layer of primary spermatogonia and there were no sign of spermatogenesis (Fig.52). A few cytoplasmic fragments were seen in the lumen of some tubules. Brain showed slight congestion in some cases.

40,50 and 60 DPE The histopathological changes observed at these intervals were somewhat chronic in nature, and varied slightly in degree in different groups. Thickening of the alveolar septa with diffuse or focal mononuclear infiltration, hyperplasia of the bronchiolar epithelium, thickening of the arteriolar wall, perivascular oedema and perivascular infiltration of mononuclear cells were the salient changes in lungs.

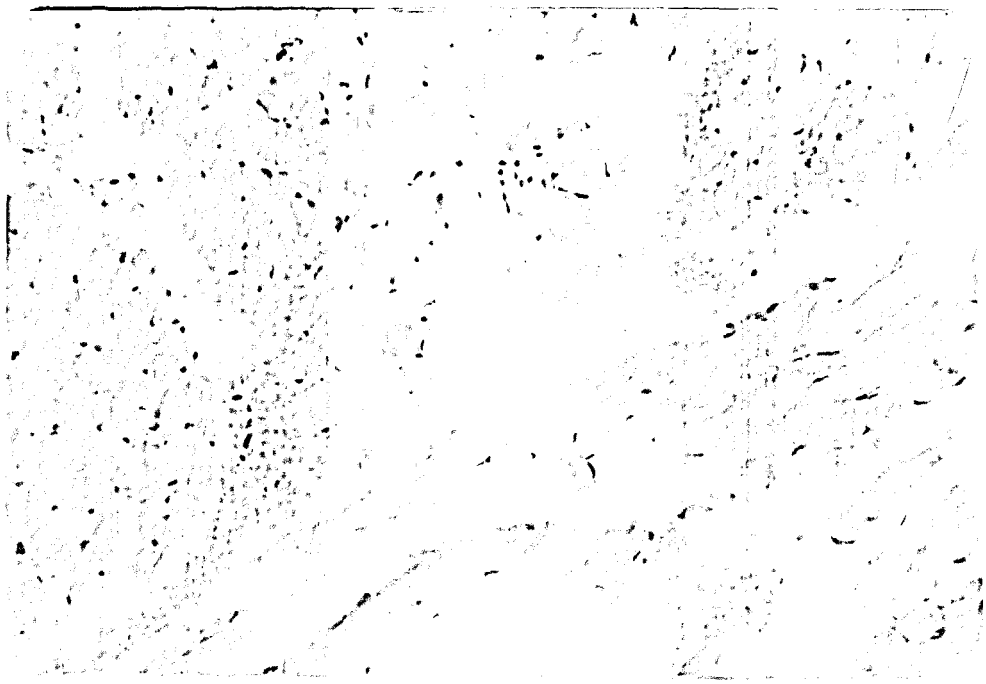
Besides varying degree of vascular changes, degeneration and necrosis of hepatic cells, mononuclear cell infiltration, hyperplasia of the intrahepatic bile duct epithelium etc. were noticed in the liver.

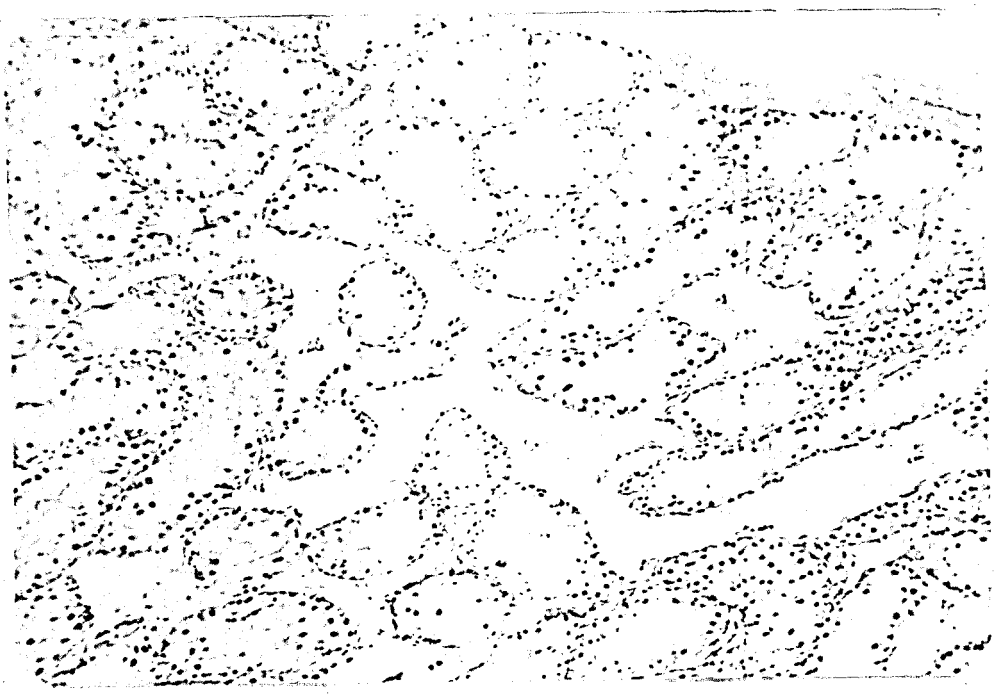
Fig.51 Section of skeletal muscle to show necrosis and haemorrhages in a naturally dead guinea-pig of group D (21 DPE).

H & E x200

Fig.52 Section of the testis from a killed guinea-pig of group B (30 DPE) to show hypoplasia. The tubules are lined by a single layer of spermatogonia with no signs of hyperplasia.

H & E x100





Heart showed foci of coagulative necrosis, congestion, slight mononuclear infiltration and, at places, fibroblastic proliferation.

Haemorrhage, tubular degeneration and necrosis with hyaline casts in the renal tubules were observed in kidney. Atrophic changes with depletion of lymphoid elements were seen in the spleen. Degeneration and desquamation of the lining epithelium and submucosal haemorrhages were the microscopic changes in gastro-intestinal tract.

DISCUSSION

CHAPTER V

DISCUSSION

The use of selenium in the prevention of carcinogenesis in human and nutritional muscular dystrophy in animals, in the recent days, has drawn the attention of research workers to investigate the toxicological aspects of this essential micronutrient because toxicity may develop due to excessive therapeutic use of this element. The industrial uses of selenium are more recent (Goyer and Mehlman, 1977) which may cause atmospheric and water pollution from industrial wastes. Moreover, the agro-chemical practices adopted now a days change the soil profile and contribute towards accumulation of selenium in plants and grains which are grown on such soils. Study of an individual pathologic process in different animal species enhances the understanding of pathogenesis of disease in human (Migaki and Capen, 1984) and provides new dimensions for the investigation of such conditions. With these objectives in mind, the present studies were undertaken to investigate the clinical manifestations, haematological and biochemical indices and pathology of experimentally induced organic and inorganic selenium toxicity in recently weaned guinea-pigs. Attempt has also been made to study the protective efficacy of sodium arsenite in cases of inorganic selenium toxicity.

For the induction of organic selenium toxicity, the guinea-pigs were fed on selenium-enriched barley which was

obtained from an artificially selenized land. Before growing barley, the selenium-treated land was utilized for the cultivation of paddy. Five days after treatment of the land with sodium selenite, the soil sample was found to contain 0.465 ppm of selenium and the straw from paddy grown on this soil contained 4.26 ppm of selenium. After harvesting the paddy, the selenium content of soil dropped to 0.15 ppm (Jakhar, 1984) but the barley grains grown thereafter on this land and used for this study were found to contain 5.10 ppm of selenium. This indicates that barley crop accumulates more selenium than the rice straw, in spite of the fact that selenium level of the soil at the time of barley sowing was lower. The difference in the selenium content of rice straw and barley grown on the same land may be due to difference in the species of plant as reported by Ammerman et al.(1978). According to Beath(1937), when barley was grown in an area where the selenium indicator plants (Astragalus spp.) grew, the grains of barley contained 20 ppm of selenium. Moxon et al.(1950) found that the selenium content of barley remained fairly constant at all stages of maturity.

The clinical signs of selenium toxicity in guinea-pigs, as observed in the present studies, are almost similar to those described in rats (Franke,1934; Smith et al.,1937; Smith,1941; Rosenfeld and Beath,1947), mice and rats (McConnell and Portman, 1952), rabbits (Berschneider et al.,1977b), guinea-pigs (McCollum et al.,1946; Ray and Ray, 1982) and Syrian hamsters (Julius et al.,1983; Birt et al.,1986). However, there were

no signs of diarrhoea or stiffness of joints as recorded by Pathak and Datta (1983) in goats and Jakhar (1984) in buffalo-calves. Loud crying and pressing of the head against the cage-wall in early stages of toxicity might be the result of abdominal pain due to severe haemorrhagic lesions in the stomach and small intestines. Blood et al. (1983) have reported these signs in blind staggers. Paleness of the visible mucous membranes and laboured breathing could be attributed to anaemia which has been evidenced by haematological studies.

The nervous signs like paralysis of hind legs and at times, convulsions might be the result of severe vascular changes in the central nervous system observed in the present study. The occurrence of paralysis of hind legs has been reported in rats (Franke, 1934) and the convulsions in mice (McConnell and Portman, 1952) and buffalo-calves (Jakhar, 1984). Oedematous swelling of the head region and ascites observed in selenium toxicity in this study may be attributed to hypo-proteinemia as the estimation of total serum proteins also indicated lower values in the toxicoses groups. Smith et al. (1937), Smith (1941) and Rosenfeld and Beath (1947) have also observed ascites and oedema in cases of subacute and chronic selenosis in rats.

As observed in the present studies, the mean feed consumption decreased significantly in both the organic and inorganic selenium toxicity which is identical with the findings of McCollum et al. (1946), Pathak and Datta (1983), Julius et al. (1983), Jakhar (1984) and Birt et al. (1986) in different

species of animals. As reported by McCollum et al.(1946), experimental animals were able to differentiate between diets of varying toxicity and the pathology in selenium toxicity according to them was caused by the toxicant while the inanition was due to voluntary restriction of food intake. The data generated in the present studies also indicate decreased feed consumption but it is difficult to say whether the decrease was the result of toxicity or voluntary restriction.

Decreased weight gain or loss of body weight in selenium toxicity has been reported by several workers in different species of animals (Smith et al.,1937; Smith,1941; Rosenfeld and Beath,1947; Schroeder and Mitchener,1971; Herigstad et al., 1973; Palmer and Olson,1974; Ray and Ray,1982; Pathak and Datta,1984; Jakhar,1984; Goehring et al.,1984; Jenkins and Hidiroglou,1986; Birt et al.,1986) which is in accordance with the findings of present study. Glenn et al.(1964) observed that the body weight was unaffected in sheep unless these developed clinical signs of toxicosis. Failure to weight gain or loss of weight observed could be ascribed to loss of appetite and decreased feed consumption. However, the possibilities of decrease in cellular metabolism in selenosis can not be excluded as Brown and de Wet (1962) have reported that selenium replaced the sulphur in essential proteins and inhibited the enzymes concerned with tissue respiration.

The clinical signs of anorexia, lassitude, oedema, nervousness, convulsions and paralysis were observed in animals of both the organic (group A) and inorganic (group B)

selenium toxicity but these were comparatively more marked in inorganic toxicosis. This may be due to higher selenium concentration in the diet of these animals. The patchy alopecia was, however, more in inorganic toxicity.

Although the selenium content of feed supplied to animals of group A (organic selenium) was low (5.10 ppm) in comparison to group B (30 ppm of sodium selenite for first 23 days and 15 ppm for the remaining period in addition to 0.86 ppm of organic selenium present in the ordinary barley) but the overall mortality during the experimental period of 60 days in the two groups was same (55 per cent). This indicates that selenium was comparatively more toxic when present in organic rather than in inorganic form. This is in conformation with the findings of several other workers (Fitzhugh et al.,1944; Clarke et al.,1981; Blood et al.,1983; Prasad and Arora,1984b) who reported that organic selenium was more toxic than the inorganic selenium. Rhian and Moxon (1943) and Fitzhugh et al. (1944) have observed that the organic selenium from the wheat and corn possessed about twice the toxicity of the selenites and selenates. In spite of the fact that the concentration of sodium selenite in the treated group (group D) was kept at 30 ppm throughout the experimental period, the overall mortality in this group was 37.5 per cent. The clinical signs exhibited by the animals of this group were also less marked. This may be the result of sodium arsenite which was added in the feed of this group, thereby indicating that sodium arsenite had some protective effect on inorganic selenium toxicity in guinea-pigs. Minyard et al.(1960) reported reduction in

symptoms of selenium toxicity in beef cattle when 0.01 per cent arsenilic acid was added to a ration containing 12 ppm selenium. According to Levander (1972) and Hill (1975), the arsenic diminished selenium toxicity by stimulating the excretion of selenium into the bile.

Haematological investigations in the experimental guinea-pigs revealed a significant drop in Hb and PCV but the TEC remained almost within normal range. Accordingly, there was decrease in MCV and MCH (not MCHC) thereby indicating that the anaemia was microcytic and hypochromic in nature. This anaemia was probably the result of excessive destruction of red blood cells as evidenced by wide spread haemorrhages and marked haemosiderosis in the spleen and lungs. A progressive anaemia with continuous decrease in haemoglobin values has been reported in selenium toxicity by several investigators (Franke and Potter,1934; Smith et al.,1937; Rhian and Moxon, 1943; Mahalanobis and Ray,1954; Birt et al.,1983 and 1986; Pathak,1984; Jakhar,1984) in different species of animals. Smith et al.(1937) and Moxon and Rhian (1943) recorded a similar type of microcytic, hypochromic anaemia in rat and dog, and the animals which died had the Hb values as low as 2.0 g/dl. Jakhar (1984) observed anaemia in buffalo-calves which was normocytic and normochromic in nature. On the basis of radioactive iron study (Fe^{59}) in rat, Halverson et al. (1970) concluded that anaemia in selenosis was caused by haemolysis rather than a defect in red blood cell synthesis. Excessive haemosiderosis in spleen in the present study also

indicate excessive destruction of erythrocytes. Though a decrease in the mean value of Hb, PCV, MCV and MCH was noticed in the treated animals (group D) but it was not so severe as in group A and B. This may be due to addition of sodium arsenite which is reported to reduce haemolysis (Halverson et al.,1970).

A significant decreased in total leucocyte counts (TLC) was observed in both the organic and inorganic toxicosis but the values did not differ significantly in the treated group, in comparison to control. This observation is similar to the findings of Pathak (1984) in goats. However, Jakhar (1984) did not observe any significant difference in the TLC, DLC and absolute lymphocytic values in buffalo-calves. This may be due to either low doses of selenium and/or short duration of the experiment or the species differences. Hogan (1986) noticed a dramatic decline in circulating leucocytes in 8 to 16 days following intraperitoneal injection of sodium selenite in mice. The decline was the result of decrease in neutrophilic granulocytes. Pathak (1984) recorded lower percentage of lymphocytes in experimentally induced selenosis in goats. A relatively lower percentage of lymphocytes and a significant decrease in absolute lymphocyte counts have been observed in both group A and B (toxic groups) but not in the treated group. Histopathological evidence of lymphoid depletion also corroborate with the haematological observations and indicates that selenium toxicity may be causing depression in lymphocyte production. In the organic selenium toxicosis, the decrease was more in lymphocytes whereas in inorganic toxicosis,

neutrophilic count decreased more. Thus, there was both neutropenia and lymphopenia, the degree of which depended upon the type of selenium toxicity.

There was significant drop in blood glucose values both in organic and inorganic toxicities as compared to the controls. According to Potter et al. (1939), decreased blood glucose in chronic selenosis was secondary to decreased food intake. This may also hold true in the present studies as the feed consumption was significantly reduced in both the toxicoses groups in comparison to control. But the possibility of failure of gluconeogenesis because of hypoadrenocorticism can not be excluded as severe congestion, haemorrhage and degenerative changes were observed in zona fasciculata of adrenal cortex (Jones and Hunt, 1983). However, the higher blood glucose levels observed by Schroeder and Mitchener (1971) in rats can not be explained. It may be due to low selenium dose (2 ppm in drinking water) employed and moreover, they did not study the effect on feed consumption. The blood glucose value in the treated group was significantly higher than the toxicoses groups but lower than the controls. Similarly, the average feed consumed by animals of this group was higher than the toxicoses groups and a mild degree of degenerative changes were noticed in the adrenal gland.

A state of hypoproteinemia was observed in the selenium toxicity in the present study. This was associated with the clinical signs of ascites and oedema of head which are identical with the findings of Rosenfeld and Beath (1964) and

Jakhar (1984). Blood et al. (1983) have described several causes of hypoproteinemia. Among them, renal diseases which lead to protein loss, liver damage causes failure in the synthesis of plasma proteins and congestive heart failure are important. Though varying degree of vascular and degenerative changes have been observed in different organs of guinea-pigs, further studies are required to identify the exact cause of hypo-proteinemia.

There appears to be no agreement in regard to SGOT activity in selenium toxicity. In the present studies, the SGOT activity in both the organic and inorganic selenium toxicity was significantly lower than that in control group. This is in contrast to the observations of Buck et al. (1961) and James and Kampen (1974) who observed increased SGOT activity in sheep and other livestock following ingestion of Locoweed. However, no appreciable elevation of SGPT values were observed by them which is identical with the findings of present study. Pathak and Datta (1984) also recorded marked increase in SGOT and slight increase in SGPT values in goats. In pigs, an initial elevation of SGOT activity upto 24 hrs followed by decline to normal on 6th day has been observed by Diehl and Mahan (1973). Madan Mohan et al. (1976) observed decline in SGOT and SGPT activities in sheep; the decrease being more with the increase of selenium dioxide administration. Jakhar (1984) observed lower SGOT activity in both organic and inorganic selenium toxicity in buffalo-calves, but there were no significant alterations in SGPT values among different groups. The

decrease in SGOT may be attributed to renal failure as reported by Martin (1981). According to him, renal failure caused inhibition of pyrodoxal kinase which in turn ensured a deficiency of co-enzyme pyridoxal phosphate necessary for transamination reaction. Although severe vascular and degenerative changes were noticed in the kidney during the present study but further work is desired to confirm renal failure in selenium toxicosis.

The seleno-enzyme GSH-PX, is reported to contain 4 g atom of selenium per mole of the enzyme (Oh et al.,1974a). This enzyme is usually incorporated into erythrocytes during erythropoiesis (Hoffman et al.,1978). The exact role of GSH-PX in mammalian cells is not fully understood but it might be playing role in protection of cells by destroying oxidising agents such as hydrogen peroxides and lipidperoxides, which are capable of causing irreversible denaturation of essential cellular proteins which leads to degeneration and necrosis (Blood et al.,1983). Estimation of erythrocytic GSH-PX activity in the selenium toxicosis, in the present study, revealed a significant increase in the concentration of this enzyme in organic and inorganic toxicity, the increase being comparatively more in the inorganic toxicosis. No significant increase was, however, observed in the treated group. This may be due to the action of sodium arsenite which has been reported to facilitate excretion of selenium in bile and thus it may not be available for incorporation into the erythrocytes. Several investigators have reported increase in GSH-PX activity in selenium toxicosis, depending upon the selenium concentration,

in man and different species of animals (Oh et al.,1974a; Thompson et al.,1976; Little et al.,1979; Thompson et al., 1980; Birt et al.,1983; Julius et al.,1983; Prasad and Arora,1984a,b; Jakhar,1984; Goehring et al.,1984; Thomson et al.,1985; Birt et al.,1986; McClure et al.,1986).According to Pierce and Tappel (1977), the effects of selenite and selenomethionine administration on GSH-PX activity were similar but Prasad and Arora (1984b) have observed that the erythrocytic GSH-PX activity was higher in buffalo-calves which were given selenomethionine as compared to those given sodium selenite. In the present studies, GSH-PX activity was comparatively higher in inorganic selenosis group which might be due to higher concentrations of selenium in that group. The highest enzyme activity was observed on 40, 30 and 40 DPE in group A, B and D, respectively. This was followed by a gradual decrease in GSH-PX activity. This decrease to some extent corresponded with the development of microcytic and hypochromic anaemia which is considered typical in iron deficiency. A depressed erythrocytic GSH-PX activity in iron deficiency anaemia has also been observed by Rodvein et al. (1974) in rabbits. It may, however, need to be investigated whether selenium toxicoses leads to iron deficiency or there is some interaction between iron and the selenium.

A number of factors are known to affect the distribution and retention of selenium in chronic and subacute selenosis. These included daily dose, route of administration,experimental animal used, and the form of selenium administered to produce

the toxicity. The organic form of selenium is known to accumulate in higher quantities and persist for longer period in tissues as compared to inorganic selenium (Rosenfeld and Beath,1964). Hurlbut and Martin (1972) found that the selenium concentration of tissues in mice fed on selenomethionine was two to eight times higher than that in animals fed sodium selenite or selenium methylselenocysteine. Estimation of selenium concentration in various tissues like liver, kidney, spleen and hair revealed a highly significant increase in its concentration; the increase was, however, more in inorganic toxicosis as compared to the organic. This may be due to relatively higher concentration of selenium in the diet of this group. Stosic (1974) reported that the increase in dietary selenium significantly increased plasma, muscle and liver selenium concentration in guinea-pigs. The increased concentration of selenium in various tissues of the body has been reported by several workers (Dudley,1936; Rosenfeld and Beath, 1945,1964; Glenn et al.,1964; Gardiner,1966; Morrow,1968; Bhatia et al.,1982; Gupta et al.,1982; Birt et al.,1983; Jakhar,1984; Anderson et al.,1985; Hiridoglou et al.,1985). The selenium concentration in the liver, kidney and spleen was maximum on 20th DPE in group B and thereafter, it had a decreasing trend. This is in accordance with the observations of McBride (1987) who reported that the inorganic forms of selenium did not store in the tissues particularly well.

Herigstad et al.(1973) observed that the pigs with toxicosis had greater selenium concentration in liver than

kidney, and those who did not develop toxicosis had greater concentration in kidney than liver. On the other hand, the selenium concentration of kidney, in the present study, was always found higher than that of liver and these findings simulate with the observations of Birt et al. (1983) in Syrian hamsters.

The selenium concentration in hair continued to increase till the end of experiment. Hurlbut and Martin (1972) observed that except for hair, the selenium content of liver, kidney and pancreas returned to normal levels after one week of the withdrawal of selenium-rich feed. Gupta et al. (1982) have recorded high selenium concentration (7.5 to 16.32 ppm) in hair of buffaloes, cattle and goats suffering from natural chronic selenosis.

The gross changes observed in the present studies included varying degree of congestion and haemorrhage in stomach and intestine; congestion, haemorrhage and oedema in lungs; congestion with foci of necrosis in liver; atrophy of the spleen; soft and flabby heart with congestion and patechial haemorrhage; congestion and haemorrhage in the kidney; distended gall and urinary bladder; and slightly enlarged but haemorrhagic adrenal gland. Microscopically, besides congestion and haemorrhages in various tissues, there was oedema and infiltration of mononuclear cells in the lung parenchyma. The bronchial epithelium revealed hyperplasia. There was periarteriolar oedema and marked infiltration of lymphocytes around blood vessels and in later stages,

haemosiderosis. Liver revealed focal areas of necrosis, hyperplasia and goblet cell metaplasia of the intrahepatic bile duct epithelium and focal areas of mononuclear infiltration. There was depletion of lymphoid elements in spleen and payer's patches. The vascular changes in urinary bladder were coupled with foci of epithelial hyperplasia. There was severe congestion, haemorrhage and degenerative changes in adrenal gland and hypoplasia with lack of spermatogenesis in the testis. These changes are more or less similar to those described by Duhamel (1913), Smith et al.(1937), Lillie and Smith (1940), Fitzhugh et al.(1944), Rosenfeld and Beath(1948), Pathak and Datta (1983) and Jakhar (1984) in different animal species. The changes in the animals of group B were more or less similar to that in group A except that the initiation of changes were earlier and the vascular changes were of greater intensity. Moreover, in group B the mononuclear infiltration was mainly focal and nodular in nature; fibroblastic proliferation was little and no goblet cell metaplasia was noticed in the intrahepatic bile ducts. Depletion of lymphoid elements from the payer's patches with evidence of necrosis was observed in group B. The changes in group D were similar to that of group B with late initiation and milder in nature. However, the overall damage to the liver parenchyma, as observed in the present studies was less than that reported by other investigators. This could be due to the vitamin C which was always added in the feed during these studies. Zannoni (1986) has reported that vitamin C at higher doses prevented the liver cirrhosis in guinea-pigs and human.

The exact mechanism of the toxic action of selenium is not known. The possible mechanism of intoxication is that, selenium may replace sulphur of the sulphur-containing amino acids which are the precursors of glutathione. The glutathione maintains the sulph-hydral groups in the cells necessary for tissue respiration (Brown and de Wet,1962; Blood et al.,1983). The degenerative changes and necrosis,in selenosis, results due to the lack of glutathione and in turn sulph-hydral groups in the cells.

Severe vascular and degenerative changes observed in the adrenal gland during the present investigations have not been reported earlier. However, haemorrhage in capsule, congestion in cortex and haemorrhage in medulla have been reported in buffalo-calves (Jakhar,1984). The microscopic changes in the testis and payer's patches are also not very well studied. A mild degree of epithelial hyperplasia and goblet cell metaplasia of intrahepatic bile ducts in group A might be due to irritating effect of selenium which is reported to be excreted in bile (Levander,1972; Hill,1975).

In general, the vascular changes were comparatively more pronounced in inorganic selenium toxicity which may be attributed to higher doses of sodium selenite. Comparatively mild changes observed in the treated group are suggestive of partial protective effect of sodium arsenite on inorganic selenium toxicity in guinea-pigs. As mentioned earlier, the sodium arsenite exterts its protective action by facilitating the excretion of selenium in the bile.

SUMMARY

CHAPTER VI

SUMMARY AND CONCLUSIONS

In the present investigations, an attempt has been made to study the clinical manifestations, some haematological, and biochemical indices and pathology of experimentally induced organic and inorganic forms of selenium toxicity in the recently weaned guinea-pigs. The study also included estimation of selenium levels in certain body tissues and examination of the protective effect of sodium arsenite, if any, on inorganic selenium toxicity in guinea-pigs.

A total of 131 recently weaned guinea-pigs of an English breed were randomly divided into four groups. For induction of organic selenium toxicity, the guinea-pigs in group A were fed on selenium-enriched barley (selenium content 5.10 ppm) whereas the inorganic toxicity was produced by feeding ordinary barley (selenium content 0.86 ppm) mixed with sodium selenite @ 30 and 15 ppm. The control group (C) continued to receive ordinary barley and the treated group (D) was given sodium selenite (30 ppm) alongwith sodium arsenite (10 ppm) throughout the 60 days of experimental period. Vitamin C at the recommended doses was given alongwith feed to all groups of animals.

The earliest signs of toxicity were noticed in group B (8 DPE) followed by those in group A (11 DPE) and D (16 DPE). In general, these included loud crying, slight lacrimation

and/or salivation, dullness, depression, progressing weakness and slight nervousness. In later stages, there was restlessness, pressing of head against cage-wall, oedema of the head region, paleness of visible mucous membranes, laboured breathing, patchy alopecia and thinness of the hair coat, convulsion, parlaysis of the hind legs and death. However, the oedema, nervousness and convulsions exhibited by animals of group B were more pronounced. Oedema, ascites and patchy alopecia were not observed in group D.

The mortality was first recorded on 11th day in group B followed by 14th day in group A and 17th day in group D. The overall mortality in the two toxicoses groups was same (55 per cent) but it was low (37.5 per cent) in the treated group. The average feed consumption and the body weight gain were significantly reduced in the guinea-pigs fed on selenium-enriched barley (group A) or barley mixed with sodium selenite (group B) as compared to controls (group C). No significant difference was, however, observed in the treated group (group D). Average rectal temperature remained within normal range in all the experimental groups.

The haematological studies revealed reduction in Hb, PCV, MCV, MCH (but not TEC and MCHC) thereby indicating a microcytic hypochromic anaemia in the toxicoses groups. Though the values of different erythrocytic indices were lower in treated group but the difference was not significant. A significantly lowered TLC was noticed in the toxicoses groups (but not in the treated group) and the leucopenia was the result of lymphopenia in organic selenosis but both lymphopenia

and/or salivation, dullness, depression, progressing weakness and slight nervousness. In later stages, there was restlessness, pressing of head against cage-wall, oedema of the head region, paleness of visible mucous membranes, laboured breathing, patchy alopecia and thinness of the hair coat, convulsion, parlaysis of the hind legs and death. However, the oedema, nervousness and convulsions exhibited by animals of group B were more pronounced. Oedema, ascites and patchy alopecia were not observed in group D.

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and neutropenia in inorganic toxicity.

A significant drop in blood glucose level, in comparison to controls, was observed in group B followed by group A and D. However, the difference between organic and inorganic toxicity groups was not significant. Estimation of total serum proteins revealed a significant drop in all the three experimental groups.

The SGOT activity in group A and B was lower as compared to the control group C. However, SGPT values did not differ significantly amongst various groups of animals. The erythrocytic GSH-PX activities were significantly increased in inorganic selenosis followed by organic one as compared to controls. However, the increase in the treated group was not significant.

A well marked elevation in selenium concentration, in comparison to control, was noticed in the liver, kidney, spleen and hair in all the three experimental groups; the values being highest in group B followed by that in group A and D. A continuous increase in selenium concentration of hair was noticed throughout the experiment in all groups. The liver also followed the same pattern in group A and D but a slight decline after an initial increase at 20 DPE was noticed in group B. The selenium level in the kidney was always found higher than that of liver in both the toxicoses.

The gross lesions included congestion, focal haemorrhages and oedema in the lungs; congested liver with necrotic foci; congestion and haemorrhage in stomach and intestines; atrophy with thickening of capsule in spleen, soft and flabby heart

with congested vessels and petechial haemorrhage; congestion and haemorrhage in kidney; distended gall and urinary bladder; and slightly enlarged but haemorrhagic adrenal gland. The vascular changes, in general, were more pronounced and their initiation was earlier in group B as compared to A. Although the changes in group D were similar to that in B but the initiation was late and the changes were less severe.

Microscopically, besides congestion and haemorrhages in various tissues there was oedema and infiltration of mononuclear cells in lung parenchyma. The bronchiolar epithelium was hyperplastic. There was periarteriolar oedema and marked infiltration of lymphocytes around blood vessels and in later stages, haemosiderosis. Liver revealed focal areas of necrosis, hyperplasia and goblet cell metaplasia of the intrahepatic bile duct epithelium and focal areas of mononuclear infiltration. There was depletion of lymphoid cells in spleen and payer's patches. The vascular changes in urinary bladder were coupled with patches of epithelial hyperplasia. Severe congestion, haemorrhage and degenerative changes were observed in the zona fasciculata and medulla of adrenal gland and the testis revealed hypoplasia. The changes in animals of group B were more or less similar to that in group A except that the initiation of lesions was earlier and the vascular changes were of greater intensity. Moreover, in group B the mononuclear infiltration was mainly focal and nodular in nature; fibroblastic proliferation was less and no goblet cell metaplasia was noticed in the intrahepatic bile ducts. Depletion of

lymphoid tissue and necrosis was observed in the payer's patches in group B. The changes in group D were similar to that of group B but these were late in initiation and milder in nature.

It may be reasonable to infer that the selenium concentrations employed in both the organic and inorganic toxicity produced appreciable adverse effects on feed consumption, body weight gain, haematological indices, total serum proteins and blood glucose levels in recently weaned guinea-pigs. Selenium at toxic doses caused increase in erythrocytic GSH-PX activities and increased levels of selenium in various tissues. Sodium arsenite (at the dose level used in the present studies) could partially protect the animals from inorganic selenium toxicity. Gross and histopathological changes generally comprised the congestion, haemorrhage, cellular infiltration, degenerative and necrotic changes in different organs, and depletion of lymphoid cells, but the epithelial hyperplasia and fibroblastic proliferation was more common in organic selenium toxicity.

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